Poster Session abstracts

Investigation of cilia during early stages of heart regeneration in zebrafish (Danio rerio)
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Cilia are cellular appendages crucial for embryonic development, and they are known to be present in zebrafish hearts. These hearts are capable of regeneration, and evidence from the Morris and Yin labs showed that cilia were upregulated on hearts during the process of regeneration from 0 to 30 days post amputation (dpa). Recent qPCR data suggested that cilia gene expression is regulated immediately following heart injury. Immunofluorescent staining of different structural components of cilia allowed application of a new protocol to measure abundance and lengths of cilia on heart cells at 0, 3 and 7 dpa. The clear presence of cilia and their possible elongation from 0 dpa lengths were observed, which may suggest they play an important role in early heart regeneration. This could provide crucial insight for future studies of human regenerative medicine.

Identification of candidate regulatory regions in ciliary genes \textit{CCDC39} and \textit{DNAH5}
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Cilia are hair-like organelles that project from the surface of cells. These organelles are responsible for proper development. When cilia are dysfunctional, diseases called ciliopathies can occur. Primary ciliary dyskinesia (PCD) results from genetic mutations affecting motile cilia, which can cause chronic obstructive airway disease, infertility, and left-right body asymmetry. \textit{CCDC39} and \textit{DNAH5} are ciliary genes which directly affect dynein arm function and structure. This makes them good candidates to study, since inner and outer dynein arm defects most typically result in PCD. For this project, computational tools were used to identify candidate regulatory regions for \textit{CCDC39} and \textit{DNAH5}. These regions are identified by finding candidate transcription factor binding sites in the noncoding regions surrounding the gene of interest, and then identifying clusters of these candidates, which form candidate regulatory modules. Computational methods of deducing these modules are important for selecting high confidence targets for experimental validation.
Monitoring Ocean Acidification Through Sediment pH in Eelgrass Areas in Frenchman Bay, Maine
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Zostera marina, eelgrass, is an inter-tidal marine ecosystem that can prevent erosion, mitigate wave action, shelter commercially important species, and act as a carbon sink. We are primarily interested in eelgrasses ability to mitigate climate change and ocean acidification. During photosynthesis eelgrass removes carbon dioxide from the surrounding water column and deposits carbon into the sediment. When anthropogenic carbon is taken out of the water column, it is unable to bond with carbonate to form carbonic acid, therefore raising water column pH, and allowing organisms with calcium carbonate structures to grow more easily. This has a large effect on commercially important species including: clams, mussels and oysters. We have made comparisons of pH levels within and around eelgrass beds. These data may provide a compelling argument for protection and restoration of eelgrass beds, which may be conferring some protection for shellfish from impacts of near shore ocean acidification.

Triclosan effects on phospholipase D activity in RBL-2H3 mast cells
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Triclosan (TCS), an antimicrobial agent, exists in various consumer products at concentrations ~10 mM. Mast cells are central components in many physiological processes, including the immune system. Phospholipase D (PLD) is involved in FcεRI signaling. Previous research in the Gosse lab showed that TCS inhibits both degranulation and membrane ruffling of antigen-stimulated mast cells and the target of TCS is likely downstream of Ca²⁺ influx (Palmer et al., Toxicol Appl Pharm., 258: 99-108, 2012). Since PLD activation occurs downstream of Ca²⁺ influx, we hypothesized that TCS will affect PLD’s function. The effects of TCS on both purified and in-cell PLD activity were determined by the Amplex® Red PLD Assay Kit. Our results show that TCS does not affect the activity of purified PLD in vitro; cellular experiments are ongoing. This research provides information on how TCS affects mast cell signaling and TCS toxicity on human health.

Microtubule Morphology and Paclitaxel Induced Peripheral Neuropathy
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Paclitaxel is a chemotherapeutic agent commonly used in breast, ovarian and lung cancer treatment. Paclitaxel induces cell cycle arrest and apoptosis of cancer cells by stabilizing microtubules. Despite its anti-proliferative effects it also primarily causes sensory axon degeneration, called paclitaxel induced peripheral neuropathy, which occurs in about 70% of patients. Evidence from our lab suggests that epidermal damage is an underlying cause of axon degeneration. The epidermis is innervated by unmyelinated “free” nerve endings, which are highly susceptible to environmental changes. Given the microtubule-stabilizing effects of paclitaxel, we hypothesized that stabilization may also be present in epidermal keratinocytes, which may contribute to epidermal damage. We assessed stabilization by staining for detyrosinated α-tubulin, which is a modification that is predominant in stabilized microtubules. Initial evidence suggests that paclitaxel increases microtubule stability specifically in keratinocytes, confirming our hypothesis.

A study of age-related effects on zebrafish phagocyte function
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As the pace of world population ageing accelerates, it is important to understand more about age-related effects on the immune system. Neutrophils and macrophages are white blood cells that serve as phagocytes, which internalize (phagocytose) and kill pathogens. Studies with elderly humans have shown an age-dependent reduction in phagocytic capacity. In mice, phagocytosis in aged individuals is not decreased unless protein deficiency is present. To determine whether age-related differences exist in the zebrafish model, we performed phagocytosis assays on cells from younger (7 months) and older (>2 years) fish. Our preliminary results show that the percentage of phagocytes participating in a moderate level of phagocytosis was actually higher in older fish, while there was no difference between the old and young fish in the percentage of cells performing high levels of phagocytosis. There are also interesting differences between the phagocytic activity in male and female fish in both age groups.

A computational tool to determine corresponding candidate regulatory regions in two different species
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Multiple closely located transcription factor binding sites can work as a unit to regulate the expression of nearby genes. This functional unit is called a cis-regulatory module. We can computationally identify candidate cis-regulatory modules by looking for groups of short sequences that are conserved across evolutionarily diverse species. Once identified, candidate modules must be biologically validated in a model species. The genetic position of modules in
different species can vary widely, and the order of the transcription factor binding sites in these modules can differ. As a result, the existing tools used to identify genetically similar regions across species, such as multiple alignments, cannot effectively find corresponding modules. To address this limitation, I developed a novel computational tool that can identify the corresponding modules in two different species.

**Effect of Botanical and Synthetic Compounds on the American Dog Tick *Dermacentor variabilis***

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The American dog tick, *D. variabilis*, is a tick species of medical and veterinary importance. Although not known to transmit diseases in Maine, *D. variabilis* spreads rickettsial diseases in other parts of the United States. In this study, we tested botanical and synthetic compounds on *D. variabilis* and noted mortality rates from chemical exposure. In addition, we examined the different effects these compounds had on male and female *D. variabilis*. Both IC3 (botanical) and Talstar (synthetic) killed 100% of ticks the first day. Another botanical, IC2, killed 100% of ticks over two days. Cedar oil killed 0% of ticks over six days. Savory Sun, a novel tobacco-based compound, killed 44.4% of ticks over six days. Savory Sun was the only compound tested that impacted male and female *D. variabilis* differently. Over six days, 33.3% of females and 55.6% of males were killed by Savory Sun.

**The involvement of microRNAs in preeclampsia**

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Preeclampsia is a disease which affects 8% of pregnant women and influences both the mother and the child. Decreased levels in VEGF is a major finding in preeclampsia. However, the pathology is not completely understood. In an earlier examination microRNAs regulating the VEGF system were found in urine samples of patients with preeclampsia. Therefore, we investigated the glomerular role of these microRNAs in a transgenic zebrafish model with different assays. After overexpression of microRNA-10b, -26a, and -489, the zebrafish developed edema and loss of plasma protein. A VEGF A protein could ameliorate these effects. Thus, microRNAs might be involved in impairments of the glomerular filtration barrier in preeclampsia. These findings could have an impact on future diagnostic or therapeutic options in preeclampsia.
Eelgrasses Capacity to Mitigate Ocean Acidification: Frenchman Bay, Maine

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\textit{Zostera marina} (eelgrass) composes a key marine ecosystem found in temperate latitudes. Eelgrass is able to sequester and store carbon through photosynthesis by taking carbon dioxide from the atmosphere or water column and storing it within sediments and below ground biomass. The ability of eelgrass to act as a carbon sink may play an important role in mitigating near shore ocean acidification. Eelgrass has declined dramatically in the past 20 years around Mt. Desert Island, ME, particularly in upper Frenchman Bay. We have analyzed the inter-annual carbon stocks in remaining eelgrass areas. In addition, we have examined the carbon sequestration rate by dating sections of sediment cores, and the derivation of carbon in the sediment through isotopic analysis of carbon. These findings will be used to raise awareness of the capacity of eelgrass to help mitigate the effects of climate change and garner support for eelgrass protection in Maine.

The neural dependence of caudal fin regeneration in zebrafish (\textit{Danio rerio})

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While most vertebrates have some regenerative capacity, few are able to regenerate entire limbs. Zebrafish (\textit{Danio Rerio}) rapidly regenerate limbs and several organs after injury or amputation. Previous studies found that fin regrowth is dependent on the presence of nerves (Simoes et al., Dev.Bio. 14(49), 2014). By exposing zebrafish, post caudal fin amputation, to Schwann cell inhibitor AG1478 or a control, we studied the effect of AG1478 on regrowth, axons, and Agr2\textsuperscript{+} gland cells. Fish were incubated in the treatment or control for 11 hrs/day for 5 days. Fish exposed to AG1478 showed reduced caudal fin regeneration compared to controls (p < 0.001). Confocal analysis revealed that regenerated tissue of AG1478 fish tended to have more peripheral axons than the controls (p = 0.06). There was no difference in Agr2\textsuperscript{+} counts between the groups. These results suggest that caudal fin regeneration is not dependent on the number of peripheral axons.

Genomic variation and host range diversity in mycobacteriophage

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Mycobacteriophage are diverse and abundant viruses that infect bacteria of the genus \textit{Mycobacterium}, including \textit{M. tuberculosis}. Mycobacteriophage are sorted into clusters based on genome sequence similarity. There are 27 clusters comprising 7,130 mycobacteriophage isolates. Nearly 88% were isolated using \textit{M. smegmatis}. This limits our ability to understand mycobacteriophage diversity and host range. To increase isolation diversity, we optimized
isolation procedures for pathogenic hosts *M. chelonae* and *M. marinum*. We have not yet isolated novel phage using these hosts. To explore host range, we applied 32 mycobacteriophage from clusters A–X to lawns of *M. chelonae* and *M. marinum*. Twelve mycobacteriophage infected *M. chelonae*. No phage infected *M. marinum*. The cluster K phage, Mynx was recently isolated and infects *M. chelonae*. The complete annotation of the Mynx genome is presented and future work includes identifying genes that may play a role in host range expansion and lysogenic conversion.

**Lactic acid bacteria reduce Salmonella Javiana-induced epithelial cell cytotoxicity and decrease pathogen virulence gene expression**

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*Salmonella enterica* Javiana is an emerging foodborne pathogen that causes severe gastrointestinal infections. Little is known of how S. Javiana interacts with intestinal epithelial cells, or whether S. Javiana infections can be mitigated by probiotic lactic acid bacteria (LAB). Using the HT29-MTX human intestinal epithelial cell line and lactate dehydrogenase release assays, we observed a strong cytotoxic effect of S. Javiana, and found that cytotoxicity was reduced when cells were exposed to LAB prior to infection. Adhesion assays showed no reduction in S. Javiana binding to host cells in the presence of LAB, but qRT-PCR analysis revealed that LAB exposure decreased S. Javiana toxin gene expression. Data suggest that LAB might be useful if taken as prophylactics to reduce incidence or severity of S. Javiana infection, and that probiotics may work by preventing S. Javiana from producing key toxins that would otherwise damage host intestinal cells.

**Role of MyD88 in Neutrophil response to Candida albicans infection of Zebrafish Swimbladders**

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Neutrophils are crucial in human defense against fungal pathogens, such as the opportunistic *Candida albicans* that colonizes mucosal membranes (Weindl et al., J Dent Res., 89(7):666-675, 2010). MyD88, an adaptor protein in the Toll-like receptor pathway of innate immune response, links transmembrane receptors to a signaling cascade. This cascade causes the release of the cytokines IL-8 and IL-1β, a chemo-attractant for neutrophil recruitment and a neutrophil activator, respectively (Lionakis et al., PLoS Pathog., 9(1):e1003079, 2013). In this experiment we assess the role of MyD88 in neutrophil recruitment. We inoculated four-day old zebrafish (*Danio rerio*) swimbladders with *Candida albicans*. Using confocal microscopy and Sudan Black staining, we quantified *Candida* and counted neutrophils in the swimbladder. Fish were then genotyped to identify strictly MyD88 mutant and wild-type fish. These results will
illuminate MyD88’s contribution to innate immune response by neutrophils. They will provide useful information on opportunistic nosocomial disease in immuno-compromised individuals.

Understanding the role of collagen triple helix repeat containing 1 (cthrc1) in arthritis: CAIA models.
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The collagen triple helix repeat containing 1(cthrc1) is a novel gene induced in remodeling tissues including arthritic joints where it is expressed by the activated fibroblast-like synoviocytes that form the joint-destructing pannus. To determine the role of cthrc1 in arthritis, we analyzed bones from a mouse model of collagen antibody induced arthritis (CAIA) performed on Cthrc1 null mice and wildtype mice. Tissue sections from the decalcified lower limbs were stained with hematoxylin & eosin and the pan-leukocyte marker CD45. The area of inflammation and pannus formation was quantified by image analysis. Inflammation was significantly increased in the joints from Cthrc1 nulls. To test the effect of CTHRC1 on the major signaling pathway regulating inflammation, we performed NF-kB reporter assays in RAW264 cells stimulated with LPS in the presence or absence of Cthrc1. In this assay we were unable to see an effect of Cthrc1 on NF-kB activation.

Biomimetic assessment for the efficacy of drug cocktails
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Heart regeneration is a complex process that requires intercellular communication from cardiac and noncardiac cells. New findings suggest the epicardium, the outer tissue layer of the heart, plays a crucial role in coordinating the injury response in adult zebrafish. Upon injury, the epicardium rapidly migrates to envelop the injury environment and secretes trophic factors that stimulate cardiomyocyte dedifferentiation and proliferation. The goal of this study is to mimic this biological response by independently establishing an explant culture of epicardial tissue and a primary culture of cardiomyocytes. To date, we have identified culture conditions that enable migration of epicardial cells and permit survival of cardiomyocytes. With these approaches, we seek to perform small molecule screens to identify potential drugs that will enhance heart regeneration.

Impact of reduced initiation, elongation, and ribosome biogenesis on global translation and health in C. elegans
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Past research identified that attenuation of translation-linked genes increases longevity. The effect of reduced translation on healthspan, however, is less characterized. To determine healthspan effects, these genes, which are responsible for translation initiation, elongation, or ribosome biogenesis, were knocked down using RNAi during adulthood. Post-RNAi, resulting changes in size or quantity of ribosomes and its subunits were determined by polysome profiling, and healthspan over time was determined by measuring locomotive capabilities. Preliminary results indicate that knockdown of transcription initiation complex components yields profiles heavy in 80S monosomes, whereas knockdown of elongation factors yields profiles heavy in polysomes. Additionally, the unique polysome profiles generated by each knockdown may be used in comparison to future gene queries of translation. Once determined using our locomotion metrics, the different healthspans can be compared; beneficial knockdowns may be candidates for therapeutic strategies to retain youthful locomotion in humans for an extended duration.

**Modulation of VRAC functional properties by variation of subunit expression levels induced by hypertonic stress**

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Cellular volume homeostasis is an intricately and tightly regulated process that is essential for cell development, growth, survival and even death. The volume regulated anion channel (VRAC) is activated by cell swelling and plays a central role in the volume regulatory release of Cl\(^-\) and organic osmolytes from cells. The biophysical and cell physiological properties of VRAC have been studied extensively. However, until recently, the molecular identity of VRAC was unknown. Voss et al. (*Science*, 344, 634-638, 2014) have shown that five LRRC8 genes, LRRC8A-E, are essential for VRAC activity. Assembly of VRAC from different combinations of subunits gives rise to channels with distinct functional properties. We are testing the hypothesis that acute and chronic hypertonic stress alters LRRC8A-E expression levels and that these altered expression levels in turn give rise to unique VRAC channels that are specifically tuned to cellular physiological needs.

**Characterization of tyrosinase activity and UV effects in wild-type and albino *F. heteroclitus* embryos**

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Killifish, *Fundulus heteroclitus*, spawn in estuaries at high tides. As the tide ebbs, some embryos are stranded and aerially incubate for up to fourteen days. During this time, embryos are exposed to UV radiation and desiccation stress. Our research focused on these stresses and their effect on wild-type and albino embryos. Embryos from fish collected at Northeast Creek (MDI) were assayed for tyrosinase activity (which leads to melanin synthesis) and embryo’s ability to tolerate UV-B radiation (280-320 nm). Preliminary experiments using a newly developed single embryo tyrosinase assay indicated that albinos exposed to UV-B radiation had lower tyrosinase levels, compared to non-irradiated controls. Spectrophotometric microassays developed to measure the chorion’s UV-B absorbance suggest that wild-type embryos are capable of absorbing twice the levels of radiation compared to albino embryos. These mechanisms of stress resistance to UV radiation in killifish may be relevant to embryos of other fish species.

**The involvement of Tryptophan Pathway enzymes in the maintenance of the glomerular filtration barrier**

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Most of the hundreds of thousands of Americans who develop renal failure yearly undergo proteinuria, the loss of essential high molecular weight proteins from the blood into the urine. Recently it has been discovered that they also experience an increase in tryptophan pathway metabolite levels. We hypothesized that the pathway might be important for the glomerular filtration barrier (GFB) of the kidney to function properly. To observe the effects on the GFB when key pathway enzymes KMO, TDO, and IDO, are not produced, we knocked down the genes encoding for the enzymes in zebrafish. We then performed several assays on the fish to see if the loss of the enzymes resulted in proteinuria. Our results revealed that the concentration of proteins in the blood was lower in the enzyme deficient fish. This suggests that the loss of the enzymes affects the GFB’s ability to function effectively.

**Studying the presence and effect of Labyrinthula zosterae in Eelgrass**

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Eelgrass (*Zostera marina*) has played a vital role in the marine ecosystem of Frenchman Bay, and has been declining since 2007. In 2013 eelgrass suffered a complete loss in both restored and naturally occurring areas. Members of the Community Environmental Health Laboratory sampled ten sites around the island, taking a total of 100 samples. These samples were tested for upregulated expression of genes associated with heat stress, disease, and general stress. A higher
level of HSP70 was detected at Hadley Point, Jordan River, and Stave Island and the presence of *Labyrinthula zosterae*, an eelgrass pathogen, was also detected at Stave Island. I performed an ethanol precipitation to clean and concentrate the DNA samples and tested for the presence of *Labyrinthula zosterae* in all 100 samples. Here I report the results of the PCR regarding the presence of *Labyrinthula zosterae* and its possible correlation to an upregulated stress gene in eelgrass.

**A novel, high-throughput method for assaying thermal nociceptive withdrawal response in *D. melanogaster***

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Chronic pain is the leading cause of disability in the United States, affecting about 30% of the U.S. population. Current medications for treating chronic pain are associated with significant risk including dependence and tolerance. Chronic pain is perpetuated by pain sensitization, which we study using multiple injury models in *D. melanogaster*. However, because current methods for assaying thermal nociception in *Drosophila* are time-consuming and technically challenging, this research project sought to develop a novel, high-throughput nociceptive response assay. Using a modified PCR machine to maintain constant temperatures, the ventral surfaces of 3rd instar larvae were subjected to a 5ºC range, and their nociceptive threshold was determined by recording the frequency of their characteristic withdrawal response. This identification of baseline thermal sensitivity is necessary for downstream sequencing analysis of various states of pain sensitization. Our overall goal is to identify novel targets for better drugs for the treatment of chronic pain.

**The Role of Mechanical Stress in Paclitaxel Induced Peripheral Neuropathy**

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Paclitaxel is an effective cancer treatment drug that induces tumor cell apoptosis by stabilizing microtubules. The majority of patients who are treated with paclitaxel also develop a condition of axon degeneration called Paclitaxel-induced peripheral neuropathy, which affects primarily the sensory nerve endings innervating the epidermis. Currently, no treatments exist and the mechanisms are largely unknown. One hypothesis is that axon degeneration is caused by epidermal stress induced by stabilized microtubule, leading to increased reactive oxygen species (ROS) formation and MMP-13 (Collagenase-3) activity. To assess stress formation in the epidermis of DMSO vehicle and paclitaxel-treated zebrafish, we are utilizing an engineered mechanical stretching device that permits the application of defined mechanical stretch to the skin. As readout for stress induction we are imaging an NF-κB-GFP reporter line. First results will be presented.
Optimizing the study of interactions between two RNA-binding proteins in the pathogenic fungus Candida albicans

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Candida albicans is an opportunistic fungal pathogen found in the majority of the population that lives commensally or causes mild infection in healthy individuals but that seriously threatens the health of immunocompromised patients. One factor influencing the spread of infection is the ability to switch between budding yeast and filamentous hyphal forms. Transport of mRNA to the hyphal tip may play a role in this switch and thus influence Candida virulence. RNA-binding proteins She3 and Slr1 both localize to the hyphal tip under certain conditions and are important for proper hyphal growth, potentially by transporting mRNA to the hyphal tip. To test whether these proteins might function in the same complex, tagged proteins were purified from hyphal cells under different conditions. Preliminary evidence does not indicate that She3 and Slr1 are in a stable complex, although low levels of each protein may prevent detection of She3-Slr1 copurification.

Characterizing the effects of paclitaxel dosage in peripheral sensory axon degeneration

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Paclitaxel, a microtubule-stabilizing agent, is commonly used in the treatment of many prevalent cancers. Paclitaxel chemotherapy also causes severe side effects, such as peripheral neuropathy. The Rieger lab has established a zebrafish model to study paclitaxel-induced peripheral neuropathy (PIPN) mechanisms in live animals to identify potential new therapeutic avenues with which to treat this condition. Concentrations inducing PIPN in zebrafish were determined to be ~100 times below those administered in humans. My project defines whether low dose-mediated PIPN in zebrafish is comparable to the mammalian condition. I injected adult fish with various paclitaxel concentrations once daily for four consecutive days and determined the LD50 as well as axon branch number in the caudal fin using an antibody against anti-acetylated tubulin to label axons. So far, I found that the LD50 approximated previously used concentrations, suggesting that species-specific differences in paclitaxel metabolism likely exist, and that the utilized concentrations are comparable.

Identifying candidate cis-regulatory modules for miRNAs involved in zebrafish heart and caudal fin regeneration

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MicroRNAs are a class of small, highly conserved, noncoding regulatory RNAs that silence messenger RNA through translational arrest or transcript degradation. In zebrafish, mir21-1 helps regulate caudal fin and heart regeneration. A better understanding of mir21 regulation may contribute to regenerative therapies in humans. To understand the difference in regulation of mir21, several candidate regulatory regions were computationally identified. The regions were found using Genetic Algorithms for Motif Inference (GAMI), a computational tool that analysed the noncoding regions surrounding mir21, looking for conservation of motifs across multiple species. These motifs serve as candidate transcription factor binding sites (TFBS), and where they are clustered are candidate conserved cis-regulatory modules. These modules were compared against the data on the UCSC genome browser, looking for regions that aligned with histone modifications, known TFBS, and DNase clusters, based on the ENCODE project. The analysis has identified several candidate enhancers, an insulator, and core promoter region for mir21.

**Inner ear development in zebrafish (Danio rerio) under conditions of oxidative stress**

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Reactive oxygen species play an important role in the developing embryo, but an over-abundance can have damaging effects. We hypothesized that oxidative stress caused by exposure to tert-butylhydroperoxide, would affect the inner ear of developing zebrafish. Using confocal and scanning electron microscopy morphological changes were scored at 48hpf, 72hpf, and 96hpf in the Brn3C:GFP line which expresses GFP within neuromast cells. The expression of two genes known to be up-regulated with oxidative stress, cfap70 and dhx40, was also determined using quantitative real-time PCR. We found observable differences in the morphology of neuromast cells in treated embryos when compared with controls, although they were not statistically significant. There were also no statistically significant differences in the expression of cfap70 and dhx40 at 48hpf in treated versus control animals. We conclude that while oxidative stress likely plays a role in otic vesicle development, sample size needs to be larger for significant results.

**Characterization of Semaphorins 1a and 2a in the cricket Gryllus Bimaculatus**

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The adult cricket *Gryllus bimaculatus* exhibits a robust compensatory response to central nervous system injury. Upon unilateral damage of an auditory afferent, downstream interneurons grow and form connections with the remaining auditory afferent. This response may be mediated by the semaphorins, a conserved chemotropic guidance protein family. In order to better characterize the roles of semaphorins in the cricket, mRNA *in situ* hybridizations were used to assay gene expression in embryos and adults. In order to test the function of semaphorins, double stranded RNA was synthesized and injected early in development. Expression patterns during development appear to be conserved. Semaphorin 2a does not appear to play a critical role during nervous system establishment, however, semaphorin 1a appears to be important for CNS and PNS development.