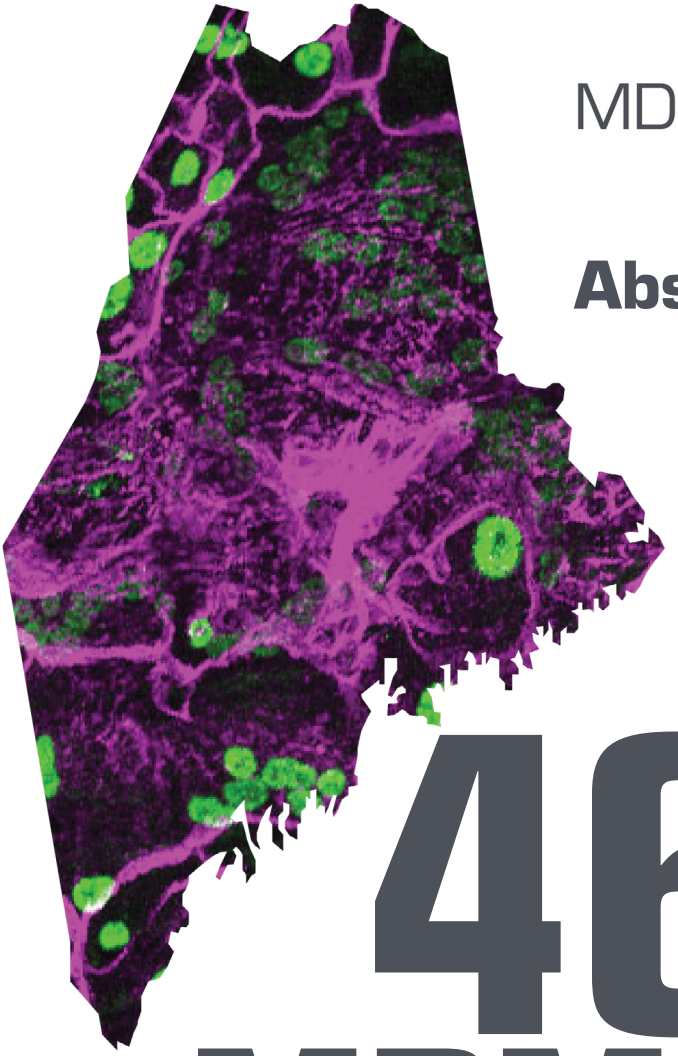

MDI Biological Laboratory

Abstracts

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Cover image credit: Losick Lab (2018), Polyploidy, Drosophila

Honors 350 CFTR Knockdown and the Innate Immune Response

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Cystic Fibrosis (CF) is a genetic disease that not only affects the lungs of patients, but also has a global effect on many processes throughout the body, including the function of the innate immune response. CF affects more than 70,000 individuals globally, with about 1,000 new individuals diagnosed each year [1]. This disease results from a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which inhibits the normal function of an ion channel that allows the passage of Na+ and the flow of water. Patients affected by this mutation are more susceptible to infection, particularly to the pathogen P. aeruginosa , which becomes prevalent in adult patients. Our goal was to examine the effect of the CFTR mutation on the immune response using the zebrafish infectious disease model. Genetic knockdown of the cftr gene in zebrafish simulates CF individuals with defective ion channels and allows for the analysis of the innate immune response to infection. We observed the innate immune response to infection with P. aeruginosa in the zebrafish with a knockdown of the CFTR protein (CFTR MO) compared with control fish. This was investigated through visualizing neutrophil location among the site of infection/wound with confocal microscopy, and by conducting bacterial burden assays. Individuals with the CFTR knockdown displayed less neutrophil migration to the site of infection than the controls, as well as an increased average bacterial burden. These results suggest that CF patients may have a weakened innate immunity, although these results were not statistically significant.

Searching for C. albicans virulence factors that alter phagocyte response

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Candida albicans is the 4th of the most common hospital acquired blood stream infection in the U.S. and can cause serious life-threatening infections (Deveau, Piispanen, Jackson, & Hogan, 2010). In order to more effectively treat these infections, it is important to understand the different virulence factors that C. albicans employs to help it survive and evade the host immune response and be a successful pathogen. Strong early phagocyte recruitment has been seen previously to be important in controlling the infection and increasing survival in larval zebrafish (Brothers, Newman, & Wheeler, 2011). Fish that did not have a strong early phagocyte response typically developed more hyphal growth and succumbed to the infection. Previous work in our lab suggests that C. albicans has a mechanism of limiting one mechanism of this early phagocyte recruitment. This ability to limit phagocyte recruitment seems to be tied to C. albicans ability to grow in the hyphal form (Brothers et al., 2013). In order to determine factors that may be limiting the phagocyte response we are screening C. albicans mutants for virulence and the phagocyte response in the larval zebrafish. Determining these factors may provide insight into targets for more effective treatment of these infections.

CpG island mediated chromatin architectural changes during aging

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Aging is an inevitable process of life accompanying various cellular and physiological degenerative changes. One outcome of aging is the 3D architectural change of chromatin. Normal young nuclei contain silent heterochromatin which associate with the nuclear lamina (underlying the nuclear envelope). In contrast, euchromatin congregate to the nuclear center. Aging disrupts the nuclear lamina leading to the decondensation of heterochromatin. These changes are also observed in premature aging diseases, such as Hutchinson-Gilford Progeria Syndrome (HGPS) and Werner syndrome. However, the cellular and physiological consequence of these chromatin architectural changes are not very clear. Through experimentation and large-scale computational analyses, we present that CpG islands (CGIs) are a crucial component for gene regulation. CGIs are genomic regions with rich Cytosine-phosphate-Guanine dinucleotides. 60% of total human genes contain CGIs at their promoters (CGI+ genes) and are broadly expressed throughout the body. However, the other 40% of genes do not have CGIs (CGI- genes) and are expressed in a tissue-restricted manner. Our results show that for normal young nuclei, CGI- genes resided within lamina-associated heterochromatin when transcriptionally inactive, while CGI+ genes associate with euchromatin, even when repressed. Results further demonstrate that aging triggers global misregulation of CGI- genes that in turn increases noise in gene transcription that leads to the loss of cellular identities. In conclusion, our studies suggest that CGI-mediated chromatin architecture changes play critical roles in age-associated degenerative changes.

Progression of Cardiomyogenesis from Embryonic Stem Cells in a Three-Dimensional Gel Matrix

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Cardiovascular disease (CVD) accounts for about one in three deaths in the United States. Traditional therapies aim to minimize damage caused by CVD, but do little to address the loss of healthy heart tissue following acute injury. Engineered cardiac tissue has emerged as a promising therapy for cardiac tissue repair. In this project, HM1 mouse embryonic stem cells suspended in a three dimensional gel matrix are differentiated in vitro into functional cardiac tissue with a highly reproducible, complex spatial cooperation of multiple cardiac cell types. A field of proliferative fibroblasts grows out from the cell-seeded matrix onto a glass substrate at approximately day 3 of differentiation. A spontaneously contracting network of myosin heavy chain (MHC)-positive cardiomyocytes self-organizes on top of the fibroblasts directly outside the original matrix at approximately day 10.5. This self-organization holds the key for the mechanism behind in vitro cardiomyogenesis. We hypothesize that boundary cues and cell-cell interactions signal the organization and outgrowth of fibroblasts from the matrix onto the glass substrate, followed by Oct4-/Nkx2.5+ cardiomyocyte precursors which require the fibroblast outgrowth in order to further differentiate. Short term specific aims are to map the spatiotemporal development of cell types in the tissue, identifying self-organization induced by boundary conditions. Long term goals are to use these mechanisms of in vitro cardiomyogenesis to engineer cardiac tissue for in vitro drug testing and in vivo cardiac tissue grafts.

Development of a High-throughput Screen for Analysis of JC Polyomavirus Infection

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JC polyomavirus (JCPyV), a human-specific virus, persists in up to 80% of the population as a dormant infection of the kidneys in healthy hosts. In severely immunocompromised individuals, the virus can migrate to the central nervous system (CNS) and cause a fatal disease, progressive multifocal leukoencephalopathy (PML). Challenges in studying JCPyV include the narrow host range of the virus and limited permissible cell lines for in vitro studies. Microscopy-based techniques have been traditionally used to quantify JCPyV infectivity in vitro . However, these techniques present obstacles to timely and impartial determination of infectivity under experimental conditions. These limitations render these techniques unsuitable for high-throughput applications. A new method for analyzing in vitro JCPyV infectivity, the In-cell Western (ICWTM) assay, provides a solution to reduce these limitations through use of an automated imaging system capable of rapid, consistent, and impartial determinations of viral infectivity in cell culture. Our work has focused on the adaptation of this methodology for use in identifying drugs and compounds with anti-viral properties. This optimization of the ICW assay for use in the study of JCPyV will allow for a reliable method for quantifying JCPyV infectivity and will significantly enhance the rate of discovery. The assay will enable high-throughput screening of panels of potential antiviral compounds and the generation of large sets of data for more effectively targeted research.

Characterizing the impact of Mycobacterial prophage by curing M. chelonae of McProf through overexpression of the excise gene

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Mycobacterium tuberculosis is the leading cause of mortality among all infectious agents to date (Fan, et al. 2015. J Biomol Struct Dyn. 34:2, 233-238). It is responsible for 1.3 million deaths per year and is the leading cause of death among HIV-positive individuals. Effective treatment for Tuberculosis remains elusive as the number of multidrug-resistant M. tuberculosis strains are ever increasing (Global Tuberculosis Report 2018, WHO). All pathogenic strains of the M. tuberculosis complex carry prophage that are hypothesized to play a role in host fitness, although without an obvious virulence factor. In M. chelonae , a pathogen related to M. tuberculosis , we have demonstrated drastic changes in bacterial gene expression in the presence of prophage BPs. M. chelonae also carries a naturally occurring prophage, McProf, that has not previously been characterized, nor has its impact on bacterial gene expression been determined. The McProf genome appears complete and encodes 99 genes. RNA-sequencing analysis of M. chelonae indicated expression of 16 McProf genes. Three membrane proteins were identified that could prevent bacteriophage superinfection. To further study the role of McProf in bacterial gene expression and fitness, the McProf genome needs to be cured from M. chelonae . We have cloned the McProf xis gene and are overexpressing it in M. chelonae to isolate cells in which the prophage genome has been excised. By studying the interactions between naturally occurring prophage and mycobacteria, we increase our understanding of how prophage impact bacterial fitness and virulence and can potentially identify new targets for treatment.

The role and regulation of age-induced polyploidy in Drosophila

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Polyploid cells frequently arise during normal aging and with age-associated diseases, including cancer. It remains poorly understood whether age-induced polyploidy functions as a beneficial tissue repair strategy or a driver of disease. The adult abdominal epithelium of Drosophila is composed of post-mitotic, diploid cells that become polyploid with age. We have characterized multiple Drosophila melanogaster strains and discovered that polyploid cells begin to form by 20 days of age with approximately 20% of the epithelial cells becoming multinucleated. By 40 days of age, large multinucleated cells, some with 30+ nuclei, are evident and appear to help to maintain epithelial integrity. In addition, we found that epithelial nuclear DNA content does not change and diploidy is maintained, indicating that age-induced polyploidization is a result of cell fusion, and not endoreplication. Taken together, these results and our on-going studies will provide novel insights into how polyploid cells emerge with age and affect tissue function.

Developmental programming of the HPA axis by chronic cortisol

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Adapting to a changing environment requires the ability to modulate biological processes to make efficient use of finite resources. To this end, the hypothalamus-pituitary-adrenal (HPA) axis integrates multiple inputs including predictable circadian cues as well as acute and perceived stressors, thereby regulating homeostasis while remaining poised for potential challenges. Glucocorticoid (GC) hormone signaling is a major output of the HPA axis. GC signaling is by nature highly dynamic, while chronic levels of elevated GC are associated with a number of well documented but incompletely understood disease states. Our lab has shown that treatment of zebrafish embryos with chronic GC leads to long-term dysregulation of immune and inflammatory genes, and we are now working to describe the mechanisms connecting chronic developmental GC with long-term health problems. Here, we show that chronic GC during development changes the expression dynamics and epigenetic profiles of genes involved in the GC regulatory circuit, which may indicate altered programming of this adaptive network. Altered development of the HPA axis under chronic GC may also contribute to long-term dysfunction in the axis itself, and RNA-seq data reveals genes and processes that may mediate pathogenesis in key tissues.

Optical Exploration of the Adipose Tissue Microenvironment using 2-Photon Microscopy

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Obesity often leads to neuropathy, a comprehensive term for damage of the peripheral nervous system which can be a severe source of discomfort. Previous work by the UMaine Townsend group has shown that neuropathy extends into adipose tissue resulting in significant peripheral nerve damage. In an obese state, adipose tissue becomes fibrotic, resulting in excess collagen deposition. Collagen aids in the organization and myelination of the nervous system. We propose a 2-photon approach to image the spatial relationship between collagen and nerve in the adipose microenvironment during obesity to gain a better understanding of neuropathy pathways and mechanisms. Our current analysis suggests that an obese diet results in greater colocalization. We aim to extend our analysis into a threshold-independent regime using a recently adapted astrophysics technique, the Metric Space Technique (MST). MST will return four output functions for each collagen and nerve image. We will calculate the metric distance between the nerve and collagen images as a means of determining how structurally related collagen and nerve are in healthy and obese adipose tissue.

Photic regulation of voluntary ethanol intake in mice: possible role for melanopsin signaling?

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We recently found that constant light (LL) or constant darkness (DD) reduces voluntary, free-choice ethanol intake in mice, relative to maintenance in standard light-dark (LD) conditions. Thus, the current study examines whether photic effects on ethanol intake are mediated by melanopsin signaling through ipRGCs. Voluntary ethanol intake was assessed under LD, LL, and DD conditions in two different engineered mutant lines and in wild type (WT) male and female mice of the same genetic background (B6x129). One mutant line carries a transgene that functionally deletes the melanopsin peptide (Opn4CRE/CRE), while the second line carries a construct that results in the developmental deletion of the ipRGCs (Opn4aDTA/aDTA). Animals were maintained individually in running-wheel cages and were offered free-choice of 10% ethanol and plain water while maintained under a series of lighting conditions for 3 weeks per condition (either LD-LL-LD or LD-DD-LD). WT mice showed stable entrainment in LD, shorter than 24-hour periods under DD, and longer than 24-hour periods and loss of rhythmic coherence in LL. As in prior studies, Opn4CRE/CRE mice displayed apparent reductions in circadian photosensitivity, characterized by less stable entrainment and shorter periods in LL. Both WT and Opn4CRE/CRE mice showed reduced ethanol intake in both LL and DD, but smaller effect sizes were seen in mutants. These results support the hypothesis that effects of lighting regimen on voluntary ethanol intake, like photic effects on the circadian clock, are mediated, in part, by melanopsin-dependent photoreception. We hypothesize that both effects will be completely abolished in Opn4aDTA/aDTA mice.

mTERT marks adult neural stem cells that give rise to neurospheres in vitro

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Renewal of neurons within the adult brain depends upon the differentiation and migration of adult neural stem cells (ANSCs). Quiescent ANSCs (qANSCs) reside within neurogenic niches of the brain and allow for long-term production of neurons throughout life. Neurodegenerative disorders and qANSC diseases are poorly understood, and there is currently no specific and unique marker for these qANSCs, hindering our understanding of these critical cells. Mouse telomerase reverse transcriptase (mTERT) is a telomere-extending nucleoprotein complex that prevents cells from becoming senescent after repeated divisions, and was previously shown to mark slowly-cycling stem cells in the adult mouse intestine. Our lab previously revealed that mTERT+ cells reside within the adult mouse brain in known neurogenic areas. Additionally, these cells express markers of ANSCs, but not transit amplifying cells, neuroblasts, or mature neurons (part of the differentiation pathway), indicating they are quiescent in nature. Here, we show that mTERT+ cells from reporter and lineage tracing mouse lines display stem cell properties. Specifically, mTERT+ cells from direct reporter and lineage tracing mice were separated from mTERT- cells via FACS sorting and formed neurospheres in culture. The presence of mTERT+ cells in known neurogenic niches of the adult mouse brain, paired with the ability of these cells to form neurospheres in vitro , led us to hypothesize that mTERT marks qANSCs in the adult mouse brain.

Characterization of miR-7 and miR-147b expression during CD8 T cell differentiation

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T cell activation through metabolic induction of aerobic glycolysis is required for efficient effector function of cytotoxic T cells (CD8 T cells). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been shown to be involved in CD8 T cell effector function through the binding of certain cytokine transcripts and altering their translation. GAPDH is a non-canonical RNA-binding protein (RBP) that can bind many RNA scaffolds, like transfer RNAs (tRNAs), cellular RNAs, ribozymes, and viral RNAs. We found through an RNA-seq screen of effector CD8 T cells that GAPDH had an extremely high affinity to microRNAs (miRs) 7 and 147b; miR-7 in a low glycolytic condition and miR147b in a high glycolytic condition (8 times log and 10 times log level respectively). To study the roles of these miRNAs in the differentiation of CD8 T cells into effector and memory phenotypes, we examined their levels in differentiated cells by qPCR. MiR-7 had high expression in naïve cells compared to effector and memory cells, while miR-147b was expressed at high levels in effector cells. These results suggest that these miRs may play a prominent role in T cell differentiation. Further experiments need to be conducted to study and validate the effect of these miRNA targets on GAPDH in regulating the effector and memory function of CD8 T cells.

Keywords: GAPDH, microRNA, CD8 T cell

The CompuMAINE Laboratory

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The CompuMAINE Lab is a computational lab dedicated to digital science and data driven outcomes . By developing and implementing novel signal processing and image analysis techniques, CompuMAINE integrates mathematics, physics, artificial intelligence, machine learning, data mining, and computational engineering approaches to study a wide variety of applications. The ever-increasing demand for state-of-the-art big data analytics expertise combined with our rapidly expanding visibility in the US and beyond has led several research labs and institutions seeking out CompuMAINE?s collaborative help. The word CompuMAINE is an acronym for Computational Modeling, Analysis of Images, and Numerical Experiments.

Focused research projects are centered on Radiomics , a new field of medical study that aims to extract large amounts of quantitative features from medical images using data-characterization algorithms. At CompuMAINE this is done via the analysis of biomedical big data from mammography and patient medical / pathological information to help improve breast cancer diagnostic accuracy. Other application examples include Medicine (cancer, with soft (lymphoma/leukemia) and solid tumors (e.g. breast, pancreatic), neurological diseases (epilepsy, Huntington?s disease, Lou Gherig?s disease), neurogenetics (Down syndrome), muscular dystrophy); Biophysics (neurodevelopment, cell nucleus architecture); Biomedical Engineering (artificial bone implants, protein modeling, astrobiology); Physics / Geophysics / Astrophysics (climate change (glaciology), surface science (ultra-thin gold surfaces), solar physics, interstellar medium, cosmology); Pure Mathematics (fractal structures in Pascal?s Triangle).

Decrease in the Histone Acetyltransferase KAT6B with Aging Regulates Differentiation of Hematopoietic Stem Cells

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As lifespan is increasing globally, there is a critical need to identify strategies to extend health span and prevent chronic diseases into older age. With age, hematopoietic stem cells (HSCs) undergo altered lineage priming and differentiation, skewed towards production of myeloid cell types at the expense of lymphoid cell types. This correlates with decline in immune function observed in aging. Comprehensive knowledge of gene regulatory and epigenetic mechanisms underlying this defect is a barrier to developing therapies to ameliorate aging associated decline in HSC function. We performed an shRNA screen focused on epigenetic factors, representing targets amenable to small molecule inhibitors. We found that knockdown of Kat6b replicated aging-associated hematopoietic phenotypes of myeloid lineage bias at expense of lymphoid lineage. We demonstrate that KAT6B is significantly decreased in expression in old HSCs at the transcript and protein levels. By using an in vitro myeloid differentiation assay we further show that Kat6b knockdown leads to increased myeloid output and enhances serial re-plating capacity of colony forming units (CFUs). In addition, we show that decreased expression of Kat6b in vivo leads to enhanced myeloid differentiation and decreased B-lymphoid and red blood cell differentiation. Together, this demonstrates that decline in KAT6B levels as observed in old HSCs causes impaired differentiation to B-lymphoid and erythroid cell types, while increasing myeloid differentiation. Thus, targeting Kat6b or its target histone modifications is a novel strategy to ameliorate aging-induced decline in HSC function.

Zebrafish as a model to unravel the impact of strength training on Duchenne Muscular Dystrophy

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Skeletal muscle plasticity is imperative for functional adaptation to changing demands. Increased activity initiates muscle growth and decreased activity initiates muscle wasting. Although a great deal is known about the structural and functional plasticity of healthy skeletal muscle, far less is known about plasticity in diseased muscle. Here, we combined the power of the zebrafish model with the adaptability of neuromuscular electrical stimulation (NMES) to study the impact of strength training on muscle architecture, muscle function, and survival in the zebrafish model of Duchenne Muscular Dystrophy (DMD). Four NMES programs, defined by their frequency, delay, and voltage, were designed to emulate each type of strength training: endurance, hypertrophy, strength, and power. Three sessions of endurance NMES improve muscle architecture, increase swim velocity and distance traveled, and extend survival. In contrast, three sessions of hypertrophy NMES worsen these outcome measures. Three days of inactivity (immobilization) worsen muscle architecture and decreases survival. Strikingly, inactivity followed by a single session of NMES (endurance or power) further worsens muscle architecture and obliterates its ability to regenerate. Our data provide a new methodology with which to study muscle plasticity in healthy and diseased muscle. In addition, our data clearly indicate that, at least in the zebrafish model, some strength training (endurance) is beneficial whereas inactivity (which is currently prescribed to human patients) is deleterious.

A modern general carbohydrate force field model captures the conformational properties of iduronic acid

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Proteoglycans are protein-carbohydrate conjugates found in extracellular matrix and associated with cell membranes. An example of the carbohydrate component of proteoglycans is the glycosaminoglycan heparan sulfate (HS). HS is composed of repeating disaccharide units of iduronic acid (IdoA) and N-acetylglucosamine. IdoA is a highly flexible hexose sugar, and therefore impacts the conformational properties of HS. The difficulty in generating pure samples and the inherent flexibility of HS continue to pose barriers in conformational analysis of HS at the atomic level. Therefore, we are using atomic-resolution molecular simulations to this end. In contrast to conflicting reports in the literature between experimental and simulation studies on HS ring pucker, we show that a modern general carbohydrate force field produces HS ring puckering preferences in line with the most recent experimental data. Therefore, this force field approach can be applied toward the problem of computational analysis of HS conformation.

TAp63 deficient mice maintain fertility after sterilizing dose of irradiation

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TAp63 is a checkpoint marker for DNA damage in germ cells. In response to radiation induced double-strand breaks, TAp63 is phosphorylated by CHK2 kinase resulting in apoptosis of oocytes within primordial follicles (PFs). In female mice deficient in TAp63 (TAp63KO) or expressing inactive TAp63 (TAp63AA), oocytes survive low-dose irradiation (0.5Gy) and fertility is maintained compared to irradiated controls. In ex vivo organ cultures of TAp63KO and TAp63AA ovaries, high-dose irradiation (3Gy) results in almost complete oocyte depletion. Surprisingly, when TAp63KO and TAp63AA mice were exposed to 3Gy irradiation, fertility was sustained. Analysis of oocyte survival one- and two-weeks post-irradiation revealed that only a small number of PFs survived. We suspected the observed fertility in p63 mutants could be a result of either de-novo generation of oocytes from unidentified stem cells promoted by the absence of functional p63 or survival of a subset of oocytes sufficient to maintain fertility. Immunohistochemistry of ovarian sections from one- to seven-weeks post-irradiation revealed the absence of stem cell markers in the ovary. While stem cell markers were not present, a small population of PFs remained, potentially sufficient to maintain fertility. Based on this preliminary data, we propose that the absence of functional TAp63 allows for preservation of oocytes in PFs upon DNA damage induced by irradiation in a dose dependent manner. Understanding how fertility is maintained in mice that lack functional TAp63 after irradiation will allow us to develop ovarian-protective therapies to preserve ovarian function and fertility in female cancer patients undergoing radiation therapy.

Novel ROR mutant displays characteristic “high-stepper” gait phenotype and retinal abnormalities.

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Murine retinoid-related orphan nuclear receptor (Rorb) has two splice variants with alternative first exons and encodes a transcription factor thought to play a role in neuronal cell fate determination. Rorb-203 is expressed in layer IV of the somatosensory cortex and in the retina. Rorb-201 is expressed only in the retina, and mutations in the two isoforms have been observed in spontaneous mutants that display the high-stepper gait phenotype. Some of these mutants also show photoreceptor degeneration dependent on the exact nature of the mutational event and the isoforms impacted (Tadenev et al., Unpublished). For instance, a high-stepper mutant (Hstp1) on the C57BL/6J background with a duplication impacting exon 1 of Rorb-203 showed the gait phenotype with no retinal abnormality. Histological and immunofluorescence approaches performed on a novel high-stepper mutant with a DBA/1J background retrieved by deviant search at The Jackson Laboratory depict photoreceptors lacking outer segments.Sequencing of each Rorb isoform revealed a single nucleotide substitution causing a missense mutation in the start codon of Rorb -203. This result suggests that mutation of Rorb -203 is sufficient to induce both the gait phenotype and photoreceptor abnormality on the DBA/1J background and that genetic background may modulate the severity of phenotypes associated with Rorb mutations.

Using Neonatal Zebrafish to Model a Bacterial Meningitis Infection Caused By Streptococcus agalactiae

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There is an overwhelming need for alternative treatments to bacterial infections than the antimicrobial treatments currently available. Use of modern antibiotics comes with the risks of antibiotic resistance development and the killing of the gut?s natural flora. To develop alternative treatments, we must have a better understanding of how the immune system works. The innate immune system is the body?s first line of defense against invading pathogens and is relied upon in organisms that lack a fully developed adaptive immune system, like newborns. While some aspects of the innate immune system are well characterized, there are still many aspects that are uncharacterized. To do this, we have developed a model to simulate the effects of a bacterial infection on the innate immune system of a newborn using a neonatal zebrafish model of bacterial meningitis. Meningitis caused by Streptococcus agalactiae is a devastating infection to newborns that can lead to sepsis or death. Infection survival can also result in long-term neurological effects like seizures, blindness, deafness, or cognitive defects. Juvenile zebrafish are an ideal model organism because they have well-developed innate immune systems and are transparent, allowing live imaging by microscopy in real time. We will use well-established methods like RT-PCR and RNA-sequencing to identify immune factors that show changes in expression at different time points in the infection. Being able to identify these factors opens the door for development of alternative treatments to antibiotics through exploring the use of these immune factors as therapeutics or biomarkers of infection.

The Effect of Biophysical Properties of Proteins on their Localization and Spatial Distribution

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There are many physical and chemical processes governing physiological cellular function and the distribution of molecular proteins in the cell for efficient functioning. While research in cell and molecular biology has largely focused on biochemical processes of protein interactions, this study is aimed at elucidating one of these many important factors that determine the localization and movement of molecular proteins in their native characteristics in the cell. The Stokes-Coulomb effect and cellular radially directed electric field, is described here mathematically in a novel dimension using a multi-pronged strategy. First, it examined the original distribution or native arrangement of proteins based on their isoelectric point and mass in the cellular pH gradient. Second, it discusses the relationship between Stokes' frictional force and charged protein Coulomb interactions. Third, it demonstrates the dynamic stability of protein and the effect of Brownian motion on them. This effect also determines the location of one protein relative to another. The biophysical results derived here indicate that charged proteins are specially localized to where their function is vital allowing for more efficient spatiotemporal cellular function. Taken together, this work proves that there is a strategic dynamic localization of protein molecules and that protein movement is not entirely determined by random walk. Understanding these biophysical mechanisms can further shed light on the advancement of research of oncogenic cells, aberrancy in the function of excitable cells such as neurons and cardiomyocytes

Role of Arsenic and CFTR in Neurological Development and Regulation of Immune Processes

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Arsenic exposure has been shown to negatively impact neurological function and innate immunity. In parts of rural New England, 10% of private wells have arsenic levels that exceed the federal safety limit of 10 ppb. However, previous research indicates that levels of arsenic below this threshold have a substantial negative impact on innate immune functioning and neural cell development. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), a gene mutated in individuals with cystic fibrosis, has been linked to decreased innate immune function. Discovering differentially expressed genes in response to low-level arsenic exposure and the compounding effects of CFTR antisense morpholino knockdown will provide new hypotheses for future research in arsenic neurotoxicity and innate immunity. We analyzed an existing RNA sequencing dataset to discover such differentially expressed genes in 24 hpf zebrafish embryos. These data characterized zebrafish subjected to the experimental factors of low-level arsenic exposure, CFTR knockdown, and infection with the opportunistic pathogen Pseudomonas aeruginosa at 6, 12 and 18 hpi. Genes imperative for proper neurodevelopment, such as those that function in the notch signaling pathway and oligodendrocyte progenitor development, were down-regulated in zebrafish exposed to low-level arsenic. Also, CFTR knockdown affected various immune cell receptors and decreased expression of CXCR3.2 , a protein vital for macrophage recruitment. These data reveal what are likely key factors in understanding how environmental stressors and genetic perturbations impact neurological function and innate immunity. Our study was funded by National Institutes of Health grant P20 GM103423 and the RNA-Seq study was funded by P20 GM103534.

Incidence of Avian Pox DNA in Mosquitoes of South and Central Maine in 2016

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Avian Pox Virus is a pathogen devastating to wild and domestic birds. Although some transmission through direct contact has been reported, Avian Pox is primarily transmitted by mosquito vector. DNA from 450 pooled mosquito samples from traps set in southern and central Maine in 2016, were tested for a 578nt fragment of the 4b core gene of avian pox virus (fowlpox). PCR of the samples showed 24 bands near the 580 molecular weight marker. From the 24 possible positives, 9 bands were sequenceable. Using a BLAST nucleotide search, 5 of 9 sequences were positive matches for a fragment of the 4b core gene in fowlpox with >99% similarity. Avian Pox is a chronic disease that causes mild to moderate skin lesions on the featherless regions of birds causing severe disfigurement. The lesions appear on the face, legs and feet. However, when the mucous membranes (such as found in the oral cavity or respiratory tract) are infected, respiration is debilitated, and death occurs. The transmission of Avian Pox can eliminate entire flocks, causing severe impairment to the ecological system and upset the natural balance of the environment.

Dynamic regulation of G-protein signaling in S. cerevisiae

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G protein-coupled receptors (GPCRs) are involved in numerous signaling processes ranging from neuronal growth to immune cells tracking invaders. GPCR signaling plays a role in many human diseases and thus GPCRs are important drug targets. Yeast respond to mating pheromone using a GPCR signaling system homologous to those used in humans to polarize their cytoskeleton toward the pheromone source. This is accomplished by initializing a MAPK signaling cascade to arrest the cells in mitosis and upregulate expression of chemotropic proteins. Pathway desensitization is accomplished by the Regulator of G-protein Signaling (RGS). RGS abrogates signaling by binding to the active GPCR and downstream effectors, accelerating hydrolysis of GTP bound G-proteins to bring them to an inactive state. Previous studies have found the RGS undergoes feedback phosphorylation by the MAPK, though the effect of this modification on RGS function was not determined. We examined the spatiotemporal dynamics of the RGS using fluorescent live cell imaging in a microfluidics gradient chamber and performed computational image analysis of single cells. We have found that RGS changes in localization during the pheromone response are controlled by phosphorylation, removing the RGS from barrier proteins, known as septins. Furthermore, our data suggests this phosphorylation site may regulate the transition from mitosis to chemotropic growth, allowing the cell to finish mitosis prior to entering the pheromone response.

Prophage BPs drives differential gene expression in pathogenic M. chelonae

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Pathogenic bacteria cause lower respiratory infections, diarrhoeal diseases, and tuberculosis and are the cause of death for nearly six million individuals worldwide each year (www.who.int). Nearly all pathogenic bacteria contain integrated bacteriophage genomes, or prophage, which contribute to the virulence and fitness of the host cell. One example is the enhanced virulence of E. coli O157:H7 which carries a shiga toxin-encoding prophage (Muniesa et al., Infect Immun ., 68:4850?4855, 2000) All pathogenic strains of the Mycobacterium tuberculosis complex likewise contain prophage regions, most often phiRv1 and phiRv2 (Fan et al., J Biomol Struct Dyn ., 34:233?238, 2015). However, the impact of mycobacterial prophage on host gene expression is poorly understood. Nontuberculous Mycobacterium species, such as M. chelonae , are fast-replicating and effective models for studying the effects of prophage on mycobacterial gene expression. RNAseq analysis of M. chelonae with and without prophage BPs reveals significant differential expression of 7.4% of the host genome. This includes genes whib7 , tap , and eis , which are related to antibiotic resistance and enhanced intracellular survival of M. tuberculosis in macrophage (Burian et al., Expert Rev Anti Infect Ther ., 10:1037-1047, 2012). We hypothesize that changes in gene expression will translate to phenotypic differences in BPs-M. chelonae as compared to wild-type M. chelonae . Antibiotic sensitivity and macrophage survival assays are being conducted to quantify these proposed phenotypic differences. As M. tuberculosis and M. chelonae are clinically-relevant pathogens, understanding their mechanisms of virulence is critical in the development of novel treatment strategies.

Understanding the role of CpsA in Streptococcal infection

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Group B Streptococcal (GBS) infections pose a great threat to mortality in neonates. Neonates are often exposed to GBS both before, during and after delivery, which can cause a range of health problems including meningitis, sepsis, or even stillbirth. One of the major virulence factors that contributes to the infectivity of the pathogen is the bacterial capsule. The capsule is a polysaccharide matrix surrounding the cell which helps in the evasion of host defenses, and penetration deeper into normally sterile sites like the bloodstream. The highly conserved GBS protein CpsA has been shown to regulate expression of the capsule [1]. The objective of this study is to identify protein-protein interactions with CpsA, as well as truncated versions of CpsA, using co-immunoprecipitation protocols to pulldown CpsA binding partners. Research has previously shown that deletion of the LytR domain, which is an extracellular domain following three membrane-spanning domains and an accessory domain, has a negative impact on capsule production, therefore indicating an extracellular binding occurrence in this domain [1]. Further evidence shows that a portion of the extracellular domain potentially binds at the septum of the cell, however the specifics of this interaction remain unknown. By demonstrating an interaction with other proteins, further targets could be identified for treatment of GBS infections.

Genomic and proteomic effects of Red Raspberry (Rubus idaeus) consumption on inflammation in perivascular adipose tissue of the obese Zucker rat, a model of human metabolic syndrome

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The Metabolic Syndrome (MetS) affects 35% of U.S. adults and is an indicator of early death. While pharmacological treatments have been developed for the majority of MetS risk factors, obesity-induced inflammation remains to be addressed. Dysfunctionaladipose tissue is a source of inflammation, and perivascular adipose tissue (PVAT) is critical in its pathogenesis.This study investigates the effects of red raspberry (rubus idaeus) diet-enrichment on inflammation of PVAT. The obese Zucker rat (OZR) model of MetS and the lean Zucker rat (LZR) control model were used. Rats received an eight-week control or red raspberry-enriched diet (8% w/w red raspberrypowder). RT-PCR was performed on LZR and OZR PVAT homogenates to determine gene expression of pro-inflammatory markers (IL-1?, IL-6, MCP- 1, NF- ?B, TNF- ?) and anti-inflammatory markers (adiponectin and IL-10), and ELISAs were performed to determine concentrations of a subset of these markers (adiponectin, IL-1?, IL-10, MCP-1). RT-PCR analyses of PVAT indicated a significant down-regulation of pro-inflammatory marker NF-kB in obese control (OC) versus lean control (LC) models. ELISA analyses indicated a significant decrease in anti-inflammatory marker IL-10 concentration in OC versus LC models, a significant decrease inpro-inflammatory marker IL-1B concentration in OC versus LC models, and a significant elevation in anti-inflammatory marker adiponectin concentration in obese raspberry (OR) versus OC models. Findings suggest that red raspberry enrichment does not have a consistent genomic or proteomic effect on PVAT inflammation status. Further investigations are needed to elucidate themolecular mechanisms dictating the pro-inflammatory and anti-inflammatory effects observed.

Investigating SR-like RNA-binding protein 1 phosphorylation and links to mRNA transport complexes in the pathogenic fungus *Candida albicans*

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The transition of the opportunistic fungal pathogen Candida albicans from budding growth to filamentous hyphal growth is implicated in virulence. Multiple pathways influence the hyphal-specific expression of virulence factors, and our work focuses on understanding mechanisms involved in directional transport of mRNAs to allow on-site translation of proteins at the hyphal tip. Predominantly nuclear SR-like mRNA-binding protein Slr1 and cytoplasmic mRNA transport protein She3 are both required for normal hyphal growth (Ariyachet et al., Infect Immun, 81:1267-76, 2013; Elson et al., PLoS Genet, 5:e1000664, 2009). The hyphal tip localization of an Slr1 protein with serine-to-alanine mutations that block phosphorylation, slr1-6SA, is decreased in the absence of She3 (Ariyachet et al., Mol Microbiol, 104:499-519, 2017), suggesting that phosphorylation and interaction with She3 affect Slr1 localization and function in hyphae. We now show that She3-FLAG co-immunoprecipitates with Slr1-GFP and slr1-6SA-GFP from hyphal cell lysates, supporting a possible role for Slr1 in She3-directed mRNA transport. To dissect the impact of phosphorylation on Slr1 localization, additional deletion and substitution mutations were introduced in the C-terminal SR domain of Slr1, and wildtype Slr1-GFP was expressed in cells lacking one or both SR protein kinases, Sky1 and Sky2. Analysis of phosphorylation by gel electrophoresis and protein localization by epifluorescence microscopy reveals an intriguing complexity of Slr1 phosphorylation. Future work will explore the impact of Slr1 and its phosphorylation on hyphal mRNA transport in C. albicans.

Role of the transcription factor Nfe2 and pro-oxidant exposure in inner ear development in zebrafish

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Millions of people worldwide suffer from hearing loss. While several mechanisms have been associated with this loss, the role of oxidative stress remains underexplored. NFE2, a transcription factor in zebrafish and other vertebrates, is localized to the otic vesicle during development and has been shown to mediate oxidative stress. Therefore, it was hypothesized that the absence of Nfe2 would promote otolith deformities, especially upon exposure to pro-oxidant chemicals. This hypothesis was tested by transiently decreasing Nfe2 expression using a morpholino and subsequently measuring otolith and neuromast development. Nfe2 knockdown in the Brn3c transgenic line significantly decreased otolith distance at 24 and 72 hours post fertilization (hpf), increased otic vesical width at 24, 48, 72 and 96hpf, and increased vesicle length at 24, 72, and 96 hpf compared to control ($p<0.05$). However, there was no significant difference in the phenotypes of neuromast cells between wildtype and nfe2 morphants. Previous studies have shown that the pro-oxidant tBOOH induces changes in wildtype neuromast morphology, but the role of Nfe2 in these changes is unknown. In the wildtype these changes were thought to be due to upregulation of the cilia and flagella associated protein Cfap70 in our nfe2 knockout model. However, morpholino knockdown of cfap70 in wildtype and nfe2 knockout larvae did not produce significant differences in otolith distance, vesicle width or length. These results demonstrate a role for Nfe2 and oxidative stress in inner ear development, but future studies are needed to further elucidate the molecular basis of the response.

Steps towards identification of antimicrobial compounds from macroalgae against MRSA and other gram-positive bacteria

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As the effectiveness of current antibiotics are steadily on the decline there is a growing need to explore our environment in search for new antimicrobial agents. The antimicrobial activity of three local macroalgae species (Ahnfeltia plicata , Chondrus crispus , and Fucus vesiculosus) was investigated, and steps were taken towards identifying the responsible compound(s). The antimicrobial activity was assessed by testing four gram-positive human pathogens, deemed by the World Health Organization as priority pathogens with regards to the need for antibiotic development. This includes Staphylococcus aureus strain USA300 and Newman, Listeria monocytogenes, and Bacillus cereus . Methanolic extractions were performed, the activity of extracts confirmed and the minimum concentration required to inhibit bacterial growth determined. This was done using an antimicrobial disk assay as well as a minimum inhibitory concentration (MIC) assay. A. plicata was generally the most active, followed by C. crispus and thenF. vesiculosus. Using high pressure liquid chromatography (HPLC), extracts were fractionated, and the active fraction determined by antimicrobial disk assays. Preliminary results indicate the most polar fraction is responsible for the antimicrobial activity. This has implications about the active compound(s) and gives direction for further research.

Mechanisms of Defective Neuromuscular Development in a Novel Zebrafish Model of GMPPB -Associated Dystroglycanopathy

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Muscular Dystrophy (MD) affects ~250,000 individuals in the United States and is a debilitating group of diseases characterized by progressive skeletal muscle degeneration. Individuals with mutations in a gene required for dystroglycan glycosylation, GMPPB (GDP-mannose pyrophosphorylase B), clinically present with variable MD phenotypes and ages of onset ranging from birth to adulthood. In order to determine the underlying mechanisms for variable phenotypes in GMMPB -associated dystroglycanopathy, we studied gene expression in a novel zebrafish model where gmmpb was mutated using CRISPR/Cas9 and compared to wild-type (normal) zebrafish. Embryonic gmmpb-/- mutant zebrafish exhibit mild to severe neuromuscular phenotypes. We hypothesize that the severity of GMMPB dystroglycanopathies are due to differential expression of key regulatory genes and pathways involved in neuromuscular development. This interdisciplinary research collaborative aimed to better understand GMMPB dystroglycanopathies by integrating developmental biology, genomic, and computational approaches to identify dysregulated pathways in gmmpb-/- mutants. RNA Sequencing was performed ongmppb mutants and corresponding wild-type controls at 4 days post fertilization (dpf) to identify differentially expressed genes, including mmp9 and mmp13 which have known roles in MD. Ongoing studies of coding and non-coding genes expressed at 4 and 7 dpf seek to characterize how gene regulatory networks differ in gmmpb-/- mutants with either mild or severe neuromuscular development phenotypes. A better understanding of the pathways misregulated in GMMPB dystroglycanopathies is essential to identifying new targets for therapeutic drugs.

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Impact of Antileprosy Medication Dapsone on Neurodegenerative Diseases Modeled in Caenorhabditis elegans

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Huntington's disease and Alzheimer's disease are two different neurodegenerative diseases that are associated with aging. Neurodegeneration occurs due to loss of function within the nervous system and often involves the inability of a neuron to signal. Huntington's disease is an autosomal dominant genetic disorder resulting in polyglutamine expansions on the huntingtin protein leading to misfolding and protein aggregation within neurons. This aggregation is associated with neurological malfunction and ultimately leads to uncontrollable movements and cognitive impairment. Alzheimer's disease is a progressive dementia disease that is characterized by the accumulation of amyloid plaques between the neurons in the brain. Dapsone (DDS) is a current therapy for the treatment of leprosy but is also known to extend lifespan in aging models such as Caenorhabditis elegans . DDS is rapidly absorbed in the gastrointestinal tract and can be seen in all tissues after absorption. DDS is known to act as an anti-inflammatory in humans but also decreases bacterial cell synthesis. Since the inflammatory pathway does not exist in C. elegans it is predicted that the overall lifespan benefits are coming from a reduction in protein synthesis which has a lifespan and healthspan promoting effect. This study hypothesized that Dapsone would delay the disease pathogenesis in two C. elegans models of neurodegeneration. Results of this study indicated that chronic treatment of DDS improves health in C. elegans models of Huntington's disease and Alzheimer's disease.

McKinley: An Isolated, Annotated DE1 Cluster Bacteriophage

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Bacteriophages are good model organisms to help develop our understanding of viruses. This study was conducted to identify a new bacteriophage, and annotate its genome. Gordonia terrae phage McKinley was found in a water sample in Farmington, Maine. It was isolated, purified, amplified, and DNA was extracted. The phage found was a subcluster DE1 siphoviridae phage with 87 genes and 59,022 bp in its genome. McKinley contains a MerR-like helix-turn-helix DNA binding protein, which helps regulate transcription. McKinley will later be added to a global database for assistance in viral research.

Polyploid cell growth restores tissues mechanics post injury

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Polyploidy frequently arises in response to injury, disease, and age-related tissue degeneration. Despite its prevalence, major gaps exist in our understanding of how polyploid cells emerge and alter tissue function. In the adult Drosophila epithelium, wound healing is dependent on the generation of multinucleated polyploid cells, which results in a permanent change in the epithelial architecture. Here, we study how wound-induced polyploid cells affect tissue function by altering tissue mechanics. We have found that the mechanosensor, non-muscle myosin II (Sqh) is activated (phosphorylated) and upregulated during wound healing. Sqh activation is dynamic and required not only early to facilitate wound closure, but also persists in the polyploid cells after healing completes. The upregulation and phosphorylation of Sqh are known to correlate with enhanced tissue tension, suggesting that polyploid cell growth alters tissue mechanics. Using laser microsurgery, we found that the relative tissue tension is significantly enhanced in polyploid epithelial cells and dependent on Sqh activity. Injury to the abdomen also damages the underlying muscle fibers, which are permanently severed. Remarkably, we found that the enhanced polyploid epithelial tension mimics the relative tension of the lateral muscle fibers. Therefore, polyploid cell growth enables the epithelium to adapt and become muscle-like to compensate for lost tissue mechanics.

Injury-induced nociceptive sensitization in larval and adult Drosophila

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Nociceptive sensitization underlies and perpetuates chronic pain, a condition that affects millions of people nationwide. With many of the treatment options for chronic pain, such as opioid analgesics, carrying numerous side effects including the potential threat for addiction, research into safer and more effective treatment options for chronic pain relief is crucial. An effective approach toward this understanding is investigation into the mechanisms and pathways of nociceptive sensitization. Recently, an injury-induced nociceptive sensitization model was developed using the larvae of the fruit fly, Drosophila melanogaster , and a novel pathway that produces this nociceptive sensitization, the Bone Morphogenetic Protein (BMP) pathway, was revealed. At present, we propose to further our investigation into new mechanisms and biochemical pathways that may also underlie chronic pain. We intend to explore additional components within multiple biochemical signaling pathways and their possible injury-induced effects on Drosophila nociceptors. We are conducting pathway analysis of RNA isolated via Translating Ribosome Affinity Purification from the nociceptors of injured larvae in order to uncover new components. Because the larval stages of fruit fly development are relatively brief, we are also developing a methodology that allows longer term experimentation of nociceptive sensitization after injury in adult fruit flies. To this end, we have started mapping the adult fruit fly nociceptor distribution by confocal microscopy and are developing injury and thermonociception methods for use with adult flies. Our ultimate aim is to provide a more relevant model in our quest to understand chronic pain so that better treatment options may be revealed.

Determining Key Residues of the LytR Domain in the Streptococcal CpsA Protein

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Streptococcus agalactiae or Group B Streptococcus (GBS), is a Gram-positive commensal bacterium that is harmless in healthy adults, yet causes systemic diseases in neonates, the elderly, and immunocompromised individuals. Neonates are at risk of GBS infection in utero or during delivery due to the colonization of the organism in the vaginal canal of between 15-30% of adult females. GBS can cause severe neonatal sepsis and meningitis, as well as chorioamnionitis, which can cause premature birth and stillbirth. GBS infection is greatly facilitated by the presence of a bacterial capsule; a protective, polysaccharide matrix surrounding the cell that plays a key role in the pathogen?s ability to evade host immune responses. Antibiotics are effective in reducing the chances of neonatal infection by GBS, however, they also increase the likelihood of the organism developing antibiotic resistance. An approach to manipulate GBS and reduce its functionality would be beneficial to counter the potential of antibiotic resistance developments, while avoiding the cytotoxic effects that antibiotics can impose on the host.
The GBS CpsA protein, a putative transcriptional regulator of the capsule locus within the GBS genome, plays a significant role in capsule production. Without CpsA, GBS displays reduced capsule production, and thus, reduced virulence. In this study, we are targeting specific amino acids in an extracellular domain of CpsA that is proposed to be responsible for ligation of capsule to the cell wall of GBS. This work will provide insight into which amino acids are the key residues required for the function of CpsA.

Neurogenic muscular degeneration in SMARD1-like mouse model em3

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Neuromuscular degenerative (NMD) diseases are devastating conditions that can lead to pre-mature fatality or life-long suffering. Autosomal recessive mutations in IGHMBP2, a ubiquitously expressed DNA/RNA helicase, have been linked to childhood NMDs. C57BL/6J-Ighmbp2 em3Cx is a SMARD1-like strain, or Spinal Muscular Atrophy with Respiratory Distress, created via CRISPR-Cas9 targeting of the IGHMBP2 gene and hereafter referred to as em3 . SMARD1 is characterized by muscle weakness starting in the distal extremities and diaphragmatic paralysis leading to respiratory failure. Most patients are diagnosed in early infancy and die in early childhood. We have preliminary muscle fiber area and neuromuscular junction innervation data comparing wild type, em3 , and a historical SMARD1-like model (nmd2J) in the hindlimb, diaphragm, and intercostal muscles. These preliminary results suggest that em3 mice suffer from more muscle atrophy than the nmd 2J mice especially in the intercostal muscles. However, the intercostal muscles and diaphragm are not significantly denervated despite the muscular atrophy in these muscles. Other observational differences are perceivable in these muscles such as unusual nerve branch patterning suggesting that the innervation in these muscles are still affected. Characterizing the degree and range of neurogenic muscular atrophy in the em3 model will allow us to identify how mutations in Ighmbp2 create these tissue specific phenotypes.

Sending Signals: adipose sensory nerves may communicate with the brain via lipid metabolites

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Obesity and related co-morbidities, such as type II diabetes mellitus, have reached pandemic proportions affecting millions worldwide. Current research aims to identify molecular mechanisms that may provide treatment and/or prevention options for these diseases. Diet is one contributor to energy balance. If energy intake exceeds energy output, individuals become susceptible to metabolic disease. Furthermore, the type of energy intake is crucial and certain dietary fats, such as n-3/n-6 polyunsaturated fatty acids, have been shown to promote beneficial metabolic activity. Through a lipidomics LC-MS approach, in which adipose tissue compositions were compared, we determined changes in signaling and structural lipids between subcutaneous white adipose tissue (scWAT) and interscapular brown adipose tissue (iBAT). Interestingly, mice fed an n-6 enriched diet produced higher concentrations of pro- and anti-inflammatory signaling lipid metabolites in scWAT, as well as iBAT, when compared to n-3 or saturated fat enriched diets. A robust increase of a specific octadecanoid, 13-HODE, was found in both adipose tissues following the n-6 enriched diet. Some octadecanoids are known to activate transient receptor potential (TRP) channels located on sensory nerves, which we have shown to be present in scWAT and iBAT using NaV1.8 reporter mice. 13-HODE is capable of activating transient receptor potential vanilloid 1 (TRPV1) channels, which are TRPs that are known to be expressed in scWAT. Therefore, we utilized both intravital fluorescent microscopy of calcium imaging in mouse scWAT after 13-HODE delivery, as well as in vitro studies using differentiated PC-12 cells to determine if 13-HODE can stimulate sensory nerves directly. If 13-HODE is a dietary-derived lipid that is signaling to sensory nerves in scWAT, it may be mediating brain-adipose communication regarding fuel stores.

Characterization of ReMo, a novel Gordonia phage

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The bacteriophage (viruses that infect bacteria) population is one of the most ubiquitous within the biosphere. With an estimated global population of 1031 phage particles the study, characterization and utilization of bacteriophage represents untapped potential in the field of microbiology (Hendrix et al., Theor Popul Biol ., 61(4):471-80, 2002). However, there is still much unknown about the bacteriophage genome and the unique proteins it may encode. Through the characterization of phage, we gain an understanding of biological functions, identify unique proteins and classify though cross-comparison to other characterized phage. ReMo, a novel bacteriophage, was isolated from a soil sample using enriched isolation and was characterized using electron microscopy, host range assays, and an immunity assay. Its genome was extracted, sequenced, annotated and compared to other sequenced bacteriophage genomes. It was determined that ReMo is a temperate, meaning that inserts its genome into host chromosomes. It is classified as a cluster A15 Siphoviridae with a genome of 52,601 bp that has a GC content of 61.9%. The genome is made up of approximately 98 putative genes and 4 tRNAs. ReMo has a gene with very strong sequence similarity to a known toxin (VIP-2) found in bacterial genomes that targets insects. The ReMo bacteriophage is unique with many uncharacterized proteins and suggests potential for insecticidal applications that grant further study.

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Systematic profiling of tissue-specific CTCF binding patterns by integrating epigenome signatures.

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Chromatin architecture is an important component of gene regulation, and its disorganization results in various diseases. CTCF (CCCTC-binding factor) is a DNA-binding protein that allows chromatin interaction by forming DNA loops, thus plays a central role in the formation and maintenance of chromatin architecture as well as tissue-specific gene expression. In this context, understanding the underlying mechanism has been a great interest in gene regulation. Here we present a novel approach to profile CTCF binding patterns by using DNA methylation data as an interpreter of CTCF binding. Firstly, we collected all available published human CTCF ChIP-seq (chromatin immunoprecipitation followed by parallel sequencing; 882 data) and bisulfite sequencing data (8,455 data) generated from various tissues/cell types. By combining CTCF ChIP-seq and DNA methylation data, we defined global tissue-specific CTCF binding sites that are specific in multiple tissues, such as blood, brain, and pluripotent stem cell. We also defined constitutive CTCF binding sites that are essential for maintaining regular cell functions. Notably, tissue-specific CTCF binding sites were enriched near the tissue-specific genes and often surrounded tissue-specific transcription factor binding sites. This result suggests that CTCF regulates tissue-specific gene expression through the interaction with tissue-specific transcription factors. Furthermore, we found that genetic mutations in the tissue-specific CTCF binding sites are often associated with various tissue-specific diseases. Taken together, our comparative approach provides an advanced methodology for accurate profiling of tissue-specific CTCF binding patterns in detail, and revealed the mechanism of how disorganized chromatin architecture can cause human diseases.

Beta-arrestin is a Major Cellular Determinant for JC Polyomavirus Infection

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JC polyomavirus (JCPyV) infects healthy individuals residing asymptomatically within the kidney and does not require intervention. However, in immunosuppressed individuals, JCPyV infection can result in the onset of progressive multifocal leukoencephalopathy (PML) within the central nervous system, a fatal neurological disease. Unfortunately, limited therapies are available to treat PML, highlighting the critical need to better understand this disease and develop effective treatments. Currently, the most promising therapeutics to treat or prevent PML are those that block JCPyV interactions with receptors and thus prevent attachment, entry, and resultant infection. JCPyV attaches to host cells by binding to alpha-2,6 sialic acid containing lactoseries tetrasaccharide c (LSTc) though viral entry requires 5-hydroxytryptamine (5-HT)2 serotonin receptors. However, the proteins that mediate internalization of JCPyV with this receptor, and how this process drives viral infection within the host remains poorly understood. Using biochemical and molecular biology approaches our findings demonstrate that JCPyV requires the activation and function of specific serotonin receptor-associated proteins to facilitate viral entry via the clathrin-mediated endocytic pathway, specifically usurping the cellular proteins clathrin, beta-arrestin, AP2, and dynamin in this process. Further, we have identified specific beta-arrestin binding sites in the serotonin receptor that are necessary for viral entry, suggesting that JCPyV requires beta-arrestin scaffolding to the serotonin receptor to mediate viral entry and infection. Together, this research identifies specific targets for novel antiviral therapeutics, contributing to the current clinical focus in the treatment or prevention of PML, through the blockage of viral attachment and entry.

RogerDodger: Novel Gordonia Bacteriophage

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Bacteriophage are viruses that attack bacteria and are the most numerous entity on Earth with an estimated 1031 phage in the biosphere. Actinobacteriophage are phage that infect Actinobacteria, including Gordonia bacteria. Gordonia is one of the many genera within the Actinobacteriophage group. Gordonia is a valuable species that degrades environmental pollutants, xenobiotics or other slowly biodegradable natural polymers. Studying the phage that infects Gordonia helps us better understand the physiology of this important genus of bacteria. These biological entities are isolated from a variety of sample sources and their genomes are sequenced in a lab. RogerDodger, a Gordonia specific bacteriophage, was isolated from soil and sequenced. RogerDodger is a temperate phage with a Siphoviridae particle morphology. This phage belongs to cluster DC and has a genome length of 59,122 base pairs, containing 97 putative genes and a GC content of 67.9%. RogerDodger encodes genes necessary for a temperate lifestyle including two distinct integrases, which is unusual. The genome itself is circularly permuted, meaning it has terminally redundant genome ends. Future research of RogerDodger includes further analysis of gene function and identifying regulatory sequences such as the phage attachment site, attP.

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Determining the Role of Prophage, Cuke, Gene Expression in Mycobacterium smegmatis

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Mycobacteriophage (phage) are viruses that infect mycobacteria, including non-pathogenic Mycobacterium smegmatis (Hatfull et al, An Rev Microbiol, 64:331-356, 2010)and pathogenic M. tuberculosis , the leading cause of Tuberculosis (Tuberculosis (TB), CDC, 2018). Nearly all pathogenic species of Mycobacterium contain prophage (viral genomes that are integrated into the host genome), includingM. tuberculosis (Canchaya, Pro Geno, 67.2:238-276, 2003).The role of prophage in bacterial fitness and virulence is not yet understood, however, understanding how phage establish a prophage state (lysogeny) and how they impact bacterial virulence could lead to the development of new treatments. Cuke is a cluster AC phage and forms lysogens in M. smegmatis; however, it is not understood how Cuke integrates into the genome and maintains lysogeny as there are no obvious integrase and immunity repressor genes. To determine where the Cuke genome integrates into the host genome, we sequenced lysogen genomes. To determine which Cuke genes are expressed during lysogeny, we performed RNAseq analysis of RNA isolated from Cuke lysogens of M. smegmatis. Four prophage genes are expressed during lysogeny, including putative integrase proteins, gp65 and 66 and two highly expressed genes gp75 and gp78. This project aims to determine if putative integrase proteins gp65 and 66 have integrase activity and if either of the highly expressed genes, gp75 and gp78, act as the immunity repressor.

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Localization based Dynamics of 5-HT 2 Receptor Subtypes in JCPyV Infection Using Super-Resolution Localization Microscopy

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JC polyomavirus (JCPyV) is a human-specific virus that can cause a devastating demyelinating disease in humans called progressive multifocal leukoencephalopathy (PML). JCPyV asymptomatically infects most of the healthy adult population but in immunocompromised individuals, the condition leads to PML which remains incurable. Exploring the early viral infectious events such as attachment and entry into host cells remains crucial for understanding viral disease pathogenesis. Previous studies have shown that the attachment of JCPyV to host cells is mediated by ?2,6-linked sialic acid receptors while viral entry is mediated by 5-hydroxytryptamine 2 (5-HT2) receptor subtypes. However, the mechanisms by which JCPyV interacts with 5-HT2 receptors is not well understood due to limitations of resolution in conventional fluorescence microscopy techniques. The goal of this project is to characterize the spatial and temporal dynamics of 5-HT2 receptors during JCPyV challenge utilizing super-resolution microscopy techniques. Our preliminary data shows that JCPyV localizes with 5-HT2 receptor subtypes that are expressed in photoactivatable Dendra2 constructs within transfected cells. Further, our data indicates that viral colocalization varies between the different 5-HT2 receptor subtypes. Using fluorescence photoactivatable localization microscopy (FPALM) we will be able to define how the 5-HT2 receptors mediate JCPyV entry. These data would improve our knowledge and understanding of JCPyV attachment and invasion of host cells, which would help discover new targets for potential antiviral therapies for PML.

The Role of the Leucine Zipper Motif in CpsA Protein Function

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Streptococcus agalactiae, also known as Group B Streptococcus (GBS), along with other Strep strains, have been identified as important contributors to systemic disease. GBS causes potentially deadly conditions such as meningitis and sepsis in neonates, immunocompromised adults, and the elderly. A vital strategy used by Strep pathogens, including GBS, to protect themselves from the host immune system, is the polysaccharide capsule. Previous studies have confirmed multiple proteins are involved in the production of the polysaccharide capsule. CpsA, the first protein in the capsule operon, has been shown to be involved in regulatory functions associated with capsule production and possibly cell wall processing and stability. Preliminary results revealed that there is a leucine zipper motif in the N-terminal, transmembrane region of CpsA. The N-terminal region has also been shown to be involved in DNA binding. Leucine zippers are found predominantly in eukaryotic proteins and are involved in DNA binding, dimerization, and protein-protein interactions. The goal of my project is to confirm that the leucine zipper motif in the N-terminal region of the GBS CpsA protein is required for proper protein function. This will be accomplished through several methods, including co-immunoprecipitation assays, western blot, capsule and morphology assays and the role of the leucine zipper in virulence. Ultimately, these findings will help develop new therapeutics to target Group B Strep and other strep pathogens.

Discovery of Novel Long Non-Coding RNAs during Zebrafish Caudal Fin Regeneration

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Humans have limited regenerative capacity as many tissues cannot regenerate following injury. Modern medicine uses tissue transplantation, but need outweighs supply. Developing therapies to promote and enhance regenerative capacity may be a solution.Danio rerio , the zebrafish, is a powerful vertebrate model for tissue regeneration as they can completely regenerate tissues and organs following injury or amputation, with the caudal fin being a model for appendage regeneration. Regeneration requires formation of a highly proliferative tissue, the blastema, early in regeneration that persists during outgrowth. Appendage regeneration also requires positional memory; the ability of regenerating cells to use spatial information to regenerate only lost tissues and alter regeneration speed depending on injury location. What genes encode positional memory remains largely unknown. Our goal was to discover novel noncoding genes that regulate positional memory by analysing a RNA sequencing data set where the expression of genes were measured at 0, 2, 4 and 14 days post injury at three amputation planes. We examined genes with a significant interaction between the factors of time (days post injury) and amputation plane. Among the differentially expressed noncoding genes, we specifically focused on long noncoding RNAs (lncRNAs) as they participate in gene regulation. LncRNAs can regulate genes by several mechanisms, but specific functions for lncRNAs are understudied, and many are unidentified.

Characterization and Genomic Analysis of Novel Bacteriophage Whack

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Bacteriophage (phage) are highly diverse viruses that infect bacterial hosts in order to replicate and produce progeny. Phage are the most populous biological entities on the planet with an estimated 1031 particles world wide. By isolating and characterizing new bacteriophage, we increase opportunities to develop medical and industrial applications. Of the 15,050 isolated phage, genomes have been sequenced for only 15 % (Russell et al., BMC Bioinformatics 33(5):784?786., 2017). A mere 55 of these have been isolated usingRhodococcus species. The singleton Rhodococcus erythropolis phage Whack is a novel, Siphoviridae bacteriophage with a icosahedral head 80 nm in diameter and a tail length of 280 nm. Gene locations and functions were called based on Glimmer and Genemark automated annotations in conjunction with data from multiple sources found on PECAAN (Besemer et al., Nucleic Acids Res., 33:W451-W454, 2005;Cresawn S.G. et al., BMC Bioinformatics., 12:1, 2011;Delcher et al., Nucleic Acids Res., 27(23):4366-4641, 1999). The Whack genome is 49,660 nucleotides long encoding 77 putative genes. Thirty-three of these genes are orphams, genes not found in any other known genome. Host range testing revealed the ability of Whack to lyse but not infect Gordonia terrae . In studying Whack through wet lab and bioinformatics techniques, we have developed strong foundations in research and data analysis as undergraduates.

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The Vasa DEAD-box helicase GLH-1 promotes differential translation of sperm genes

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P granules are a heterogeneous mix of RNA and protein that reside at the nuclear periphery of germ cells.1 Here they receive nascent transcripts as they exit the nucleus.2 Like germ granules in other animals, P granules in C. elegans contain core Vasa DEAD-box helicases called GLHs (germline helicases).3 Vasa proteins have been implicated in both small RNA amplification4 and translational regulation5 , but how this conserved multipotency factor works is unclear. GLH-1 and GLH-2 function redundantly and are the only germline helicases in C. elegans that contain all the domains that distinguish Vasa proteins from other DEAD-box helicases. Loss of both glh-1 and glh-2 causes sterility, but glh-1 single mutants are fertile at permissive temperatures, providing a way to compare relatively healthy germlines from both wild-type and glh-1 mutant worms.
mRNA-seq was performed, in triplicate, on total and polysome-associated RNA from a wild-type strain and a strain carrying a precision deletion of glh-1 . Like previous microarray studies in glh-1 mutants, very few changes in transcript levels were observed. However, results comparing polysome fractions showed a dependence on GLH-1 for the selective translation of hundreds of sperm-enriched transcripts. In contrast, a handful of histone variant transcripts lose association with polysomes in the glh-1 deletion. To validate these observations, translational reporters of histone and sperm genes are being created in wild-type and glh-1 deletion backgrounds and changes in reporter expression area being compared to the spatial expression and abundance of their transcripts using single molecule FISH. These results are providing insight into the male-specific sterility of Vasa/Mvh/DDX4 mutants in mammals and the mechanism of Vasa function across species.

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Biomedical applications of marine algal extracts against human pathogens

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Secondary metabolites in macroalgae have ecological functions in their marine environment, but their biomedical applications against human pathogens are still underexplored. We investigated three macroalgal species for their antimicrobial activity on an array of WHO priority pathogens. The brown alga Fucus vesiculosus , and red algae species Chondrus crispus and Ahnfeltia plicata were collected from the intertidal zone of the Gulf of Maine and their identity confirmed by DNA barcoding. Algae were extracted with solvents of different polarity including methanol, dichloromethane and pentane and their antimicrobial activity was investigated against four gram positive pathogens (methicillin-sensitive Staphylococcus aureus strain Newman (MSSA), methicillin-resistant Staphylococcus aureus (MRSA) strain USA300, Bacillus cereus , and Listeria monocytogenes) as well as five gram negative pathogens (Pseudomonas aeruginosa , Proteus mirabilis , Salmonella Typhimurium, Klebsiella pneumoniae , and Escherichia coli). Antimicrobial activity was tested in a disc diffusion assay followed by a Minimum Inhibitory Concentration (MIC) assay. All three algal species tested showed antimicrobial activity against several human pathogens. Extracts of the red alga Ahnfeltia plicata showed the highest potential, followed by Chondrus crispus and Fucus vesiculosus . Of the three solvents tested, extractions with the most polar solvent, methanol, showed the highest antimicrobial activity. Of nine human pathogens tested, five were inhibited by algal extracts. Those pathogens include two gram positive microbes, methicillin-sensitive Staphylococcus aureus strain Newman (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA) strain USA300, as well as three gram negative pathogens, Proteus mirabilis , Salmonella Typhimurium, and Klebsiella pneumoniae . Antimicrobial compounds from algae may help combat the increasing lack of effective antibiotics.

Corneal neuron morphology and sensory function in dry eye disease

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Corneal neurons require tear film to maintain homeostatic functioning. Reduction in aqueous tears results in pain and irritation on the corneal surface and affects approximately 15% of the world population (Galor et al. Eye, 29(3):301-312,2015). Dry eye disease (DED) is characterized by pain, unusual regeneration morphology and altered sensory properties. This study examines morphological changes and genetic contributions that occur in a lacrimal gland excision DED model. This study examines the effects of corneal nerve injury in DED using the Nav1.8Cre;tdTomato-Sox11 knockout transgenic mouse. Sox11 is a transcription factor previously reported to impair nerve regeneration and alter expression of regeneration genes following peripheral injury (Jankowski et al., Brain Res., 1256:43-54, 2008). This study hypothesized that corneal afferent neurons would have impaired regenerative abilities and would express an altered transcriptome in DED and transgenic animals. Parameters in this study were measured using sensory function behavioral assays, image analysis, immunohistochemistry and fluorescent in situ hybridization. This study found decreased innervation and sensory functioning in DED and DED transgenic animals when compared to controls. Novel treatments can be developed through the discovery of various pathophysiological changes occurring to corneal neurons in subjects with chronic DED.

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Investigating the contribution of prophages in the pathogenesis of Streptococcus agalactiae

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Approximately 50% of babies born through vaginal delivery from women infected with Streptococcus agalactiae develop meningitis or septicemia within the first hour of life. The pathogenicity of S. agalactiae has been linked to infection by bacteriophages (viruses that infect bacteria), however the mechanism by which this occurs is poorly understood. Lysogenic conversion, where phages integrate into the bacterial genome and change their phenotype is a phenomenon that occurs in most pathogenic bacterial species such as E. coli and increases their pathogenicity. We hypothesized that phage integration into the S. agalactiae genome may enhance pathogenicity and play a role in neonatal-associated infection. Throughanalysis of the genome sequences of seven S. agalactiae strains using the software PHASTER we detected intact phage sequences in three out of the seven strains, which are A909, CNCTC 10/84, and 2603V/R. We have successfully annotated the genome of the detected CNCTC 10/84 phage and initial analysis suggests that it is a lysogenic phage. Further analyses are currently underway to identify genes which may be associated with pathogenesis of S. agalactiae . Comprehensive analysis of the phage genome and analysis of changes in bacterial gene expression due to the integration of phage will illuminate the contribution of phages to S. agalactiae infection. Ultimately, these findings may lead to the identification of potential targets for an alternative therapeutic approach.

The effects of prophage BPs on intrinsic macrolide and aminoglycoside antibiotic resistance in Mycobacterium chelonae

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Multidrug resistant tuberculosis is a global health concern because treatment is dangerous, expensive, and often ineffective (Tang, et al. 2011). Bacteriophage play a key role in the virulence of most bacterial pathogens (Rusconni, Eppinger, 2016). In fact, all pathogenic strains of Mycobacterium tuberculosis are lysogens, bacterial cells which act as hosts for integrated viral genomes, or prophage. Mycobacterial prophages are hypothesized to increase host fitness. Elucidating how mycobacteriophage BPs contributes to intrinsic antibiotic resistance in non-tuberculosis pathogenic M. chelonae is a critical step to find new drugs and therapies for mycobacterial diseases. Previous studies in the Molloy laboratory indicate that prophage induce differential gene expression of 7.8% to 24.1% of the mycobacterial genome (Figueroa-Bossi, et al. 2001). RNAseq data on pathogenic strains of M. chelonae , which possess prophage BPs, indicates an upregulation of whib7, eis and tap orthologs which are members of the whib7 regulon (Figueroa-Bossi, et al. 2001). The whib7 regulon expresses genes involved in antibiotic efflux, ribosomal protection, and other defense mechanisms (Siringan, et al. 2014). Minimum Inhibitory Concentration assay protocols were optimized for M.chelonae to determine intrinsic sensitivity to different macrolides and aminoglycosides. BPs M. chelonae yielded higher antibiotic resistance than WT strains suggesting that the presence of prophage increase host fitness. These results implied that M.chelonae is very sensitive to macrolides, likely due to its lack of the erm operon which promotes macrolide resistance. Future research could help identify the mechanisms by which BPs increases the transcription of whib7 .

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Defining infection strategies of an elusive and deadly virus in primary human astrocytes

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JC polyomavirus (JCPyV) establishes an asymptomatic, persistent infection in the kidneys of 50-80% of the population worldwide. However, in individuals who are immunocompromised, JCPyV reactivates and traverses from the kidneys to the brain where it establishes a lytic infection in the central nervous system causing the disease progressive multifocal leukoencephalopathy (PML). Due to the rapid progression and devastating outcomes, this disease is fatal in 1-2 years and there are currently no treatment options for PML. To understand PML pathogenesis and thus make progress on developing therapeutic strategies to combat this virus, it is important to understand the mechanisms of infectivity in an animal model. Unfortunately, JCPyV is a human-specific virus, and research is limited to studying JCPyV in vitro with cell culture strategies utilizing a mixed fetal glial cell line, SVG-A cells. However, as these cells are comprised of immortalized attributes to help better recapitulate JCPyV infection, the mechanisms of infection may not be accurately portrayed. Therefore, we have established a unique approach to studying JCPyV: utilization of primary normal human astrocytes (NHAs). By analyzing infectivity assays and responses to inhibitor and siRNA treatments, JCPyV utilizes clathrin-mediated endocytosis as an entry mechanism in NHAs, similar to that of SVG-A cells. Future research will define downstream signaling pathways after viral entry, further characterizing JCPyV infection in a primary cell line and thus building a better understanding of JCPyV infection and PML pathogenesis.

Triclosan Disrupts Mast Cell Plasma Membrane Electrochemical Potential

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Triclosan (TCS) is a popular wide-spectrum antimicrobial agent. While TCS was recently banned from several consumer products such as hand soaps, it remains in a top-selling toothpaste and other household items. TCS inhibits the function of both mast cells and mitochondria. Mast cells are found in most human tissues and play important roles in a wide variety of biological processes and diseases. Through investigating the mechanisms by which TCS disrupts mitochondrial and mast cell signaling, the Gosse lab found that TCS depolarizes the mitochondrial membrane and disrupts cellular Ca2+ dynamics. However, triclosan?s effects on mast cells and mitochondria disappear when its ionizable hydroxyl group is not present. These findings suggest TCS to be a proton ionophore capable of not only uncoupling mitochondria, but also possibly capable of disrupting the plasma membrane electrochemical potential (PMP). In this study, we have utilized a fluorescent, genetically-encoded voltage indicator named ArcLight A242 to measure TCS effects the PMP of mast cells. Utilizing this method for the first time, in mast cells, we have observed plasma membrane depolarization in the presence of gramicidin, using confocal microscopy coupled with image analysis. Using these newly-developed methods we have observed an inhibitory TCS effect on mast cell PMP. TCS disruption of PMP could provide a mechanistic explanation of triclosan?s disruption of Ca2+ influx, mast cell function, and a host of other cellular processes dependent on PMP.

Brain expression analysis of candidate endocrine receptors in black sea bass (*Centropristis striata*) during sex change

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Hermaphroditism is a common reproductive strategy among teleost fishes. Black sea bass (Centropristis striata) is a commercially important fish that can change sex from female to male, but relatively little is known about brain gene expression patterns associated with sex reversal. The present study focused on five endocrine receptors expressed in the brain that were previously associated with sex differences, behavioral changes, or vertebrate brain development. These genes included: AVT receptor 1a (avtr1a), GABAA receptor gamma 2 subunit (gabrg2), G protein-coupled receptor 157 (gpr157), G protein-coupled receptor 49 (lgr5), and G protein-coupled receptor 85 (sreb2). Genes were identified in a previously sequenced black sea bass brain transcriptome, confirmed using traditional sequencing, and expression was measured in male, female, and sex changing fish using qPCR. These genes were also combined with 10 previously studied candidate genes in the black sea bass brain and used in principal component and cluster analyses. Once confirmed, sequence fragments for genes of interest were deposited in the NCBI nucleotide database. No significant differences among reproductive stages or sex change were detected in any gene of interest. However, in combination with other candidate genes, most expression patterns were overall highly similar, except for a slight trend of lower expression in sex changing fish. In addition, elevated avtr1a expression (15 fold) was detected in three individuals and may be associated with behavioral changes, but this requires further research using larger sample sizes and behavioral studies.

Characterization of novel Gordonia phage Sidious

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Bacteriophage (phage), viruses that infect bacteria, have a highly vast and genetically diverse population (Suttle., Nat., 5.10:801, 2007). While phage isolated from the genus Gordonia are more diverse than phage from Mycobacteria , they are less characterized and thus give us broader insight on how these viruses infect bacteria (Russell et al., Bio., 33:57:784-786, 2017). A phage isolated from Gordonia terrae , called Sidious, was sequenced using Illumina high-throughput sequencing. Sidious was analyzed and characterized to determine the start and function of each putative gene using the databases and programs: DNAMaster, PhagesDB, Phamerator, and PECAAN. It is a Siphoviridae phage with an 80-nm diameter icosahedral head and 480-nm tail. Sidious is a temperate phage and forms 1-mm turbid plaques on a lawn of G. terrae . The temperate lifestyle of Sidious is supported by the presence of an integrase and a repressor in its genome. Sidious was assigned to cluster CZ based on high sequence identity with other CZ phage, Yeezy and BaxterFox (CZ3) but with enough unique sequence to warrant assignment to a new subcluster CZ7. The genome is 51,789 base pairs in length, has a GC content of 66.6%, and encodes 84 putative genes. Twelve of the genes are orphams, genes that lack homologs in the database, including a toxin-antitoxin system, RexAB, which has yet to be described in CZ phage. Further research will focus on novel genes of the CZ cluster, including the RexAB-like gene as well as regulatory sequences in Sidious.

RNA interference identifies genes involved in ECM signaling and the generation of polyploid cells during wound healing in Drosophila

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Drosophila is considered a model organism for studying potential pathways in human health and disease. Many genes are conserved between both species. One area of interest in conserved genes is wound healing and polyploidy cells. In response to damage, Drosophila will induce a polyploidy response to heal. This effect on wounds in Drosophila has also been observed in specific organ of humans, such as liver and heart tissue. A group of students from Southern Maine Community College (SMCC), under the guidance of Vicki Losick and her lab, looked at three genes of interest involved in wound healing response. The Losick lab bred three strains of Drosophila, each with an RNAi (RNA interference), which knocked down function of a specific gene of interest. The genes examined, Grainyhead (GRH), Parvin, and Pvr, are all found in the pathway of extracellular matrix (ECM) signalling. Through deliberately wounding of adult Drosophila and subsequent examination of surrounding tissue using expression of GFP-tagged PCNA and immunofluorescence, we examined how reduced expression of these genes of interest potentially affected polyploidy and wound healing.

Induction of the MAPK cascade is required by JC polyomavirus infection for viral transcription

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JC polyomavirus (JCPyV) infects the majority of the human population resulting in an asymptomatic persistent infection in the kidney. In immunocompromised individuals, JCPyV can spread to the central nervous system and cause the incurable and fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). Through biochemical analysis of JCPyV-induced signaling pathways, our work has demonstrated that JCPyV infection induces the mitogen-activated protein kinase (MAPK) cascade in order to hijack host-cell transcription machinery. In particular, JCPyV infection requires the presence of the core MAPK proteins including the extracellular signal-regulated kinase (ERK). Using confocal microscopy, we have determined that upon JCPyV-induced MAPK activation ERK translocates into the nucleus in order to promote viral gene transcription through the recruitment and activation of host-cell transcription factors such as cMyc. These findings demonstrate how JCPyV activation of the MAPK signaling cascade drives viral infection and enhances our understanding of JCPyV pathogenesis.

Race to Lysis; Characterizing Novel Gordonia Phage MagicMan

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Bacteriophage (phage) are viruses which infect and kill bacteria. Phage are the most plentiful organisms in the universe, with an estimated 1031 particles in the ecosystem. Novel Gordonia phage MagicMan was isolated from soil in Orono, ME using Gordonia terrae as a host. Of the 15,050 known Actinobacteriophage, only 2.5% are Gordonia phage which have been sequenced (PhagesDB). MagicMan particles have a head diameter of 150 nm and a tail length of 700 nm. MagicMan is a temperate, Siphoviridae cluster DB phage which is closely related to cluster DB Gordonia phage Schwabeltier. MagicMan is a temperate Gordonia phage with a GC content of 67.1% encoding 70 putative genes, of which 5 are orphams (genes with no known homologues). Bioinformatic tools are used to analyze and determine the location and function of genes in the MagicMan genome, including PhagesDB, PECAAN, Phamerator, and DNA Master. MagicMan is a unique Gordonia phage, which demonstrates the mosaicism and diversity present in bacteriophage. Genetic diversity and simplicity of phage genomes provide opportunities to learn about bioinformatics and phage biology. Phages targeting G. terrae have been isolated from industrial sludge and can destroy the biofouling products formed by Gordonia species.

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Determining the impact of prophage BPs viral gene expression on bacterial host Mycobacterium chelonae gene expression

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Mycobacterium tuberculosis is the leading cause of death by an infectious disease. In 2017, 10 million people developed tuberculosis and 558,000 of these cases were resistant to antibiotics (Global tuberculosis report 2018, WHO). All members of the M. tuberculosis complex carry prophage, viruses that infect bacteria and integrate themselves into the host genome through a process called lysogeny (Fan, et al., J Biomol Struct Dyn., 34:2, 233-238, 2015). The non-pathogenic vaccine strain for tuberculosis lacks the prophage, which suggests a prophage role in virulence (Fan, et al., J Biomol Struct Dyn. 34:2, 233-238, 2015). Because the prophage do not encode obvious virulence genes, we hypothesized that prophage impact bacterial virulence by altering bacterial gene expression. By studying gene expression patterns in Mycobacterium chelonae , a close relative of M. tuberculosis , in the presence and absence of prophage BPs, we determined prophage impact mycobacterial gene expression. Through RNAseq analysis of M. chelonae cells with or without prophage, we detected changes in expression of 7.74% of M. chelonae genes, including genes involved in antibiotic resistance. During lysogenic infection of M. chelonae the most highly expressed genes in BPs were the repressor (gp33) and a putative mobile element (gp58). To determine if viral gene products drive changes in gene expression, we will construct strains of M. chelonae that express either gp33 or gp58 and measure changes in gene expression and antibiotic resistance.

Genetic Screening as a Tool for Personalized Medicine

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Genetic screening can be used as a tool for personalized medicine. Various genes exhibiting frequent mutations were analyzed by genetic sequencing. The CYP2C19 gene encodes an enzyme which is responsible for the metabolism and activation of many drugs. Mutations in this gene cause inactive forms of the enzyme which affects drug metabolism and activation. In the CYP2C19 gene, two point mutations were screened, one in exon 4 and one in exon 5. Both of these mutations result in premature stop codons that lead to truncated proteins, which do not metabolize proteins. The mutation in exon 5 was more common than that in exon 4 (90.9% to 9.1% respectively). Studies were also conducted to examine mutations in exon 10 of the CFTR gene. The first was a point mutation that changes a methionine to valine at the 470th amino acid position (M470V), which contributes to infertility. The second was a 3 base pair deletion, resulting in the deletion of a phenylalanine residue at position 508, which causes Cystic Fibrosis when homozygous. No examples of an F508 deletions were found in the 13 samples, yet significant variation in the genotype at the M470V loci was observed (20% of samples were homozygous for methionine, 40% were homozygous for valine and 40% were heterozygous for valine). Our data suggest that genetic screens for individual gene mutations may improve clinical care by providing information that will inform clinical care paradigms.

Development of Non-Linear Microscopic methods to investigate Osteoconduction of Polysaccharide-based scaffolds

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Over the past decade, the use of polymers as host matrices or scaffolds for biomedical applications has been of rising interest. Recent work specifically utilizing naturally derived polysaccharides as three-dimensional scaffolds for cellular loading and tissue growth has shown promising material characteristics, however, due to complexities such as composition and morphology, material optimization is required for each application. Conventional quantitative methods such as fluorescence microscopy, AFM, and flow cytometry used to optimize these types of biomaterial systems often either require destructive sample analysis or struggle to garner sufficient material information while simultaneously capturing the three-dimensional nature of cell ingrowth. Multiphoton microscopy has emerged as a viable tool for performing such quantification, permitting greater imaging depth, minimizing un-favorable scattering, and producing high-resolution images of optical cross sections. Here, we present a method utilizing multiphoton microscopy to non-invasively image cellular growth into three-dimensional cellulose nanofibrillar-based scaffolds.

Sensory gene expression after knockout of primary cilia function in Ephydatia muelleri

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Sponges are one of the earliest branching animal phyla and prove to be valuable in studying the earliest components of animal sensory systems. Several genes and regulatory pathways that have been identified to possess sensory function in other organisms are currently being studied for their roles in receiving and transmitting sensory information in freshwater sponges. Here, we are studying the potential sensory function of two of the identified sensory genes, Bsh and Msx , in addition to the Wnt pathway component GSK3 in the freshwater sponge E. muelleri . We test the sensory functions of these genes by observing their expression changes after function of the primary cilia within the aquiferous system is knocked down using the TRP channel blocker gadolinium (III) chloride and chased with L-glutamate. We find that Bsh and GSK3 are upregulated while there is no significant change in Msx expression. These data suggest potential roles of Bsh and GSK3 in transmitting sensory signals originating from external stimuli detected by primary cilia. Additionally, we find that treating sponges with GdCl3 causes reorganization of the canal system where there appears to be canal expansion in addition to disappearances of the sneeze response. The changes in gene expression and canal morphology after primary cilia function knockdown indicate the presence of genetic precursors in freshwater sponges that have formed complex sensory systems in more evolved organisms.

The effects on healthspan of the glucocorticoid prednisone in Huntington?s disease modeled in Caenorhabditis elegans

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Prednisone, a glucocorticoid, has been previously shown to reduce cellular degeneration in C. elegans modeling muscular dystrophy. In humans, prednisone is known to act on anti-inflammatory pathways, but these pathways do not exist in C. elegans ; therefore, it is predicted that prednisone produces these effects by acting directly on the muscle cells themselves. In Huntington's disease, a neurodegenerative protein-aggregation disorder, the abnormally expressed huntingtin gene overproduces polyglutamine, leading to misfolding of the protein and subsequent pathology impacting both the nervous system and the muscles. Prednisone has not yet been applied to the treatment of Huntington?s disease. In this study, we attempt to characterize the effects of prednisone treatment in C. elegans which model Huntington's by exhibiting intramuscular polyglutamine aggregation. We hypothesized that prednisone will act directly on the muscle tissue of the model animals and maintain health as measured by movement in a thrashing assay. Results thus far indicate that prednisone applied to the animal after reaching adulthood does show a significant delay in disease onset and maintenance of healthspan in these models after only a few hours of treatment.

Investigating the Antiviral Effects of Lobster Hemocyanin on JC Polyomavirus Infection

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Human JC polyomavirus (JCPyV) infects up to 80% of the population and establishes a persistent, lifelong infection in the kidneys. In individuals with severe immunosuppression, JCPyV can spread from the kidneys to the central nervous system (CNS), resulting in the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML), for which there are no effective treatments. This research explores the potential antiviral effects of hemocyanin using established protocols and seeks to define the mechanism of inhibition. Hemocyanins are biological macromolecules, acting as oxygen-transporting proteins in arthropods and mollusks, that promote host defenses against bacterial, fungal, and viral infections. Hemocyanin derived from Homarus americanus , the American lobster, has been demonstrated to exhibit antiviral properties against a number of viruses, including herpes simplex virus 1 (HSV-1). To determine whether hemocyanin could inhibit JCPyV infection, cells were treated with hemocyanin at various intervals and analyzed for infectivity. Treatment of cells with hemocyanin at times consistent with JCPyV attachment and entry results in decreased JCPyV infectivity, suggesting that either viral attachment or entry are inhibited. Treatment of cells with hemocyanin following JCPyV attachment and entry does not impact JCPyV infectivity, further suggesting that hemocyanin blocks either JCPyV attachment or entry into host cells. These findings are consistent with published data showing that hemocyanin blocks HSV-1 entry into host cells. Future research will examine the mechanism by which hemocyanin reduces JCPyV infection. Findings from this research could serve as the platform for the future development of a novel antiviral therapy for PML or other viral-related diseases.

Macroalgae from the Gulf of Maine, and their antimicrobial properties against human pathogens

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Marine macroalgae are an unexplored source of antimicrobial compounds against human pathogens. Algae are able to survive and even thrive in harsh environments, surrounded by infectious bacteria, suggesting they have antimicrobial properties. This research focuses on the antimicrobial properties of three macroalgae species collected in the intertidal zone of the coast of Maine: the brown alga Fucus vesiculosus , and red algae species Chondrus crispus , and Ahnfeltia plicata . Extracts from these three different macroalgae were tested against a variety of gram negative human pathogens. These include Pseudomonas aeruginosa, Proteus mirabili s, Salmonella enterica serovar Typhimurium (S. Typhimurium), Klebsiella pneumoniae and Escherichia coli. These pathogens were selected from the World Health Organization high priority list for Research and Development of new antibiotics. Extracts were prepared with three different solvents of varying polarity including methanol, dichloromethane, and pentane. The effects of algal extracts were measured using a disk diffusion assay. In addition, a minimum inhibitory concentration assay (MIC) was performed for extracts that showed the greatest growth inhibition. Of the three solvents tested, methanol extracts were the most effective followed by dichloromethane and pentane. All algal extracts that were tested showed inhibition of bacterial growth of P. mirabili s, S. Typhimurium and K. pneumoniae. However, the extracts of the red algae A. plicata showed the highest antimicrobial activity against all three pathogens. These are very encouraging results, as the overuse of antibiotics has caused antibiotic therapies to become less effective.

Gene expression profile analysis during cardiomyocyte differentiation of premature-aging induced pluripotent stem cells

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The overarching goal of this Lab?s research is to understand the biology of aging. Aging causes the global disorganization of the nuclear chromatin structure, which contributes to the gene expression changes associated with aging. Structural changes within chromatin are commonly observed in general aging. It is also observed with premature aging diseases; Hutchinson-Gilford Progeria syndrome (HPGS) and Werner syndrome (WS). HGPS and WS are caused by mutations (in lamin A and WRN genes, respectively) and replicate various aging-related degenerative changes, thus providing powerful models for aging research. Lamin A and WRN genes remain silent during early development, and are activated upon differentiation. We hypothesize that the premature aging diseases disrupt the gene expression in differentiated tissues which in turn, causes various degenerative disorders such as heart problems. To verify this, we are performing RNA sequencing analysis through the use of induced pluripotent stem cells (iPSC) containing the mutations that cause premature aging diseases, as they differentiate into cardiomyocytes (CM). In this project, we generated prematuring-aging iPS cells by introducing mutations in WRN and LMNA genes using CRISPR-Cas9 genome-editing technology. In addition, we have differentiated normal and premature aging-iPSC directly into cardiomyocytes. To validate our RNA-seq data analysis pipeline, we compared a published dataset showing differentiation of embryonic stem cells into cardiac progenitor cells and cardiomyocytes. The data shows many changes in gene expression and enrichment.

Discovery and Analysis of Subcluster DE3 Phage Roadkill from Wastewater Treatment Facility

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Bacteriophages are useful tools when studying antibiotic resistance and understanding protein function. A bacteriophage able to infect Gordonia terrae was isolated from a wastewater treatment facility in Farmington, Maine, named Roadkill.Roadkill is a subcluster DE3 siphoviridae phage, produced medium clear round plaques. Roadkill?s genome consisted of 55,939bp and 84 genes. The circularly permuted genome in Roadkill contains genes that code for terminase small and large subunits. This genome was being annotated and gene functions will be discussed. Roadkill was added to a bacteriophage database that may be utilized when doing research on antibiotic resistance and phage therapies.

Identification of Novel Regulatory Genes with Roles in Muscular Dystrophy

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Muscular Dystrophy (MD) causes progressive muscle weakness and loss of muscle mass, affecting 250,000 individuals in the United States. Between the 30 different forms, disease severity and age of onset varies drastically, and even individuals with the same form can vary. Dystroglycanopathic MD is a form of MD for which there is no cure. It is characterized by dysfunctional dystroglycan: a transmembrane protein that requires GDP-Mannose Pyrophosphorylase B (gmppb) for proper glycosylation and function. Several mutations in gmppb have been identified in individuals with dystroglycanopathies. To better understand molecular mechanisms that underlie MD variability, we have characterized genome-wide gene expression by high-throughput RNA sequencing in wild-type and mutant zebrafish embryos where gmppb was mutated using CRISPR/Cas9 technology. We hypothesize that variable phenotypes in MD are due to differences in genetic regulation by a class of genes called long non-coding RNAs (lncRNAs). These genes are known to play regulatory roles in various physiological processes and diseases such as development and cancer, yet they are currently unexplored in MD. We have identified approximately a dozen putative novel lncRNAs that are differentially expressed in the mutants. We aim to characterize these putative lncRNAs using sequence analysis to determine k-mer content, protein alignment, and repetitive elements. As some lncRNAs regulate protein coding genes that are antisense or in close genomic proximity, we will also characterize the nearby protein coding genes. These ongoing studies seek to improve our understanding of the many roles regulatory genes have in controlling MD severity and onset.

Truncated form of CpsA reduces capsule on the surface of Streptococcus agalactiae

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Streptococcus agalactiae , also known as Group B Strep (GBS), is a Gram-positive bacterium that asymptomatically colonizes the vaginal and genitourinary tracks. During pregnancy, GBS may ascend the vaginal tract and infect the fetus in utero or the neonate during vaginal birth. Infections result in still birth, pneumonia, or meningitis depending on time of infection. Virulence of GBS is largely dependent on capsule production. Current research has pointed towards one virulence factor, CpsA, as a likely target for developing novel therapies. CpsA is a protein that belongs to the LytR-CpsA-Psr (LCP) family and facilitates the incorporation of polysaccharides into the cell wall. CpsA is comprised of an intracellular domain, three transmembrane domains, and a large extracellular domain. The large extracellular domain is comprised of an accessory and LCP domain which are connected by a region with no defined structure. The LCP region actively facilitates ligation of the capsule to the cell wall. Previous work demonstrated that a truncated version of the extracellular domain of CpsA expressed in the wild type strain resulted in drastically reduced capsule. The current project utilizes a small peptide created from the unstructured region between the two extracellular domains of CpsA. When this peptide is added to the culture of GBS, capsule production is reduced. Future therapies utilizing truncated forms of the CpsA protein could provide a more effective method for treating GBS infection without altering neonate microflora or developing antibiotic resistance.

A human NEMF variant results in novel mouse model of neurodegenerative disease

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Background: Ribosomal stalling has been implicated in neurodegenerative disease. NEMF helps to correct ribosomal stalling by adding alanine and threonine tails to push the stalled nascent proteins out of the ribosome, as well as assisting in proteins being ubiquitinated for degradation (Shen et al., Science , 347(6217):75-78, 2015). NEMF dysfunction has been implicated in neurodegenerative disease based on the phenotype of mutant mice characterized in the Cox lab.

Methods: In C57BL/6J mice, we induced a mutation based on a rare human variant observed in an ALS patient (I98T) by CRISPR-mediated targeting. The I98T variant was predicted to be deleterious to the NEMF protein. Unaffected (wild type and mice heterozygous for the mutation) were compared to affected mice (homozygous for the mutation). We recorded the weights of the mice each week and tested their grip strength using a wire grid hang test. We assessed nerve health by counting myelinated axons in femoral motor and sensory nerves.

Results: We found no difference in weights of affected and unaffected mice up to 8 weeks. Starting at 6 weeks, affected mice display a reduced ability to hold onto the wire grid. At the 8-week time point, affected animals have significantly fewer myelinated axons in their femoral motor nerves compared to unaffected animals.

Conclusions: Mice with the I98T amino acid substitution in NEMF begin to display progressive muscle weakness and neurodegenerative pathology that suggests the variant discovered in an ALS patient is damaging to the protein and may be associated with their ALS symptoms.

Isolation and Characterization of Novel Bacteriophage SleepyHead

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Bacteriophage (phage) are viruses that infect bacteria. There are an estimated 1031 phage particles in the biosphere, making them the most abundant biological entity. Research has led to advances in the understanding of their impact on antibiotic resistant bacterial strains, as biocontrol agents in respect to agriculture, and in the creation of vaccines. Phage SleepyHead was isolated using Rhodococcus erythropolis . Electron microscopy showed Sleepyhead was part of the Siphoviridae family, with a a 350-nm tail and 50-nm icosahedral head. Through lysogen isolation, SleepyHead was determined to be a temperate phage with 17.7% lysogeny efficiency. Genomic DNA was isolated from SleepyHead and sequenced. The SleepyHead genome is 43,943 base pairs in length, has 61% GC content, and encodes 67 putative genes, including 37 orphams, genes unrelated to any other sequence in the database. SleepyHead was clustered as a Singleton because 55% of genes are novel. Future research includes analysis of the integrase cassette to better characterize how lysogeny is established and maintained. Further characterization of genes with unique functions expressed during lysogeny would help us to better understand how SleepyHead could impact host fitness. In addition, future tests to determine a potentially larger host range which could be important for medical treatments, or other industrial applications.

Stress and Immune Regulation in Zebrafish

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Adaptation to stress is controlled by the hypothalamic-pituitary-adrenal (HPA) axis in mammals and the homologous hypothalamic-pituitary-interrenal axis in fish. Glucocorticoids are major stress hormones produced by the adrenal gland which bind to the glucocorticoid receptor (GR) to regulate gene expression in target cells. This neuroendocrine system influences energy metabolism, inflammation and homeostasis. Early exposure to chronic stress has been shown to have detrimental effects in later life that may involve altered dynamics of HPA activity. Cortisol is the active glucocorticoid hormone in both humans and zebrafish, and its levels fluctuate on a daily basis. Expression of two GR target genes, fkbp5 and klf9 , was observed to decrease during the day, which correlates with the known circadian behavior of cortisol levels. Using a knock-out mutant of the GR gene in zebrafish, we tested to what extent GR signaling is involved in this dynamic expression pattern by comparing expression at 6:45 am and 10:15 am in 5-day-old larvae. We observed decreased levels of expression of the two target genes in the GR knock-out mutant and the two-time points showed the same low level, indicating that the diurnal expression pattern of these genes relies on the GR. Because GR signaling is involved in immune cell development and regulation, we also observed development of transgenic zebrafish with fluorescent neutrophils exposed to either dexamethasone (a potent synthetic glucocorticoid) or mifeprestone (a GR inhibitor). Preliminary counts of these immune cells using computerized image analysis revealed no significant difference between treatments and control at 3 days post-fertilization.

Surface Extractions of Antimicrobial compounds from Seaweed of the Gulf of Maine

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Macroalgae, commonly known as seaweed, are exposed to constant microbial advances in their marine environment. Recent studies have shown that macroalgal secondary metabolites, possibly produced in defense of microbial and predatory advances, were more effective at inhibiting human pathogens than commercial antibiotic counterparts. Considering that macroalgae create a chemical gradient barrier and that macroalgal surfaces act as a primary defense against microorganismal advances, antimicrobial compounds are hypothesized to reside on these surfaces. Attempts to isolate samples of these bioactive metabolites commonly involve the crude extraction of many inactive intra- and extracellular compounds, which may dilute the concentration of targeted compounds. Yet, few alternative extraction methods have been explored. Here, two novel methods were employed to investigate the antimicrobial properties from the surface of macroalgal tissue. Three local macroalgal species from the rocky intertidal zone of Biddeford Pool, Maine, were examined including one brown alga, Fucus vesiculosus , and two red algae, Chondrus crispus and Ahnfeltia plicata . Results indicate that surface antimicrobial activity was present in Fucus vesiculosus when tested against Staphylococcus aureus strain Newman (MSSA) and Methicillin-resistant Staphylococcus aureus (MRSA) cultures through a direct contact method. Inhibition resulting from macroalgal tissue surfaces suggest that whole tissue extraction methods may dilute and decrease effectiveness against human pathogenic bacteria.

Duck assisted seed dispersal and natural history: Assessing the invasive potential of Najas minor in Maine

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Waterfowl have long been considered a mechanism for the dispersal of aquatic plants between spatially isolated habitats through the consumption and subsequent deposition of seeds. Najas minor (brittle naiad), a relatively new invasive aquatic plant to the state of Maine, reproduces annually by seeds and is a good candidate for waterfowl endozoochary. However, no previous studies have assessed the effectiveness of waterfowl as vectors for the dispersal of N. minor . This research was conducted in order to assess if waterfowl, in addition to human activity, could transport N. minor seeds from an infested waterbody to an uninfested one. Najas minor seeds were fed to captive domesticated mallards and their excreta was subsequently collected and searched for surviving seeds. Seed viability was then assessed using 2,3,5-triphenyltetrazolium chloride. A median of 10.7% of seeds were recovered from the excreta (Q1= 7.1%, Q3= 18.7%) and 50% of those seeds were viable (Q1= 31.2%, Q3 = 61.7 %), suggesting waterfowl can disperse N. minor in the field. A spatial probabilistic model was then created using R and ArcGIS to model the potential future distribution of N. minor in Maine in twenty five and fifty years. While wildlife can disperse invasive species to new areas, human activities are still the biggest pathway for the spread of invasive species and preventative actions should be taken whenever possible.

A program to generate biologically-relevant atomic-resolution models of chondroitin sulfate biopolymers of arbitrary length

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Chondroitin sulfate (CS) is one of the most abundant proteoglycan biopolymers in the body and may contain hundreds of repeating disaccharide units. Atomic-resolution simulations of large systems are computationally taxing so we aimed to find a more efficient method of generating biologically-relevant CS models. We developed a program that applies conformations from unbiased all-atom explicit-solvent molecular dynamics (MD) simulations of non-sulfated chondroitin 20-mers. Our program was used to generate chondroitin 20-mer models using conformations from MD-generated chondroitin 20-mers. We applied phi/psi dihedral angles of glycosidic linkages from MD-generated ensembles to our program and noticed a subtle difference in end-to-end distance distributions of MD-generated ensembles and those generated using our program suggesting that there are other factors contributing to backbone flexibility. Next, we applied monosaccharide ring dihedrals from the MD-generated ensembles to our program and found that these contribute to end-to-end distance. Bond lengths and angles are also contributing factors and application of these data from MD-generated ensembles is necessary to produce biologically-relevant configurations. Each ring and linkage conformation was treated independently in our program so we checked for interactions between different linkages and rings by incorporating short energy-minimizing simulations of each configuration and examining resulting bond lengths. To validate application of 20-mer conformations to generate polymers of different lengths, we generated chondroitin 10-mer ensembles and compared to 10-mer simulations. For each polymer length (20-mer and 10-mer), constructed and MD-generated ensembles had matching conformations and end-to-end distances indicating that our program can efficiently generate biologically-relevant chondroitin biopolymer models of arbitrary length.

Age-Related Peripheral Neuropathy in the Adipose Organ

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Peripheral innervation of adipose tissue, both white (energy storing) and brown (energy expending) depots, is crucial for maintaining healthy metabolic function and energy balance. Important processes such as lipid breakdown, production of new adipocytes, thermogenesis, and browning (conversion of white adipocyte to a healthier inducible brown adipocytes) all require regulation via peripheral sympathetic nerves. Without sufficient regulation from the nervous system adipose tissue can become diseased, hypertrophic and inflamed. Peripheral neuropathy (the dying back of nerves) in extremities has been well documented particularly in the diabetic population as well as with aging. We have shown for the first time that age-related peripheral neuropathy extends beneath the skin to the subcutaneous white adipose depots of mice. This decrease of innervation was most pronounced around the vasculature of the adipose depots. Following from these findings, we have further investigated adipose vascular neuropathy in the longevity mouse line HET3 in an attempt to answer the following questions: Do HET3 mice replicate the neuropathic phenotype? Are there sex differences in age-related neuropathy? Are all nerve types affected or just specific subsets? Are more than just vascular associated nerves effected?