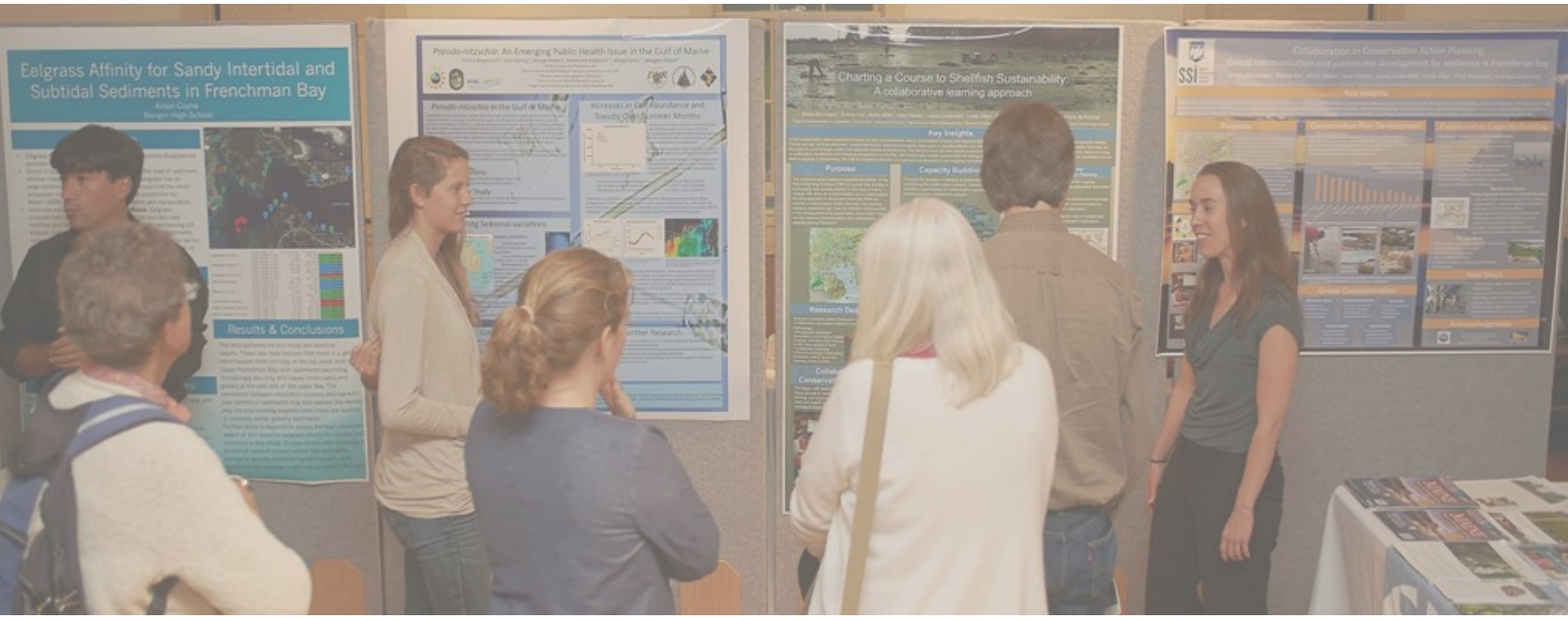

Student Symposium Abstracts

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A Study of the Importance of Syndecan and Versican Expression for the Glomerular Filtration Barrier, Proteinuria and Edema using zebrafish embryos

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Nephrotic syndrome is a kidney disorder related to the disruption of the glomerular filtration barrier, proteinuria and edema. Zebrafish are used as models for the human kidney in this experiment because they have very similar glomeruli structures to humans. Previous studies have shown that an up regulation of microRNA 143 (which inhibits Syndecan/Versican expression) occurs within the urine of patients suffering from nephrotic syndrome. This experiment aims to establish the connection between the lack of Syndecan and Versican expression in the glomerular filtration barrier and the occurrence of proteinuria and edema. Transgenic zebrafish embryos that express fluorescent plasma proteins are injected with microRNA 143 and morpholinos designed to knockdown Syndecan and Versican expression. The embryos are then observed to look for signs of edema or lowered eye fluorescence, which are correlated with proteinuria. The results show that the knockdown of Syndecan 2 and Versican, as well as the overexpression of microRNA 143 produces leads to a nephrotic phenotype whereas the knockdown of Syndecan 3 does not.

Measurement of neutrophil extracellular trap formation in zebrafish, (*Danio rerio)*

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Neutrophils are key cells of the innate immune system that serve as a first line of defense from invading pathogens. In addition to the well-characterized process of phagocytosis, neutrophils can also respond to microbes by neutrophil extracellular trap (NET) formation. This process is characterized by the release of neutrophil DNA wrapped around antimicrobial molecules. The web-like structures of NETs then physically trap and neutralize pathogens. Unlike mammalian models, zebrafish yield relatively small numbers of neutrophils for *in vitro* study of NET formation. Therefore we are modifying an existing whole-kidney based protocol to one using pooled cell suspensions and fluorescent detection of extracellular DNA. We also are optimizing our cell purification methods. In our preliminary investigation, we have used a plate-based assay to compare the kinetics of NET production by neutrophils from untreated zebrafish to those having undergone acute exposure to ethanol.

Assessment of Leukocyte Mobilization in *Leucoraja erinacea* Using AMD3100

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Elasmobranchs are cartilaginous fishes that lack the endosteal niche present in mammalian bone marrow. These animals possess hematopoietic tissues (the Leydig and epigonal organs) composed of only a vascular niche, where hematopoietic stem and progenitor cells (HSPCs) are maintained and produced. Inhibiting the connection between the chemokine ligand, CXCL12, and its receptor, CXCR4, leads to HSPC mobilization. AMD3100 is a CXCR4 antagonist used to mobilize cells in transplant donors. In this study, tissues were collected from four control and four experimental *Leucoraja erinacea*, and the efficacy of AMD3100 was assessed via serological, histological and immunoistochemical staining methods. We anticipate that these results will support findings from previous studies demonstrating significant mobilization of leukocytes. In addition, these data should clarify the source of mobilized leukocytes, which we believe to be the epigonal and Leydig organs. Lastly, differential leukocyte counts may provide further insight into the types of cells mobilized by AMD3100.

Uncoupling Lifespan and Gerospan in *Caenorhabditis elegans* Short-lived Mutants

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The emerging term gerospan describes the period of decline that lasts from the end of healthspan until death. Recent healthspan research with *C. elegans* longevity mutants found four life-extending genetic pathways resulted in a long, robust healthspan but consequently extended gerospan. We hypothesized that short-lived mutants would have a condensed gerospan compared to wild-type. We tested health over time for wild-type and two strains with mutations in heat stress (*hsf-1*) and insulin-like signaling (*daf-16*) transcription factors shown to reduce lifespan. Healthspan was assayed using pharyngeal pumping rates, age-related auto-fluorescence, heat stress, oxidative stress, and movement coordination. Regression analysis revealed pharyngeal pumping declined faster in both mutants compared to wild-type. Additionally, average movement speed declined faster in *daf-16* and wild-type compared to *hsf-1*. Contrary to expectations, our results suggest these short-lived mutants have a condensed healthspan compared to controls, indicating their reduced lifespan may correspond with an extended gerospan.

Analyzing the molecular components of injury response in *Gryllus bimaculatus* using single-cell RNAseq

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While a large amount of research has broadened our understanding of axonal regeneration, little is known about the ability of dendrites to regenerate after injury or deafferentation. Crickets possess unusually robust responses to neuronal injury. Deafferented cricket auditory neuron dendrites grow across the midline, a boundary they usually observe, and form functional synaptic connections with the auditory afferents from the opposite ear. The goal of our research is to understand the molecular control of dendritic growth and plasticity in response to deafferentation in the adult central nervous system. Previous work identified differentially expressed genes in control and deafferented prothoracic ganglia using subtractive hybridization, but this whole-ganglion approach may lack sufficient specificity to elucidate the molecular changes responsible for the compensatory response. Instead, this project will use RNAseq performed on single cells analyzed in reference to *de novo* assemblies of control and deafferented prothoracic ganglion transcriptomes to find differentially regulated genes.

Cilia in Regenerating Zebrafish Cardiac Tissue

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Cilia are hair-like cellular appendages typically with two functionalities, either sensory or motile. We have observed cilia in the regenerating cardiac tissue of zebra fish, *Danio rerio,* and endeavor to understand their purpose in the complex processes of tissue regeneration. We hypothesize that cilia form in response to injury and that they may play a sensory role, as either mechanical or chemical sensors that resemble cellular antennae, to assist injured tissue through the process of regeneration. We used immunocytochemistry to determine the stage and localization of cilia in sectioned regenerating heart tissue as compared with uninjured tissue. Our results suggest that while cilia are not observed in uninjured cardiac tissue, cilia are found in the injury site, epicardium and outflow track of regenerating tissue 7 days post-injury. Understanding the mechanisms of cardiac tissue regeneration in zebrafish may lead to practical prevention and cures for cardiac injury in humans.

Determination of the role of Nox1 in basal keratinocyte migration after puncture wounding

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H_2O_2 is critically important for wound repair and regeneration due to its role as a secondary messenger. Previous studies showed that low-level H_2O_2 production post-puncture wounding in zebrafish promotes basal keratinocyte migration. The underlying mechanisms remain unknown. A candidate enzyme for H_2O_2 production is NAPH oxidase 1 (Nox1) because Nox1 is expressed in human skin and a subunit required for activation of Nox1-4 (p22phox) impairs migration when knocked down in zebrafish. To test this, we performed *in situ* hybridization and morpholino-mediated mRNA knockdown. Preliminary results demonstrate the functionality of the morpholino and loss of H_2O_2 production upon *nox1* knockdown. *In situ* staining revealed *nox1* mRNA expression around puncture wounds. We plan additional co-localization studies with keratinocyte-specific markers to confirm this result. We will further monitor migration upon *nox1* knockdown using time-lapse confocal imaging. These studies will reveal a previously unknown *in vivo* function of Nox1 in H_2O_2-dependent keratinocyte migration.

To Identify Small Molecules that Stimulate Tissue Regeneration

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Unlike humans, zebrafish can regenerate much of their lost tissue. In preliminary studies, we identified the naturally occurring compound ZF143 as a stimulator of fin regeneration, through inhibition of the PTP1B phosphatase. Now, research is being done to identify additional compounds that could enhance regenerative capacities in zebrafish caudal fin. We blindly tested several different compounds. We injected four microliters of the compounds into seventy fish (35 fish per round). We amputated 50% of their caudal fins and injected their assigned compound daily for four days. We imaged the caudal fin and quantified fin regeneration. In the first round, we found that regeneration was impaired and not enhanced in compound one. In the second round, compounds seven, eight, ten, and twelve enhanced fin regeneration significantly over the control. Our hope is that we will find at least one compound which can potentially aid the limited regenerative capacities of humans.

Carbon Stocks in Eelgrass Areas around Mt. Desert Island: Implications for Eelgrass Protection in Maine

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Zostera marina, which is commonly known as eelgrass, is an important part of marine ecosystems around the world. This plant is able to store carbon in a process known as carbon sequestration, resulting in the removal of carbon from the atmosphere and storage of it in sediments. This plant has experienced a significant worldwide decline, including the area around Mt. Desert Island, ME. We have begun determining the percent total carbon and dry bulk density in the above ground biomass, below ground biomass, and sediments in five remaining eelgrass areas. This involved sampling shoots, rhizomes, and ocean sediment with a coring device at each sample site. In addition we mapped the extent of the eelgrass beds in these areas. We will be able to calculate the total carbon stock with these data. With this information, we will be able to build a compelling case for protecting eelgrass in Maine.

Using immunofluorescent staining and imaging to identify cilia in the regenerating tube feet of green sea urchins *Strongylocentrotus droebachiensis*

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The crucial role of cilia in embryonic development suggests their potential involvement in the process of regeneration. Sea urchins, an excellent model for studying development, rapidly regenerate their tube feet. To test whether cilia are present during regeneration as they are during development, we collected tube feet from 15 urchins at 0, 7, 14, and 21 days post-amputation (dpa), and used immunofluorescence to label cilia. Confocal imaging of non-regenerating 0 dpa samples revealed small cilia within the tube foot disc, while preliminary data indicates large, prominent cilia on 7 dpa regenerating tube feet. We hypothesize that this trend will continue through 14 and 21 dpa tissue samples, showing a consistent timecourse of cilia expression during regeneration. These data have the potential to advance research concerning the role of cilia in regeneration, and could suggest potential treatments for humans undergoing tissue regrowth.

Sex differences in hypothalamic-pituitary-adrenal axis and their role in neuroendocrine responses to stress

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Using rats as experimental models, this research is centered on gonadal hormones such as estrogen and testosterone and their ability to modify neuronal activity in brain while in the presence of a stressor. Ethanol, a central nervous system depressant, is used as a stressor in this experiment and administered to the animals based on their body weight. The hormone secretion rate is found to be significantly higher in females than in males in presence of Ethanol, especially in the phases of their reproductive cycle when the estradiol levels are relatively high. In summary, the goal is to investigate the underlying neuronal mechanism driving sex differences in hypothalamic-pituitary-adrenal axis responses to stress, and doing so using dual labelling immunofluorescence and quantitative PCR.

Identifying ciliated cells in regenerating zebrafish heart tissue

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Cilia are centriole-derived cell protuberances with a microtubule skeleton that play a role in cell communication and embryonic development. Because of their role in the generation of tissue in zebrafish embryos, we hypothesize that cilia may play a role in the regeneration of heart tissue in zebrafish adults. To test this hypothesis, we looked for the presence of cilia on uninjured, resected, and cryoinjured zebrafish hearts. The tissue was labeled by immunofluorescence to identify cilia specifically. Also, using a transgenic reporter strain, we will be able to identify regenerating cardiomyocytes that are upregulating the cardiogenesis transcription factor gata4 and thereby test whether these regenerating cells are ciliated. Finding an increase of cilia, either in length or number, would suggest that cilia participate in the regeneration of heart tissue and could elucidate means by which we may treat degenerative diseases in humans.

Effects of Cortisol on the Dynamics of Immunocytes in Regenerating Tissue of Zebrafish Larvae

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The purpose of this study was to determine how chronically elevated cortisol affects immunocyte (macrophage and neutrophil) dynamics in regenerating tissue of zebrafish larvae. Macrophages and neutrophils were examined using transgenic zebrafish lines in which those cells are respectively labeled with yellow and green fluorescent proteins. Tailfins of larvae treated continuously with 1 micromolar cortisol and untreated control larvae were amputated 5 days post-fertilization, and the movement of immunocytes to the wound was imaged 1, 4, 7, and 24 hours post-amputation using fluorescence microscopy. The results showed that in cortisol-treated larvae, more macrophages and fewer neutrophils infiltrated the regenerating fin compared to control embryos. The data also shows that cortisol has a significant impact on macrophage dynamics, but not a significant impact on neutrophils dynamics. Going forward, these results could shed light on how neutrophils and macrophages respond to chronic stress in humans.

Expression of tyrosinase activity in wild-type and albino embryos of the killifish, *Fundulus heteroclitus*

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Aerial incubation of stranded *Fundulus heteroclitus* embryos is evolutionarily advantageous and results in faster growth rates and avoidance of aquatic predators. The occurrence (~6%) and types of natural mutations (~20) in these embryos have been categorized previously in this laboratory. Albinism, a pigmentation disorder, is the most commonly occurring mutation (~1.6%). Spectrophotometric microassays were used to measure tyrosinase activity via dopachrome formation to determine its expression in wild-type and albino embryos. Dopachrome production in wild-type embryos exceeds that in albino embryos using rate and endpoint analysis. Tyrosinase assay methods utilizing single embryo methods are in development. These data support the hypothesis that decreased tyrosinase activity underlies the albino phenotype. Albino embryos may be more susceptible to UV damage and to increased levels of predation. This is the first characterization of naturally occurring mutations in embryos from the Northeast Creek population.

Identification of novel germ-granule regulators in the germline.

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Germ granules are ribonucleotide organelles found in the germ-line cytoplasm of most sexually reproducing organisms. The germ granules of *C. elegans*, known as P granules, are required to maintain germline integrity. The *sam16* mutant, isolated previously by the Updike Lab, is sterile with a very distinct P-granule phenotype. In our studies, a genetic recombination frequency was used to determine the distance of the mutation from the *dpy-5* marker. Our results show that the *sam16* mutant is 1.107 map units from *dpy-5*, which corresponds to a non-synonymous mutation in the *npp-12* gene. NPP-12 is a highly conserved protein in the nuclear pore complex known as Gp210 in humans. P-granules, which reside at the nuclear periphery, directly contact and cluster nuclear pore complex components; one possibility is that the *sam16* mutation directly compromises the NPC/P-granule association, causing the large P-granule phenotype. We have designed CRISPR constructs to create a transgenic line to determine whether the *npp-12* mutation in *sam16* can reproduce the large P-granule phenotype, and will report on our progress. By identifying *sam16*, we will better understand the role that P-granules play to maintain stem cell-like properties in the germline.

Investigating intra-laminar heterogeneity of gene expression in the main olfactory bulb

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The peripheral sensory inputs to the main olfactory bulb are labeled and topographic (Mombaerts et al. 1996), defining a system of molecularly heterogeneous modules. An important and longstanding question is whether the intrinsic bulbar circuits composing these columns are similarly specialized. Is the bulb a system of functionally distinct columns, or is it one invariant circuit, iterated many times in parallel? As a preliminary investigation into this, we have begun quantifying the areal patterning of the potassium channel superfamily across the bulb. Using the Allen Brain Atlas (Lein et al 2007) – a compendium in-situ-hybridization experiments for the mouse genome, we have extracted expression profiles for 33 potassium channel genes across the mitral cell layer. Our preliminary investigations show that expression of these genes is non-uniform, with evidence of spatial periodicity in some cases. We are also applying principal components analysis to investigate whether potassium channels are organized as spatial cliques.

Optimizing culture conditions for *Calliobothrium verticillatum*

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Long-term *in vitro* maintenance of metacestodes is essential for *in vivo* studies of host-pathogen interactions. *Calliobothrium verticillatum,* a tetraphyllidean cestode, is a common parasitic tapeworm found in the hermit crab*, Pagurus pollicaris*. Metacestodes of *C. verticillatum* were isolated from the foregut of *P. pollicaris* and placed into elasmobranch cell culture media to replicate the conditions in their final hosts, the sharks, skates and rays. Media with and without trimethylamine N-oxide (TMAO), an osmolyte in elasmobranch tissues that counteracts the destructive effects of urea, was used to assess survival rates over time. Results suggest that the survival of metacestodes over 4 days was greater in media with TMAO than in media without TMAO. These findings provide a preliminary optimization that will reduce constraints in complex *in vivo* immunological experiments.

Evaluation of the maltose-binding protein fusion system in the isolation and purification of DTX3L E3 ligase

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Picornaviral replication coordinates many processes between the virus and the host cell machinery. Hepatitis A virus and Encephalomyocarditis virus 3C proteases are critically active in polyprotein processing during picornaviral replication. These 3C proteases are targets for ubiquitilation by host cell E3 ligases, which tag them for degradation by proteasomes. Further understanding of these E3 ligases' function may expand our knowledge of picornaviral replication. This study tests the InFusion® HD Cloning and maltose-binding protein (MBP) fusion systems to isolate the DTX3L E3 ligase. Restriction enzyme digests of the vector constructs visualized on a 0.8% Agarose gel and DNA sequencing show that InFusion® HD Cloning produced the pMAL-c5X-DTX3L fusion vector. SDS-PAGE and protein immunobloting inconclusively show that the MBP-DTX3L fusion protein was not successfully produced. Although the MBP fusion system is known for its success in producing soluble and functional protein products, other approaches must be explored to isolate DTX3L.

A survey of stress gene expression in ten eelgrass (*Zostera marina*) populations in Frenchman Bay

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Eelgrass creates a vital ecosystem and habitat for a wide array of marine life. Thus its decline in Frenchman Bay over the last 20 years, and its complete and sudden loss in the upper bay in 2013, despite ongoing restoration efforts, are cause for concern. Most hypotheses involve biotic or abiotic stressors, including changes in water quality, predators, and fishing practices. By comparing the levels of expression of genes associated with different kinds of stress, we seek to determine if plant stress is linked with the decline of eelgrass. I am comparing the expression levels of genes associated with heat stress, disease, and general stress between and among ten populations of eelgrass in upper and outer Frenchman Bay. I will present RT-qPCR expression profiles from these genes, normalized to the expression of an endogenous housekeeping gene, and explore any correlations with patterns of eelgrass health and decline in Frenchman Bay.

Constructing barcoded *Candida albicans* isolates for competitive fitness assays

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Candida albicans is the most common fungal pathogen in humans. Its genome plasticity allows it to quickly adapt to its host environment and even become resistant to the newest antifungal drugs. Therefore, understanding the mechanisms underlining host-pathogen interactions is of great interest. An approach to this problem is passing strains of *C. albicans* through murine models and compare their fitness before and after the infection. To efficiently track the variety of strains, it is necessary to introduce unique barcode sequences into their genomes. The barcode sequence is fused with other necessary components into a DNA construct via fusion PCR and inserted into *C. albicans* via transformation. The strains that are successfully transformed will be used for fitness competition assays. These studies would ultimately allow to identify a genetic basis for the phenotypic changes observed in the strains passed through murine models.

Behavioral effects of the injury-induced compensatory growth response seen in the auditory system of the field cricket, *Gryllus bimaculatus*

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In the field cricket, *Gryllus bimaculatus*, the auditory interneuron AN2 demonstrates a unique compensatory growth response after injury to the auditory system. Since AN2 is responsible for detecting high frequency sounds known to elicit negative phonotaxis behavior, this research aims to determine how the compensatory growth response of AN2 affects negative phonotaxis after deafferentation by amputation of the auditory organ. Crickets are flown in a laminar air stream and exposed to short durations of 20 kHz pure-tone pulses in order to provoke negative phonotaxis. Behavioral responses are video taped and analyzed using Tracker software. Preliminary analysis using an alternative assay indicates that at 14 days post-deafferentation, female deafferented crickets' turn intensity in response to ultrasound seems to weaken when compared to both male deafferents and controls. This weakened turn intensity may provide an evolutionary benefit for female crickets, who theoretically spend more time in flight than do male crickets.

The function of heparanase 2 and its connection with kidney function

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Zebrafish are good models to study kidney diseases like proteinuria because they develop rapidly and have a similar glomeruli structure to humans. Heparan sulfate is a component of the glomerular basement membrane, which constitutes the filtration barrier of the kidney. We are investigating the potential role of heparanase2 in proteinuria because the related protein heparanase1 is responsible for the cleavage of heparan sulfate on cell surfaces of endothelial cells. A morpholino targeting heparanase2 mRNA and thus downregulating heparanase2 production is injected into eggs of the transgenic zebrafish line l-fabp:DBP-eGFP, which produces a fluorescence plasma protein. Measuring the fluorescence of the fish in the eye (at 96 and 120 hours post fertilization) and the amount of edema enables us to classify the extent of proteinuria. The injected fish show severe edema and lose their eye-fluorescence, indicating proteinuria.

Effect of antibacterial agent Triclosan on reactive oxygen species generation in RBL-2H3 Mast Cells

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Triclosan (TCS) is an antimicrobial found in many hygiene and household products at concentrations ~10mM. Our published data show that TCS, at concentrations of 2μM and higher suppress mast cell degranulation (Palmer 2012). Mast cells release histamine and inflammatory agents in the process of degranulation, due to antigen stimulation. Our published data also indicates that TCS suppresses oxidative phosphorylation by acting as a proton ionophore mitochondrial uncoupler at low-micromolar concentrations in several cell types, including human and rat mast cells and primary human keratinocytes (Weatherly, 2015). Mitochondrial respiration naturally produces reactive oxygen species (ROS); also ROS are required for degranulation. We have found that ROS production is increased by low-micromolar doses of TCS in rat basophilic leukemia (RBL-2H3) mast cells, both with and without antigen stimulation. Uncovering the effects of TCS on ROS will narrow the scope of possible inhibitory mechanisms employed by TCS.