Lily Helfrich
Smith Lab, Marine Biological Laboratory, Woods Hole, MA
Project Contributors: Joel Smith (PI), Antje Fischer (Postdoc), and Kimberly Johansson (Undergraduate Student, Harvard University)
EDEN Undergraduate Internship: June 17, 2013 to August 30, 2013

Visit Narrative:

The effect of MAPK-signaling on *Crepidula fornicata* during spiral cleavage: using a reference transcriptome to investigate axis development by *in situ* hybridization

I spent my summer in Dr. Joel Smith's lab at the Marine Biological Laboratory, studying the embryonic development of *Crepidula fornicata*, the slipper snail. This Gastropod mollusk is an emerging model organism that can provide insight into animal body plan evolution. *Crepidula* develops via a spiral cleavage pattern, unique to Lophotrochozoans–a largely understudied phylum, but it establishes the symmetrical body axes common to bilaterians. I wanted to explore the molecular mechanisms that govern spiralian development, which are not yet well understood.

This project was motivated by my experience in the Smith lab last summer, where I worked with Dr. Antje Fischer and two other undergraduates to collect *Crepidula* embryos across an early developmental time-course for RNA extraction followed by deep-sequencing, from which we built a reference transcriptome. We also treated embryos with the MAP kinase inhibitor, U0126, for deep-sequencing.

I wanted to learn a new, complementary approach that takes advantage of the transcriptome: whole mount *in situ* hybridization (WMISH). WMISH provides spatial and temporal information about gene expression. It has been proposed that the 4d quadrant acts as the organizer in *Crepidula*, which parallels MAP kinase activation in 4D. Additionally, treatment of *Crepidula* embryos with the MAPK inhibitor U0126 induced a loss of dorsoventral axis formation, further suggesting a critical role for MAPK in establishing the body plan.[1,2] Therefore, I prioritized members of the MAPK signaling pathway and other major pathways, possibly induced by MAPK, implicated in axis development: Delta-Notch, TGF-β, and Wnt. To inform possible relationships between these genes, I planned to also perform WMISH with U0126-perturbed embryos.

This summer, I mastered the *in situ* hybridization protocol. To develop probes, I performed local BLAST searches against the transcriptome, extracted RNA, synthesized cDNA, and cloned genes of interest by PCR. These initial steps often involved extensive troubleshooting, yet I was able to obtain DIG-labeled probes for 15 of the 17 genes for which I designed primers.

I then performed several rounds of WMISH to optimize the protocol for this project. I found that young *Crepidula* embryos are extremely sensitive to this protocol, as nearly all of the embryos were breaking apart. I modified the protocol so that the embryos remained intact throughout the lengthy procedure, and I would now feel very comfortable troubleshooting the protocol for other model and non-model organisms. Unfortunately, I did not have the time to perform WMISH with any staged or with U0126-treated animals, and I have not yet seen successful staining outside of *Nematostella* controls. In hindsight,

one change I would make to my approach is to target fewer genes initially in order to efficiently develop the protocol.

Although I was not able to see the project through to completion, I gained a great deal of wet lab experience and carried out challenging but useful protocols for the first time. I had the chance to interact with visiting summer scientists and attend the lectures offered by the MBL's Embryology Course. My second summer in the Smith lab provided a fantastic educational experience, and I am grateful to EDEN for giving me this opportunity.

References:

[1] Henry, J.J. and K.J. Perry. 2008. MAPK activation and the specification of the D quadrant in the gastropod mollusc, *Crepidula fornicata*. Developmental Biology 313: 181–195.

[2] Henry, J.Q., K.J. Perry, and M.Q. Martindale. 2006. Cell specification and the role of the polar lobe in the gastropod mollusc *Crepidula fornicata.* Developmental Biology 297: 295–307.