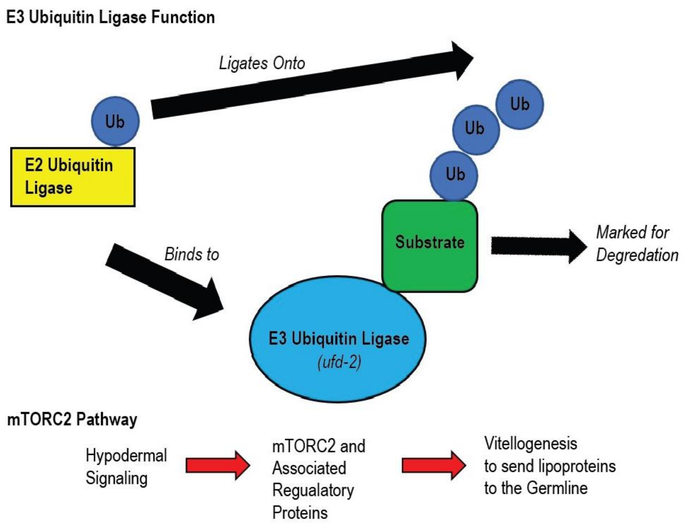
Potential *ufd-2* control of vitellogenesis in *C. elegans*

Mercy L. Neal[1,2] and Robert H. 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

Background

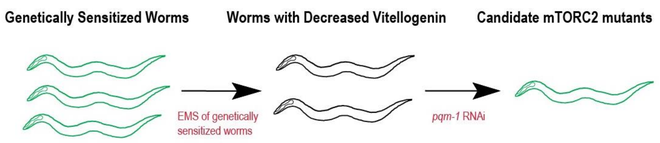


- The mTORC2 pathway in *C. elegans* is a highly conserved metabolic pathway present in many organisms, including humans in the homologous MTOR pathway which in part controls the process of vitellogenesis.
- The allotment of lipoproteins to this process has impacts on many cellular and developmental processes, and the degree of vitellogenesis occurring in a worm can affect numerous factors such as body size, lifespan and reproduction efficacy. (Saxton & Sabatini, 2017).
- *ufd-2*, shorthand for Ubiquitin Fusion Degradation protein 2, is a ubiquitin ligase that appears to act in the MTOR pathway. In *C. elegans*, ubiquitination proteins can serve several functions, the most common of which is ligation. (Kipreos, 2005)
- Other unpublished work in our laboratory, particularly around FBOX proteins suggests that ubiquitination plays a significant role in signaling in the mTORC2 pathway, and so elucidation of *ufd-2* function in the pathway would be a significant step towards understanding the overall mTORC2 signaling pathway and would provide significant insight into the human *ufd-2* homolog, UBE4B's, function.
- The mutation of hedgehog signaling proteins *grd-3* and *grd-4* upstream of the mTORC2 pathway has been proven to reduce vitellogenesis and enhance the effect of other mutations resulting in loss of vitellogenesis in the mTORC2 pathway. (Dowen, unpublished)

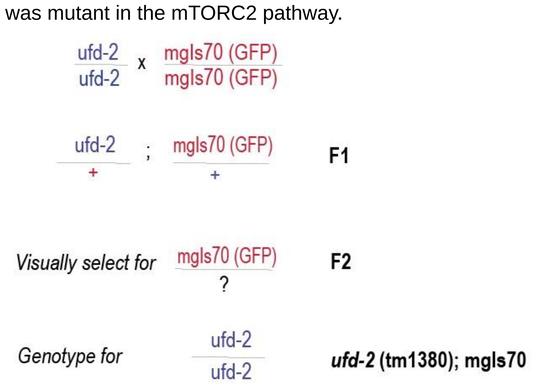
Objectives

- To isolate the *ufd-2* mutation in an *mgIs70[Pvit-3::GFP]* vitellogenesis promoted fluorescent reporter background.
- To characterize the effect of *ufd-2* mutations on mTORC2 dependent growth and ageing pathways.
- To characterize the genetic interaction between hedgehog signaling mutants and the *ufd-2* mutation
- Determine where the UFD-2 protein functions within the mTORC2 pathway in *C. elegans* nematodes.

Methods



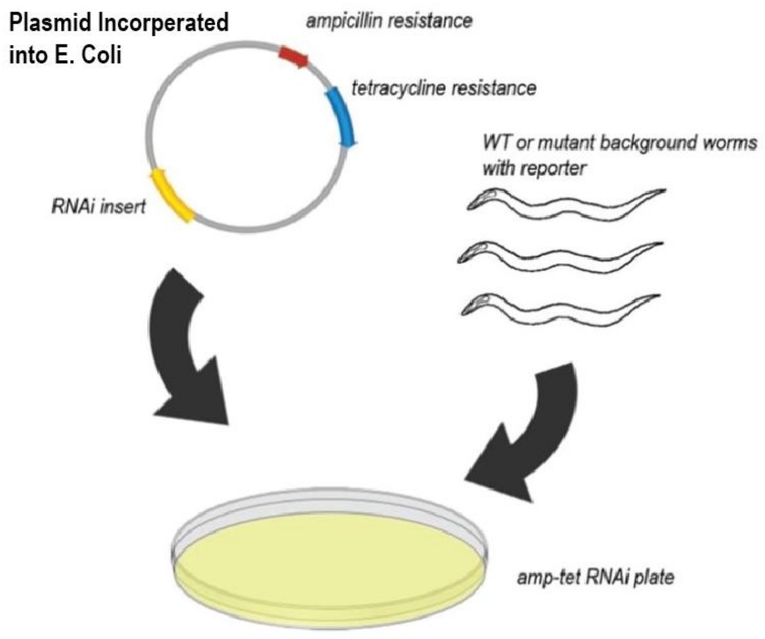
A. Screening for Candidates. Pqm-1 RNAi was used to identify candidates with loss of vitellogenesis phenotypes that likely resulted from a mutation in the mTORC2 pathway. Recovery of vitellogenesis after pqm-1 treatment was used as an indicator that a worm was mutant in the mTORC2 pathway.



B. Crossing the *ufd-2(tm1380)* mutation into the *mgIs70[Pvit-3::GFP]* fluorescent reporter background. The mutation ordered was a 900 bp deletion from the center of the *ufd-2* locus. F2s were isolated that were both homozygous and heterozygous for GFP expression, which is dominant, and then plates were selected that were uniformly homozygous.

Methods

C. RNAi was used to determine UFD-2 knockdown phenotype. ***ufd-2*** RNAi was used to knock down levels of the UFD protein in order to disrupt the pathway as an alternative genetic approach to assess function. The results used to guide the isolation of genetic mutants, which is a significantly lengthier process. After the RNAi was introduced into the worms, the worms were fluorescently imaged in order to determine their phenotype. The plasmid insert was transformed into *E. Coli* bacteria and allowed to incubate to allow for duplication of the plasmid. The bacteria were then fed to the worms to incorporate the RNAi plasmid through the gut.



Results

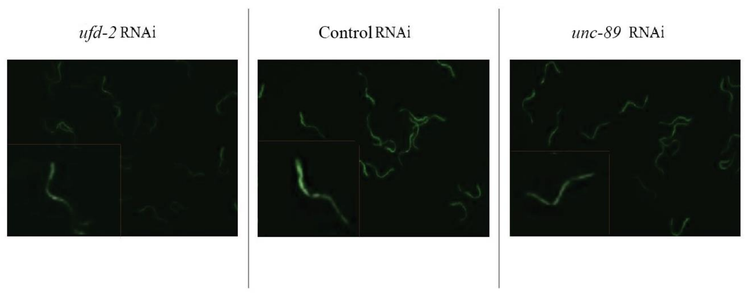


Figure 1. *ufd-2* RNAi produces substantially reduced vitellogenesis. The comparison of several candidates isolated after the EMS mutagenesis and pqm-1 RNAi screen, compared in a mgIs70; grd-3; grd-4 background by RNAi. The loss of vitellogenesis phenotype can be observed most dramatically in the *ufd-2* genotype. L4440 can be considered wild type expression.

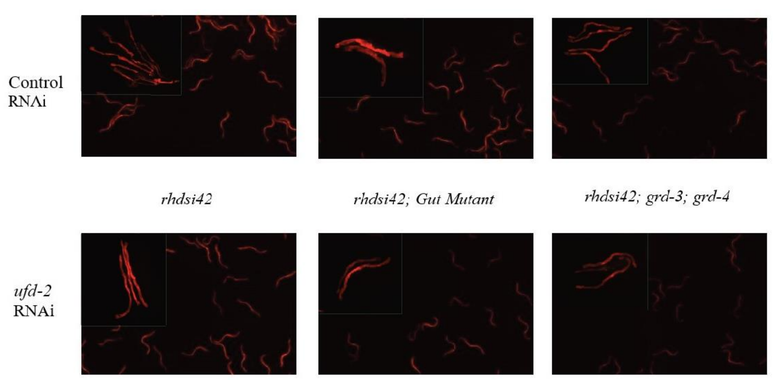


Figure 2. *ufd-2* RNAi produces a more dramatic loss of vitellogenesis in mutant backgrounds. Comparison of control RNAi worms against *ufd-2* RNAi worms in the *rhdSi42[Pvit-3::mCherry]* single-copy reporter background, which produces increased sensitivity over the multi-copy *mgIs70[Pvit-3::GFP]* reporter. This RNAi experiment was carried out in wild type worms (left), intestine-specific RNAi worms (middle), genetically sensitized (*grd-3; grd-4*) mutant worms (right). A significant loss of vitellogenesis reporter signal is observed in both the gut-specific RNAi and the *grd-3; grd-4* mutant background.

Results

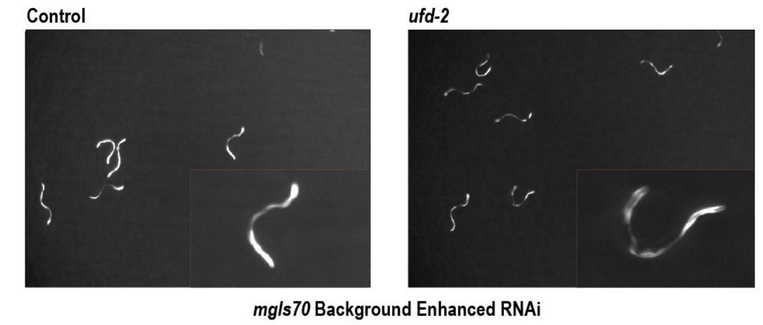


Figure 3. Enhanced *ufd-2* RNAi produces a strong vitellogenesis phenotype even in a wild-type background. An enhanced RNAi experiment with *mgIs70[Pvit-3::GFP]* and control and *ufd-2* RNAi results in a significant phenotypic difference between the two strains. This occurs even though there's no background sensitization mutation (*e.g.*, *grd-3; grd-4*).

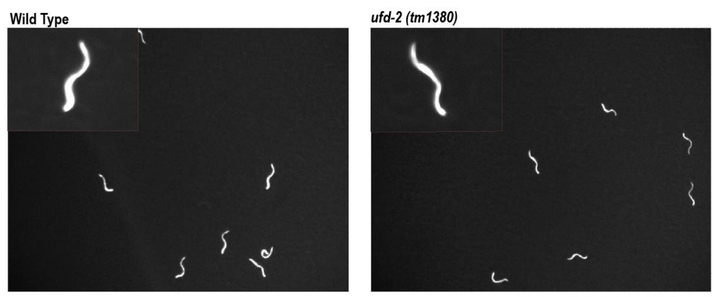


Figure 4. *ufd-2(tm1380)* mutant worms show a modest reduction in vitellogenesis. The introduction of the *ufd-2 (tm1380)* mutation into an *mgIs70 Pvit-3::GFP]* reporter background shows a modest reduction in vitellogenesis between wild type worms and mutant worms.

Conclusions

- Conducting RNAi experiments to reduce levels of UFD-2 protein and mimic loss of function mutation of *ufd-2 (tm1380)* consistently produces a loss of vitellogenesis phenotype, particularly in a genetically sensitized or gut specific background.
- RNAi analysis of *ufd-2* knockdown in wild-type worms produces a modest vitellogenesis reporter defect, which is enhanced in as sensitized background.
- Similarly, a *ufd-2(tm1380)* genetic mutant reduces *mgIs70* reporter expression.

Future Directions

- Further phenotypic analysis of the *ufd-2(tm1380)* mutant, considering the effects of the mutation in the mTORC2 pathway.
- Introduction of the *ufd-2(tm1380)* mutation into a sensitized *grd-3; grd-4* background in order to determine *ufd-2* mutations enhance weak Hedgehog mutations.
- Determine whether a strong *ufd-2* mutation is sufficient to produce a vitellogenesis reporter phenotype using the *rhdSi42* background
- Determining if the *ufd-2* mutation genetically interacts with other key hedgehog pathway genes.
- Understanding the mechanism of UFD-2 within the mTORC2 pathway and determining which of the components of that pathway are ubiquitinated by the action of UFD-2.

References

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