High Throughput Identification of Developmental Regulators in Early C. elegans Embryos

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INTRODUCTION

The objective of this project was to create and image strains of c. elegans with markers on previously unexplored early developmental genes. The genes of interest included the tbx-31, tbx-32, tbx-33, and tbx-39 tbox genes, as well as the ceh-32 and ceh-76 homeotic genes. Using state of the art imaging software, the expression of these genes was tracked in individual cells of developing embryos from the 2 cell stage to the larval stage of young C. elegans.

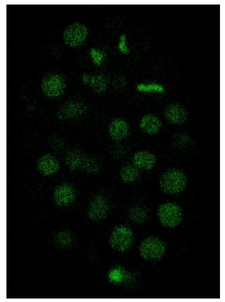
METHODS

The new strains were created by crossing existing strains with the requisite RFP marker on the gene of interest into a strain with GFP histone lineaging markers in order to track cell expression in developing embryos. Using ACETREE imaging software, the expression of RFP tagged genes was tracked over time. This was used to develop an ancestral tree of cell lineages, highlighting the individual cells in which the genes of interest were expressed.

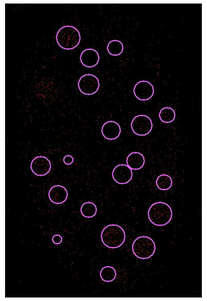
RESULTS

Viable strains were created for the tbx-31, tbx-32, tbx-39, and ceh-32 genes through multiple rounds of backcrossing the RFP-tagged genes of interest into a GFP lineage reporter strain.

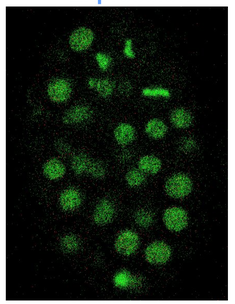
GFP lineage reporter



RFP tbx-39 reporter

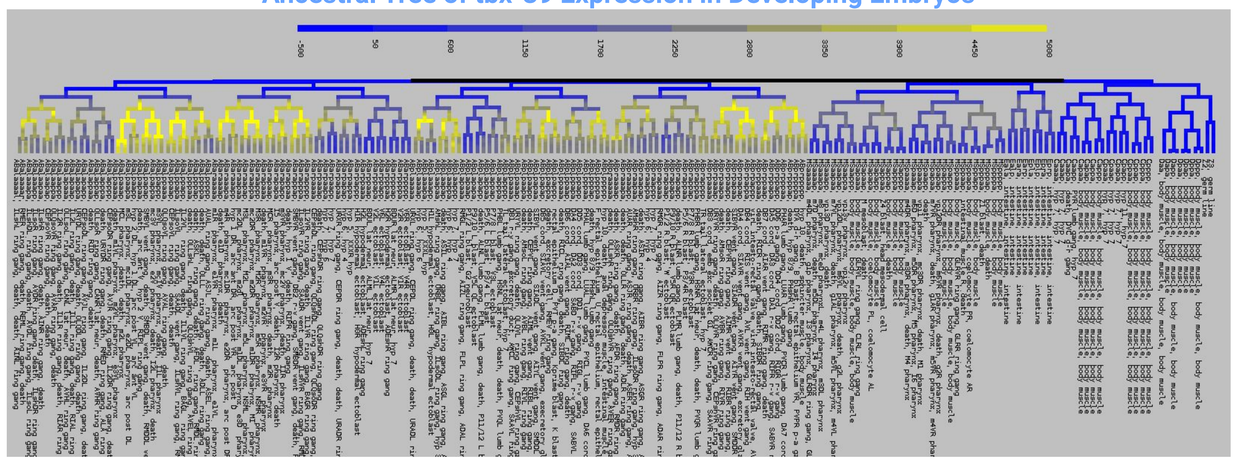


Combined Expression



Pictured above is the expression data for a tbx-39 embryo. The green lineage marker tracks all cell positions over time. The red marker shows where tbx-39 was expressed in the developing embryo.

Ancestral Tree of tbx-39 Expression in Developing Embryos



The ancestral tree below shows the results of the tbx-39 imaging. Cells highlighted in yellow showed tbx-39 expression. The tbx-39 gene appears to be expressed exclusively in the AB cell lineage. It appears that while tbx-39 is not tissue specific, the plurality of cells in which it is expressed become pharyngeal cells.

CONCLUSION

The development of these new worm strains for the imaging of early developmental regulators elucidates the temporal and spatial expression of several early tbox and homeotic genes. Specifically, the data gathered on tbx-39 opens the door for further research into its interactions with other genes, in the hopes of understanding new signaling pathways in development.

FUTURE RESEARCH

The other newly developed strains with RFP tagged tbox and homeotic genes will need to be imaged and an ancestral trees of gene expression will be determined. Additionally, mutants for these genes are in the process of being developed through CRISPR knockouts, which, combined with ACETREE, will help determine the function of these early developmental regulators.

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