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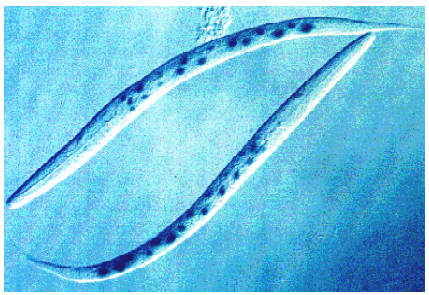
Raising Transgenic Lines of *Caenorhabditis elegans* for the Study of Stress-Inducible Genes

Topic Overview

•*C. elegans* can be used for the study of stress-inducible proteins and as biomarkers for environmental contamination

-The overall goal of the research is to raise several different transgenic lines of *Caenorhabditis elegans* in which the promoter of a stress-inducible gene is fused to the gene for GFP. When *GFP* is fused to a promoter of a *C. elegans* gene, the resulting transcription and translation product will be expressed in cells as if it were the full gene. Once transgenic lines of *C. elegans* containing the promoter-*GFP* fusion products are raised, we hope to study the effect of certain toxicants on gene expression (for example, Cd) and to use the worms as biomonitors for environmental contamination. Work in this field has been performed using B-galactosidase (see picture below).



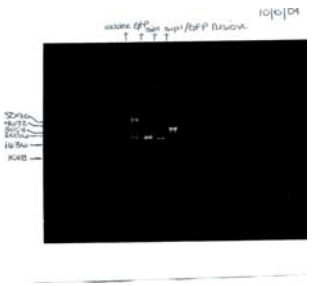
Left: Two *C. elegans* containing B-galactosidase reporter genes respond to cadmium.

Picture from: Freedman, J.H., Slice, L.W., Dixon, D., Fire, A. and Rubin, C.S. The novel metallothionein genes of *Caenorhabditis elegans*. Structural organization and cell-specific transcriptional activation. *J. Biol. Chem.* 268, 2554-2564 (1993)

Left: Amplification of *mrp-2* and *mtl-2* promoters through PCR of genomic DNA. *GFP* was also amplified from the 95.67 plasmid.

Right: Fusion of *mrp-2* and *mtl-2* promoters to *GFP* via PCR.

Left: Amplification of *sip-1* promoter through PCR of genomic DNA.



Right: Fusion of *sip-1* promoter to *GFP* via PCR.

Scientific Approach

•Research Plan

Create promoter-*gfp* transgenes through polymerase chain reaction

-Promoter-*GFP* fusion products were created as described by Hobert[1] for three *C. elegans* genes: *mtl-2* (metallothionein-2), *mrp-2* (multi-drug resistance protein-2) and *sip-1* (stress-induced protein-1). The resulting amplicons from each PCR were elucidated via 1% agarose gel electrophoresis, using ethidium bromide and UV light for visualization (at left). The fusion products were then purified and quantified in preparation for microinjection.

Microinject adult *C. elegans* with promoter-*gfp* fusion product

-Adult *C. elegans* worms will be injected in the gonad prior to egg-laying. This will result in the F1 generation containing the transgenes, as well as the F2 and subsequent generations.

Expose transgenic *C. elegans* to stressors

-The main stressor that will be focused on is cadmium.

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[1]Hobert, O. PCR Fusion-Based Approach to Reporter Gene Constructs for Expression Analysis in Transgenic *C. elegans*. *Biotechniques* 32:728-30 (2002).

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