GENETIC ANALYSIS OF A NUCLEAR RECEPTOR GENE AND A CYTOCHROME P450 GENE IN *CAENORHABDITIS ELEGANS* AND THEIR EFFECT ON DAUER RECOVERY

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Abstract

We hypothesized that both the nuclear receptor gene *nhr-85* and the cytochrome p450 gene *cyp-14A5* are involved in dauer recovery in *Caenorhabditis elegans*. *C elegans* is a non-parasitic nematode with a life cycle consisting of four stages: L1, L2, L3, and L4. However, when conditions are not favorable, for example in the induction of starvation, the L2 stage molts to the dauer form, which is better capable of withstanding unfavorable conditions than the normal L3 larva. When conditions become favorable, the dauer larva undergoes molting into the L4 stage, and finally into the adult. We are interested in the downstream signaling pathways that are involved in responding to environmental conditions as the worms recover from the dauer stage. We used mutant forms of *nhr-85* and *cyp-14A5* to test the role of these genes in dauer recovery. The results obtained so far show a slight but inconsistent difference between the mutant worms and the wild type in the ability of dauer to molt successfully into the L4 stage. We are currently outcrossing these worms to cancel any effect of other mutations that may contribute to dauer recovery. After outcrossing is complete, we will again force the worms into dauer and monitor their recovery. We predict that the wild type worms should molt at a higher rate than the mutant worms. Researching the genes that control dauer recovery can be of pharmaceutical importance in fighting parasitic nematode infections in humans because the dauer is considered analogous to infective stage parasites.

C. elegans Life Cycle

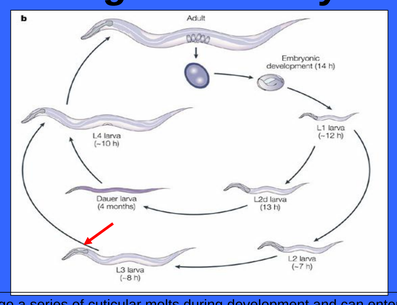


Figure 1: *C. elegans* undergo a series of cuticular molts during development and can enter an alternate life cycle stage called dauer (arrow) when food is scarce and/or crowding is high. Recovery from dauer is triggered by the presence of food and/or reduced crowding. Image adapted from: Jorgensen and Mango. 2002. Nature Reviews Genetics 3:356-369

nhr-85

- One of ~284 nuclear receptor transcription factors in *C. elegans.*
- required for development of the egg-laying system and formation of SDS-resistant dauer larvae

cyp-14A5

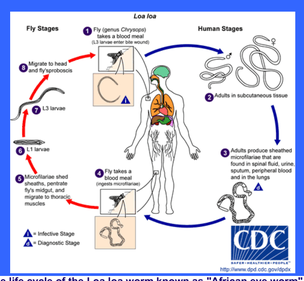
- one of ~80 *C. elegans* cytochrome P450s: membrane-associated, heme-containing NADPH-dependent monooxygenases that catalyze the oxidative metabolism of a variety of exogenous compounds and endogenous substrates.
- Expression is induced during dauer recovery

Hypothesis

nhr-85 and cyp-14A5 play a role in dauer recovery in *C. elegans*

Significance

A better understanding of the genetic basis of dauer recovery could be potentially applicable to understanding the ways in which parasitic nematodes respond to the environment of a host during infection. This is because the dauer form worms are similar to the infective stage of parasitic nematodes. Knowledge of the genes that are expressed during dauer recovery may suggest ways to block molting in parasitic nematodes, thereby reducing infection.



The life cycle of the Loa loa worm known as "African eye worm" as in its migrations one or more worms may find their way into the eye. Vision is not threatened but it may be very painful as it moves around
http://www.darwinsgalapagos.com/animals/nematoda_roundworms.htm

Outcrossing mutants

Cross homozygous mutant L4 hermaphrodites with *him* males

↓

Cross male offspring (heterozygotes) with wild type L4 hermaphrodites.

mut/+; him/+ males X +/+; +/+ hermaphrodites

↓

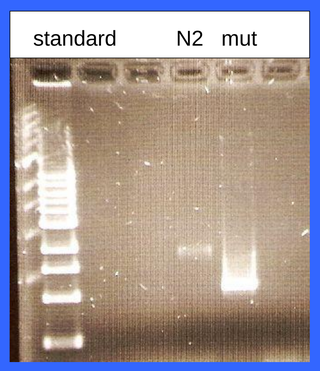
Self cross 10-20 individual offspring and run PCR analysis on their offspring.

Possible genotypes of individuals being selfed:
1/4 mut/+; +/+
1/4 mut/+; him/+
1/4 +/+; him/+
1/4 +/+; +/+

Plates that contain worms with the mutation will have a smaller product in the PCR results

↓

Self cross individual worms from PCR positive plates and use PCR to screen for plates containing only homozygous mutants.



N2: wild type
Mut:*nhr-85* mutant

Figure 3: Example of PCR results for a mutant gene with the wild type used as control. Here, *nhr-85*(rb1661) is used.

Dauer Recovery Assay

Allow worms to form dauers by starvation.

↓

Wash worms off plate and incubate in 1% SDS to kill all worms except dauers.

↓

Remove SDS and put 20-40 worms onto plates with fresh food.

↓

Monitor dauer recovery (molting to L4, then adult) for three days.

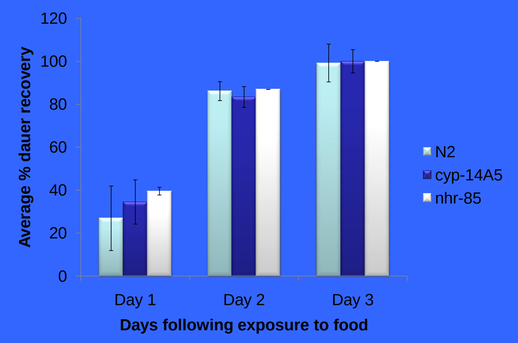


Figure 4: ***nhr-85* and *cyp-14A5* are not required for dauer recovery.** *nhr-85 (RB1661) and cyp-14A5 (VC249)* mutant worms were scored for their ability to recover from dauer. Average percent recovery is shown. Wild type (N2) worms are used as control. Error bars show standard deviation.

Conclusions

- *nhr- and cyp-14A5* appear not to function in the pathway for dauer recovery.

Future Directions

- complete outcrossing of mutants and repeat dauer recovery assays.

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