Characterization of the Novel Fluoride Resistant Gene *flr-3* in *C. elegans*

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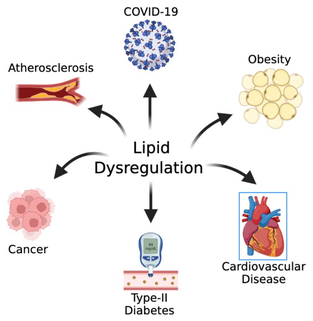
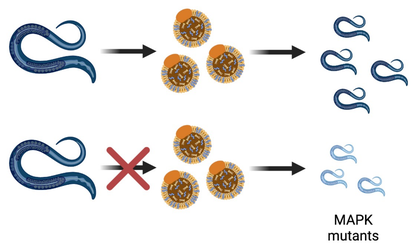
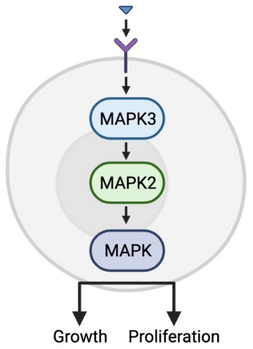
Background

- In *C. elegans*, vitellogenesis is the process by which lipid-rich particles are transported from adult intestinal cells to the germline oocytes, a mechanism analogous to low density lipoprotein (LDL) transport in humans
- Dysregulation of lipid homeostasis underlies human metabolic disease
- Vitellogenin production is noted by the P*vit-3*::*mCherry* reporter in *rhdsi42* strains







- Previous work in the Dowen Lab has identified DRL-1 as a Mitogen Activated Protein (MAP) kinase responsible for proper growth and vitellogenesis in *C. elegans*
- *flr-3* genocopies *drl-1*, though its molecular identity remains unknown

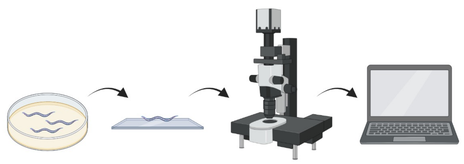
Objectives

1. Characterize the *flr-3* mutation phenotypically. Does *flr-3* genocopy *drl-1*?
2. Determine the genetic identity of *flr-3*.

Methods

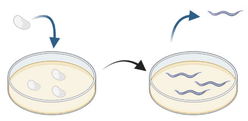
Body Size Assay and Fluorescent Signal Quantification

- Approximately 30 L4 animals were picked to *E. coli* OP50 seeded Normal Growth Media (NGM) plates and imaged 24 hours later as day one adults
- Animals were traced in Fiji and the two-dimensional outline was used to calculate the body size and fluorescent signal intensity of each animal



Developmental Growth Rate Assay

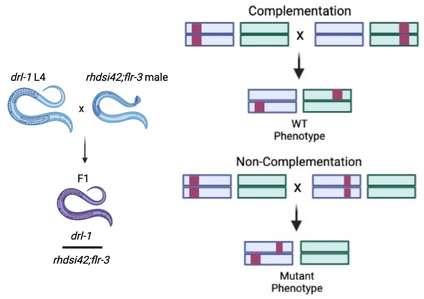
- Approximately 100 eggs were picked to *E coli* OP50 or HT115 seeded NGM plates
- Every 24 hours, gravid adults were picked off and recorded as having reached adulthood



Methods

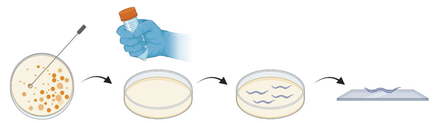
Complementation Tests

- To determine if the *flr-3* mutation is located within the *drl-1* locus, complementation tests were performed
- *rhdsi42; flr-3* L4s were heat shocked to produce males
- *rhdsi42; flr-3* males were plated with *drl-1* and *flr-3* hermaphrodites
- F1 cross-progeny were picked as L4s and imaged 24 hours later as day one adults



RNA Interference (RNAi)

- *pmk-1* and *fshr-1* RNAi clones were streaked out on plates containing ampicillin and tetracycline
- Bacterial colonies were grown in Lysogeny Broth (LB) and seeded on Isopropyl β-D-1-thiogalactopyranoside (IPTG) plates
- Synchronous populations of L1 animals were dropped on plates and imaged as day one adults



Results

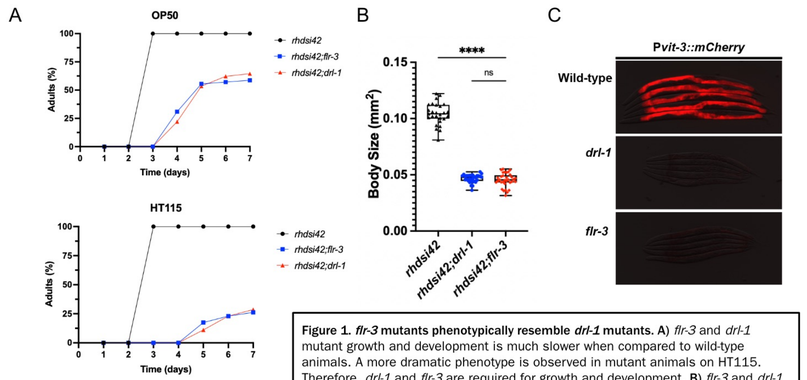


Figure 1. *flr-3* mutants phenotypically resemble *drl-1* mutants. A) *flr-3* and *drl-1* mutant growth and development is much slower when compared to wild-type animals. A more dramatic phenotype is observed in mutant animals on HT115. Therefore, *drl-1* and *flr-3* are required for growth and development. **B)** *flr-3* and *drl-1* mutants are much smaller in body size when compared to wild-type animals, indicating that *drl-1* and *flr-3* are required for normal body size. **C)** The reduction in *vit* gene expression in *drl-1* mutants is similarly observed in *flr-3* mutants, as noted by the absence of the vitellogenesis reporter.

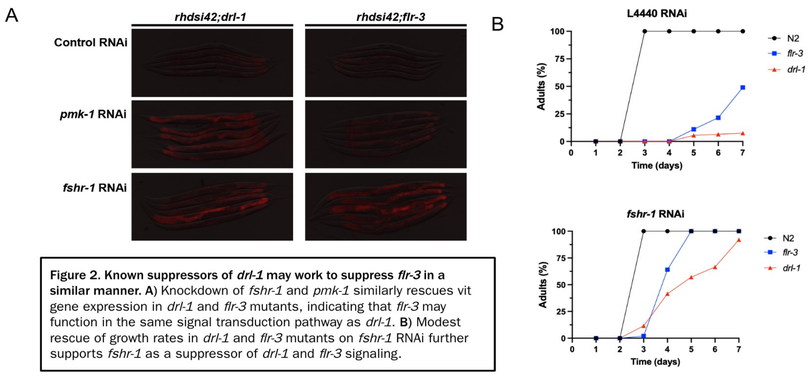


Figure 2. Known suppressors of *drl-1* may work to suppress *flr-3* in a similar manner. A) Knockdown of *fshr-1* and *pmk-1* similarly rescues vit gene expression in *drl-1* and *flr-3* mutants, indicating that *flr-3* may function in the same signal transduction pathway as *drl-1*. **B)** Modest rescue of growth rates in *drl-1* and *flr-3* mutants on *fshr-1* RNAi further supports *fshr-1* as a suppressor of *drl-1* and *flr-3* signaling.

Results Cont'd

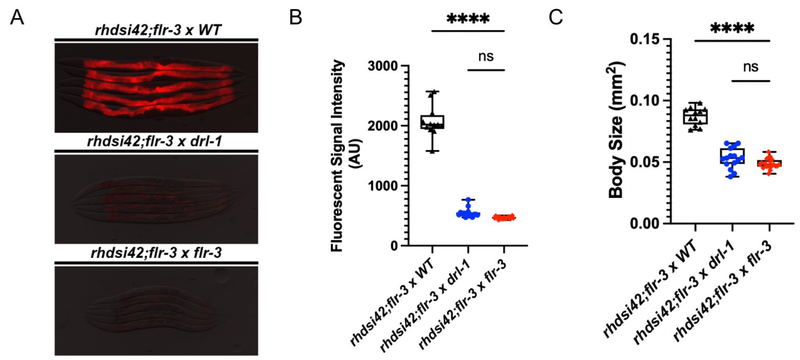


Figure 3. *drl-1* and *flr-3* fail to complement. A) F1 progeny from a cross between *rhdsi42;flr-3* and *drl-1* mutants display reduced vit gene expression which is characteristic of both the *drl-1* and *flr-3* recessive mutations, indicating that *drl-1* and *flr-3* fail to complement. **B)** The reduction in *vit* gene expression in *rhdsi42;flr-3 x drl-1* mutants is similarly observed in *rhdsi42;flr-3 x flr-3* mutants, as noted by the reduction in fluorescent signal intensity. **C)** Small body size phenotypes are similar in *rhdsi42;flr-3 x flr-3* and *rhdsi42;flr-3 x drl-1* strains.

Conclusions

1. *flr-3* mutants have a phenotype that is small in body size, slow growing, and lipid devoid, much like *drl-1* mutants.
2. Known suppressors of *drl-1* also suppress *flr-3*, indicating that *flr-3* may work within the same pathway as *drl-1*.
3. The *flr-3* mutation fails to complement the *drl-1* mutation, suggesting they may be located within the same locus.

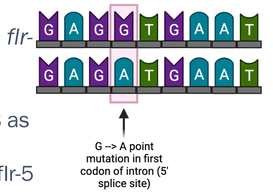
Future Directions

To confirm that *flr-3* is an allele of *drl-1*, the *drl-1* locus is to be sequenced in *flr-3* mutants via Polymerase Chain Reaction (PCR) and Sanger sequencing

- Previous attempts have resulted in unsuccessful binding of *drl-1* primers in *flr-3* mutants, suggesting that the causative mutation for *flr-3* may be a large deletion or complex rearrangement

Additionally, the same forward genetic screen for mutations conferring fluoride resistance also identified *flr-5;* its genetic identity similarly remains unknown

- *flr-5* is thought to genocopy *flr-2* and suppress *drl-1*
- Whole genome sequencing of *flr-5* identified T23B12.8 as a candidate for the causative mutation
- PCR and Sanger sequencing of the T23B12.8 locus in flr-5 mutants identified a G→A point mutation at the 5' splice site
- Future experimentation utilizing the CRISPR/Cas9 system to recapitulate the *flr-5* mutant phenotype would confirm T23B12.8 as the causative mutation of *flr-5*



References

[1] Chamoli, M., Singh, A., Malik, Y., & Mukhopadhyay, A. (2014). A novel kinase regulates dietary restriction-mediated longevity in *Caenorhabditis elegans* . *Aging Cell*, *13*(4), 641–655. https://doi.org/10.1111/acel.12218
[2] Chung, K. W. (2021). Advances in understanding of the role of lipid metabolism in aging. Cells, 10(4), 880. https://doi.org/10.3390/cells10040880
[3] Katsura, I., Kondo, K., Amano, T., Ishihara, T., & Kawakami, M. (1994). Isolation, characterization and epistasis of fluoride-resistant mutants of Caenorhabditis elegans. *Genetics*, *136*(1), 145–154. https://doi.org/10.1093/genetics/136.1.145
[4] Mortality in the united states, 2020. (2021). National Center for Health Statistics (U.S.). https://doi.org/10.15620/cdc:112079
[5] Perez, M. F., & Lehner, B. (2019). Vitellogenins–Yolk gene function and regulation in caenorhabditis elegans. Frontiers in Physiology, 10, 1067. https://doi.org/10.3389/fphys.2019.01067
[6] Van Sinay, E., Mirabeau, O., Depuydt, G., Van Hiel, M. B., Peymen, K., Watteyne, J., Zels, S., Schoofs, L., & Beets, I. (2017). Evolutionarily conserved TRH neuropeptide pathway regulates growth in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*, *114*(20). https://doi.org/10.1073/pnas.1617392114

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