# LIN-61 regulates growth and lipid homeostasis via *drl-1* suppression in *C. elegans*

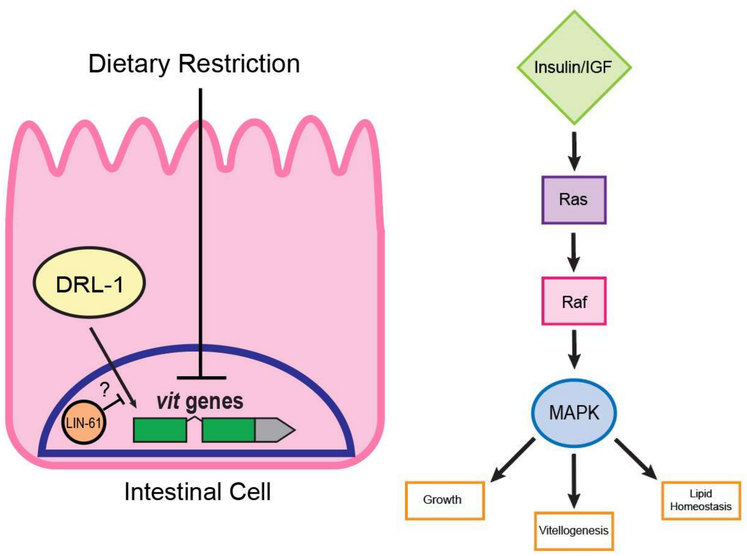
Sarah Torzone[1,2] and Dr. Robert Dowen[1,2]

[1]UNC-CH School of Medicine — Department of Cell Biology and Physiology, Chapel Hill, NC, USA | [2]UNC-CH College of Arts and Sciences — Department of Biology, Chapel Hill, NC, USA

## Abstract

Dietary (caloric) restriction in mammals is associated with reduced risk of disease/cancer for incompletely understood reasons[1]. DR can be induced in a *C. elegans* model to better examine the molecular controls of the metabolic tradeoffs between growth, reproduction, and lipid homeostasis. Mutation of *drl-1,* a MAP kinase orthologous to human MEKK-3, mimics a dietary restricted state in *C. elegans*[1]. *drl-1* mutants have been previously demonstrated in the Dowen lab to produce a slow-growing, lipid-devoid animal with down-regulation in vitellogenin expression[6]. *lin-61* is a chromatin factor identified in an EMS screen of *drl-1* mutants which is a genetic suppressor of DRL-1. Genetic mutation and RNAi knockdown of *lin-61* in *drl-1* mutants suggest that LIN-61 antagonizes *vit* gene expression and growth rates in *C. elegans*. Further experimentation will be required to elucidate the exact mechanisms by which LIN-61 interacts with DRL-1 to regulate the metabolic tradeoffs between these key processes.

## Background




Vitellogenesis is the process by which mature hermaphrodite *Caenorhabditis elegans* transport lipids from its intestines to its germline[3,8]. Vitellogenin genes which encode yolk lipoproteins regulate the process of vitellogenesis. While yolk provisioning may come at a high metabolic cost to the mother, it serves to improve the rate of development and longevity of its progeny[7].

Dietary restriction is a process which can modulate metabolic tradeoffs between reproduction and longevity. In mammals, this is correlated with reduced risk of cancer, heart disease, and type II diabetes[1].

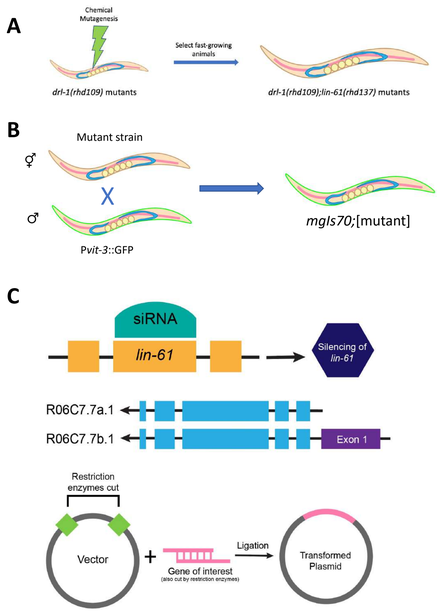
*C. elegans* are a model organism to study the molecular and genetic processes that regulate dietary restriction. Knockdown of some MAP kinase genes in *C. elegans* can mimic dietary restriction — a state marked by reduced fat stores in the intestines and hypodermis. Knockdown of the *dietary restriction like 1* gene, a serine-threonine kinase orthologous to human MEKK-3, induces dietary restriction in *C. elegans*[1].

The MAPK proteins encoded by *drl-1* are necessary for vitellogenesis, lipid homeostasis, and proper growth, however the signaling transduction pathways that regulate the metabolic tradeoffs between these processes are incompletely understood. Identifying key molecular regulators of these processes is crucial to understanding lipid homeostasis. The chromatin factor *lin-61*, which was identified in a suppressor screen for *drl-1,* is being explored as a suppressor of dietary restriction.

## Objectives

- Identify the effects of LIN-61 on growth and vitellogenesis in *drl-1* mutants via observation of body size, growth rates, and *vit* gene expression
- Evaluate the candidacy of *lin-61* as a suppressor of dietary restriction
- Explore the role of other molecular regulators such as *flr-3* in the lipid homeostasis of *C. elegans*

## Methods




**Generation of mutant strains:** *lin-61(rhd137)* was previously identified as a potential suppressor of *drl-1(rhd109)* in a chemical mutagenesis screen by the Dowen lab.

**Generation of transgenic strains:** L4 hermaphrodite animals from mutant strains were crossed with *mgIs70* males which contain a GFP-tagged *vit* gene promoter. Transgenic strains were used to assess vitellogenin expression in mutant strains.

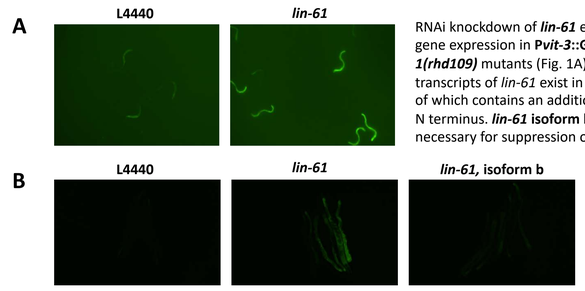
**Gene Silencing:** RNA interference (RNAi) was conducted to silence *lin-61* by destroying mRNA transcripts in mutant *C. elegans* strains.

Two transcripts of *lin-61* exist in *C. elegans.* R06C7.7a.1 is "Isoform A" and R06C7.7b.1 is "Isoform B". Knockdown of *lin-61* using an Ahringer RNAi clone silences both transcripts. To determine the specific role of *lin-61* isoform b which contains an additional exon at the N terminus, restriction enzyme cloning is required to create a plasmid that selectively silences isoform b.

**Restriction Enzyme Cloning:** The restriction endonuclease *Pst1* acts as molecular scissors to cut both the pL4440 vector and exon 1 of *lin-61* isoform b (amplified via polymerase chain reaction) to create "sticky" overhangs in the DNA. The vector and insert were ligated at a 4:1 insert to vector ratio and verified by Sanger sequencing. The plasmid was then transformed into the HT115 RNAi-competent bacterial strain via heat shock. This RNAi clone was used to achieve specific knockdown of *lin-61* isoform b.

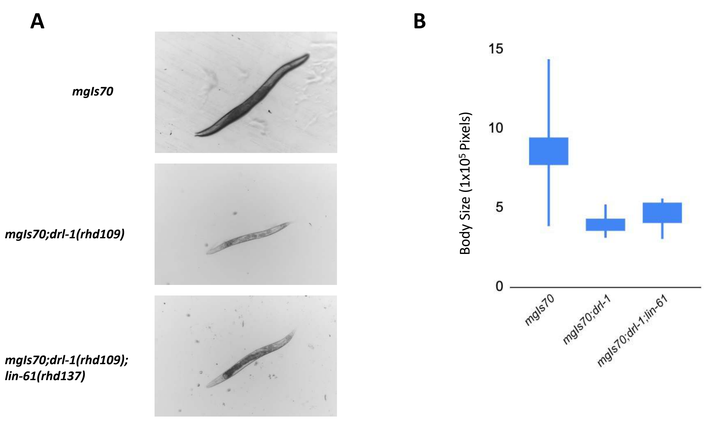
## Results

### Figure 1



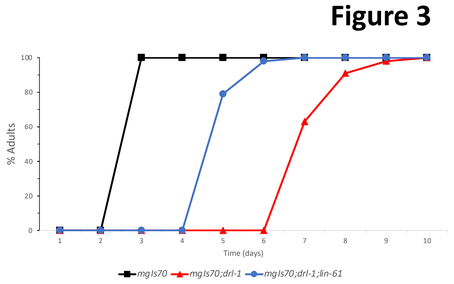

RNAi knockdown of ***lin-61*** enhances *vit* gene expression in ***Pvit-3*::GFP;*drl-1(rhd109)*** mutants (Fig. 1A). Two transcripts of *lin-61* exist in *C. elegans*, one of which contains an additional exon at the N terminus. ***lin-61* isoform b** alone is not necessary for suppression of *drl-1* (Fig. 1b).

### Figure 2




Images of adult *C. elegans* strains (Fig. 1a) suggest ***lin-61(rhd137)* has a modest effect on body size** and lipid content of *drl-1(rhd109)* mutants. *C. elegans* strains were grown on OP50 seeded NGM plates, selected at the L4 stage, and imaged 24 hours later. Loss of *lin-61* mildly suppresses the body size defects in *drl-1(rhd109)* mutants (Fig. 1b). The *P* value obtained from t-test of *mgIs70:drl-1(rhd109)* and *mgIs70;rhd(109);lin-61(rhd137)* body size assay is <.01 which signifies that the difference in body size is statistically significant.

### Figure 3



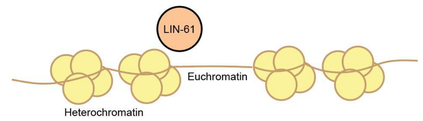

Growth rate assay suggests ***lin-61* is a suppressor of growth rate defects** observed in *drl-1* mutants. Embryos from each *C. elegans* strain were picked to OP50 seeded NGM plates and observed each day. Animals not carrying embryos were separated from the population and the remaining gravid animals were recorded as having reached adulthood.

## Conclusions

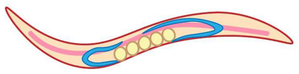
- *lin-61* mutations suppress vitellogenin gene expression defects in dietary restricted (*drl-1*) *C. elegans* mutants
- *lin-61* isoform b alone is not necessary for vitellogenin gene suppression in *drl-1* mutants
- The *mgIs70;drl-1(rhd109);lin-61(rhd137)* triple mutant animal modestly suppresses body size defects of *drl-1* mutants
- The *lin-61* mutation suppresses growth rates of *drl-1* mutants but does not revert growth rate to wild-type

## Future Directions

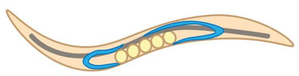
- Perform chromatin immunoprecipitation sequencing in *drl-1* mutants to determine the genomic loci LIN-61 acts on to regulate gene expression



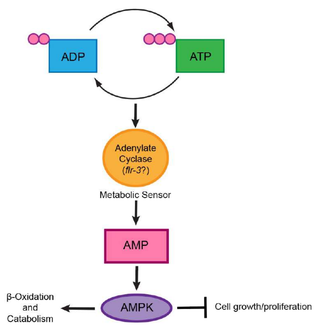

- Generate *rhdsi42;drl-1(rhd109)* and *rhdsi42;drl-1(rhd109);lin-61(rhd137)* mutants to confirm vitellogenin suppression with a P*vit-3::mCherry* reporter which is a more accurate reflection of endogenous gene expression



- Knockdown *lin-61* only in the intestinal cells of dietary restricted mutants by RNAi to confirm the intestine as the location in which LIN-61 functions



- We found that an uncharacterized mutant *flr-3* genocopies *drl-1;* explore the role of the now sequenced *flr-3* mutant in dietary restriction and lipid homeostasis via RNAi knockdown of *flr-3* candidates and CRISPR genome editing




**FLR-3 Candidates**

*chrI 9534665 splice region variant & intron variant C41G7.7
*chrIV 6801329 splice region variant & intron variant C17H12.4
*chrV 461774 nonsynonymous variant nhr-203 p.Trp409* (strong mutation)
*chrV 3085199 frameshift variant srt-71 p.Ile364fs (pseudogene)
*chrX 3572496 nonsynonymous variant vit-3 p.ValAlaLeuAlaIleAla10GluAspProArgAspTrp
*chrX 5330686 nonsynonymous variant C26B9.5 p.Pro198Leu (enriched in intestine; serine protease 16)
*chrX 10671788 nonsynonymous variant F13E6.2 p.Trp221*
*chrX 13408086 nonsynonymous variant cyp-13B1 p.Phe273Leu
*chrX 14083598 splice donor variant & intron variant C33G3.6

## References

[1] Chamoli, M., Singh, A., Malik, Y., & Mukhopadhyay, A. (2014, August). A novel kinase regulates dietary restriction-mediated longevity in Caenorhabditis elegans. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4326946/?tool=pmcentrez
[2] Katsura, I., Kondo, K., Amano, T., Ishihara, T., & Kawakami, M. (1994, January). Isolation, characterization and epistasis of fluoride-resistant mutants of Caenorhabditis elegans. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1205766/
[3] Kimble, J., & Sharrock, W. (1983, March). Tissue-specific synthesis of yolk proteins in Caenorhabditis elegans. Retrieved from https://pubmed.ncbi.nlm.nih.gov/6825952/
[4] McMurchy, A., Stempor, P., Gaarenstroom, T., Wysolmerski, B., Dong, Y., Aussianikava, D., . . . Huang, N. (2017, March 15). A team of heterochromatin factors collaborates with small RNA pathways to combat repetitive elements and germline stress. Retrieved from https://pubmed.ncbi.nlm.nih.gov/28294943/
[5] Morrison, D. (2012, November 1). MAP kinase pathways. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3536342/
[6] Park, A., & Dowen, R. (2020). *MEKK-3 regulates growth and lipid homeostasis in C. elegans.*
[7] Perez, M., & Lehner, B. (2019, August 21). Vitellogenins - Yolk Gene Function and Regulation in *Caenorhabditis elegans*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6736625/
[8] Telfer, W. (2009). Vitellogenesis. Retrieved from https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/vitellogenesis

## Acknowledgements

I would like to thank Dr. Robert Dowen for his guidance and mentorship of this project. Special thanks also to Natalie Cohen and Aaron Park for their assistance with laboratory techniques.