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Project Title: Comparative early development of cephalopod embryos versus spiralian mollusks

Visit Narrative:

I spent my summer at Ragsdale Labs exploring aspects of cephalopod embryonic development at a molecular level. Through the EDEN internship, I was able to gain an opportunity to work in a lab that is currently conducting research on *Octopus bimaculoides*, an emerging cephalopod model organism. Work is being done on sequencing the genome, and on learning more about the embryonic development of this species. My work was focused on the evolutionarily derived developmental program in cephalopods.

Cephalopods, though part of the mollusk phylum, differ from their cousins in many respects. Not only do these organisms resemble vertebrates in their highly developed brains and camera-type eyes, but they also utilize a different method of early embryonic development. While other mollusks undergo spiral cleavage, cephalopods develop from a yolky egg using bilateral, meroblastic cleavage. My goal was to examine this novel developmental program on a molecular level, starting with one of the major developmental signaling pathways present in most animals– the MAPK (Mitogen Activated Protein Kinase) signaling pathway. Previous researchers have shown MAPK to be active only the D quadrant of the spiralian embryo, which is known to act as a signaling center that is crucial for patterning of the dorsal-ventral axis of the embryo. The Ragsdale Lab has conducted previous research on this pathway in *O. bimaculoides* and has obtained data that suggests there to be a gradient of activation along the anterior-posterior axis of the embryo during early cell division. My goal was to expand on this research by exploring the distribution of RTKs (Receptor Tyrosine Kinases), which act upstream of MAPK, at crucial stages of early embryo development. Because variations in the types or concentrations of RTKs could be indicative of different MAPK pathway configurations, I was particularly interested in seeing if the gradient of MAPK activation was somehow expressed in RTK variation on different parts of the embryo.

In order to look at patterns of RTK expression, in situ hybridization was necessary. For the in situ hybridization to be possible, probes needed to be designed to bind to a variety of RTK sequences in the octopus. Therefore, I started out my work over the summer with a degenerate PCR sample survey for three different stages (gastrulation, axis patterning, and organogenesis) of the octopus embryo in order to examine the variety of RTKs present at these stages. Having found variety, I then proceeded to clone out the genes for a handful of RTKs of interest such as Fibroblast Growth Factor Receptor, Insulin-like Growth Factor Receptor, and Epidermal Growth Factor Receptor. From here, probes were made of the RTKs, which would then be used for a series of in situ hybridizations on octopus embryos of the three aforementioned developmental stages.

Before I could conduct these in situs on octopus, however, it was necessary to practice the procedure on chick embryos using the neuronal markers SNAP-25, NeuN, and Islet 1. I was able to practice and obtain the skills needed to conduct my experiments on cephalopods by cloning out, making probe, and conducting an in situ on these chick genes.

This internship was an extremely valuable experience to me because it allowed me to think beyond the procedures that I was conducting in the lab. My previous experience in labs had not been as fulfilling because I was simply conducting the procedure with minimal knowledge of the purpose behind the experiments, and of the questions that the research is attempting to discover the answers for. My experience this summer has taught me a lot about the thought process that goes on behind scientific research. Unfortunately, I was unable complete my experiments before the summer ended: I have yet to conduct the in-situs on the octopus embryos and have therefore not yet gotten the data on RTK patterning that I had hoped to find. However, since my host lab is the same as my home lab, I hope to be able to continue research throughout this school year.