Mitochondrial membrane translocases are required for progeny production in *C. elegans*

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Abstract

Genetically identical *Caenorhabditis elegans* roundworms naturally differ in the number of progeny that they produce, and these differences are correlated with gene expression differences in conserved genes involved in protein transport through the inner and outer mitochondrial membranes. This experiment strives to test whether two of these genes, *tomm-20* and *timm-17B.1,* causally affect brood size in *Caenorhabditis elegans*, by knocking them down at the mRNA level using RNA interference (RNAi). These genes have human orthologs, allowing my results to be relevant to human conditions. RNAi knockdown of the target genes as well as positive and negative controls was performed on groups of *C. elegans*. Then progeny numbers were counted, totaled, and compared. My results have indicated a relationship between brood size and the knockdown of the genes *tomm-20* and *timm-17B.1,*with both genes exhibiting reduced brood size compared to a control. The effect of *tomm-20* knockdown was particularly strong, indicating that the inhibition of the outer mitochondrial membrane translocase has drastic effects on the ability of *C. elegans* to produce a normal brood size. Knockdown of *eef-1A.2* (a known positive control gene) exhibited lower numbers of progeny compared to both *tomm-20* and *timm-17B.1,* suggesting that progeny production is not inhibited to the same degree in these translocase-related genes. Because both *timm-17B.1* and *tomm-20* reduced brood size it can be ascertained that translocase of the outer and inner membrane of the mitochondria plays a role in maximizing normal gamete production and fertilization.

Figure 1: Heatmap of brood associated genes displaying positive and negative association (Webster *et al.* 2025)

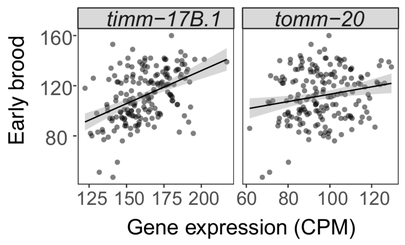

Figure 2: Positive association of target genes *timm-17B.1* and *tomm-20* early brood and gene expression (Webster *et al.* 2025)

Background

C. elegans is a nematode worm that is often used in genetics research. It is small (1mm), has a short life span (1 month) and has large brood size (about 250), making it a perfect model organism. With 40% of its genes having human orthologs the applications of research in this organism is highly significant in genetics research (Kim et al, 2018). *tomm-20* is a gene *in C. elegans* that encodes an important complex in the translocase of the outer mitochondrial membrane and is an ortholog of human TOMM20. It is integral to homeostasis of the mitochondria allowing for transport between the cytoplasm and the inside of the mitochondria (Ahier et al. 2018). *timm-17B.1* is a gene that specifically codes for a messaging receptor in the translocase of the inner membrane and is an ortholog of human TIMM17A. This allows materials to be signaled from the intermembrane space into the matrix of the mitochondria. This is integral for the use of the electron transport chain as well as other processes of maintaining homeostasis such as the UPRmt in the mitochondria (Shpilka et al. 2020). While there is research on the effects of knockdown of *tomm-20* and *timm-17B.1* genes on longevity and mitochondrial function there is little to no research on reproduction. To this end I strive to ascertain the effects of RNAi knockdown of the genes *tomm-20* and *timm-17.B* on brood size of *C. elegans*. I hypothesized that knocking down the genes *tomm-20* and *timm-17.B* would significantly lower brood size because of the important role that those genes play in stable mitochondria function.

Results

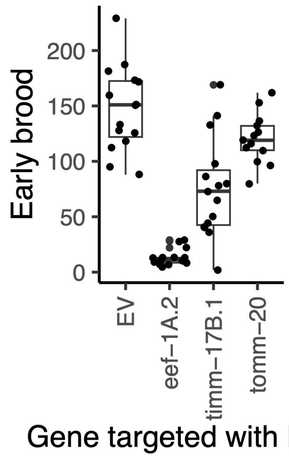

Figure 3: RNAi knockdown of *tomm-20* and *timm-17B.1* reduces early brood relative to empty vector (EV).

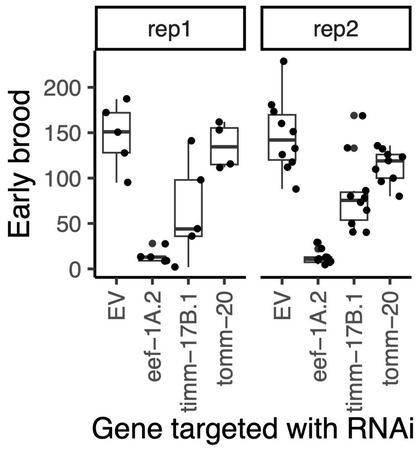

Figure 4: Robust effects of both genes of interest are apparent across two biological replicates.

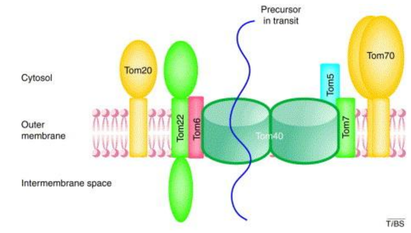

Figure 5: Visualization of the translocase of the outer mitochondrial membrane (Rapaport 2002)

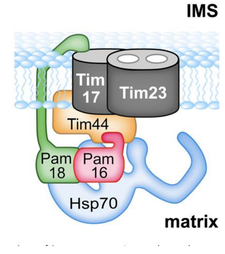

Figure 6: Diagram of the translocase of the inner mitochondrial membrane (Shpilka et al 2021)

Methods

To conduct the experiment, I began by labeling a plastic test tube for each RNAi that would be used, and I filled that tube with 5 mL of LB, then adding carbenicillin to kill any possible other contaminants. Finally, I used a pipet tip to select a bacterial colony from the specific LB plate and ejected it into each test tube. Those test tubes I then placed in the shaker at 37 degrees Celsius and 180rpm and left overnight. I checked if the tubes were cloudy and labeled RNAi plates that were seeded with 2-3 drops of bacteria then left overnight on the lab benchtop. Then I transferred five L4 larvae from stock plates to one of each type of the RNAi plates. 16 hours later I singled the worms each to their own corresponding RNAi plate. Exactly 24 hours later I removed the parent worm leaving any embryos. Two to three days after I counted the larvae on each plate using a grid and microscope as well as a clicker counter. Then I used R coding to analyze and present these brood sizes collected from each RNAi gene in comparison to EV and a known gene (*eef-1A.2*) in box plots.

Discussion

When looking at our collected data comparing the empty vector to our collected results for *tomm-20* and *timm-17B.1* we see that the RNAi knockdown was highly effective at decreasing brood size in (Fig.3). Comparatively to EV there is a significantly large decrease in the amount of progeny seen in these two genes knock-down, indicating the importance of these translocases (Fig.3). The signaling pathways of transductions, controlled by these translocases, of the mitochondrial membrane will have a direct effect on mitochondrial production and thereby an indirect effect on brood size. This could be a result of increased mitochondrial stress and decreased overall energy production preventing normal brood sizes. Further, when comparing *tomm-20* and *timm-17B.1* we can see that the *timm-17B.1* knocked-down worms experienced lower average brood sizes than those of *tomm-20* in both replicates (Fig. 4). This was unexpected as the translocation of both the outer and inner membrane are needed to maintain homeostasis of the mitochondria. This suggests that the signaling pathway for the translocase on the inner membrane coded for my timm-17B.1 more greatly disrupts the production of energy and thereby the size of the brood the worm can produce.

Resources

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