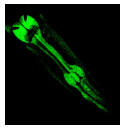

Global Gene Expression Analysis in the Absence of a Non-Receptor Tyrosine Kinase During Post-Embryonic Development of the Nematode *C. elegans*.

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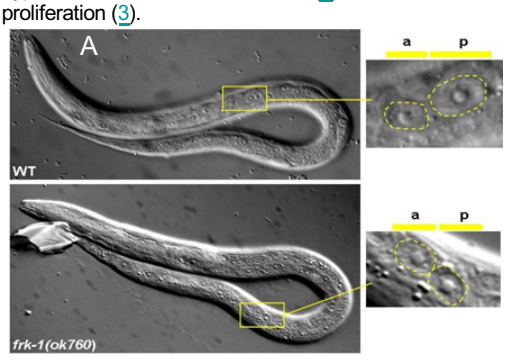
Abstract

FRK-1 is a non-receptor tyrosine kinase that regulates cell proliferation and differentiation during embryonic development of the nematode *Caenorhabditis elegans* (1). During the embryogenesis, the movement of hypodermal cells (skin cells) allows for enclosure of the embryo. Fer-related kinase -1 (FRK-1) limits asymmetric Wnt signaling dependent for specification of a subset of hypodermal cells called seam cells (2) and for endoderm proliferation (3).



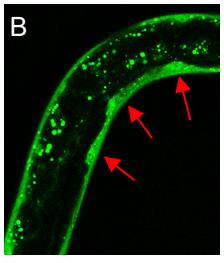


Figure 1. A. *frk-1(ok760)* mutants arrest at the L1 stage with an apparent loss of asymmetric division of the seam cell lineage (shown here by DIC). **B.** *frk-1::GFP* fusion (via CRISPR) shows *frk-1* promoter activity and localization in hypodermal cells (shown) as well as muscle types: pharyngeal, vulval, and anal depressor (but not body wall).

RNA Seq: Isolation and Amplification of Transcripts

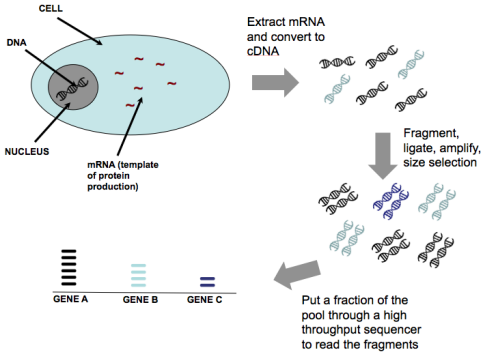


Figure 2. RNA Seq Process and Analysis. Worms were synchronized developmentally, and *frk-1(ok760)* mutants isolated from wildtype larvae. Total RNA was isolated and prepared for high throughput sequencing (whole transcriptome shotgun sequencing (WTSS) to quantify gene expression levels. Transcript abundance and differential expression was further analyzed through the DNA Subway and refined further using Bioconductor algorithms.

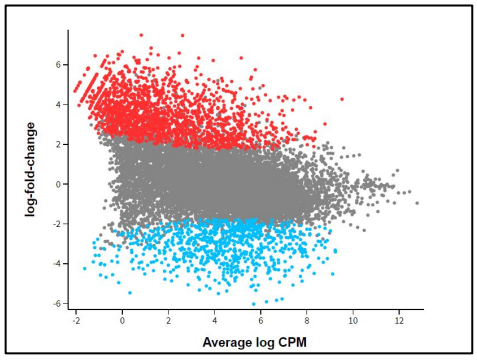


Figure 3. Gene Expression Threshold Analysis. Glimma plot showing up-regulated (red) versus down-regulated (blue) genes. Grey dots represent genes with no significant change. A total of over 2000 genes were differentially regulated either up or down greater than 2-fold.

Sample intersection plot showing most differentially regulated genes with no *frk-1*

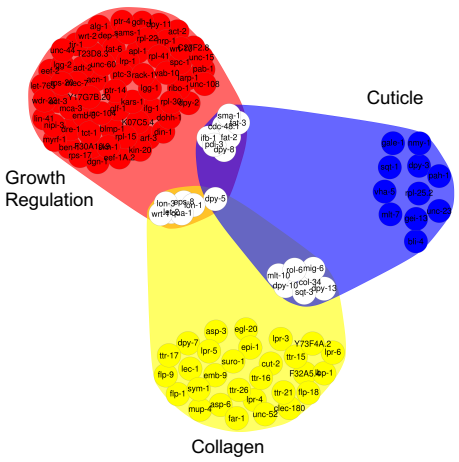


Figure 4. Major gene clusters related to larval progression. Three clusters from the list of seven (in the abstract) were analyzed and overlap graphs were formed in order to aid in the discovery of interacting genes. (Red: Regulation of growth, Blue: Cuticle development, Yellow: Collagen)

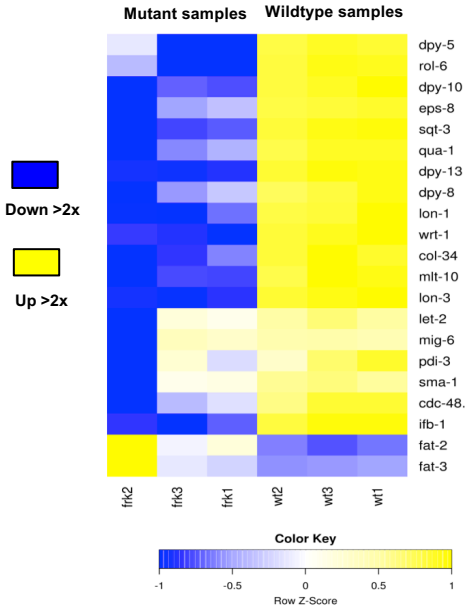


Figure 5. Heat map of differentially expressed genes chosen from the intersection region of the FEA analysis in Figure 4. Genes identified were collagen metabolism pathways and other cuticle components, which were down regulated in the mutant (blue) compared to wildtype (yellow).

In vivo validation of RNA Seq Analysis

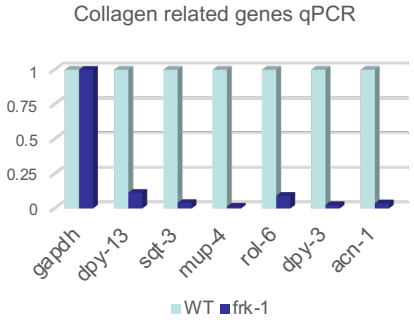
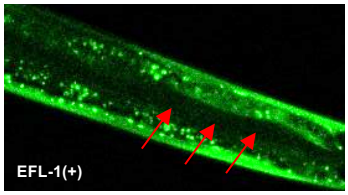
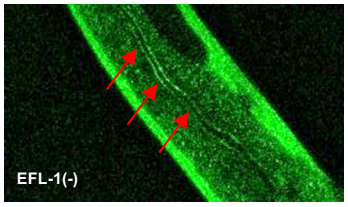


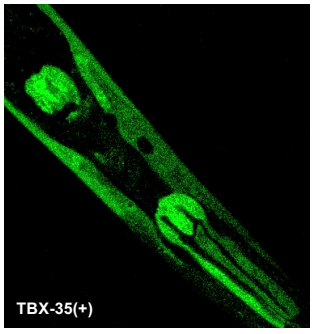
Figure 6. Quantitative PCR validation of collagen metabolism genes. A subset of genes were selected to validate on separately isolated mRNA from *frk-1(ok760)* mutant and wildtype larvae to verify in vivo what was determined via RNA Seq analysis.

Discovery of *frk-1* regulation from RNA Seq Analysis



FRK-1 localizes to the lumenal surface of intestinal cells in the absence of EFL-1.





Loss of *frk-1* expression in the absence of TBX-35.

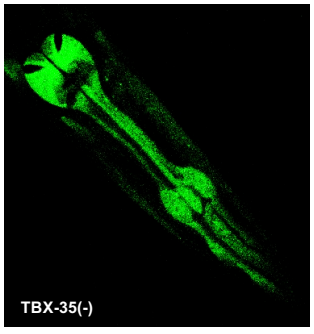


Figure 7. *frk-1* expression and localization is regulated by transcription factor activity in a tissue specific manner.

Conclusions and Continuing Work

- From our RNA Seq analysis we conclude that major pathways affecting larval progression are affected, especially those related to collagen metabolism and cuticle formation.
- We are now pursuing RNAi and tissue expression characterization of genes associated with these subsets that have not been reported in the literature beyond association with collagen and cuticle pathways during development.
- Furthermore, we are investigating whether the genes responsible for affecting larval progression are involved in asymmetric cell division.

Acknowledgements

- The RNA Seq raw data sets are archived publicly through NCBI Bioproject, ID # PRJNA391722.
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