**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Other unpublished work in our laboratory, particularly around FBOX proteins suggests that ubiquitination plays a significant role in signaling in the mTORC2 pathway, and so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, UBE2B’s function.

The mutation of hedgehog signaling proteins significantly affects numerous factors such as body size, lifespan and reproduction efficacy. As a result of this, studying the function of ufd-2 in this pathway could provide significant insight into the human MTOR 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, UBE2B’s function.

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**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**

RNAi analysis of the vitellogenesis reporter. This RNAi experiment produced substantially reduced vitellogenesis. Other unpublished work in our laboratory, particularly around FBOX proteins suggests that ubiquitination plays a significant role in signaling in the mTORC2 pathway, and so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, UBE2B’s function.

**Conclusions**

- Conducting RNAi experiments to reduce levels of UFD-2 protein and mimick 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) mutant genetic mutant reduces mgs70 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 rhdr42 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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