Peroxisomal Gene Knockdown Influences Fertility in Caenorhabditis elegans

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Abstract

Fertility is a complex biological process that relies on tissue-specific communication between germline and somatic tissues. Gene regulation can be used to manipulate gene expression, allowing identification of key reproductive contributors. The nematode roundworm *Caenorhabditis elegans* serves as a unique model organism due to its highly conserved human orthologs and genetic manipulability. We previously identified peroxisomal genes *prx-5* and *prx19* as possible regulatory genes of fertility using RNA-seq. However, it is unknown whether these genes causally regulate fertility and in which tissues they may act. This study investigates the tissue-specific roles of the peroxisomal genes *prx-5* and *prx-19* using whole-organism and tissue-specific RNA interference (RNAi). Early brood production is used as a proxy for fertility. Whole-organism RNAi knockdown of both genes resulted in an overall decrease in early brood production in comparison to the control. However, when RNAi was conducted at the germline and epidermal level, brood production (fertility) significantly decreased, in comparison to intestinal levels. Our findings suggest that intestinal tissue may serve as a regulatory tissue capable of activating compensatory fertility mechanisms in response to peroxisomal dysfunction or metabolic stress. Ongoing work is examining whether this intestinal phenotype is sex-specific and aims to identify the molecular pathways underlying this response. Collectively, these results highlight a previously underappreciated role for peroxisomal genes in reproductive regulation and emphasize the importance of tissue-specific signaling in fertility.

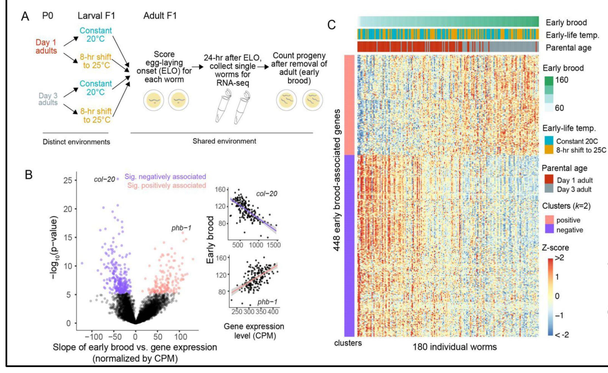


Figure 1: Heatmap of genes associated with positive and negative brood production characteristics (Webster et al. 2025)

Background

Peroxisomes are specialized organelles involved in lipid metabolism, cellular detoxification, and intracellular import. They play a critical role in maintaining homeostasis and oxidative reactions. Dysfunctional peroxisomes often lead to detrimental outcomes, including organ dysfunction, neurodegeneration, and multiple neonatal developmental defects. However, the effects of peroxisomes on fertility continue to remain poorly understood. *Caenorhabditis elegans* serves as an excellent model organism for studying peroxisomes and epigenetic effects due to its conserved orthologs and well-established fertility assays. This research aims to investigate how various peroxisomal genes (*prx-5* and *prx-19*) affect fertility, and which molecular pathways mediate these reproductive outcomes.

Methods

Early Brood Assay:

- EV (empty vector Escherichia coli), *prx-5, prx-19* bacterial gene vectors were obtained
- RNAi bacteria prepared in LB + carbenicillin and incubated 12+ hours at 37 °C.
- RNAi plates seeded and dried; six plates per gene per cycle.
- Five N2 L4 C. elegans placed on each plate for 16 hours at 20 °C.
- Each worm singled onto new RNAi plates for 24 hours.
- Worms removed; plates left 72 hours at 20 °C for progeny development.
- Progeny counted; plates discarded.
- Data analyzed using R Studio.

Tissue Specific RNAi Assay:

- WM45 control and tissue-specific strains: WM45 (RNAi deficient), DCL569 (germline), IG1839 (intestine), IG1846 (epidermis) were used instead of N2, with the same early brood assay procedure.

Results

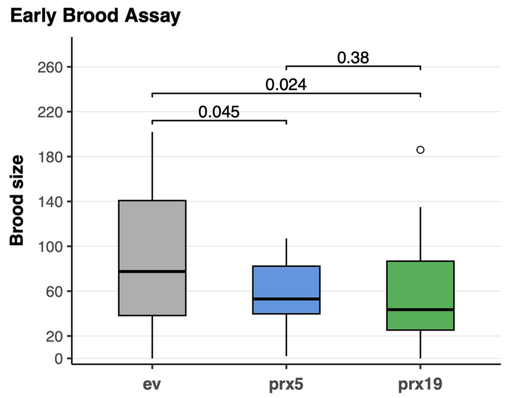
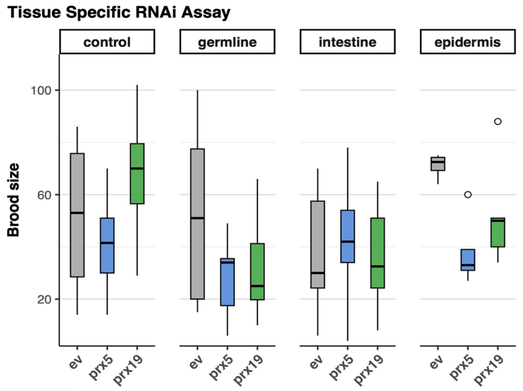


Figure 2: Early Brood Assay. Brood size is shown for *C. elegans* exposed to RNAi knockdown with ev, *prx-5*, or *prx-19* conditions.

Figure 3: Tissue-Specific RNAi Assay. Brood size is shown in response to a tissue-specific RNAi experiment. Within this experiment, a WM45 strain was used as a control, DCL569 for the germline, IG1839 for the intestine, and IG1846 for the epidermis



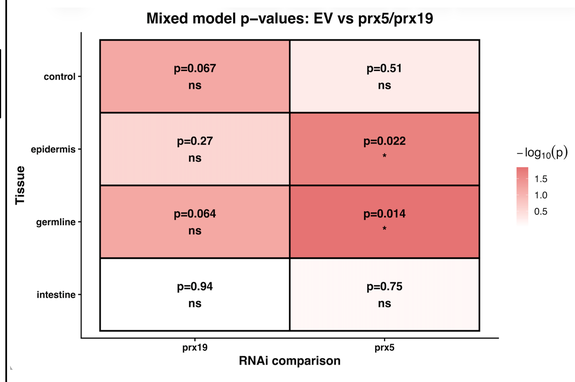


Figure 4: Tissue-specific RNAi p-value heatmap. p-value statistical test comparisons were performed using ev, *prx-5*, and *prx-19* conditions.

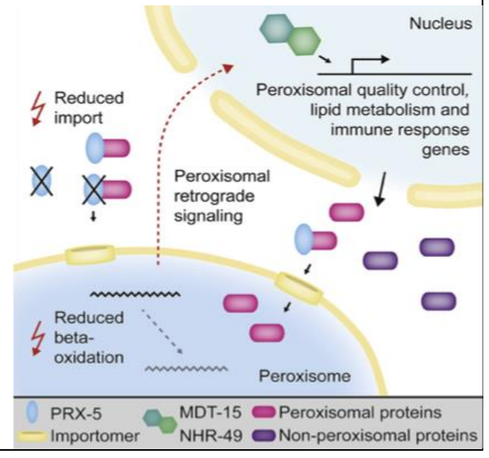


Figure 5: Diagram depicting peroxisomal retrograde signaling (Rackles et al., 2021)

Discussion

Knockdown of *prx-5* and *prx-19* through RNAi resulted in significantly reduced early brood production (Figure 2), affirming that peroxisomal genes play an important role in fertility and progeny production in *C. elegans*. One possible explanation for this reduction is the cumulative stress model, which proposes that defective peroxisomal genes lead to increased levels of reactive oxygen species (ROS) and cellular stress (Wei et al., 2025). Elevated ROS may disrupt cellular homeostasis and reproductive processes, providing a potential explanation for the reduced progeny observed in the early brood assay. While previous studies have linked peroxisomal dysfunction to oxidative stress and hypothesized reproductive defects, the tissue-specific assays revealed a more nuanced pattern. Specifically, the reproductive effects of *prx-5* knockdown varied depending on the tissue in which gene function was disrupted (Figure 3). Knockdown in the germline and epidermis resulted in significantly reduced brood sizes, which is to be expected, whereas intestinal knockdown produced brood sizes relatively similar to the control condition (Figure 3; statistical comparisons shown in Figure 4). In contrast, prx-19 knockdown did not produce statistically significant differences across tissues in this analysis. Together, these findings demonstrate that the reproductive consequences of peroxisomal gene disruption are not uniform across tissues. The preservation of brood production following intestinal knockdown suggests that intestinal tissue may possess adaptive or compensatory responses that buffer the reproductive effects of peroxisomal dysfunction, highlighting a potential tissue-specific role for the intestine in maintaining reproductive output in *C. elegans*.

Implications

Epigenetic work can provide great insight into *C. elegans* reproductive biology. Through RNAi gene knockout and tissue-specific RNAi, this study provided preliminary results that show intestine-specific peroxisomal gene knockout can increase brood production. These findings may help guide future research towards intestinal tissues, as the intestine may serve as a central regulatory system for fertility. This research also highlights both *prx-5* and *prx-19* as key peroxisomal genes of interest. Future work is needed to understand and solidify the specific mechanism through which this phenomenon functions. Nonetheless, these preliminary findings serve as a strong and promising foundation for subsequent work exploring the connection between peroxisomal dysfunction and reproduction in *C. elegans*.

Resources

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