Unlocking the role of the symbiotic community in the calcification process of

The impacts of temperature changes on the coral *Astrangia poculata*

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With fluctuating temperatures due to climate change, corals worldwide are experiencing massive coral bleaching events. Corals lose the symbiotic relationship they have with the dinoflagellate Symbiodinium sp. that provide energy, and eventually starve and die. *Astrangia poculata*, a wide ranging coral species, can tolerate large temperature fluctuations unlike their more tropical cousins. They naturally exist in both a symbiotic state with zooxanthellae and aposymbiotic state without zooxanthellae. This project analyzes the physiological attributes of *A. poculata* at warm, cold, and changing temperatures to determine how these corals adapt to temperature fluctuations of the climate which can help research in the conservation of coral species that are suffering from climate change. We measured critical parameters (buoyant weight, photosynthetic yield, and density of symbionts) before and after a temperature change. This experiment showed, despite some exceptions, an overall decrease in the weight, photosynthetic yield, and symbiont density after a temperature change, whether the corals experienced warming or cooling. However, warming the corals seemed to have more of an impact, with a mortality rate of 100% after 29 days. Preliminary confocal microscopy imaging of the corals also allowed us to analyze the degree of stress on the corals by demonstrating fluorescence of reactive oxygen species and chlorophyll A. This project will help future scientists by understanding how *A. poculata* can withstand widely fluctuating temperatures and the how temperature changes might impact corals in general.

Biological Discovery in Woods Hole at the Marine Biological Laboratory NSF REU Award #1659604

Site-Directed RNA Editing Using TadAs

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Site-Directed RNA Editing (SDRE) is a strategy to modify genetic information at the mRNA level. The SDRE system developed by the Rosenthal Lab catalyzes adenosine (A) to inosine (I) conversion using the Deaminase Domain (DD) of ADAR2 linked to λN peptides. The λN peptides interact with boxB hairpins located in guide RNAs (gRNA) that direct the DD to the target As. The system can precisely drive A-I conversion in mRNAs, but it has many limitations including sequence context dependency and off-target editing. In this study, we replaced ADAR with TadA, a different adenosine deaminase that catalyzes A-I conversion in E. coli tRNAs. This change will allow us to use random mutagenesis in bacteria to select for TadA variants with improved editing efficiency and novel substrate recognition. To investigate

Phosphorylated Tau Activates Signaling Pathways That Inhibit Fast Axonal Transport in Squid Axoplasm

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Tau tangles, a hallmark of Alzheimer's Disease (AD), are formed when tau, a microtubule associated protein, misfolds and aggregates in neurons. Previous work has shown that aggregated tau inhibits fast axonal transport (FAT), a process essential for maintenance of axons and synapses, via a specific phosphatase activating domain (PAD) at the N-terminus of tau. The PAD, which is not exposed in tau monomers, activates protein phosphatase 1 (PP1), which activates the protein kinase GSK3β via dephosphorylation. GSK3β phosphorylates the motor protein kinesin, leading to the release of its vesicle cargoes. Although aggregated tau in AD has no mutations, it is known to be highly phosphorylated. A closer look at tau phosphorylation has identified sites that are phosphorylated in normal and AD brains. Using vesicle motility assays in squid axoplasm, the current study aims to understand potential roles of these sites in regulating the PAD exposure. Three phosphorylation sites located in the proline-rich domain of tau are S199, S202, and T205. We found that concurrent pseudophosphorylation of these sites inhibits anterograde FAT, imitating the effect of tau aggregates. This effect was blocked by a PP1 inhibitor, suggesting the PAD was exposed. Additionally, each of the phosphorylation sites were tested individually. S199E inhibited both anterograde and retrograde FAT, while T205E inhibited only anterograde transport and S202E showed no effect. Further experiments used an antibody, TNT1, which binds specifically to the exposed PAD. TNT1 successfully blocked inhibition of anterograde FAT by T205E tau, but did not prevent inhibition of FAT by S199E tau, suggesting that T205E exposes the PAD but S199E does not. These results show that phosphorylated tau can inhibit FAT and suggests different phosphorylation sites can expose different biologically active domains of tau, a notion consistent with a potential function of tau for regulating FAT in both disease and non-diseased brains.

NINDS, Tau Consortium, Hunter College Institutional Overhead Funds

Training *Octopus bimaculoides*: Isolating Arm and Sucker Movements to Evaluate Sensory Capabilities

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Octopus arms are capable of performing a wide range of flexible motions due to their advanced musculature. Four distinct muscle groups (transverse, longitudinal, and two oblique) allow for elongation, shortening, bending, and twisting. Suckers lining each arm allow octopods to both manipulate objects and obtain physical and chemical cues from their environment. To study the sensory capabilities of octopus arms and suckers, we developed a protocol to train *Octopus bimaculoides* to reach through a hole in a plexiglass divider and grasp an object. By associating successful touches with a food reward, in three weeks we were able to achieve a rate of successful touches of ~75%. This classical conditioning represents a key first step in isolating the movements of a single octopus arm and its suckers to experimentally test their range of sensory capabilities.

Partial funding for this project was provided by ONR Grant N00014-19-1-2445 to R. Hanlon.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Differences in Axonal Growth of Dopaminergic Neurons Exhibited in Familial Parkinsonôs Disease Mouse Model

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Familial Parkinson’s Disease (PD) is a neurological disorder which results in tremors, bradykinesia, and stiffness of movement. PD is caused by the degeneration of dopaminergic neurons, which are nerve cells responsible for the production of dopamine. The dysfunction and death of dopaminergic neurons results in a lack of dopamine and disrupts normal motor function. How this degeneration of dopaminergic neurons occurs, however, is still not fully understood; PD can be due to either environmental or genetic conditions. One cause of familial PD is mutation of the gene for the protein DJ-1. Wild type DJ-1 functions to decrease the uncoupling of the mitochondrial inner membrane by binding to the β subunit of the ATP synthase and thus increasing the efficiency of ATP production. It is hypothesized that this increase in efficiency results in an increase in neuronal process outgrowth. Therefore, it is expected that tyrosine hydroxylase (TH +) dopaminergic neurons of model mice lacking DJ-1 will have impaired dopaminergic neuronal outgrowth. Comparing the intensity of TH staining in substantia nigra neuron axonal arbors within brain slices will determine whether this difference exists.

McCarter Metcalf Fellowship

The Proline-Rich Domain Mediates Toxic Effect of Mutant Huntingtin on Fast Axonal Transport

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Microplastics everywhere: The history of microplastic pollution in Cape Cod salt marsh sediments

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Function of reverse transcriptase-related (rvt) genes in metal stress response

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Reverse transcriptase-related (rvt) genes comprise a remarkable class of reverse transcriptases found across several domains of life, including fungi, plants, invertebrates, protists, and even certain bacteria. Among other notable properties, rvts contain highly conserved coiled-coil N-terminal domains demonstrated in bacterial rvt to confer the ability to multimerize and a C-terminal domain purportedly responsible for protein priming. Prior experimentation has shown significant transcriptional stimulation of various rvts in response to transition metal ions, particularly to Ni2+ in the ascomycete fungus *Neurospora crassa* and Fe2+ in the filamentous bacterium *Herpetosiphon aurantiacus*, suggesting that rvts may play a role in the metal stress response. Here we investigate Ncrvt in the model fungus *N. crassa* and express Harvt from *H. aurantiacus* introduced into the heterologous host Escherichia coli by transformation to explore the potential function of rvts in mediating the metal stress response. Rvt activity in *N. crassa* is monitored using the transformant T147 strain with the GFP reporter regulated by the Ncrvt promoter. Mutants of T147 obtained through random somatic mutagenesis under UV radiation are grown in different nickel concentrations and examined for GFP induction and cellular localization. E. coli strains expressing recombinant plasmids with functional mutations in key domains of HaRVT are grown across concentration gradients of various transition metals to investigate their growth and survivability. The distinct induction patterns observed in *N. crassa* implicates Ncrvt in different pathways of homeostatic control, while experimentation with E. coli suggests that Harvt may provide an advantage in iron-rich environments. Although the mechanism of rvt activity in relation to transition metals is still uncertain, our observations made with fungal and bacterial versions of rvt are consistent with their role in the transition metal stress response.

Brown-MBL Rosenthal LINK Award program

Creating Rfx6 Knock-Out Mutants of *Xenopus*

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Xenopus are an excellent model system for human disease due to their external development, their large spawning and embryo size, their relatively rapid development, fate mapping, and their accurate genome sequencing. Mutations in the rfx6 gene can cause neonatal diabetes and digestive system defects. Diabetes is a chronic disease caused by a disruption in the pancreas, where β-cells cannot properly produce insulin. To better understand what role rfx6 plays in early endoderm development, we are producing mutant Xenopus laevis frogs using genome editing. To create mutants, we used CRISPR-Cas9 (Clustered Regularly-Interspaced Short Palindromic Repeats). Cas9 is an enzyme that can cut the two stands of DNA at a specific target point. An RNA guide binds to the pre-designed sequence and directs Cas9 to the correct area on the genome. We examined the exon-intron structure of rfx6, and identified potential sgRNA target sites. Rfx6 has 19 total exons, with our specific sites in exon one and three. These sites are close to the start of the gene and thus likely to completely disrupt the protein function. We microinjected the sgRNA along with Cas9 protein into *Xenopus* embryos at the one/two cell stage to induce site-specific double strand breaks (DSBs) in genomic DNA. Inducing indels at a target site, CRISPR-Cas9 causes a frame-shift mutation. Endogenous DSB repair mechanisms are error prone and commonly introduce insertions and deletions (indels) that result in frame-shift mutations which disrupt protein structure and function. These F0 embryos would have mosaic mutations, which vary between cells. Following injection, embryos are grown and 24-48 hours later we collect genomic DNA from several embryos to ascertain whether mutations were induced. We PCR amplify the targeted region from genomic DNA and send the PCR product for sequencing to interpret whether Cas9 cut the target sequence.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Assessing the suitability of *Sepioloidea lineolata* as a genetically tractable model

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The use of genome editing reagents for the development of new genetically tractable models has received worldwide attention for its potential to offer novel insight into broad areas of biology. In cephalopods, it could uncover the molecular innovations that drive complex behavior. Accordingly, the MBL's cephalopod program is investing significant resources towards creating a genetically tractable cephalopod model. Recently, a targeted knock-out of the tryptophan dioxygenase (TDO) gene was achieved in

Driving fish wild? The effects of pile driving on black sea bass feeding behavior

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Microplastics everywhere: Watershed urbanization affects microplastic abundance in salt marsh sediment

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As worldwide plastic production and use increases, the accumulation of plastic debris in coastal ecosystems has become a major environmental concern. In particular, microplastics (plastic particles <5mm) are believed to be ubiquitous in the world's oceans. The abundance and sources of microplastics in coastal environments such as Cape Cod salt marshes have been barely studied. Here, we analyze surface sediment from six different estuaries in Waquoit Bay, representing a wide range of watershed urbanization, in order to determine (1) what relationship exists between human activity and the abundance of different types of microplastics, and (2) what these abundances suggest about sources and transport mechanisms for the different types of microplastics. Our results show that microplastic fragments are more abundant in more urbanized sites. Contrastingly, plastic microfibers are ubiquitous in the sampled sites, regardless of the degree of urbanization. These findings suggest that the larger and heavier microplastic fragments are less efficiently transported in estuaries, and tend to accumulate in proximity to land sources. Microfibers, in contrast, are less dense and have smaller dimensions. Transportation via currents or through air brings them to all sites, including areas located far from human activity. These results can inform both future research on microplastic abundance in salt marshes and pollution management.

Woods Hole Oceanographic Institute Sea Grant and the Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Assessment of particle cycling processes in the deep ocean using the carbon isotopic composition of fatty acid biomarkers

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Fatty acid (FA) molecular and carbon isotopic composition (δ13C) is useful in assessment of sources, recycling, and degrad]ĄɣonĘn re]n! ! | an tcon(mf Ŏ T D l p U ɣ ! ! c an`]i a n_ n ent]ú ecu

Optimizing a protocol for the electroporation of

The influence of Hurricane Igor on the deep ocean carbon flux

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Hurricanes exert large physical forces on the ocean, such as intense mixing and cooling of surface waters and upwelling of nutrient-