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EDEN Undergraduate Internship: Report

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All animals share a unicellular common ancestor that underwent a transition in order to evolve multicellularity. The purpose of this exchange was to better understand the origins of animal multicellularity by studying the closest relatives of animals, choanoflagellates. At the time when this project was conceived, only two genomes of the twenty-one total species of choanoflagellates currently grown in culture had been sequenced: *Monosiga brevicollis* and *Salpingoeca rosetta*. These two choanoflagellate genomes were discovered to contain cadherins, as well as a diversity of transcription factor families and protein domains previously believed to have only been animal-specific. However, those two species were chosen before a phylogenetic tree of choanoflagellates was available, and they appear to be closely related within this tree of choanoflagellates. Preliminary sequencing of two other choanoflagellates species chosen for their phylogenetic diversity, *Salpingoeca napiformis* and *Diaphanoeca grandis*, implies that a substantial number of protein domains that are exclusively shared between choanoflagellates and animals remains to be discovered. My goal, therefore, was to sequence the remaining nineteen choanoflagellate species to gain a more complete understanding of the nature of the unicellular ancestor of choanoflagellates and animals.

The project consisted of three major components: optimizing cell culture conditions, isolating total RNA for transcriptome sequencing, and isolating 18S sequence. Using mRNA sequencing is the preferred approach since it is difficult to separate choanoflagellate DNA directly from that of their food bacteria, which greatly outnumber them in culture.

I began the project by working on cell culture optimization. Because *Monosiga brevicollis* and *Salpingoeca rosetta* are the most commonly studied choanoflagellate species, many of the choanoflagellate species were in poor condition and required antibiotic treatments to reduce not only the number and variety of non-food bacteria that could potentially take away nutrients from the choanoflagellates, but also to reduce subsequent RNA degradation. Additionally, some cultures required different concentrations of media or changes in incubation temperature to optimize choanoflagellate growth. All of these conditions needed to be optimized separately for each choanoflagellate species. That culture then was grown to high volume before harvesting a pellet of the cells for RNA and DNA preparation. Culture conditions for all species have now been optimized.

In isolating RNA preparation, we initially used a TRIzol preparation. However, it soon became apparent that, despite many modifications to the procedure, using TRIzol would lead to an intolerable amount of RNA degradation, rendering our work useless. Currently, we are optimizing a protocol using RNAqueous as the main reagent, which has lead to a significant reduction in RNA degradation. Thus far, we have isolated total RNA from 15 of the 19 choanoflagellate species.

We also were required to obtain 18S sequence for all of the cultures to verify the identity of the cultures we were working with. We prepared and harvested separate pellets of cells using a phenol/chloroform genomic DNA extraction. For the cultures whose sequence has already been submitted to GenBank, directly sequencing off of the

gel extraction product after PCR has been sufficient. For the remaining species, I have cloned the DNA to ensure high quality 18S sequence. Thus far, I have obtained 18S sequence for 15 of the species.

In short, this experience has been an excellent introduction to an under-studied but emerging model organism. It has offered me not only exposure to basic biological techniques and protocols, but has also allowed me to hone my experimental analysis and problem solving skills to troubleshoot our various problems with culturing and isolation of DNA and RNA. I look forward to receiving the results of our transcriptome data and opening the doors to a greater understanding of the unicellular ancestor of choanoflagellates and animals.