Determining the effect of *uso-1* gene silencing on distal tip cell migration in *C. elegans*

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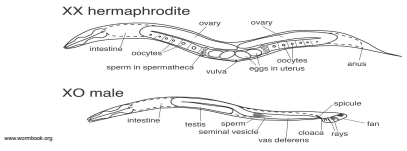
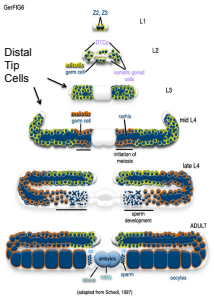
Introduction to *C. elegans*

In this project, we used the nematode *C. elegans* as a model system to investigate the genes and associated proteins that control distal tip cell (DTC) migration. *C. elegans* is an ideal system for this experiment given its relatively short life span, compact genome (6 chromosomes), ability to self-fertilize, and transparent anatomy. Though *C. elegans* exhibits two distinct sexes (male and hermaphrodite), our studies concentrate on manipulating the hermaphrodite phenotype. We specifically manipulated genes that directly affect the migration of the hermaphrodite gonad or ovary. The hermaphrodite and male anatomy including the ovary can be seen below.



The DTCs are leader cells of the ovary that receive, interpret, and direct the 'go', 'turn', and 'stop' signals.

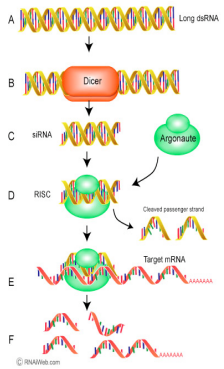


Throughout the course of embryonic development the DTCs switch to migratory cells, migrate outward from the vulva (roughly the center of the worm), take two 90° turns and migrate back towards the center of the worm. Normal migration ends when both ends of the gonad migrate back to the vulva. This migration depends on the extracellular matrix for protein signals as well as an anchoring structure. Multiple protease enzymes are also required to breakdown the extracellular matrix to allow for the migration of the DTCs.

RNAi screening has demonstrated that hundreds of genes are involved in DTC migration.

What is RNAi?

RNAi is a way of silencing gene expression by taking advantage of the worm's innate immune system. When viruses introduce double-stranded RNA into the worm, the worm's response is to use enzymes and protein complexes to break down the dsRNA. This way the viral proteins cannot be made. We can mimic this process in the lab by introducing dsRNA that is homologous to genes in the worm's genome. The dsRNA is ingested by the worm via inoculated cultures of transformed *E. coli* containing the gene of interest. Thus every time the mRNA for that gene is transcribed, it is targeted by worm enzymes and broken down prior to translation. The worm genome remains intact as it is the mRNA that is digested.



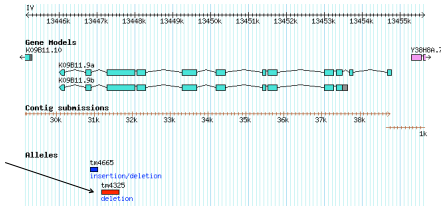
Abstract

It has been documented that the *uso-1* gene in *C. elegans* is involved in vesicle transport, necessary to dock vesicles coming from the endoplasmic reticulum to the Golgi, though little has been done to characterize the role of this gene in cell migration and worm development. By using gene suppression tools we characterized the role *of uso-1* in controlling distal tip cell migration in *C. elegans* by (1) disrupting *uso-1* gene expression using RNA interference (RNAi) in both wild-type and RNAi-sensitive strains *(rrf-3)*; (2) verifying a 600-base pair deletion in the *uso-1* gene using PCR amplification and gel electrophoresis; and (3) measuring and comparing distal tip cell migration in wild-type strains (N2) versus strains containing the deletion allele of *uso-1* (*tm4325*).

Hypothesis: Silencing the *uso-1* gene using RNA interference will result in overshoots of the distal tip cells during gonad migration.

***uso-1* gene structure**

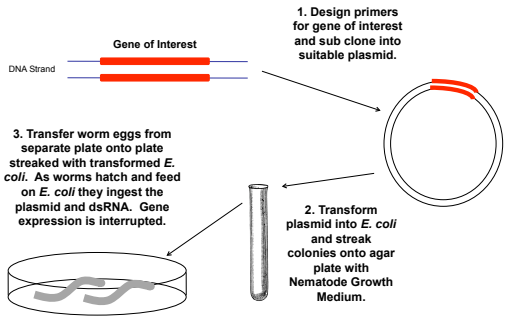
***uso-1* deletion**



Tests and Methods

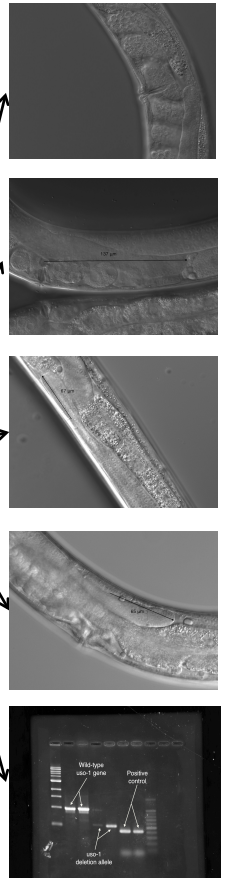
Group/Test	Method
1 (Control): Wild Type (N2)	Fed empty plasmid and observe DTC migration
2: Wild Type (N2)	RNAi of uso-1 gene and observe DTC migration
3: *rrf-3* (RNAi-sensitive strain)	RNAi of uso-1 gene and observe DTC migration
4: *tm4325* (Strain with *uso-1* deletion allele)	Grew mutant strain and observe DTC migration
5: Verify size and sequence of *uso-1* deletion (*tm4325*)	PCR Amplification and Electrophoresis

Introducing dsRNA Into C. elegans by feeding RNAi



USO-1 is a conserved vesicle tethering protein

Species	Description	BLAST E-value	% Length
C. briggsae	gene CBG01765	0	99.90%
B. malayi	SubName: Full=Uso-1 vesicle tethering protein	2.90E-219	92.80%
M. musculus	RecName: Full=General vesicular transport factor Full=Vesicle-docking protein;	2.10E-119	81.70%
H. sapiens	General vesicular transport factor	2.40E-114	92.20%
S. Cerevisiae	Essential protein involved in intracellular protein transport, coiled-coil protein necessary for transport from ER to Golgi; required for assembly of the ER-to-Golgi SNARE complex	2.50E-32	74.10%



Quantitative Results

Treatment/ Strain	Distal Tip Cells Scored (N)	% Normal Phenotype	% Overshot Phenotype Only	% Aberrant Migration Only	% Aberrant and Overshot
N2 fed empty vector	28	86%	14%	0%	0%
N2 with RNAi treatment of *uso-1*	26	73%	19%	0%	8%
rrf-3 with RNAi treatment of *uso-1*	28	75%	14%	7%	4%
tm4325 (*uso-1* deletion allele)	51	74%	16%	6%	4%

Conclusion for *uso-1* Experiments

Our results show that although there were more gonad overshoots and abnormal migration patterns observed in the worms treated by RNAi and worms possessing the deletion allele, an analysis using binomial (95%) confidence intervals shows there is no significant difference between control and experimental groups. Significance may have been increased had a greater number of distal tip cells been observed and scored. This rejects our hypothesis that silencing the *uso-1* gene will result in a greater number of DTC overshoots than that of the wild type worms. Additionally, observations of abnormal distal tip cell migration patterns were neither expected nor accounted for in our original hypothesis.

Results and Conclusion for PCR and Gel Electrophoresis

The deletion allele of the *uso-1* gene as seen in the worm strain *tm4325* was predicted to be ~ 600bp as predicted by www.wormbase.org. Prior to using the strain as a model for manipulating DTC migration the deletion was amplified and the length of the deletion confirmed via gel electrophoresis. As compared to the N2 worm the gel stain shows a difference in size of roughly 600 bp. The *pat-3* gene was used as a positive control.

Acknowledgements

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Research Experiences for Teachers at Northeastern University
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