Final Grant report for 2010-2011 Faculty Development Grant

Comparative Analysis of Key Developmental Events in Two Species of Nematode

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This project had three main goals: 1) Obtain, culture, and film early development in *C. briggsae;* 2) Induce and observe sinistral embryos in *C. elegans;* 3) Compare developmental events between *C. briggsae* and *C. elegans.*

**Materials & Methods Summary:**

Feeding RNAi is an inexpensive and convenient way to block the function of a specific gene so that its role can be understood. The ability to undergo feeding RNAi is a characteristic absent in wild type *C. briggsae.* Marie-Ann Fleix of Jacques Monod Institute in Paris sent to me a genetically engineered feeding RNAi-sensitive *C. briggsae.* These worms contain a *C. elegans* gene that makes *C. briggsae* capable of undergoing feeding RNAi.

Pierre Gönczy of École Polytechnique Fédérale de Lausanne sent to me a *gpa-16* feeding RNAi plasmid. Thus, I should be able to block *gpa-16* gene function in *C. briggsae*.

I also tested several 100X microscope objective lenses and filters from Olympus and purchased the optimal parts. The new objective is very powerful and allows me to see cellular details that are not visible with the 40X lens (image comparing 100X to 40X attached).

This project allowed me to attempt several laboratory techniques that were new to me. For example, for the first time I induced competence to take up foreign DNA in a strain of *E. coli* called HTT115 (DE3). This procedure allowed me to then place the *gpa-16* plasmid into the *E. coli* and then to feed the worms with the *E. coli* to induce the RNAi effect. Student researchers in my lab will be able to use the competent HTT115 cells to test feeding RNAi efficacy in newly isolated nematode species.

**Project Goals Summary**

Goal 1 of the project was met; I made several films of early *C. briggsae* development.

Goal 2 of the project is incomplete. I will conduct more feeding RNAi experiments to knock down *gpa-16* expression in *C. elegans* and examine embryos and adults for randomization of the Left-Right developmental axis. Mounting the embryos and adults in the proper orientation

for easy determination of axis polarity has proven to be technically difficult and I have decided to film samples in sub-optimal orientations and then compare 4-D movies to 3-D models. Interpreting the films by building clay models and rotating the models in my hands had inspired me to use modeling clay as often as possible when I teach developmental biology for the first time this fall.

Goal 3 was partially met; I was able to compare developmental events between *C. briggsae* and *C. elegans*. However, I plan to spend more time in the comparisons and record additional movies of development as I teach my summer research students how to analyze nematode development.

A sub-aim of goal 3 was to knockdown *gpa-16* in *C. briggsae* to determine whether this gene has the same role in *C. briggsae* as it doesn in *C. elegans.* However, the genetically engineered *C. briggsae* appear to have reverted back to normal *C. briggsae* as feeding RNAi is not effective against them. Reversion is common in genetically engineered nematodes, even when the foreign DNA has been inserted into the worm's genome. (I utilized two positive controls that were very effective at causing RNAi phenotypes in *C. elegans*. These controls are highly conserved genes that should cause the same effect in *C. briggsae.*)

**Final Summary**

While this project has been successful in many ways, there are several areas where I will continue to add to my findings. I hope to complete the rest of the work during the summer. If my results are sufficiently interesting, I will submit my work for presentation at a developmental biology professional meeting in summer 2012.

Techniques developed and equipment purchased for this work will provide a foundation for future work in my laboratory. I am grateful for the financial and other support from UMW.

Image stills from developing nematode embryos