THE ROLE OF CYTOCHROME P450 ENZYMES IN CAENORHABDITIS ELEGANS DAUER RECOVERY


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Abstract and Introduction

The purpose of this experiment is to determine if cytochrome P450 (cyp450) enzymes play a role in dauer recovery of *Caenorhabditis elegans.* The life cycle of this free-living nematode is divided into several larval stages. Under stressful conditions, L2 larvae molt into the dauer stage, an alternate life cycle stage designed to cope with undesirable conditions. The dauer stage may be analogous to infective stage parasitic nematodes, some of which cause human disease. Microarray analysis has identified four *cyp450* genes that are expressed transiently during dauer recovery. We are using double stranded RNA interference (dsRNAi) to knock out these genes. Using this technique in silencing the genes of interest, we will be able to observe a null phenotype in comparison to that of normal gene function in worms recovering from dauer. A *cyp-14A5* dsRNAi plasmid was purchased (Geneservice Ltd.), but gene specific dsRNAi plasmids were not available for the other three genes. We have amplified these three *cyp450* genes (*cyp-13A4*, *cyp-13A5* and *cyp-13A10)* and have cloned the genes into *E. coli* plasmids for use in the dsRNAi experiments. Using DNA sequencing, we have thus far confirmed that that the *cyp-13A10* plasmid is correct, however we are in the process of resequencing the other two plasmids. Initial results have shown delayed recovery from dauer, however, we are repeating the dsRNAi experiments and will report the results. We predict that a defect will be observed in the ability of the worms to recover from dauer as compared to the controls.

C. elegans Life Cycle

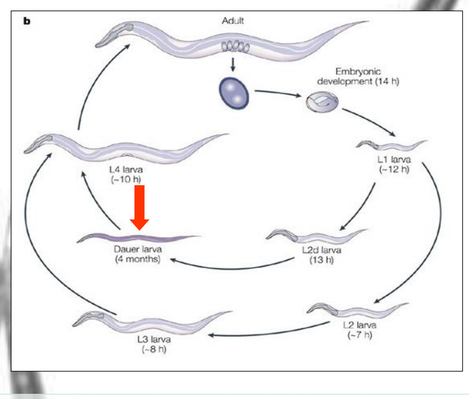


Figure 1: *C. elegans* undergo a series molts during development and can enter a phase similar to a hibernation state called dauer (arrow) when environmental conditions are harsh or nutrient supply is low. Recovery from dauer is triggered by the presence of more favorable environmental conditions or an ample supply of nutrients.

Image adapted from: http://thalamus.wustl.edu/nonetlab/ResearchF/LifeCycle

Research Significance

Recovery from the dauer stage is analogous to a developmental transition that many parasitic nematodes undergo when they infect a new host. Knowledge of the genes required for dauer recovery may potentially be useful in designing technology to combat these parasites.

Cytochrome p450

The Cytochrome p450, or CYP450, family spans numerous species from bacteria to humans.

Microarray analysis has shown that 4 *CYP450* genes (*cyp-13A4, cyp-13A5, cyp-13A10, cyp-14A5*) are briefly induced as worms recover from dauer (Wang and Kim. 2003. Development 130:1621-1634). Another *CYP450*, *daf-9*, is known to have a role in dauer formation.

CYP450 enzymes function in hormone processing and drug metabolism.

Hypothesis

Cytochrome p450 enzymes contribute to dauer recovery in *C. elegans*.

Methods Overview

Clone the dsRNAi plasmids for *cyp-13A4*, *cyp-13A5*, and *cyp-13A10* by amplifying the genes using the Polymerase Chain Reaction (PCR) and inserting into the dsRNAi vector.

Double stranded RNA interference (dsRNA) to knock out genes of interest expressed during dauer recovery and observe their function.

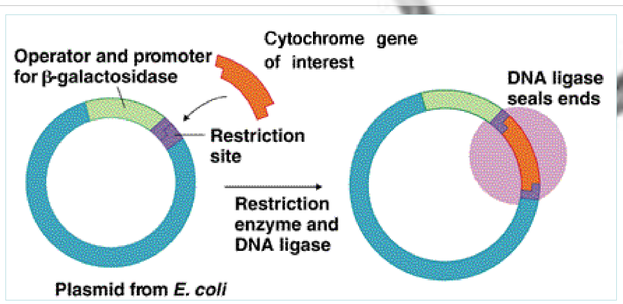
Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) to confirm expression patterns of genes thought to be induced during dauer recovery.

Cloning an Insert into dsRNAi Vector

Cloning an insert into a plasmid vector is a process by which a small piece of DNA, in this case an *E. coli* plasmid, will have a foreign piece of DNA placed into it.

By using restriction enzymes, the plasmid is cut and the insert carrying the gene or genes of interest, is inserted into the plasmid. The plasmid is then sealed via DNA ligase.

In our case, we have cloned *cyp-13A4*, *cyp-13A5*, and *cyp-13A10* into *E. coli* plasmids for use in dsRNAi.



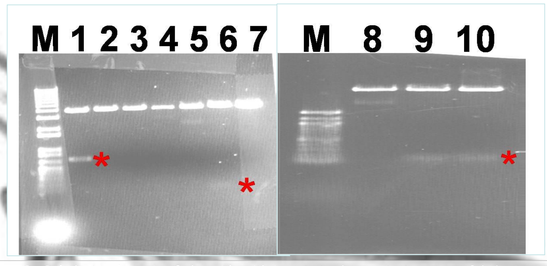


Figure 2: Gel electrophoresis of plasmids with inserts to demonstrate successful cloning. M: 100 bp ladder; Lanes 1-3: cyp13A10 plasmid clone. Lanes 4-7: cyp-13A4 plasmid clone. Lanes 8-10: cyp-13A5 plasmid clone. * Represents an expected insert fragment.

dsRNA Interference

Double stranded RNA interference is a process which inhibits gene expression for specifically chosen genes in an attempt to "knockout" the phenotype produced by that gene.

This process has been done through the "feeding" method in which *E. coli* engineered to express gene specific dsRNA are fed to the nematodes. This induces silencing in the genes of interest and allows for the observation of the null phenotype.

The dsRNAi assays are conducted in *daf-2* and *daf-7* mutant worms because they can be induced to form and recover from dauer by changing their incubation temperature.

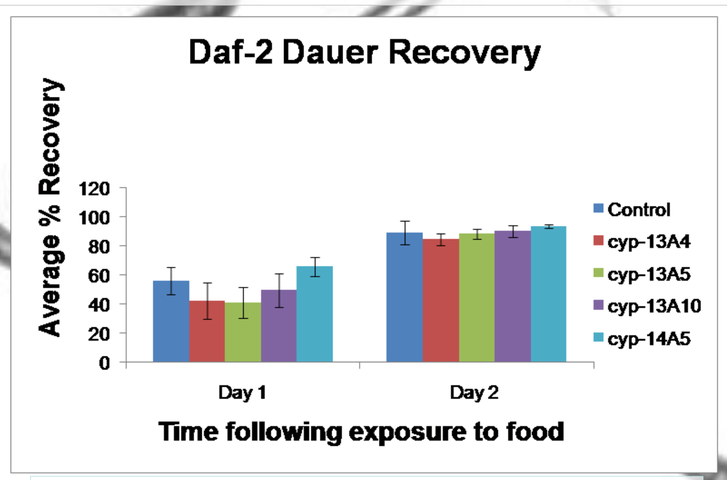


Figure 3: Dauer recovery in *daf-2* worms. *E. coli* expressing dsRNA for the control (L4440, empty vector), *cyp-13A4, cyp-13A5, cyp-13A10,* and *cyp-14A5* genes were fed to worms. Recovery from dauer was artificially triggered at 15.0°C and the average percent of adult recovery is shown for each gene of interest. Error bars represent standard error. There was no significant difference among the samples for either day of recovery (Day 1: F=0.638, df=4, p=0.641; Day 2: F=0.295, df=4, p=0.878)

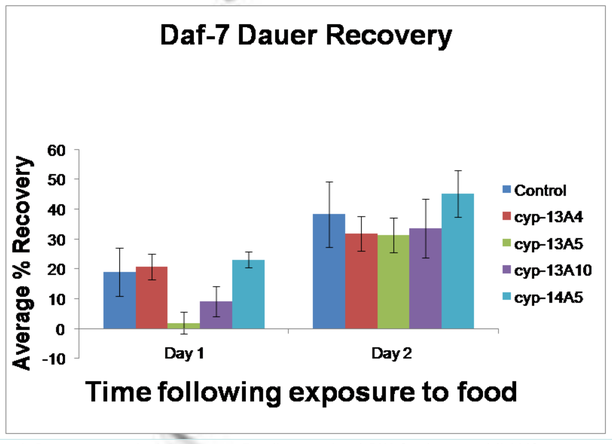


Figure 4: Dauer recovery in daf-2 worms. *E. coli* expressing dsRNA for the control (L4440, empty vector), *cyp-13A4, cyp-13A5, cyp-13A10*, and *cyp-14A5* genes were fed to worms. Recovery from dauer was artificially triggered at 15.0°C and the average percent of adult recovery is shown for each gene of interest. Error bars represent standard error. * Represents cyp-13A5, which showed significantly less recovery from dauer on Day1 as compared to the control. (Tukey's HSD, $p<0.05$).

qRT-PCR

qRT-PCR, or quantitative real time polymerase chain reaction, is a method used to amplify targeted sequences of DNA.

SYBR Green dye is included in the reactions and fluorescence is recorded with each round of amplification, allowing a quantitative measure of the amount of cDNA in a sample by comparison to an endogenous control (*ama-1)* that is known to be expressed at uniform levels.

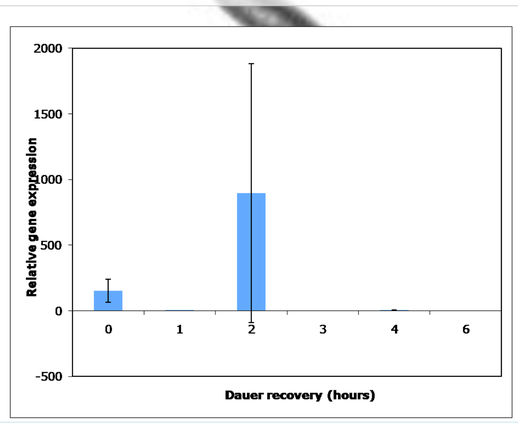


Figure 5: qRT-PCR relative gene expression of *CYP-13A10*. qRT-PCR was conducted on cDNA samples generated from worms during dauer recovery. The *ama-1* gene served as an endogenous control. Results suggest that *cyp-13A10* is active during dauer recovery, but must be repeated due to inconsistent amplification by the *ama-1* primers in the 2 hr sample. Relative quantitation was calculated using qBase software. Error bars represent standard error.

Summary of Work Thus Far

- Inserts for *cyp-13A4, cyp-13A5, and cyp-13A10* were successfully cloned into a dsRNAi vector.
- *cyp-13A10* expression peaks at 2 hours of dauer recovery, confirming microarray results.
- Recovery from dauer in *daf-7* worms is slowed in *cyp-13A5* dsRNA treated worms, indicating that the dsRNAi process has been effective.
- An unusual phenotype was observed in *daf-7* worms when fed *cyp-14A5* dsRNA. The phenotype, known as dumpy, was observed in 62.5% of the adults scored on day 1, as well as 57.0% of the adults scored on day 2.

Future Directions

- Repeat dsRNA experiments assaying *cyp-13A4*, *cyp-13A5,* cyp-13A10 and *cyp-14A5* during dauer recovery to further investigate the overall "knockout" effects.
- Conduct and repeat qRT-PCR on all CYP450 genes to confirm expression profiles during dauer recovery and to assess efficacy of dsRNAi.
- Further study of *daf-7* related phenotypes to better understand the link between *daf-7* worms, the *cyp-14A5* gene, and the 'dumpy' phenotype.

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