Characterization of a gain-of-function mutation that suppresses defects in MAP Kinase signaling in *C. elegans*

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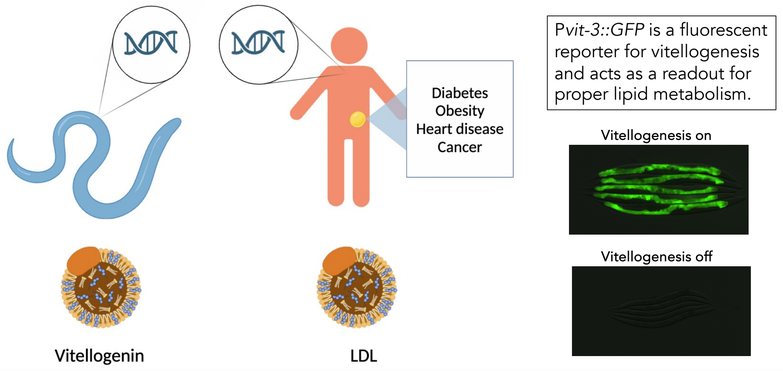
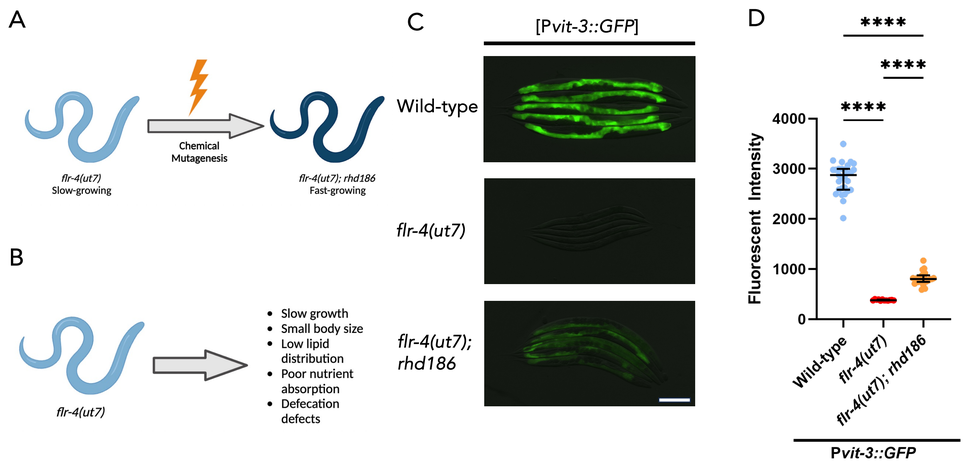
Background



Pvit-3::GFP is a fluorescent reporter for vitellogenesis and acts as a readout for proper lipid metabolism.

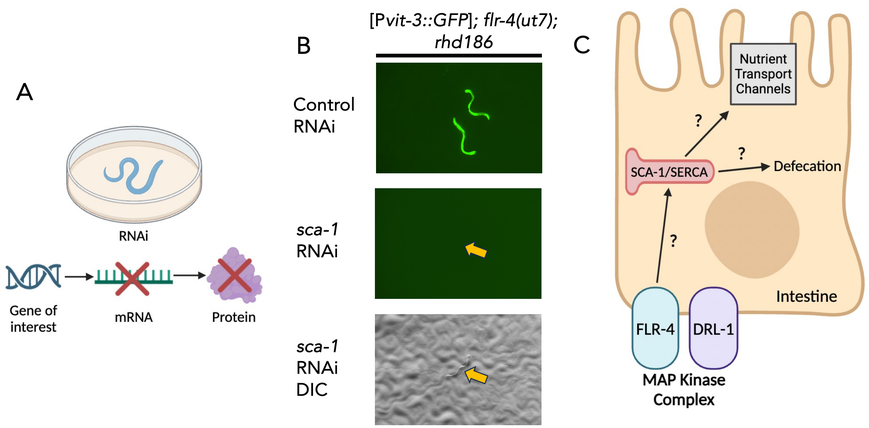
Balancing the storage and transport of lipids is essential to regulating biological processes that require large energy investments like growth and reproduction. In the model organism *C. elegans*, the process of reallocating fats from the intestine to germline for reproduction, is known as vitellogenesis. This process is coordinated, in part, by Mitogen-Activated Protein Kinase (MAPK) signaling. FLR-4 is one MAPK that balances the redistribution of lipids to promote growth signaling and reproduction, but it is poorly understood how it coordinates these processes.

Forward genetic screen reveals a mutant allele that rescues impaired FLR-4 MAPK signaling



(A) Graphic depicting the forward genetic chemical mutagenesis screen that identified the *rhd186* allele. 40 nonsynonymous mutations in protein-coding sequences were identified by whole genome sequencing as potentially causative of the phenotype that rescued the *flr-4(ut7) mutation*. **(B)** Diagram describing the phenotypes of *flr-4(ut7)* mutant animals. **(C)** Day one adult animals expressing the high copy *mgIs70*[P*vit-3::GFP*] transgene, which is a transcriptional reporter for vitellogenesis. The *rhd186* allele partially suppresses the *flr-4* mutant vitellogenesis phenotype. Scalebar is 200µm. **(D)** Quantification of fluorescent intensity of the P*vit-3::GFP* reporter and **(E)** body size of adults consuming empty vector RNAi. Animals were imaged 48 hours after the L4 larval stage (median and interquartile range, p<0.0001, one-way ANOVA).

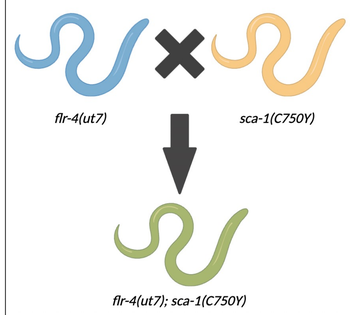
RNAi screen indicates that a gain-of-function mutation in *sca-1* rescues *flr-4(ut7)* lipid defects



(A) Graphic depicting how RNA interference (RNAi) prevents protein synthesis by degrading mRNA. RNAi screens were conducted for all 40 potential causative alleles identified by the initial EMS screen. **(B)** *mgIs70*[P*vit-3::GFP*]; *flr-4(ut7); rhd186* worms consuming empty vector and *sca-1* RNAi reveal the mutation caused by the initial EMS screen to be a gain-of-function mutation. Arrows indicate the presence of an animal. **(C)** Diagram outlining the hypothesized relationship between FLR-4 and SCA-1 in intestinal epithelial cells. SCA-1 is a Sarco-Endoplasmic Reticulum ATPase (SERCA) essential in calcium ion transport and is believed to regulate defecation rhythms. **(D)** Schematic of the CRISPR/Cas9 gene editing technology used to create the putative *sca-1(rhd186[C750Y])* gain-of-function mutation identified via whole genome sequencing of the worm strain containing the *rhd186* allele. **(E)** Illustration of the restriction enzyme cut site additionally engineered at the *sca-1* locus via CRISPR to verify the presence of successful CRISPR/Cas9 editing. **(F)** Visualization of successful CRISPR/Cas9 editing by gel electrophoresis reveals restriction enzyme cutting in two candidate strains, indicating the likely presence of the desired *sca-1(C750Y)* mutation.

Future Directions

- What are the phenotypic characteristics of *flr-4(ut7); sca-1(C750Y)* double mutants?
  - Body size
  - Lifespan
  - Nutrient absorption
  - Defecation
  - Growth rate
  - Triglyceride levels (Nile red staining)
- By what mechanism does the activation of *sca-1* suppress growth and lipid defects in MAPK mutants?



Acknowledgements

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References

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