Characterizing a Novel *sgk-1* Gain-of-Function Mutation in *C. elegans*

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Abstract

Regulation of cellular metabolism and lipid transport in *Caenorhabditis elegans* is necessary for growth, reproduction, and survival and is mediated in part by the conserved complex Rictor/mTORC2 via downstream phosphorylation of SGK-1 and Akt. Our lab previously identified a novel gain-of-function mutation in *sgk-1* that restores growth and vitellogenesis in a *rict-1* loss-of-function background. This mutation was found to demonstrate little difference in phenotype when expressed singly or in combination with a previously characterized *sgk-1* gain-of-function mutation. The novel mutation was also expressed in combination with a *pdk-1* gain-of-function mutant and in an F-box overexpression strain to further characterize its phenotypic effects. I found that expressing a gain-of-function mutation in *pdk-1* can further restore body size, but not vitellogenesis, of *sgk-1* mutants in a *rict-1* loss-of-function background. Furthermore, I found that the novel *sgk-1* mutation does not restore vitellogenesis in an F-box overexpression strain. These results suggest the need to determine if the *pdk-1* mutation acts through Akt signaling to restore body size and to identify the mechanism by which F-box suppresses vitellogenesis and growth phenotypes in *C. elegans*.

Background

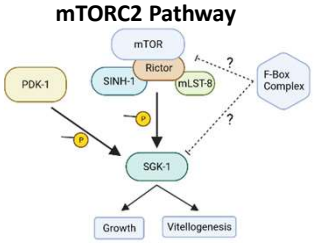
Questions:

- **How do modulators of the mTORC2 signaling pathway function to regulate organismal growth and lipid transport in C. elegans?**
  - **What effects do activating mutations have on defects in mTORC2?**

Why *C. elegans?*

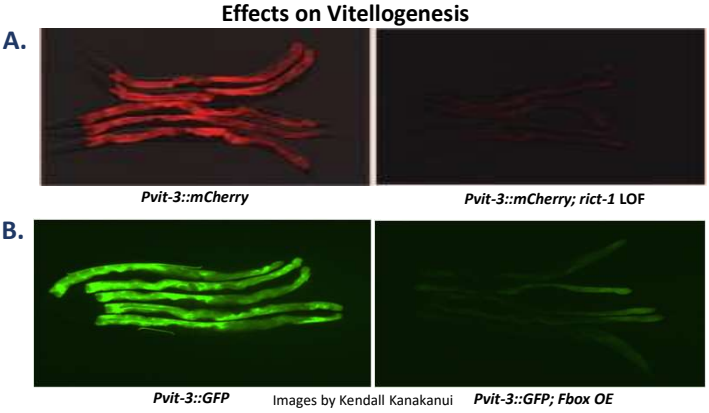
- Transparency of organism
- Fluorescent reporters for lipid transport
- Self-reproducing

mTORC2 Pathway



Regulation of cellular metabolism and lipid transport is critical for organisms to perform numerous biological processes including growth, reproduction, and survival.[1]

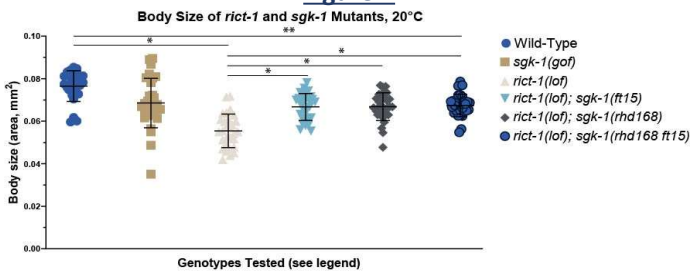
Effects on Vitellogenesis



A. Loss of function of the Rictor gene results in developmental delay and reduced vitellogenesis.[2] **B.** Our lab previously identified a complex involving an uncharacterized protein with an F-box domain (F-box) that negatively regulates mTORC2 activity (Kendall Kanakanui).

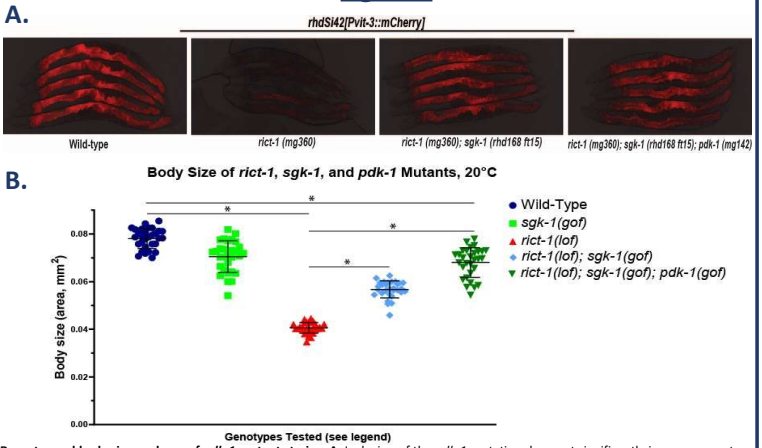
Results

Figure 1



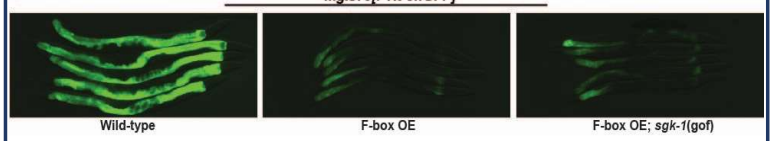
Body size analysis of *sgk-1* mutants in a *rict-1* loss-of-function background. Both *sgk-1* mutations partially restore wild-type body size to a similar degree when expressed separately or in concert. P-values were generated by one-way ANOVA with a Bonferroni's multiple comparison test ([*] P < 0.0001; [**] P = 0.0001).

Figure 2



Reporter and body size analyses of *pdk-1* mutant strains. A. Inclusion of the *pdk-1* mutation does not significantly increase reporter expression. **B.** Addition of the *pdk-1* gain-of-function mutation fails to completely restore body size, but increases it relative to the *sgk-1* mutation alone in the *rict-1* background. P-values were generated by one-way ANOVA with a Bonferroni's multiple comparison test ([*] P < 0.0001).

Figure 3



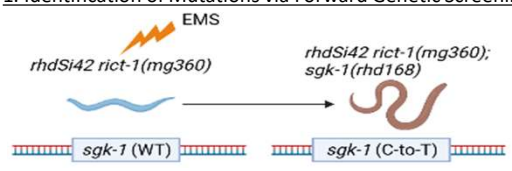
Reporter Analysis of F-box Mutants. Introduction of gain-of-function mutations in *sgk-1* fail to restore wild-type vitellogenin reporter expression in an F-box overexpression background.

Objectives

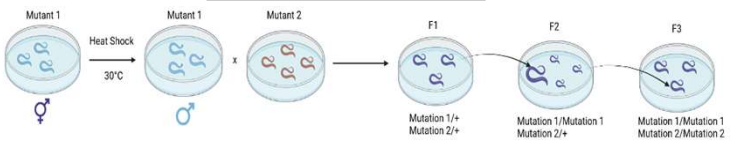
- Determine how novel (*rhd168*) and existing (*ft15) sgk-1* gain-of-function mutations genetically interact with a *rict-1* loss-of-function mutant through analysis of vitellogenin reporter expression and body size
- Determine whether a *pdk-1* gain-of-function mutation (*mg142*) further enhances *sgk-1* gain-of-function activity
- Assess the genetic relationship between F-box and *sgk-1* using gain-of-function alleles

Methods

1. Identification of Mutations via Forward Genetic Screening



2. Generation of Mutant Strains



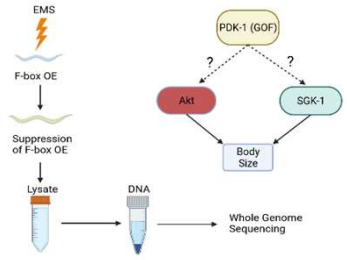
- *rict-1(mg360)*: loss of function
- *sgk-1(ft15)*: Glu-to-Lys amino acid substitution
- *sgk-1(rhd168)*: Leu-to-Phe amino acid substitution
- *pdk-1(mg142)*: Ala-to-Val amino acid substitution
- F-box: Overexpression of transgene in intestine

Conclusions

- There is no significant difference in body size between novel and previously characterized *sgk-1* gain-of-function mutations
- A gain-of-function mutation in *pdk-1* can further restore body size, but not vitellogenesis phenotype, of *sgk-1* mutants in a *rict-1* loss-of-function background
- *sgk-1* gain-of-function mutations do not suppress the F-box overexpression phenotype

Future Directions

- Perform a lifespan assay on existing strains to further characterize the effects of *sgk-1* and *pdk-1* on *rict-1* loss of function
- Quantify reporter expression to confirm effects of *sgk-1* and *pdk-1* on vitellogenesis
- Specify the downstream effects of *pdk-1(mg142)* on body size by crossing Akt mutants into existing strains
- Perform whole-genome sequencing to identify mutations suppressing F-box overexpression



References

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Acknowledgements

Thanks to Dr. Rob Dowen for sponsoring and supporting me during this project and to Kendall Kanakanui for mentoring me and taking the time to make sure I understood our research as well as everybody in the Dowen Lab for their continued assistance!