Unlocking the role of the symbiotic community in the calcification process of *Astrangia poculata*

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Calcification is the process by which reef-building corals create their skeletons. This mechanism is still not wholly understood. Symbiotic and aposymbiotic colonies of *Astrangia poculata* were reared in 15ºC, 27ºC, or ambient conditions. Scanning electron microscopy (SEM) was used to describe how these physiological and environmental conditions impact skeletal structure. Buoyant weight data over time revealed that symbiont state and temperature both significantly affect growth rates. SEM of *A. poculata* skeletons revealed that aposymbiotic colonies appear to have a lower density of calcium carbonate at growing septal spines. Quantitative analysis of roughness of septal spines revealed that aposymbiotic colonies have a rougher surface texture than symbiotic colonies. This roughness trend is strongest in the colonies reared at 27ºC, which were also the fastest growing colonies. Subsequently, scanning electron microscopy was used to examine the calicoblastic ectoderm of *A. poculata*. Initial results reveal that calicoblastic cells appear to form a fine mesh across the skeleton. SEM of both the skeleton and tissue revealed a pervasive presence of bioeroders in the skeleton. Light sheet microscopy using the L-SPI Single Plane Illumination system was used to confirm and characterize this community of bioeroders. Finally, we studied skeletons of the tropical corals *Porites astreoides* and *Acropora cervicornis* to understand how *A. poculata* skeletal structure compares to its tropical counterparts. Few studies have examined the skeleton of *A. poculata* or corals in general using SEM. These results unlock new insights into the skeletons of temperate corals and the associated community.

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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 whether TadA fused to λN could edit RNA, we performed in cellula and in vitro editing assays using recombinant enzymes that contained TadA homo or heterodimers. For both assays, we designed an RNA substrate that contained several target As within sequence contexts preferred by TadAs, including a target site mimicking the tRNA anticodon loop (ACL) naturally edited by TadAs. In cellula results suggested that TadA-λN could edit at the ACL target site in the absence of gRNAs. The in vitro assay recapitulated ACL editing at high efficiency and revealed an additional editing site within a motif that partially overlaps the ACL sequence. This suggested that TadA-λN activity is dependent on specific neighbouring contexts but does not require the full tRNA ACL sequence. In vitro and in cellula results also showed that TadA-λN editing may be independent of binding to gRNAs. These preliminary data suggest that using TadA as the enzymatic component for SDRE is promising but it may be necessary to develop new strategies to direct TadA to specific target sites.

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Differences in Axonal Growth of Dopaminergic Neurons Exhibited in Familial Parkinson’s 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

**Protein palmitoylation via a specific palmitoyl transferase facilitates Golgi dispersal observed with nicotine exposure**

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Our lab has observed dispersal occurring in nicotine treated neurons and human embryonic kidney cells (HEK) expressing α4β2-type nicotinic acetylcholine receptors (α4β2Rs). α4β2Rs bind nicotine with high affinity and initiate the additive process with nicotine binding. Typical GA morphology is observed as a set of membrane stacks in the soma of neurons. During nicotine-induced dispersal we find that the stacks disperse into mobile membranes throughout dendrites and axons. Preliminary data from our lab has implicated the palmitoyl transferase, DHHC2, in the downstream events after nicotine binding to α4β2Rs causing GA dispersal. The identification of DHHC2 as being involved in Golgi dispersal is also consistent with additional evidence from our lab that the posttranslational modification, palmitoylation, is part of the signaling that leads to GA dispersal. To further test whether DHHC is involved, we overexpressed DHHC2 in HEK cells and imaged for changes in GA morphology. Fluorescently tagged sialyltransferase 3 (eGFP-ST3) and DHHC2 (myc-DHHC2) were assayed on the Zeiss spinning disk confocal system with or without the expression of tagged α4β2Rs (HA-α4β2Rs). Preliminary results suggest that DHHC2 overexpression does facilitate GA dispersal and that the GA dispersal induced by DHHC expression occurs independent of α4β2R expression.

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**Examining symbiont selection and polyp connectivity in *Astrangia poculata***

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Given the recent acceleration in global coral reef decline, there is a growing interest in understanding resilient corals. Increasing ocean temperatures cause a breakdown of the symbiotic relationship between corals and the dinoflagellate *Symbiodinium*, leading to expulsion and eventually colony mortality due to lack of nutrition normally provided by symbionts. *Astrangia poculata* is distinctive in that it can survive in both symbiotic and aposymbiotic states, unlike most tropical corals that have an obligate relationship with *Symbiodinium*. It is therefore an exceptional model system to understand this symbiosis. Our study
focuses on the reintroduction of *Symbiodinium* following a bleaching event and the connectivity between polyps in a colony. In this study, we reintroduced symbionts in a single polyp of naturally and chemically bleached colonies of *A. poculata* using two different clades of cultured *Symbiodinium*, as well as tissue from a symbiotic *A. poculata* colony. Image analysis was used to evaluate symbiont density following reintroduction. We found that successful reintroduction of symbionts was independent of how the coral was bleached and the clade of symbiont used, showing that tissue reinfection is the most effective method. This study demonstrates that reintroduction of symbionts is possible in a laboratory setting for *A. poculata*. We also found that there was a lack of polyp connectivity in grown *A. poculata* colonies as well as connectivity with surrounding polyps. This suggests that *A. poculata* polyps lose connectivity after development, unlike those in tropical corals like Acropora and Porites, species that maintain connectivity as developed polyps. These results offer insight into the symbiosis and colonial interaction of *A. poculata* and how they relate to wholly tropical corals, providing a deeper understanding of resilient corals in the face of climate change.

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**Identifying Markers of Tergal and Precoxal Tissues in *Parhyale hawaiensis***

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The origin of the insect wing as an evolutionary novelty is a longstanding problem in arthropod biology. One school of thought suggests that the wing was modified from a dorsal lobe (e.g. gill or plate) on the leg of ancestral crustaceans, while another theory suggests that the wing is a novel projection from the body wall that is unique to insects and is not present in the crustacean ancestor. Recent work from the Patel lab supports the hypothesis that the insect wing is homologous to a lobe on the leg of the pan-crustacean ancestor. In this model, the most proximal leg segment has vestigialized, becoming flatter and broader, and now forms much of the lateral body wall of insects. This caused the lobe on that leg segment to migrate dorsally, to a position consistent with wings in modern insects. According to this model, the lateral aspect of the dorsal shell (the tergum) actually consists of the most proximal leg segment (precoxal) as well as true body wall tissue. While morphological data supports the model that insects incorporated the proximal leg segment into their body wall, genetic evidence for this model is lacking. In this study, I follow a candidate gene approach to identify markers that will distinguish true body wall from the precoxal in a crustacean model, the amphipod *Parhyale hawaiensis*. To this end, I am using fluorescent in situ hybridization (FISH) to study a panel of genes known to pattern the Drosophila notum and characterize their expression domains in Parhyale embryos. I expect to see a clear boundary between medial expression of dorsal genes and the lateral expression of proximal leg genes during embryonic limb development.

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Regulation of mitochondrial ion channel activity in Fragile X brain mitochondria by ATP synthase leak modulation

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Fragile X syndrome is caused by mutation in the X chromosome at the fmr1 gene, which controls fmrp protein expression. This protein binds to synaptic protein mRNA and inhibits their translation. In wild type (WT) brain cells, fmrp falls off the mRNA after sufficient stimulation, which allows translation to occur. It’s known that the F1 domain of the ATP synthase is regulated by fmrp in such a manner, and this regulation contributes to a significant change in F1 domain/FO domain ratio after stimulation. Previous research of Dr. Jonas’s lab shows that the FO domain can form a leak channel on the inner membrane of mitochondria, and they have found that increasing F1/FO ratio helps close the leak and enhances the efficiency of ATP production. However, fmrp is absent in Fragile X (FX) brain cells due to mutation at the fmr1 gene. Without fmrp regulation, mRNAs are continuously being translated, and this disrupts the desired F1/FO ratio during development. It’s possible that pharmacological intervention could ameliorate the leak and normalize rates of protein synthesis. We propose that Dexpramipexole (dex) is a promising drug for improving brain development in Fragile X syndrome due to previous evidence that it can bind to the F1 domain and promote closure of the FO domain channel in WT mitochondria. Nevertheless, its effects on leak channel activity in the Fragile X mitochondria remain unknown. Therefore, this project explores the effect of Dex on decreasing channel activity on FX brain mitochondria. Membrane channel activity is recorded before and after dex is added to the bath in both WT and FX brain mitochondria using patch clamp technique. The result confirms that dex is able to down-regulate channel activity in WT and FX mitochondria. These findings are encouraging in that dex may show therapeutic potential in Fragile X syndrome.

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Effect of pthalates on tentacle regeneration in sea anemone, Nematostella vectensis

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Nematostella vectensis is a species of sea anemone that has recently been adopted as a model organism for tissue regeneration studies due to its ability to rapidly regenerate amputation tentacles within a week. Pthalates are a group of compounds that are derivatives of pthalic acid and are used as plasticizers to make plastic products more elastic. Despite their known effects on the human reproductive system and nervous, they are widely used in everyday products from erasers to PVC pipes and could potentially leach
into water bodies. This could potentially harm a variety of aquatic organisms. In this project I tested the effects of different concentrations of phalates on the ability of *Nematostella* to regenerate. Two different phthalates were used in the experiments – diocetyl phthalate and bis – ethyl, hexyl- phthalate - with the former being used in printing inks and the latter being used in PVC pipes. Young adult animals were amputated at the middle of the pharynx and were kept at the various concentrations of phthalates. A variety of phenotypes were observed after a week of regeneration, with the most prominent being the outward curling of tentacles.

Finally, used real time PCR the impact of the presence of phthalates on gene expression was studied.

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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.

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Anaerobic Salt Marsh Microbial Consortia Remain Stable Over Time When Exposed to High Nitrate Concentrations

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It has been observed that microbial communities respond dynamically to environmental perturbations with respect to metabolism, taxonomy and abundance. While competition is certainly prevalent in microbial communities, we also know that some bacterial communities work cooperatively together to effectively utilize resources in what is called division of labor. However, little is known about the stability of these cooperating sub-communities and how they respond to perturbations. We hypothesized that consortia, representing sub-populations of the whole community, would remain stable over time if isolated from other communities. For our experiment, we collected bacterial samples from Plum Island in Newburyport, MA, using 30 traps sunk 24 cm into the sediment. The resulting consortium was used to inoculate three replicate flasks that had feed mixtures consisting of marine broth with 1000 uM nitrate. A sterile flask served as control. Purging nitrogen gas through the flasks kept them anaerobic, and 300 mL samples were extracted every three days for analyses followed by the addition of 300 mL of sterile medium to maintain constant culture volume. The consortia were measured over the course of two weeks for metabolic output (through CO2 production), nitrate concentration (via chemiluminescent analyzer), cell numbers (DAPI counts), and taxonomy (16S gene identification). Preliminary results suggest that the consortia are indeed stable over time.

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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-rich waters which may trigger episodic phytoplankton blooms. While the upper-ocean response to hurricanes is relatively well-established, hurricanes’ impact on the deep ocean carbon cycle remains barely known. I focused on Hurricane Igor to research the impact of episodic events on particle flux to the deep ocean. In September 2010, Hurricane Igor tracked through the NW Atlantic Ocean passing 64 km WNW of Bermuda. This study evaluates the organic composition of sinking particles collected before, during and after Igor’s passage at the Oceanic Flux Program site offshore Bermuda. Lipid biomarkers were analyzed to characterize the relative contributions of fresh surface-derived phytodetritus, zooplankton, and bacterial biomass to the particulate organic carbon as it was transported through the mesopelagic and bathypelagic zones. Results indicate that the large total mass flux exported to the deep ocean during Igor’s passage was mainly composed of carbonates resuspended from Bermuda’s platform by internal waves, with a relatively small contribution from particulate organic
carbon. However, the organic carbon exported to 1500 m and 3200 m was rich in labile and fresh phytoplankton-derived (e.g. fatty acids 20:5ω3 and 22:6ω3, and phytosterols), zooplankton-derived (e.g. 18:1ω9 and 20:1 fatty acids), and microbial-derived (e.g. 18:1ω7 and hopanoids) lipid compounds. This indicates that Igor enhanced the transfer of surface and platform-derived organic carbon and stimulated heterotrophic activity in the deep ocean. Our results demonstrate that hurricanes can enhance oceanic carbon export and impact deep ocean ecosystems. With an increasing number of extreme weather events due to climate change, these results indicate that such events, like hurricanes, can have an impact on global carbon cycling.

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