Investigating Genetic Mechanisms that Regulate Germ Cell Fate in *C. Elegans*

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Infertility affects one in seven couples in the United States (Seli, 2011). One cause of infertility may be the improper differentiation of germ cells into gametes (sperm or egg). We use the *C. elegans* germ line as a model to study germ cell proliferation and differentiation into sperm and egg. Similar to mammalian spermatogenesis, the *C. elegans* germ line has a population of germ cells that will continue to divide until genetic signals trigger differentiation into gametes. Two genes that have been shown to work together to regulate germ cell fates, are *glp-1* and *fbf-2*. GLP-1 acts to promote the proliferation of germline stem cells, and FBF-2, an mRNA-binding protein, promotes the differentiation of germline stem cells into gametes (sperm and eggs). Previous research has shown *glp-1*; *fbf-2* double mutants exhibit a more severe phenotype than the phenotype of the *glp*-1 mutant alone (Lamont et al., 2004), indicating that the two genes are functioning in the same pathway. Despite this, the mechanistic link between the two genes is unknown. Our research is specifically focused on identifying and characterizing the link between *glp-1* and *fbf-2*.

Based on previous genetic and biochemical experiments, two candidate genes, *lst-1* and *sygl-1*, were identified as the potential genetic link between *glp-1* and *fbf-2*. *lst-1* and *sygl-1* have been shown to function redundantly meaning that loss of either gene (*lst-1* or *sygl-1*) alone will not result in a visible phenotype. Loss of both genes (*lst-1* and *sygl-1*) will result in a *C. elegans* worm that lacks germ cells (Kershner et al., 2014). Given the redundant nature of *lst-1* and *sygl-1*, we began our investigation with *lst-1* by examining the structural elements required for interaction between *lst-1* and *fbf-2.* To do this, we altered specific amino acids in the LST-1 protein and used a yeast-2 hybrid assay to determine the effect of the mutated proteins on the interaction between LST-1 and FBF-2. Through this assay, we determined that changing the leucine (L) at the 153rd residue on LST-1 (L153) to an alanine (A) prevents the interaction between FBF-2 and LST-1. We refer to this mutation as L153A. We then wanted to test the importance of L153 during germ line development in *C. elegans*.

Using the genome-editing technique, CRISPR/Cas9, we introduced the L153A mutation into a "normal" *C. elegans* worm. As expected, due to the redundancy between *lst-1* and *sygl-1*, L153A mutant worms did not exhibit a visible phenotype. Next, we used RNAi to create a double *lst-1(L153A); sygl-1* mutant to address the redundancy between the two genes.
Surprisingly, these *lst-1(L153A); sygl-1* mutants did not display the same lack of germ cell phenotype was previously observed when both *lst-1* and *sygl-1* gene function was eliminated. However, further characterization using a DNA stain (DAPI) and microscopy techniques revealed that the number of proliferating (non-differentiated) cells in the *lst-1(L153A)* mutant

was significantly reduced compared to "normal" worms. Additionally, introduction of the *syg-1* mutation in the *lst-1(L153A)* mutant resulted in an even more dramatic reduction of proliferating cells.

Our data show that Leucine 153 in the LST-1 protein is important for the binding of LST-1 to FBF-2. Furthermore, this interaction is important for germ cell proliferation. While, the L153A mutation results in a reduction of the number of proliferating germ cells, it does not result in the same sterile phenotype that occurs when all LST-1 activity is lost. Due to this, we can conclude that there is another structural component of LST-1 that is required for its function. Future experiments will be targeted at identifying these other components required for LST-1 and FBF-2 binding as it is required for germ cell proliferation and differentiation. These studies will not only further our understanding of germ cell proliferation and differentiation in C. elegans but also further our understanding of two genes, *glp-1* and *fbf-2* which are conserved throughout the animal kingdom. Therefore, our studies here in *C. elegans* will better inform human germ cell studies as they pertain to human fertility.

References:

Kershner, A. M., Shin, H., Hansen, T. J., & Kimble, J. (2014). Discovery of two GLP-1/Notch target genes that account for the role of GLP-1/Notch signaling in stem cell maintenance. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(10), 3739–3744. https://doi.org/10.1073/pnas.1401861111

Lamont, L. B., Crittenden, S. L., Bernstein, D., Wickens, M., & Kimble, J. (2004). FBF-1 and FBF-2 Regulate the Size of the Mitotic Region in the C. elegans Germline. *Developmental Cell*, *7*(5), 697–707. https://doi.org/10.1016/j.devcel.2004.09.013

Seli E. 2011. Infertility. Hoboken, UNKNOWN: Wiley]. http://ebookcentral.proquest.com/lib/ithaca-ebooks/detail.action?docID=661742.

Shin, H., Haupt, K. A., Kershner, A. M., Kroll-Conner, P., Wickens, M., & Kimble, J. (2017). SYGL-1 and LST-1 link niche signaling to PUF RNA repression for stem cell maintenance in Caenorhabditis elegans. *PLoS Genetics*, *13*(12), e1007121. https://doi.org/10.1371/journal.pgen.1007121

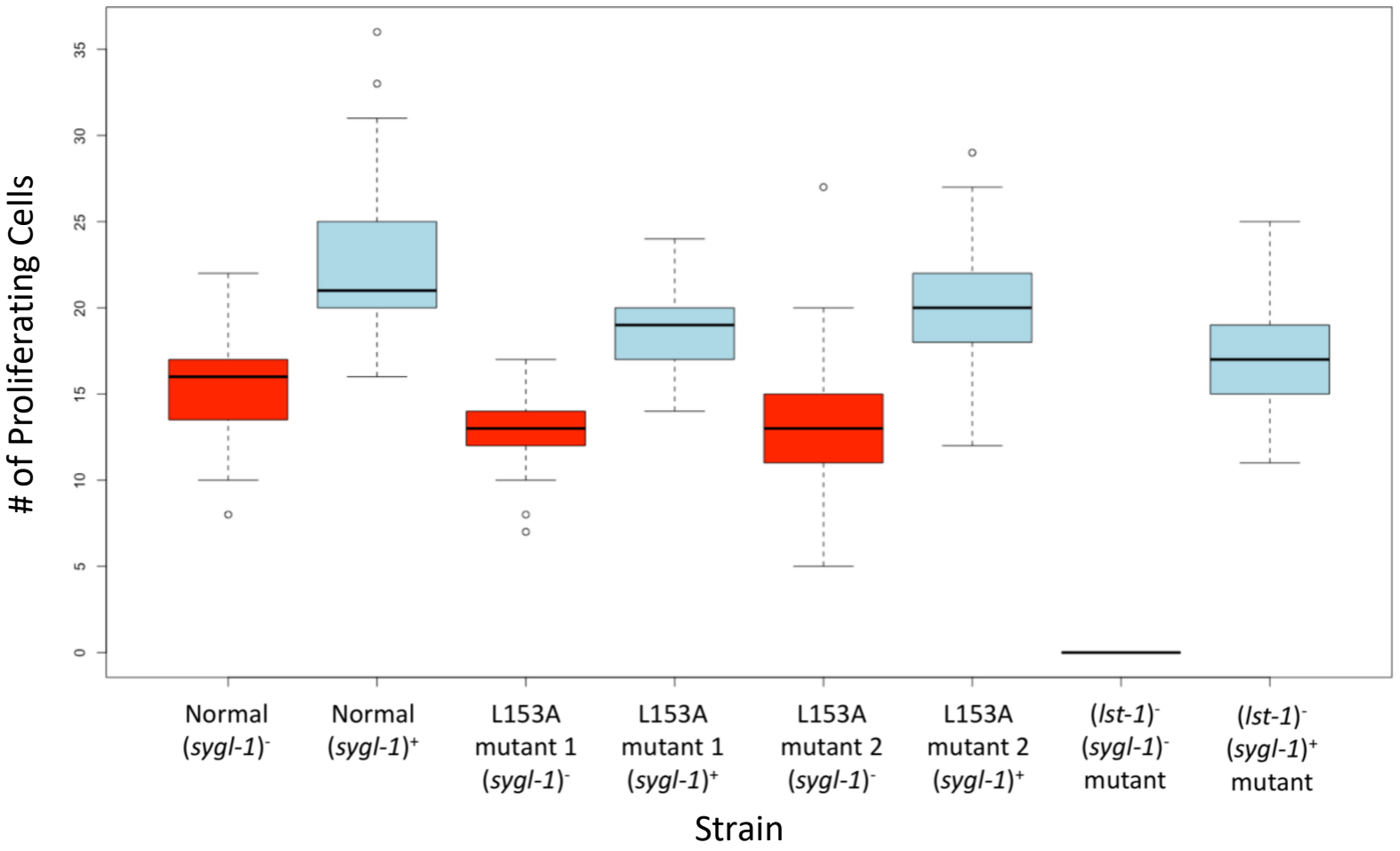

Figure 1: The *lst-1* L153A mutation decreases the number of proliferating, cells in *C. elegans* germ line. When *sygl-1* is functional (blue boxes), *lst-1* L153A mutants have fewer mitotic cells than normal worms, but do not display a total lack of germ cells like the *lst-1*;*sygl-1* double mutants. When *sygl-1* is silenced (red boxes), *lst-1* L153A; *sygl-1* double mutants possess fewer proliferating cells than any worms with functional *sygl-1*, but still do not display the *lst-1* loss of function phenotype.