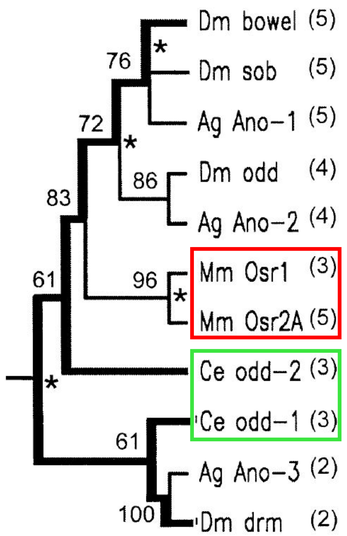
Investigating the effects of RNAi-mediated knockdown of *fos-1* on the expression of the transcription factor *odd-skipped 2* in *C. elegans*

Levi G. Duhaime, Lillian B. McCormick, Jonathan P. Rappi, Jr. and Amy C. Groth, Ph.D.
Department of Biology, Eastern Connecticut State University, Willimantic, CT

Introduction

- *Odd-skipped* genes are transcription factors that play critical roles in embryonic patterning and tissue morphogenesis [1,2]
- Mammalian homologs are associated with developmental defects, and diseases of the kidneys, heart and lungs[3,4,5]
- *Odd-1* and *odd-2* are expressed in the intestine of *C. elegans*[1]; *Odd-2* is also expressed in the rectal gland cells.[6]



Taxonomy of odd homologs in *D. melanogaster, A. gambiae, C. elegans* and *M. musculus*. Numbers in parentheses refer to the number of zinc finger motifs. (Buckley *et al.,* 2004)

Dm - Drosophila melanogaster
Ag - Anopheles gambiae
Ce - Caenorhabditis elegans
Mm - Mus musculus

Objectives

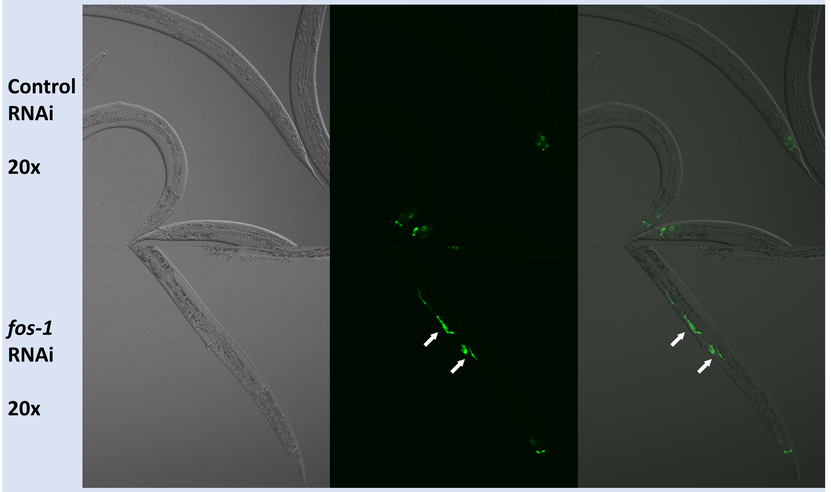
- Investigate the effects of *fos-1* on tissue-specific ODD-2 expression
- Obtain evidence for/against localization of ODD-2 to the hermaphrodite specific motor neurons (HSN) following *fos-1* RNAi

Methods

- Knocked-down *fos-1* in *odd-2::GFP* worms (JR2005) by RNAi
- Visualized ODD-2 expression by confocal fluorescence microscopy
- Generated *odd-2::GFP;rab-3::RFP* reporter strain (AG22)
- Knocked-down *fos-1* in *odd-2::GFP;rab-3::RFP* worms (AG22) by RNAi
- Visualized ODD-2/RAB-3 expression by confocal fluorescence microscopy
- Compared ODD-2/RAB-3 expression patterns for evidence of colocalization

Results

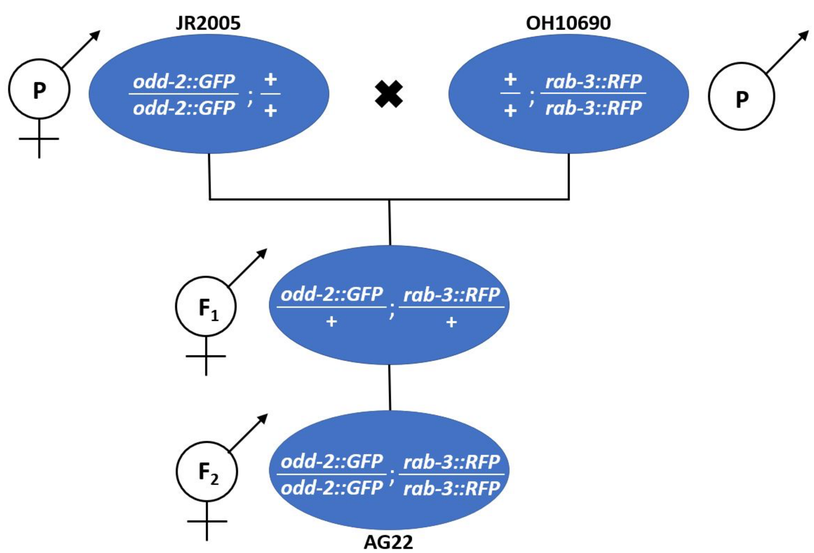
JR2005 - ODD-2::GFP Expression Following RNAi



Max intensity GFP Z-stack projections and a representative brightfield slice

- Control RNAi: L4440 plasmid vector
- *Fos-1* knockdown resulted in ectopic expression of ODD-2 in the vicinity of the germline
- White arrows indicate areas of observed ectopic expression

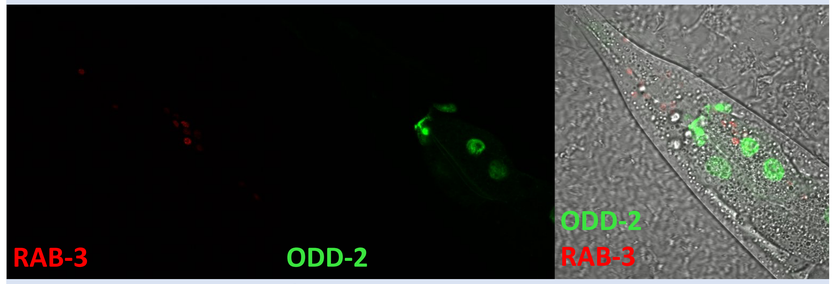
Creation of *odd-2/rab-3* Dual Reporter Strain



- JR2005: *odd-2::GFP* reporter strain (Rothman lab)
- OH10690: *rab-3::RFP* reporter strain[7]
- Males generated by heat shock-induced nondisjunction

Results (cont'd)

AG22 - ODD-2::GFP/RAB-3 Expression



Max intensity RFP/GFP Z-stack projections and composite image

Conclusions

- Inconsistent expression of *odd-2::GFP* and *rab-3::RFP* in AG22
- Unsuccessful in reproducing germline-adjacent ectopic expression of ODD-2 following *fos-1* RNAi in AG22
- Further evidence of ODD-2 expression in the rectal gland cells

Future Directions

- Vary RNAi feeding methodology in order to reproduce ectopic expression in double-reporter strain
- Alter RNAi induction temperature (*i.e.* feeding concentration)
- Create new *odd-2* fluorescent reporter strain(s) via CRISPR with greater expression consistency

References

1. Buckley, M., Chau, J., Hoppe, P., & Coulter, D. (2004). *Development Genes and Evolution,* 214(1), 10–18.
2. Katoh, M. (2002). *International Journal of Molecular Medicine*, 10(2), 221–5.
3. Stricker, S., Brieske, N., Haupt, J., & Mundlos, S. (2006). *Gene Expression Patterns,* 6(8), 826–34.
4. Tena, J., Neto, A., Calle-Mustienes, E., Bras-Pereira, C., Casares, F., & Gómez-Skarmeta, J. (2007). *Developmental Biology*, 301(2), 518–531.
5. Wang, Q., Lan, Y., Cho, E., Maltby, K., & Jiang, R. (2005). *Developmental Biology*, 288(2), 582–594.
6. Groth, unpublished data
7. Stefanakis N., Carrera I., & Hobert O. (2015). *Neuron*, 87(4), 733–50.

Acknowledgements

ECSU Department of Biology
CSU-AAUP Faculty Research Grant
The Rothman Lab, for strains

Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Program (P40 OD010440)