MicroRNA-181b influences blastema proliferation during zebrafish caudal fin regenerating

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Humans have a limited capacity for tissue regeneration. While we are able to regenerate portions of the kidney, liver, blood, hair and skin cells, we lack the ability to regenerate limbs or larger, more complex organs. Other organisms however, such as zebrafish, are endowed with the capacity to regenerate nearly all complex tissues, including appendages. Using the adult zebrafish (Danio rerio) as a model organism, we investigated the role of the miR-181b in the caudal fin during the regenerative process. Our findings reveal that miR-181b is upregulated during regeneration and its spatial expression is enriched in the regenerating blastema, the defining tissue of appendage regeneration. Importantly, depletion of miR-181b activity with antisense locked-nucleic-acid oligonucleotides inhibited regeneration by ~40% in comparison to scrambled control treated fins. Defective regeneration was accompanied with a robust suppression of blastema cell proliferation and defects in cell patterning. My findings suggest a model whereby miR-181b activation promotes blastema proliferation and tissue outgrowth.

Defining the role of IFT88 in Danio rerio caudal fin regeneration

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Intraflagellar Transport homolog 88 (IFT88) is a protein encoding gene associated with cilium that affects the position and orientation of microtubules and cytoplasmic dynein during cellular division and regulates the orientation of blastula cells during embryonic development. How and to what extent IFT88 may control the directionality and positioning of blastemal cells during regeneration has not been assessed. Here we show that IFT88 mRNA is upregulated during adult zebrafish appendage regeneration. Importantly, IFT88 depletion with an antisense locked-nucleic-acid Gapmer oligonucleotide inhibited migration of the wound epidermis, resulting in complete abrogation of blastema formation and overall caudal fin regeneration. To effectively examine the mechanism of action behind this result, novel antibody staining procedures were developed to accurately visualize cilium in the caudal fin under confocal microscopy. These procedures will be utilized to properly assess how IFT88 depletion affects the propagation and
orientation of cilium and the downstream signaling pathways. These results may allow for a far
greater comprehension of the cellular mechanisms surrounding wound healing and appendage
regeneration. Understanding the signaling circuitry that regulates tissue regeneration in zebrafish
may lead to the development of novel approaches that enhance the limited regenerative capacity
of mankind.

A study of the auxin-inducible degradation (AID) system in the germlines of C. elegans
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Germ granules are large ribonucleoprotein complexes that extend the nuclear pore complex into
the cytoplasm within germ cells. In Caenorhabditis elegans, these complexes are called P
granules, and they form an environment for post-transcriptional regulation throughout the germ
line. Prior studies have demonstrated that these P granules are necessary for the maintenance of
germin cell fate and pluripotency, but the mechanism involved has remained unclear. Among some
of the proteins shown to preserve P-granule aggregation are PGL-1, PGL-2, and PGL-3, which
were the targets of this study. Prior analyses, targeting P-granule proteins with RNAi, have been
used to show that absence of P granules results in sterility among C. elegans germ cells.
However, RNAi requires the use of multiple targets and significant amounts of time and effort,
making it a severely limited technique. This study sought to utilize the auxin-inducible
degradation (AID) system and CRISPR/Cas9 gene editing to conditionally deplete PGL-1, PGL-2,
and PGL-3 in the germline of C. elegans. The preliminary results of this study demonstrate
that the AID system is sufficient to deplete PGL-1::degron. Due to the conservation of germ
granules across different species of sexually reproducing organisms, the knowledge gained
through this research could be applied to human health in years to come.

Fkbp5 expression may contribute to pro-inflammatory phenotypes in cortisol-treated
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Stress-related inflammatory diseases are some of the leading causes of morbidity and mortality
in humans globally (Zannas et al., 2017). Zebrafish embryos treated with the stress hormone
cortisol have been found to develop a pro-inflammatory phenotype later in life, modeling the
inflammatory disease development we see in humans. Our lab is investigating the role of the
glucocorticoid receptor (GR), a cortisol-activated transcription factor, in mediating this
phenotype. This study looks specifically at the role of fkbp5, a GR-induced gene that encodes a
negative regulator of the GR. Cortisol treated zebrafish embryos were found to have the highest
level of fkbp5 expression during day 4 of development. A splice-blocking morpholino was used
knock down the expression of fkbp5, and the expression of several pro-inflammatory genes
found to be upregulated in response to cortisol treatment were measured to determine whether
fkbp5 contributes to this effect. Results of these experiments will be presented.
Tanyocyte plasticity in the hypothalamus with the regulation of energy balance
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Energy balance, including energy intake and expenditure, is primarily controlled by the hypothalamus in our brain. Recently, a niche of potential adult neural stem cells (ANSCs) called tanyocytes were discovered in the hypothalamus, lining the third ventricle and within the median eminence. Previous data from our laboratory has revealed tight co-expression of tanyocytes with the bone morphogenetic protein (BMP) receptor, BMPR1A. Based on these data, along with findings in the published literature, we hypothesized that the BMP7 ligand found circulating in the cerebrospinal fluid is taken up by BMPR1A-expressing tanyocytes in order to affect neurogenesis of the tanyocyte population. To test this hypothesis, we have taken two approaches: First, we utilized a transgenic mouse line and uncovered that hypothalamic tanyocytes express mouse telomerase reverse transcriptase (mTERT), a marker for slowly cycling, quiescent stem cells. The expression of mTERT [marked by green fluorescent protein (GFP) in transgenic reporter mice] was very dense in the median eminence and dotted along the third ventricle lining in the hypothalamus, representing expression in the beta subset of tanyocytes. This indicated that the beta- tanyocytes might be the most primitive hypothalamic stem cell. For the second approach, BMPR1A was ablated from the beta- tanyocytes using Cre-Lox technology, with the Rax-Cre driver that specifically targets beta- tanyocytes. This mouse model is currently being utilized to examine the functional relationship between BMP7-BMPR1A signaling and tanyocyte plasticity in regulating hypothalamic neurogenesis. We believe this relationship most likely plays an integral role in the ability of the hypothalamus to regulate energy balance.

Investigating the causes of meiotic arrest in the Sertoli cell-specific AR knockout testes
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Androgen receptor (AR) action in Sertoli cells is required for germ cells to complete meiosis and is important for male fertility. The Sertoli cell-specific AR knockout (SCARKO) mouse model exhibits a meiotic arrest phenotype, with germ cells failing to exit meiotic prophase I. Previous studies on the SCARKO mouse model have shown that these mice do not complete meiosis and are infertile. In this study, I used SCARKO mice with synchronized spermatogenesis to thoroughly investigate which germ cell population undergo cell death. Using the TUNEL assay to detect apoptotic germ cells at different time points of synchronized spermatogenesis, I observed a high accumulation of dying germ cell population starting at 12 days’ post retinoic acid (RA) injection in SCARKO testes. These results indicate that, in testis lacking AR signaling in Sertoli cells, apoptosis in germ cells occurs at early pachynema.
Recent research has linked early life chronic stress to the development of several pro-inflammatory conditions, including diabetes, high blood pressure, and obesity (Vanitallie, 2002). To elucidate the relationship between chronic early life stress and inflammatory diseases, zebrafish embryos were treated chronically with cortisol and examined for morphology changes and differential gene expression. Previous research has shown that this treatment results in upregulation of several pro-inflammatory genes (Hartig et al., 2016). Additionally, chronic cortisol treatment leads to significantly elevated expression of klf9, a transcriptional regulatory gene activated by the glucocorticoid receptor. To investigate klf9’s role in mediating the response of zebrafish embryos to cortisol treatment we used a morpholino antisense oligonucleotide to knock down its expression along with quantitative reverse transcription polymerase chain reaction to assess how the knockdown affects expression of several genes that are differentially expressed in cortisol-treated embryos. Initial results regarding this investigation will be presented.

Heart disease is a major problem worldwide; it causes mortality and morbidity (Lloyd-Jones et al. Circulation., 121:46-21, 2010). In humans, a common heart disease pathology known as coronary infarction (Heart attack) can lead to ischemic injury, scarring, and subsequently heart failure. Zebrafish (Danio rerio) are model organisms for cardiology research due to their molecular similarities to humans, but unlike humans, D.rerio is able to regenerate cardiac tissue after injury (Bakkers, Cardiovascular research, 91: 279-288, 2011). Researching the pathway of D.rerio heart tissue regeneration following trauma may reveal information pertinent to human illness. Organelles known as cilia are of special interest in this case due to their implicated roles in. In this project three separate approaches comprised of both molecular and microscopy techniques were used to assay the function of cilia in D.rerio heart regeneration. First, immunohistochemistry was used to label selected portions of heart sections from both injured and uninjured specimens to detect the relative prevalence of cilia in either scenario. Subsequently quantitative polymerase chain reaction (qPCR) analysis will be used to determine whether selected genes related to the growth and maintenance of cilia are upregulated following cardiac injury. Additionally, qPCR analysis was employed in order to test for the depletion of IFT 88 mRNA in fish following a knockdown of IFT 88 mRNA. Data derived from these projects will offer information on the roles of cilia in regeneration.
Importance of eIF-2α phosphorylation in regulation of stress response, health, and longevity in C. elegans.
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The primary risk factor for many diseases, including Alzheimer’s and cancer, is age. Dietary restriction (DR), a reduction in calories consumed while maintaining proper nutrition, is a potential intervention to help mitigate late-onset diseases. DR has been an effective way to extend longevity and health in multiple organisms which respond to DR by altering mRNA translation. Understanding the pathways underlying responses to DR in C. elegans will be informative in devising pro-longevity treatments in humans. In an evolutionarily conserved pathway, DR activates the protein kinase gcn-2 which phosphorylates and inhibits the translation initiation factor eif-2alpha on serine 49. While gcn-2 activity is necessary for the inhibition of global protein translation that occurs under DR, it is unknown if the phosphorylation of eif-2alpha is also required. The importance of eif-2α phosphorylation on global translation under DR was tested with polysome profiling, a technique which measures the distribution of mRNA among active and inactive ribosomes, using a mutant incapable of being phosphorylated on serine 49 of eif-2alpha. The same mutant was used to determine whether or not eif-2α phosphorylation is necessary for enhanced health, as measured by the locomotion of the worms, imparted by DR. Also, impact of the mutation on heat stress resistance was categorized through heat shock, as worms on DR have historically exhibited higher vitality post-shock of a given duration and temperature. Preliminary results suggest that the phosphorylation state of eif-2alpha is dispensable for inhibiting global translation under DR but plays a role in maintaining health.

Purification of red abalone hemocyanin subunits for alternative vaccine production
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Keyhole Limpet Hemocyanin (KLH), a respiratory protein of the marine gastropod Megathura crenulata, is a potent immunostimulant and an important ingredient in vaccine formulas and research. Purified KLH, often valued at approximately $3,000 per gram, is neither ecologically sustainable nor economically accessible, as over-fishing has endangered wild M. crenulata populations, and only one company has developed M. crenulata aquaculture. The molecule cannot be synthesized. Previous studies suggest similarities between KLH and hemocyanin from the red abalone (Haliotis rufescens). H. rufescens meat is already a delicacy, and their shell is the prized Mother of Pearl. The use of RAH as a hapten carrier in vaccine production would significantly augment the value of this already lucrative species, which has been successfully aquacultured in Maine. The focus of this study is to isolate and purify RAH from live specimens for comparative biochemical, immunomodulation, and hapten carrier analysis with KLH. Preliminary findings suggest that RAH is similar in size to KLH, which is an important factor. RAH subunits, however, may dissociate differently than KLH subunits. Market and economic research will complement the scientific data needed to turn a RAH venture into a viable pharmaceutical development endeavor in Maine.
**Drosophila** as a model to study age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause of blindness with no cure to date. One of the causes of AMD is due to the degeneration of the retinal pigment epithelium (RPE) characterized by the loss and subsequent formation of large, multinucleated cells. A major challenge for AMD studies has been the late onset of the disease, even in mouse models, where it can take several months or years for pathology associated phenotypes to arise. As **Drosophila melanogaster** ages, their terminally differentiated epithelial cells are lost resulting in the analogous formation of large, multinucleated cells within weeks. The genetic and molecular tractability of the fruit fly offers a unique opportunity to define, for the first time, the causes and consequences of these cell morphological changes. To do so, **Drosophila** orthologs of human disease-linked genes, including **alp**ha-catenin, **protein kinase C**, **adamtsl4**, **crumbs**, and **complement factor H** were knocked down in our adult fruit fly model. So far, we found that the conserved apical-basal polarity protein Crumbs plays a key role in protecting against cell loss and multinucleation with age. These findings validate the fruit fly as a novel model to determine the molecular mechanism(s) regulating degenerative cell morphological changes in the RPE. In future, the fruit fly model could provide a means to identify genetic and/or pharmacological therapies to prevent or even reverse the cell loss and multinucleation observed in human retinal dystrophies.

The effect of embryonic arsenic exposure on the sensorimotor behavior of zebrafish (**Danio rerio**)

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The optic systems of **D. rerio** are very similar to eyes of humans, and can therefore be useful in studies of human eye disease. The zebrafish optic system is also a good model to observe the response to contaminants in the environment, especially in water systems. Previous studies in our lab have shown that arsenic exposure to zebrafish embryos caused gross morphological changes in the retina. The optomotor response in zebrafish deploys swimming movements, and is dependent on the retina, which is negatively impacted by arsenic (Muto et al., PLoS Gen., 1:e66, 2005). Zebrafish embryos were exposed to 3 replicates of 4 treatments of sodium arsenite from 0 to 72 hours post fertilization: A control, 0 ppb arsenic; the current water standard, 10 ppb of arsenic; the water standard in 2000, 50 ppb; and 500 ppb, the upper environmentally relevant threshold in Maine. Behavioral testing took place at 5 days post hatch, when the eye was fully developed and the fish was visible to a camera. The behavior of individual fish was video recorded in a series of 3 tests: 1) a blank arena, 2) a black dot in the center of an arena, and 3) a black dot towards the edge of an arena. Using this assay, any effects on vision due to arsenic exposure could be detected. Data are currently being analyzed using idTracker.
Modeling the differentiation of induced pluripotent stem cells using scRNA-Seq data
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Induced pluripotent stem cells (iPSCs) are promising tools for biomedical research such as human disease model, and regenerative medicine. Yet, our insight into the gene regulatory mechanism of iPSC differentiation is still incomplete. The recently developed single-cell RNA sequencing (scRNA-seq) technology has enabled researchers to measure gene expression at single-cell resolution. However it is still challenging to identify cell-to-cell variation and cell state transitions from scRNA-seq data. This study aims to understand the regulatory mechanism underlying the iPSC differentiation via a novel combined approach that utilizes both scRNA-seq data analysis and network modeling. In this study, the scRNA-seq data measure the gene expression of iPSCs differentiating into four different cell types: mesoderms, endoderms, neurons, and trophectoderm (TEs), and at four collection times: day 1, 2, 4, and 6. Based on hierarchical clustering analysis, we first found the 640 GO embryonic developmental genes can capture major cell phenotypes. The results also show that after iPSCs differentiate into TEs, around day 4 and 6 the TEs diverge into two subpopulations. In subsequent analyses, we inferred a pseudotime of each cell and examined the time dynamics of the 640 genes in the TE cell line and clustered the genes based on their dynamical behaviors. We are planning to elucidate the mechanism underlying the decision-making of the differentiation processes within the TE cell line by constructing a gene regulatory network from these gene clusters and applying the newly developed computational method called random circuit perturbation (RACIPE) to analyze the dynamics of the constructed network.

Evolution of Fusion and Mosaic Proteins in Relation to Eukaryogenesis and Genomic Intron Insertion
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This study aims to establish phylogenetic and molecular lineages of vertebrate intron insertion pathways in large, modular, multifunctional proteins (LMMP’s) to determine origins of introns via the intron-late or intron-early theory. Specifically, this investigation focuses on three related LMMP’s: c-MET, a proto oncogene that utilizes a tyrosine kinase; HGF/SF, the ligand for c-MET; and CDH1, a cell adhesion protein. Using computational tools, protein domains were mapped and characterized to establish conserved residues (costrings) that are vital to proper protein activity and aid in establishing evolutionary relationships amongst vertebral species. Additionally, this project identified and investigated the functionality of novel regions of extreme conservation in internally duplicated protein domains (MOD-costrings) that are present in HGF/SF and CDH1. 3-Dimensional analyses were performed on these LMMP’s to show conservation, or lack thereof, in protein secondary structure, as well as to model location of costrings in the protein tertiary structure. Preliminary results support the intron-late theory, stating that intron insertion occurred simultaneously with eukaryogenesis. This study’s
identification and analysis of costrings and MOD-costrings may aid in determining specific areas for targeted mutation analyses amongst actively evolving protein domains.

**Spermatogonial stem cell self-renewal in the mouse**

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In males, germ cell homeostasis is maintained by self-renewal and differentiation of a robust stem cell population. However, it is unknown how mammalian spermatogonia accomplish this task. It has been assumed that germline stem cells divide symmetrically, whereby Aₛ spermatogonia give rise to an Aₛ cell with similar stem cell potential or a pair of cells connected by cytoplasmic bridges (Aₚᵣ) that undergo differentiation (Huckins, C., Anat. Rec., 169:533-557, 1971; Oakberg, E., Anat. Rec., 169:515-531). Braun Lab has identified a T-box transcription factor EOMES and cell cycle marker Ki67 that localizes asymmetrically in clones of GFRA1+ cells. Immunohistochemical analysis using GFRA1 antibody on wholenufort seminiferous tubules suggests that there is asymmetrical and asymmetrical localization of EOMES<sup>tdTOMATO</sup> in Aₚᵣ cells in the steady-state. Preliminary data suggests that during regeneration, there is an increase in symmetrical localization EOMES<sup>tdTOMATO</sup> in the GFRA1 paired population. Despite the increase in GFRA1 cycling population during regeneration, there was no significant change in the frequency of asynchronous to synchronous in GFRA1+ clones. To further test the hypothesis if self-renewal is asymmetrical, we would trace the progeny of EOMES+ cells with the tamoxifen-inducible Eomes knockin mouse line crossed to a dual reporter, which would allow to observe if there is a correlation between asynchrony and asymmetrical localization of EOMES and other proteins important for maintaining stem cell fate.

**Haloferax volcanii for carotenoid production**

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Carotenoids are light-absorbing pigments produced by plants and microorganisms that have been shown to have numerous health benefits. They act as antioxidants to protect animals from harmful oxidative damage and have implications in preventing heart disease and in acting as anticancer agents; however, because humans and other animals cannot synthesize carotenoids, they must be obtained through dietary intake. These health benefits have led to an increasing demand for commercially available carotenoids. We are studying carotenoid biosynthesis in the halophilic archaeon Haloferax volcanii. This organism predominantly produces the carotenoid bacterioruberin, which is not in high demand commercially compared to other carotenoids such as lycopene, β-carotene, and astaxanthin. Although H. volcanii synthesizes some lycopene, which is a bacterioruberin precursor, it does not endogenously produce β-carotene or astaxanthin; therefore, using synthetic biology and molecular cloning, we want to exploit the innate carotenoid-making abilities of H. volcanii and to re-engineer its biosynthetic pathway in order to synthesize large amounts of these carotenoids. We successfully prevented bacterioruberin production by removing the gene encoding for a key enzyme in the pathway,
thus creating a build-up of lycopene. Then, we were able to increase lycopene synthesis by 15 to 30-fold through increasing production of the enzyme catalyzing the committed step of carotenoid synthesis. Currently, we are optimizing methods of introducing enzymes from other organisms which act to synthesize β-carotene and astaxanthin from lycopene into *H. volcanii*. Optimization of these methods may lead to new approaches for the natural production of carotenoids on an industrial scale.