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 (dsRNA) 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 dsRNA plasmid was purchased (GeneService Ltd.), but gene specific dsRNA 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 dsRNA experiments. Using DNA sequencing, we have confirmed that the cyp-13A10 plasmid is correct, however we are in the process of rescuing the other two plasmids. Initial results have shown delayed recovery from dauer, however we are repeating the dsRNA experiments and will report the results.

Methods Overview

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

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

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.

C. elegans Life Cycle

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.