COLLABORATION BETWEEN A CONSERVED RNA-BINDING PROTEIN AND A SPECIFIC PROTEIN PARTNER RESTRICTS STEM CELL DIFFERENTIATION IN THE GERMLINE OF *C. ELEGANS*

by

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I dedicate this thesis to my parents. For sharing and supporting my dreams all along and also for choosing to give me the best education possible.

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by

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The University of Texas at Dallas, 2018

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An important question in stem cell biology is understanding the molecular factors that maintain stemness. The cells that remain in a proliferative state at the distal tip produce daughters that differentiate into mature gametes as they progress proximally. The stem cell niche is controlled in part by the notch signaling pathway, but the molecular details are opaque. Here we address the role of a recently discovered gene *lst-1* and its interaction with FBF-2. FBF maintains the stem cell pool in *C. elegans,* but we don't understand the mechanisms involved. LST-1 and FBF are downstream targets of Notch signaling. We studied the physical interaction between LST-1 and FBF to identify the amino acid residues that are important for interaction. Leucine at 153 in LST-1 and Tyrosine at 479 in FBF-2 are required for the interaction to take place. The site of interaction on FBF is shared between multiple protein partners. To determine if the interaction between FBF and LST-1 is relevant in animals, we applied CRISPR genome editing to incorporate the L153A mutation. Gld-1 is an mRNA target of FBF in the stem cell region. We measured GLD-1 protein abundance in the absence of either *lst-1* or *sygl-1* or both. We see that there is much higher

expression of GLD-1 protein when both *lst-1* and *sygl-1* are absent, suggesting a lack of repression by FBF. Similar results were observed in the case of our LST L153A mutant worm. This makes us believe that LST-1 facilitated repression by FBF. We envisage that this knowledge provides an essential step towards the discovery of repressive mechanisms through which LST-1 engages mRNA destabilization, decay, or localization machinery.

TABLE OF CONTENTS

CHAPTER 1 INTRODUCTION……1

1.1 *C. elegans* as a model organism……...2

1.2 *C. elegans* structure/anatomy……...3

1.3 Life cycle of *C. elegans*……..4

1.4 The germline……6

1.5 Niche for germline stem cells……..9

1.6 The Distal tip cell (DTC)……... 9

1.7 Notch signaling……..10

1.8 RNA binding proteins-PUF proteins involved in germline stem cell maintenance...13

1.9 FBF…… 14

1.10 Importance of post-transcriptional regulation in regulating the switch from proliferation to differentiation……15

CHAPTER 2 MATERIAL AND METHODS……..17

CHAPTER 3 RESULTS……...25

3.1 LST-1 region 125-175 binds FBF-2…….. 25

3.2 Lysine residue at position 153 in LST-1 is necessary for binding to FBF-2……28

3.3 FBF-2 requires Y479 and the loop region to bind LST-1……..30

3.4 LST-1 binds FBF……... 32

3.5 The interaction is specific for FBF-2……33

3.6 Gld-1 is highly expressed in the Distal tip of mutant worms…… 34

CHAPTER 4 DISCUSSION…….36

REFERENCES…….. 39

BIOGRAPHICAL SKETCH……..43

CURRICULUM VITAE

LIST OF FIGURES

Figure 1 Representation of the anatomy of *C. elegans*……. 4

Figure 2 Life cycle of *C. elegans*……. 6

Figure 3 Schematic representation of the germline of *C. elegans*……. 7

Figure 4 Notch signaling pathway in the *C. elegans* germline……12

Figure 5 PUF protein binding RNA…… 13

Figure 6 (A) schematic representation of the yeast two-hybrid assay LST-1 fused to the DNA binding domain (B) Binding data of LST-1 truncations and FBF-2 in a yeast two-hybrid assay. LST-1 truncations were fused to the plasmid containing DNA binding domain while FBF-2 was fused to plasmid containing the activation domain (C) Diagrammatic representation of the LST-1 truncations used in the experiment…….27

Figure 7 LST-1 fused to the activation domain (A) Binding data of LST-1 truncations and FBF-2 in a yeast two-hybrid assay. LST-1 truncations were fused to the plasmid containing Gal4 while FBF-2 was fused to plasmid containing the lexa DNA binding domain (B) binding affinities of LST-1 point mutants between 125-175 with FBF-2…… 29

Figure 8 (A) Binding of FBF-2 mutants to LST-1 in a yeast two-hybrid assay (B) binding affinities of FBF-2 mutants along the loop and LST-1 (C) Binding of FBF-2 variants at position 479 with LST-1…….31

Figure 9 Pull-down assay…… 32

Figure 10 LST-1 fused to DNA binding domain and FBF homologues fused to the activation domain. Graph indicating their interaction. Error bars indicate standard deviation….33

Figure 11 (A-D) GLD-1 protein expression in the distal end of the C. Elegans gonad, (A) wild type (B) lst-1(+) sygl-1(rnai) (C) lst-1(-) sygl-1(rnai) (D) lst-1(L153A) sygl-1(rnai), stained with α-GLD-1 antibody (pink) and DAPI (blue). (E) Intensity of α-GLD-1 expression across 20μm (1-5 GCD) from the DTC…….……. 35

LIST OF TABLES

Table 1 PCR reaction mix……18

Table 2 PCR reaction conditions……19

LIST OF ABBREVIATIONS

RNAi: RNA interference

E. coli: Escherichia coli

C. *elegans*: Caenorhabditis elegans

L1-L4: larval stage 1-4

Z1-Z4: zygote 1-4

PGC: Progenitor germ cell

DTC: Distal tip Cell

GSC: Germline stem cell

GCD: Germ cell diameter

GLP-1: Glucagon like peptide-1

LAG-2: Lin12 and Glp-1

PUF: Pumilio and FBF

RBP: RNA binding protein

TRM: tripartite recognition motif

UTR: Untranslated region

PCR: Polymerase chain reaction

BSA: Bovine Serum Albumin

DMSO: Dimethyl sulfoxide

LST-1: Lateral Signaling Target-1

SYGL-1: Synthetic GLP-1

GLD-1: Female germline-specific tumor suppressor gld-1

SEL-8: Protein lag-3

FBF: Fem-3 mRNA-binding factor

EV: Empty vector

PUM: Pumilio

LB media: Luria-Bertani medium

NGM: Nematode growth media

RT: Room temeperature

IPTG: Isopropyl β-D-1-thiogalactopyranoside

PFA: Paraformaldehyde

PBS: Phosphate-buffered saline

PTW: PBS+ 0.1% tween

DAPI: 4',6-diamidino-2-phenylindole

Y2H: Yeast two hybrid

Y3H: Yeast three hybrid

CPEB: cytoplasmic polyadenylation element binding protein

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

DBD: DNA binding domain

AD: Activation domain

MBP: Maltose binding protein

CPB-1: Cytoplasmic polyadenylation element-binding protein 1

CHAPTER 1

INTRODUCTION

Stem cells have the unique ability to 'self-renew'. They can also differentiate into various other cell types. The process of self-renewal in stem cells increases their number during development, ensures preservation within germline of adult tissues, and reestablishes function in the stem cell pool post injury. Failures in renewal results in developmental defects, premature aging, and cancer (He, Nakada, & Morrison, 2009). Stem cells are a pre-requisite for multicellular organisms. Understanding the nature and regulation of stem cells is key as they play an important role in development. There are two possible mechanisms by which stem cells divide. One, following the asymmetric form of replication where a single stem cell divides into a differentiated daughter cell as well as a mother cell that is identical to the stem cell itself. Or, following the symmetric replication where each stem cell produces either two stem cells or two differentiated daughter cells (Morrison & Kimble, 2006). Symmetric replication of stem cells can be observed in the germline of *C. elegans*. The larval nematodes are hatched from just 2 stem cells in the egg post which eventually increases to more than hundreds of stem cells with equal number of differentiated daughter cells in adults.

Infertility is a problem, this is why understanding the mechanisms of stem cell regulation may improve diagnosis and treatment. The *C. elegans* germline is an ideal model for function given the genetic tractability of the organism, foundation of existing work, and astonishing molecular clarity with which macromolecules can be directly imaged.

1.1 *C. elegans* as a model organism

Caenorhabditis elegans or *C. elegans* for short, is one of the most popular model organisms used in the field of development and stem cell research. Sydney Brenner was the first person to propose the use of *C. elegans* as a model organism in 1965 (S. Brenner, 1973). He was interested in the nervous system and development. *C. elegans* has a very simple nervous system made up of only 302 neurons, and is very amenable to genetic manipulations and behavioral studies. They are anatomically very simple but have sophisticated processes such as RNAi and apoptosis as seen in other organisms (Altun, Z.F. and Hall, D.H, 2018). They have been highly used in the study of stem cells because of the fact that numerous characteristics of the *C. elegans* germline are similar to the stem cell systems of mammalian adults and all cell fates have been meticulously mapped (Hubbard, 2007). *C. elegans* are among the most abundant animals present on earth. They are naturally found living in the soil feeding on bacteria, but they can be grown very easily in labs on agar plates or liquid cultures with bacteria (*E. coli*) to feed on. They are small, microscopic worms that are about 1mm in length, so a single petri dish can hold more than ten thousand. They reproduce very rapidly; a new laid egg takes only 3 days to develop into a fully grown adult worm further capable of laying more than 300 eggs. Mutant forms of *C. elegans*, where certain genes are either edited or absent, can lead to phenotypic changes in cells and tissue and can be used to study functions of the gene anatomically. Another advantage of these worms is that they have a transparent body, so you can visualize everything through the body during course of development under the microscope, *in vivo* (Joshi, Riddle, Djabrayan, & Rothman, 2010).

C. elegans development occurs in a specific pre-determined fashion, where each cell divides and specializes in a particular way that can be tracked back to the embryo, something known as

‘invariant cell lineage’. The simplicity of tissue architecture, ease of identification of stem cell niches, and tools for genetic, cellular and biochemical analysis have made *C. elegans* a powerful model organism for studying stem cell biology and understanding mechanisms of stem cell renewal, sex determination, and cellular reprogramming(Joshi et al., 2010).

1.2 *C. elegans* structure/anatomy

C. elegans have a simple anatomy. They are present in one of two sexes, either hermaphrodites (XX) or males (XO). *C. elegans* are commonly referred to as roundworms because they have an unsegmented, round body shape which tapers towards the ends. A typical nematode body plan, in either sexes, show an outer tube and inner tube, spaced by the pseudocoelom. The outer tube which is the body wall, is made up of the cuticle, excretory system, neurons, hypodermis and muscles, while the inner tube consists of the intestine, pharynx and gonads (in adults). The reproductive system in *C. elegans* is fascinating. A typical hermaphroditic adult worm consists of two U-shaped Gonadal arms that meet at the uterus (fig.1). Whereas a male adult consists of a single U-shaped gonadal arm. The hermaphrodites are self-fertile. The hermaphroditic germline starts off by producing only male gametes, then lays eggs through its uterus post internal fertilization. All the sperm are produced in the L4 stage (150 sperm per gonadal arm), after which only oocytes are produced. These sperm cells, which are initially stored in the same area of the gonad as the oocytes, are then pushed into the spermathecal (a chamber wherein oocytes and sperm fertilize). Essentially, the self-fertile hermaphrodites are just modified females capable of producing sperm for a short period at the beginning of development after which they exclusively produce oocytes. When self-inseminated, the wild-type worm is seen to lay about 300 eggs, whereas when inseminated by a male, the number of eggs laid can exceed 1,000.

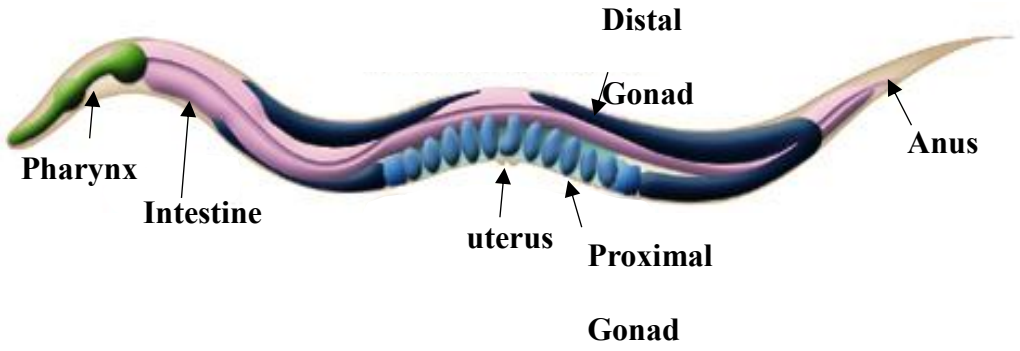


Figure 1: Representation of the anatomy of *C. elegans.*
http://www.wormbook.org

1.3 Life cycle of *C. elegans*

The average duration of reproductive development in *C. elegans* is around 3-5 days under ideal conditions. However, this can vary depending on the conditions/temperature. At 25°C it takes about 2.5 days, at 20°C it takes 3.5 days and at 15°C it takes about 5.5 days to develop into an adult (M. R. Klass, 1977). The life cycle of *C. elegans* can be categorized into three phases; a) embryonic stage, b) 4 larval stages (L1-L4), and c) adulthood (fig.2). Embryogenesis extends from fusion of the egg and sperm to form a zygote, up until hatching. This process takes about 14 hours. After the zygote (P0) is formed, a series of asymmetric divisions take place producing the primordial germ cell, P4. This enters the interior of the embryo and divides symmetrically to produce Z1, Z2, Z3 and Z4. Embryonic development takes place within the gonad, post this Z2 and Z3 divide to give rise to germ cells. Z1 and Z4 develop into all the somatic structures of a mature adult germline, such as distal tip cells (DTCs), sheath cells, spermathecal cells, and the uterus (Sulston, Schierenberg, White, & Thomson, 1983). Thus, analogous to other systems, P4

and its daughter cells Z2 and Z3 are referred to as the primordial germ cells (PGCs) that are the sole progenitors of the germline (Altun, Z. F. & Hall, D. H. 2005; Lints, R. & Hall, D. H. 2005). On hatching, it takes about 9 hours to get to the larval 1 stage, here the gonad is made up of four cells: the two germline precursor cells (Z2 and Z3) and the somatic gonadal cells (Z1 and Z4). The development from egg to L1 takes 9 hours, and L1 to L2 takes 14 hours. After the L2 stage, under conditions that are not very favorable, such as lack of food, change in temperature or overcrowding, these worms arrest and develop into a specialized L3 stage called dauer (live for 2-3 months), and once they are transferred back to favorable conditions they begin development from where they all entered. These Z2-Z3 divisions occur continuously from L1 through adulthood (J. Kimble & Hirsh, 1979).

The gonad elongates and germ cells enter meiosis during L3 and L4 stages. L4 develops into adult, which are then capable of laying eggs themselves. In adults, the germline proceeds from mitosis to meiosis. Thus, as the worms develop from L1 to adult stage, there is movement of germ cells proximally to form sperm, but the distal end remains mitotic. Adults are capable of living for about 2-3 weeks. Interestingly, *C. elegans* also go through a process called molting from L1 to L4 stage, wherein at the end of each larval stage they shed their cuticle and synthesize a new one. This helps them maintain their transparency throughout their life cycle.

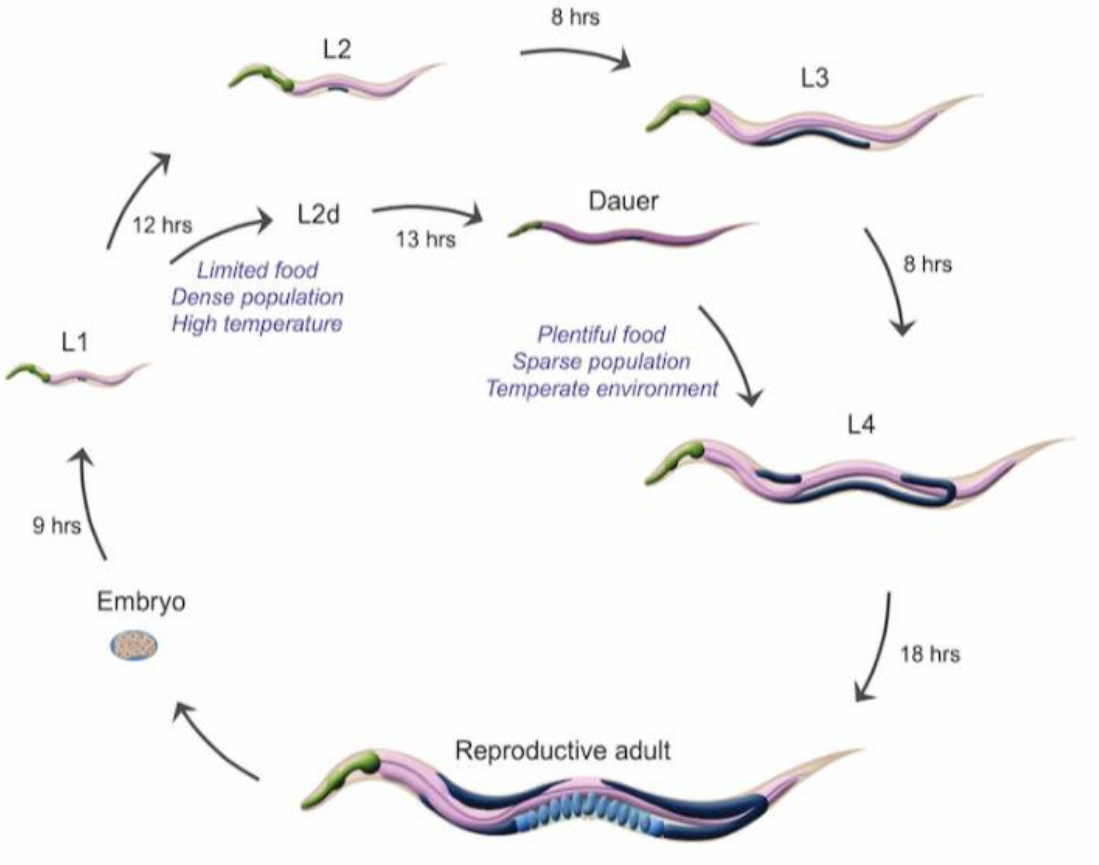


Figure 2: Life cycle of *C. elegans*
http://www.wormbook.org.

1.4 The germline

The germline is a specialized cell lineage that gives rise to both eggs and sperm. It consists of all the stages of germcell development, right from proliferating stem cells to differentiated gametes all at one time, making it a convenient tool to study the genetic and molecular regulation of stem cells. As we have seen in the previous section, the *C. elegans* germline proliferates from one primordial germ cell (PGC) present in the early embryo to over a thousand cells in the adult

(Hubbard, 2007). Germline stem cells are crucial for genome transmission to future generations. Intercellular signaling play a crucial role in the development of the soma as well as the germline in both males and hermaphrodites (Murray, Yang, & Van Doren, 2010). The adult gonad is an assembly line, with germ cells present at the distal end progressing from an undifferentiated stem cell to fully differentiated gametes (egg or sperm) at the proximal end. As new cells are produced in the distal end, they move out of the mitotic region into the meiotic cell cycle region and progress through meiotic phases towards the proximal end to ultimately mature as either sperm or oocytes (fig.3). This linear advancement of gamete formation makes the study of development and stem cell regulation very simple (Hansen & Schedl, 2013). The gonad of an adult male is made up of a single U-shaped arm that produces only male gametes (sperm). Whereas the gonad of an adult hermaphrodite is made up of 2 U-shaped arms that join at the middle of the body to a shared uterus. They produce oocytes and a restricted number of sperm (Hirsh, Oppenheim, & Klass, 1976; M. Klass, Wolf, & Hirsh, 1976). The uterus is connected to the outside through a vulva.

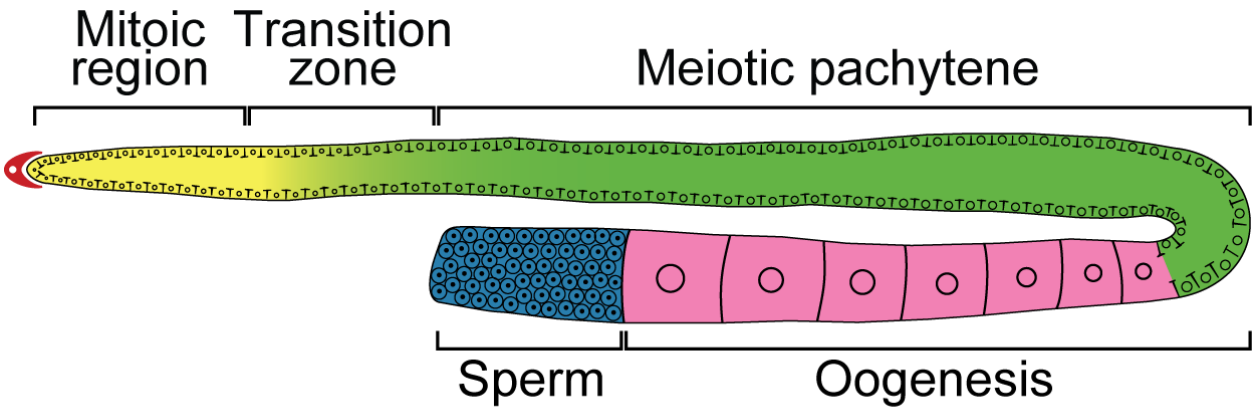


Figure 3: Schematic representation of the germline of C. elegans

Proliferative cells are present at the distal end of the gonadal arm which is also known as the mitotic zone. This zone extends ~20 germ cell diameters. Cells move out of the mitotic zone towards the proximal end and enter meiosis. They pass through a region between the two phases known as the transition zone, where large changes in chromosome morphology occur. The transition zone has been defined as the region from last mitotic zone nucleus to the first meiotic zone nucleus (Crittenden, Troemel, Evans, & Kimble, 1994; Hansen, Wilson-Berry, Dang, & Schedl, 2004). Transition zone cells are very distinctive in that their nuclei are crescent-moon-shaped, as the chromosomes pair in the leptotene/zygotene stages of meiotic prophase (Dernburg et al., 1998). Cells that enter meiotic phase progress from prophase I to pachytene stage. Not all cells enter meiotic prophase at precisely the same location in the gonad arm. Once the cells enter the pachytene region, the chromosomes disperse about the periphery, corresponding to side-by-side aligned homologous chromosomes forming a "bowl of spaghetti" phenotype. This is followed by terminal differentiation into gametes (Arur, 2017; Strome & Updike, 2015).

The *C. elegans* germline contains a fairly large population of stem cells that can give rise to an equal number of daughter cells which eventually will adopt different fates subject to various other factors such as intercellular signaling and the cells position relative to the niche signal. The distance of the stem cells from the DTC, a somatic cell present at the distal end of the gonad, plays an important role in the decision of self-renewal, in the *C. elegans* germline. It is also maintained by Notch signaling, which plays an important role in self-renewal.

1.5 Niche for germline stem cells

The distal tip cell (DTC), provides the niche for the establishment and maintenance of the *C. elegans* germline stem cells (GSCs) (Byrd, Knobel, Affeldt, Crittenden, & Kimble, 2014). Niche, in a biological sense, refers to the immediate surroundings of the cell that is crucial to the survival and regulation of the cell. One of the first stem cell niche to be identified and understood at a cellular and molecular level is that of the *C. elegans* germline. The germline of *C. elegans* has a continuously proliferating pool of cells throughout its life (Hirsh et al., 1976). The niche plays a very important role in this process, which includes the somatic cell (the DTC) and Notch signaling (Morrison & Spradling, 2008). The position of the niche is essential to generate polarity within the maturing germline. This way all the mitotic cells reside at one end and meiotic cells are at the other end of the germline. The Niche is formed when certain signaling molecules interact with one or more cellular signaling pathways in the germ cells. Mechanisms exist for proliferation of cells through the mitotic cycle and also exit of cells through meiotic prophase, but central to understanding germline development is identifying the genes and regulatory elements that help make this decision for transition from proliferative stem cells to differentiated meiotic phase (Hansen & Schedl, 2013). Regulators of stem cell self-renewal have been identified and analyzed in depth. It has been seen that along with the niche, Notch signaling and a group of RNA binding proteins plays a critical role in the stem cell regulation (Liu, Sato, Cerletti, & Wagers, 2010).

1.6 The Distal tip cell (DTC)

A key factor in the maintenance of the proliferative state of germline stem cells (GSCs), is the distal tip cell (DTC) a somatic cell that is present at the very distal end of the germline. They cap

the distal end of the gonad and extends cytoplasmic processes to about 10 germ cell diameters (GCD) from the main body of the cell (J. Kimble, 1981). The "germ cell diameter" is a representation of distance and is measured by the space occupied by cells along the gonadal periphery. This measure of distance has been used as a standard in *C. elegans* to measure distance. The DTC controls germline proliferation and cytoplasmic processes to about ten Germ cell diameters from the main body throughout the process of development, from the larval to adult stage. In one of the experiments done by Kimble and White, DTC removal by laser ablation causes GSCs to stop proliferating and they differentiate into gametes (J. E. Kimble & White, 1981). Amplification of the DTC or moving it to another position promotes proliferation of GSCs in the new position (J. E. Kimble & White, 1981) . Therefore, DTCs are both necessary and sufficient for the proliferation of stem cells. The proximity of the stem cell to the DTC largely determines if it remains in the mitotic zone or enters the meiotic zone. Subsequent work has focused on identifying the molecular factors originating at the niche which are a part of the signaling cascade that play a role in perceiving and executing these signals, factors that promote entry into meiosis, and determination of how these factors work in conjunction to balance between the proliferative differentiated state (Hansen & Schedl, 2006; Hubbard, 2007; J. Kimble & Crittenden, 2007; Seydoux & Schedl, 2001). It is established that most stem cells in the mitotic region retain their stem cell potential prior to entering the transition zone. Distance from the DTC favors differentiation.

1.7 Notch signaling

GLP-1/Notch signaling, which is responsible for maintaining the proliferative state of stem cells is relatively higher in cells close to the DTC, whereas cells further from the DTC show reduced

glp-1/notch signaling. The levels of certain proteins such as GLD-1 and GLD-2 that are part of a RNA regulatory pathways involved in controlling meiotic entry of cells, is higher in cells farther from the DTC. The Notch signaling pathway is highly conserved in most multicellular organisms. The Notch protein extends throughout the surface of the cell membrane, with certain part of it to the interior and certain part to the exterior of the membrane. Ligands bind to the extracellular domain of the receptor which leads to proteolytic cleavage and release of signals downstream to the nucleus modifying expression of the genes (Kopan, 2012). The core components of the Notch signaling pathway include a signaling ligand (LAG-2), a receptor (GLP-1), and transcription factors dedicated to this pathway (LAG-1 and LAG-3/SEL-8). Upon deletion of any of these core pathway components, germ cells leave the mitotic cell cycle and enter meiosis (Austin & Kimble, 1987; Lambie & Kimble, 1991). On the other hand, unregulated LAG-2 ligand or GLP-1 receptor leads to uncontrollable germline mitoses and generation of a germline tumor (Fitzgerald & Greenwald, 1995; Pepper, Killian, & Hubbard, 2003). The GLP-1 receptor is expressed in the distal end of the gonad and LAG-2 is expressed close to the DTC, which signals GLP-1 to promote mitosis (Austin & Kimble, 1987; Crittenden et al., 1994). One check on entry into meiosis takes place, in part, by translational repression of the Gld-1 mRNA as GLD-1 protein is responsible for transition to meiosis. It has been shown that, FBF binds directly to the 3'UTR of Gld-1 mRNA and post transcriptionally represses it in the distal tip of the gonad. Consistent with this idea, GLP-1 protein is high in distal regions of the germline in the mitotic zone, whereas in the same region there is a reduced amount of GLD-1 (Marin & Evans, 2003). Repression of GLD-1 is a very important aspect of the mitotic/meiotic switch; GLD-1 deletion mutants possess stem cells that enter meiosis rather than proliferating in the mitotic zone, despite the fact that they have high

expression of GLP-1 (J. L. Brenner & Schedl, 2016; Hansen et al., 2004; Marin & Evans, 2003). It is important to understand and identify direct molecular links of the niche, relevant signaling pathway and regulators in order to fully understand the mechanism of stem cell self-renewal. One of the key targets of GLP-1/Notch signaling that function to promote germ cell self-renewal is FBF (fig 4). Very recently, two proteins called *lst-1* and *sygl-1* have been discovered that were shown to function redundantly downstream of the notch signaling pathway. A LST-1 and SYGL-1 double-mutant show similar germline collapse phenotype to that of *glp-1/Notch* mutants (Kershner, Shin, Hansen, & Kimble, 2014).

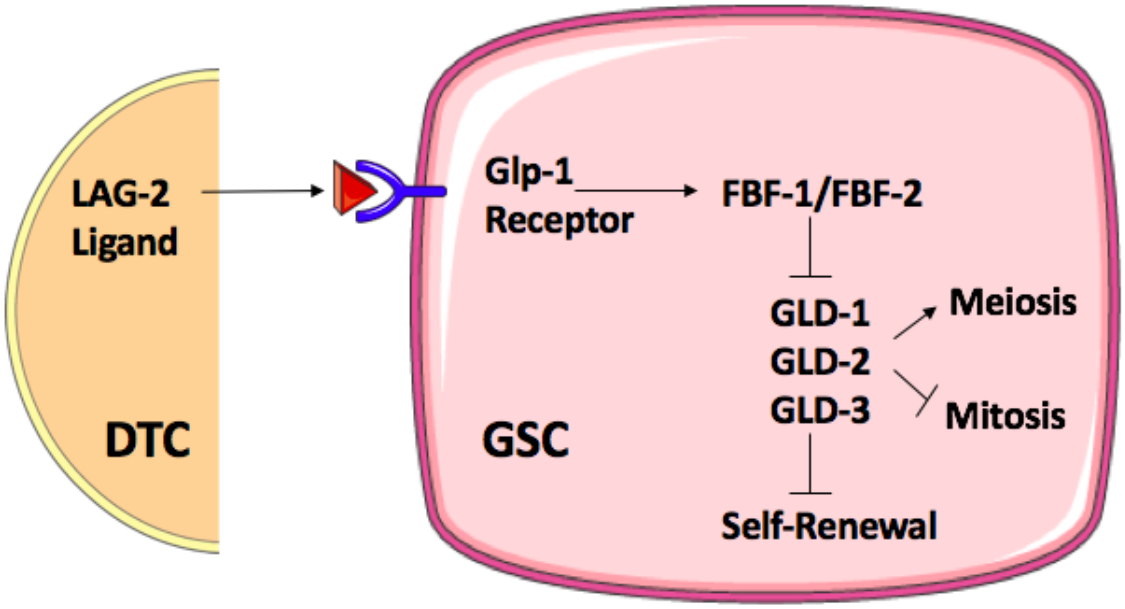


Figure 4: Notch signaling pathway in the *C. elegans* germline

1.8 RNA binding proteins-PUF proteins involved in germline stem cell maintenance

RNA control is prevalent throughout biology. Every aspect of the mRNA is subject to meticulous regulation by RNA-binding proteins also known as RBPs. They control the mRNA stability, localization and translation (Goldstrohm, Seay, Hook, & Wickens, 2007; Szostak & Gebauer, 2013). In the *C. elegans* germline the process of stem cell self-renewal or the switch from mitosis to meiosis is controlled, at least in part, by a family of RNA binding proteins called PUF proteins. PUF proteins regulate the expression of certain target mRNAs by binding with other proteins or short RNA sequences (Campbell, Bhimsaria, et al., 2012).

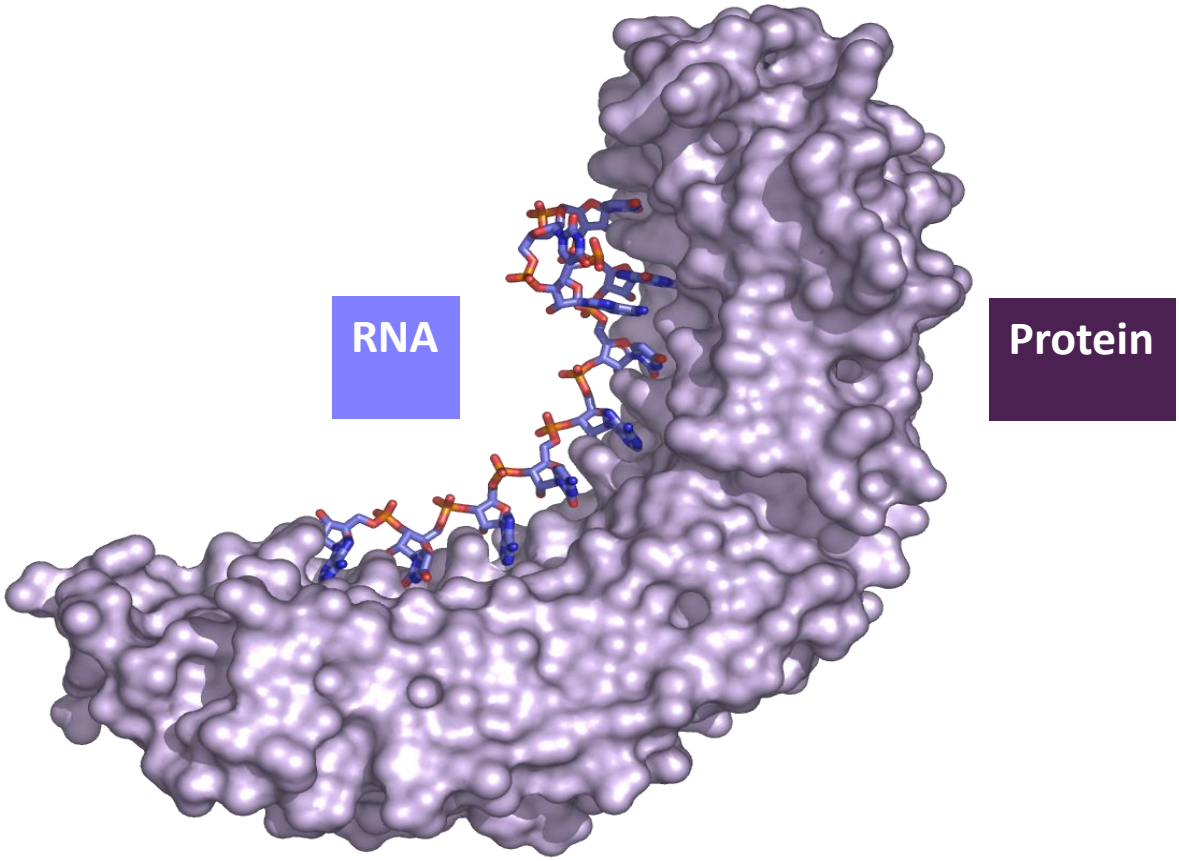


Figure 5: PUF protein binding RNA

PUF proteins are composed of eight Puf repeats which are made up of ~40 amino acids, that come together in the form of a semi-crescent. Each of these Puf repeats forms three α-helices. All the α-helices that are involved in binding to the RNA lie on one side of the PUF structure and make up the core consensus sequence. Each Puf repeat contains a distinctive central consensus sequence that binds to one of the bases in the RNA sequence (Wang, Opperman, Wickens, & Hall, 2009). An arrangement of 3 amino acid residues, known as a tripartite recognition motif or TRM dictates the RNA base that is targeted. The interaction between TRMs and RNA bases occur through a mix of edge-on and stacking interactions. The target RNA sequence is generally eight bases, one for each of the consecutive helix. The edge-on interaction involves hydrogen bonding, while one amino acid is stacked in between RNA bases forming the stacking interaction (Campbell, Valley, & Wickens, 2014). FBF is one of the first members of the PUF protein family and is known to contribute to the stem cell function in the reproductive germline of *C. elegans*. PUFs are known to function by recruiting specific protein partners. They repress translation of mRNA through recruitment of deadenylases, de-capping factors or other conserved protein factors. However, other mechanisms have been proposed such as translational repression. FBF represses mRNAs that code for proteins that promote meiosis. Additionally, FBF is a known target of Notch signaling pathway.

1.9 FBF

FBF-1 and FBF-2, are two homologous proteins that are more than 90% similar to each other. They are redundant and play a role in maintaining the stem cells in the proliferative phase (Crittenden et al., 2002). They are RNA binding proteins that bind a specific sequence and cannot be distinguished biochemically. So, they are collectively called FBF (Bernstein, Hook, Hajarnavis, Opperman, & Wickens, 2005; Zhang et al., 1997). In FBF-1 FBF-2 double mutants, the mitotic

divisions come to a halt during the L4 stage, and all stem cells enter the meiosis to differentiate into a specific cell, either egg/sperm. There is a complete collapse of the germline, proving that FBF is responsible for maintenance of stem cells in the germline. FBF belongs to the Puf family of proteins (for Pumilio and FBF) (Wickens, Bernstein, Kimble, & Parker, 2002; Zhang et al., 1997). FBF binds to specific sequences in the 3' untranslated regions (3'UTRs) of target mRNAs, and causes repression of translation, either by decapping, deadenylation or some other mechanism that is not entirely clear yet. Similar translational repression is seen in its homologs in Drosophila and yeast (Wickens et al., 2002). Many mRNAs that are said to be FBF targets have been reported, two of which are gld-1 and gld-3. (Crittenden et al., 2002; Suh et al., 2009). Both of these code for proteins that promote meiosis. Thus, FBF binds to these mRNAs to keep this level low in the distal end by repressing translation. As we move towards the proximal end we see an increase in level of GLD-1 protein as the number of cells entering meiosis increases. FBF also maintains its levels at the distal end by binding to the fbf-1 and fbf-2 mRNA causing autoregulation (Lamont, Crittenden, Bernstein, Wickens, & Kimble, 2004). Consistent with this idea, we see that *fbf* mRNA and FBF protein are localized in the distal-most end of the germline. We know that FBF-2 is responsive to Glp-1/Notch signaling, we also know that FBF contains four LAG-1 binding sites, but we don't fully understand how FBF works to repress expression of GLD-1 and promote mitosis.

1.10 Importance of post-transcriptional regulation in regulating the switch from proliferation to differentiation.

RNA–protein interactions dictate mRNA regulation. There are four known factors that control meiotic entry: GLD-1, GLD-2, GLD-3 and NOS-3 (J. L. Brenner & Schedl, 2016; Kadyk &

Kimble, 1998). While the requirement of PUFs in germline stem cells has been reported in *C. elegans*, their biochemical functions are opaque. Two recently discovered genes called lst-1 and sygl-1 have been identified as direct targets of Notch. They have also been shown to be expressed in the stem cell region. A *lst-1 sygl-1* double mutant displays the same phenotype as a Glp-1/Notch mutant phenotype, i.e., collapse of the germline. They are shown to be functionally redundant. LST-1 contains a Nanos-like zinc finger in the C-terminal, signifying the possibility of some RNA binding activity (Kershner et al., 2014; Shin et al., 2017). Both are cytoplasmic proteins that control the stem cell pool, increase in expression of either LST-1/SYGL-1 leads to tumor formation in the germline. This reinforces their role in stem cell maintenance. However, very little is known about LST-1 and its molecular mechanisms. There has been no homologue of LST-1 found outside of *C. elegans.*

I examine the hypothesis that LST-1 is a protein partner of FBF in the stem cell niche, and facilitates PUF protein repression of the Gld-1 mRNA within the stem cell pool.

CHAPTER 2:

MATERIAL AND METHODS

Site directed mutagenesis

PCR Reaction

The primers were designed as required by the experiment. A reaction mix of the following was prepared for the PCR reaction (table 1).

Table 1: PCR reaction mix

	20µl Reaction	50µl Reaction
5X GC	4µl	10µl
10Mm dNTPs	0.4µl	1µl
Forward + Reverse primers	1+1 µl	2.5+2.5 µl
Template	0.5 µl	1 µl
DMSO	0.6 µl	1.5µl
H_2O	12.3 µl	31 µl
Phusion DNA Polymerase	0.2 µl	0.5 µl
TOTAL	20 µl	50 µl

Once all the components were added into a PCR tube, the reaction was run in a thermocycler following the given settings (table 2).

Table 2: PCR reaction conditions

98°C	30s	
98°C	10s	25 Cycles
55°C	30s	
72°C	1-5:00 min	
72°C	10:00 min	
4°C	∞	

Electrophoresis gel run

Once the run was complete, about 2.5 µl of the reaction was run on a gel to observe a band corresponding to our product size.

DpnI digestion

0.5 µl of DpnI enzyme was added to the reaction tube and incubated at 37°C for 1 hour.

Bacterial transformation

The final reaction was transformed into bacterial competent cells by adding 5µl of the final reaction into 50µl of thawed competent cells. This was then incubated on ice for 30 minutes. It was heat shocked at 42°C for 45 seconds and put back on ice for another 2 minutes. This was plated on a LB-amp plate and incubated overnight at 37°C.

DNA isolation/miniprep

Next day, a colony was picked and inoculated in 5ml of LB-amp liquid media for 16-22 hours. The tubes were taken out and DNA was isolated following the 'GeneJet Plasmid Miniprep Kit'. These isolated DNA were sent for sequencing to identify/check for our mutants.

Yeast two and three hybrid assays

yeast strain ($L40^-$ or YBZ1) was inoculated in 5ml of liquid YPAD and incubated with shaking at 30°C overnight. The overnight culture was added to 50ml YPAD and allowed to grow for 4 hours at 30°C. The culture was harvested in a sterile 50ml falcon tube at 3000 x g (5000 rpm) for 5 min. The supernatant was poured off and the cells were resuspended in 25ml of sterile water and washed again. The water was poured, and the cells resuspended in 1.0 ml water. 100µl of this culture was transferred to individual 1.5 ml Eppendorf tubes. The cells were pelleted at top speed for 15 seconds and water is removed with a micropipette. 350µl of the transformation mix (240 µl PEG (50%w/v), 36 µl 1.0 M LiAc, 50 µl SS-DNA (2.0 mg/ml), 5-6 µl plasmid DNA (0.1-10 µg), 34 µl Sterile water) was added to each cell pellet and vortexed vigorously to resuspend. This was incubated in a water bath at 42°C for 1 hour. It was centrifuged at 6000-8000 rpm for 15 seconds and the transformation mix was removed with a pipette. 1.0 ml of sterile water is pipetted into each tube and the pellet is resuspended by pipetting it up and down gently. 200µl of the resuspended transformants were plated onto selective media plates and incubated at 30°C for 1-2 days. Colonies were picked and analyzed further.

Beta-glo assay

A single colony from the desired plate was picked and inoculated in a 5mL culture in the appropriate selective media. This culture was allowed to grow at 30°C for about 1.5 to 2 days. 100µl of this culture was dispensed in replicates onto a 96 well plate. Each culture was diluted by adding 1 mL of fresh selective media. These cultures were allowed to grow at 30°C for about 2 to 2.5 hrs. Approximately 50-100µl of this culture was transferred onto a transparent round bottom 96 well plate. The tecan plate reader was used to read OD at 660nm. From the same plate about 20-50µl culture was transferred to a white flat bottom 96 well. Equal amounts of β-glo reagent was added to each well. This plate was then incubated at room temperature for an hour in a drawer or under tin foil, because the β-glo reagent is light sensitive. The same tecan plate reader was used to obtain the luminescence values. The readings were analyzed on an excel spreadsheet and graph plotted.

The fusion protein

Bacteria expressing the protein were grown until it reached O.D 0.4-0.6 in 100 ml LB medium at 37 ºC. IPTG was added to a final concentration of 100µM and incubated for two hours. This was centrifuged for 5 minutes at 4000rpm at RT. The pellets were resusupended in 5ml 1X PBS+ 1X protease inhibitor. The solution was sonicated for 5 cycles- 10sec on/off repeat till it was clear. This was centrifuged for 10 min at 4ºC, 8000g. 50µl of GST/ amylose beads was added to the supernatant and incubated for 20 minutes at RT. These beads were then pulled down by centrifugation for 1 min at 1000g. The beads were incubated with protein for 10 minutes at 4ºC. These were centrifuged for 1 min at 4ºC 1000g. The washing steps were repeated thrice after which the beads were resuspended in 150µl 1X PBS.

Pull down assay

30mg of each protein were incubated either separately or together with 20µl resin(GST or Mbp) in 50µl of resuspension buffer on ice while agitating at regular intervals. This was then washed thrice in resuspension buffer. The sample was boiled with 2x crack buffer and 10µl was loaded on the gel and analyzed by coomassie staining.

***C. elegans* culture and maintenance**

Worms were cultured on 100 mm NGM (Nematode Growth Media) agar plates seeded with OP50 E. coli bacteria at Room Temperature. They were synchronized by bleaching and growing till L4 stage.

Bleaching of worms/synchronize worm

Worms were collected with M9 buffer by adding 2 ml of M9 buffer onto each plate, tilting the plate, and washing the worms by pipetting them from top to bottom of the plate. This was repeatedly done, to collect all the worms. The buffer containing worms was collected in a 15 ml falcon tube and the volume was brought up to 10 ml with M9 buffer. The worms were pelleted by centrifugation for 2 minutes at 1800rpm. The supernatant was removed and pellet was resuspended in 5 ml of alkaline hypochlorite solution/Bleach solution (1 ml of 8.25% bleach, 500 µl 5M NaOH and 3.5 ml water). The falcon tube was shaken vigorously for about 30 sec to break up the existing worm bodies. After about 2-2:30 minutes, M9 buffer was added to bring the volume to 10 ml. embryos were pelleted via centrifugation for 2 minutes at 1800 rpm. The supernatant was removed and embryos were washed with 10 ml of M9 for 2 minutes at 1800 rpm, twice. All but 100 µl of

the embryo suspension was removed and 3-4 drops of this was transferred to NGM plates containing OP50 bacteria. The plates were incubated at 20°C for ~20-24 hours for L1 offspring hatching. The plates were then placed at RT for about 3 days to reach L4 stage.

RNAi

Feeding RNAi was performed using *sygl-1* clones from Dharmacon RNAi library. Bacteria were grown overnight at 37°C in LB media containing 100μg/μl of ampicillin. Cultures were concentrated, seeded onto Nematode Growth Medium (NGM) plates containing 1mM IPTG, then induced overnight. Worms at L4 stage were picked onto the plate and let to incubate at Room temperature for 2 days.

Germline dissection and Immunofluorescence ***(***D.S. Yoon et al./MethodsX 3(2016) 378-385***)***

50 adult worms were transferred to a glass dish containing 0.25mM Levamisole using a platinum worm picker. Once worms are paralyzed (1–2 min), Their heads were cut off using a disposable /Sterilized needle under a microscope. The dissected worms were transferred to a 1.5 ml micro centrifuge tube and spun down at about 8000 rpm for 2–3 s using a mini-centrifuge. Once the pellet is spotted the supernatant was carefully removed using a micropipette. 100μl of 3% paraformaldehyde (PFA) fixation solution was added to the micro centrifuge tube and incubated for 10min at room temperature.

The dissected worms were spun at about 8000 rpm for 2–3 s using the mini-centrifuge and the PFA solution was carefully removed with a 200μl pipette tip to leave the pellet of dissected worms. The sample was washed two times with PTW solution. After the last centrifugation, the PTW

solution was carefully removed, and 200µl of ice-cold 100% methanol was added to the microcentrifuge tube, and incubated for 10 min at 20°C. (fixed gonads can be stored in cold methanol at -20°C for a few days). It was washed three times with PTW solution. After the last centrifugation, 100µl of 30% Goat serum solution was added to the micro centrifuge tube and incubated for 1 hour. Primary antibody (Chicken Gld-1 antibody at 1:1000 dilution in 30% Goat Serum) was added to the microcentrifuge tube and incubated overnight at 4°C. It was washed three times (5 min interval) with PTW. The sample was incubated with secondary antibody (α-Chicken Alexa fluor 555, 1:2000 dilution in 30%Goat serum) for 1-2 hours at Room temperature. The supernatant was carefully removed by spinning down at about 8000 rpm for 2–3 s using a mini-centrifuge. DAPI solution (100 ng/mL) was added to the microcentrifuge tube and incubated for 10 min at room temperature. The supernatant was carefully removed by spinning down at about 8000 rpm for 2–3s using a mini-centrifuge, and then washed three times using PTW solution (5min interval).

Image acquisition

2% agar was completely dissolved using a microwave and dropped at the center of a glass slide, immediately another glass slide is placed on the glass slide with agar drop. After gelation, the upper glass slide was carefully removed, and the stained germline samples were transferred onto the agar pad on glass slide. 8µl of antifade mounting solution (e.g., VECTASHIELD) was dropped onto the stained germline samples and carefully spread to avoid the overlap of samples. A coverslip was placed over the top to put the sample between the agar pad and coverslip. The coverslip was

sealed with nail polish to prevent evaporation of antifade mounting solution. Images were taken using a laser scanning confocal microscope.

Microscopy

Images were taken using a FV3000 Confocal Laser scanning microscope with a 100x oil immersion objective. Figures were prepared in Adobe Illustrator.

Gld-1 quantitative analysis

The images were quantified by calculating the intensity of DAPI and GLD-1 using ImageJ, across all samples at a distance of 0-20 μm (1-5 GCD) from the Distal end. The intensity of Gld-1 across samples was normalized to DAPI. From the values obtained we calculated minimum, maximum, Quartile 1, Quartile 3 and median. We developed the box plot in excel using the above calculated values where the bottom and top boundaries of each box is the first and third quartiles, middle line is the median, and whiskers being the minimum and maximum values.

CHAPTER 3

RESULTS

3.1 LST-1 region 125-175 binds FBF-2

LST-1 and FBF-2 are expressed in the stem cell region of the *C. elegans* germline, (in the distal end) and associate with one another *in vivo* (Kershner et al., 2014). How LST-1 physically interacts with FBF-2 and the role of this interaction *in vivo* is unclear. We first performed a yeast two-hybrid (Y2H) assay with two vectors: one containing the DNA binding domain fused to LST-1, and the other containing a Gal4 activation domain fused to FBF-2. My assays were done in a specific strain of yeast called L40 ura^- with the genotype of His^-, Trp^-, Leu^- and Ade^-. If the two proteins interact, RNA polymerase II promotes expression of the downstream reporter, which in our case was lacZ. Thus, based on expression of β-galactosidase from the lacZ gene downstream, we are able to detect interactions between LST-1 and FBF-2 (fig.6a). Full-length LST-1, which is 398 amino acids long, was divided into two truncations: regions 1-104 and 104-398. These were cloned into the vector pBTM116 containing the DNA binding domain, while FBF was cloned into the vector pGADT7, which contains the activation domain GAL4. Based on the quantitative expression of β-galactosidase from the Y2H experiment, the region from 1-104 of LST-1 does not bind to FBF-2, whereas the region from 104-398 shows binding activity (fig.6b). We further truncated the region that showed positive binding into two parts: 104-250 and 250-398. The region from 104-250 bound. we made truncations in LST-1 region 104-250 using site-directed mutagenesis and

performed yeast two-hybrid assays. We produced five additional truncations of LST-1 104-150,150-200, 200-250, 125-175 and 150-250, which we used to perform the yeast two-hybrid assay. From the data obtained, we observe that the region 250-398 is not involved in binding to FBF-2, comparable to the region 1-104. The other four parts shows that there is loss of binding in region 200-250, whereas region 104-150,125-175 and 150-200 showed positive binding to FBF-2. This led us to believe that the binding region lies between 125-175 residues.

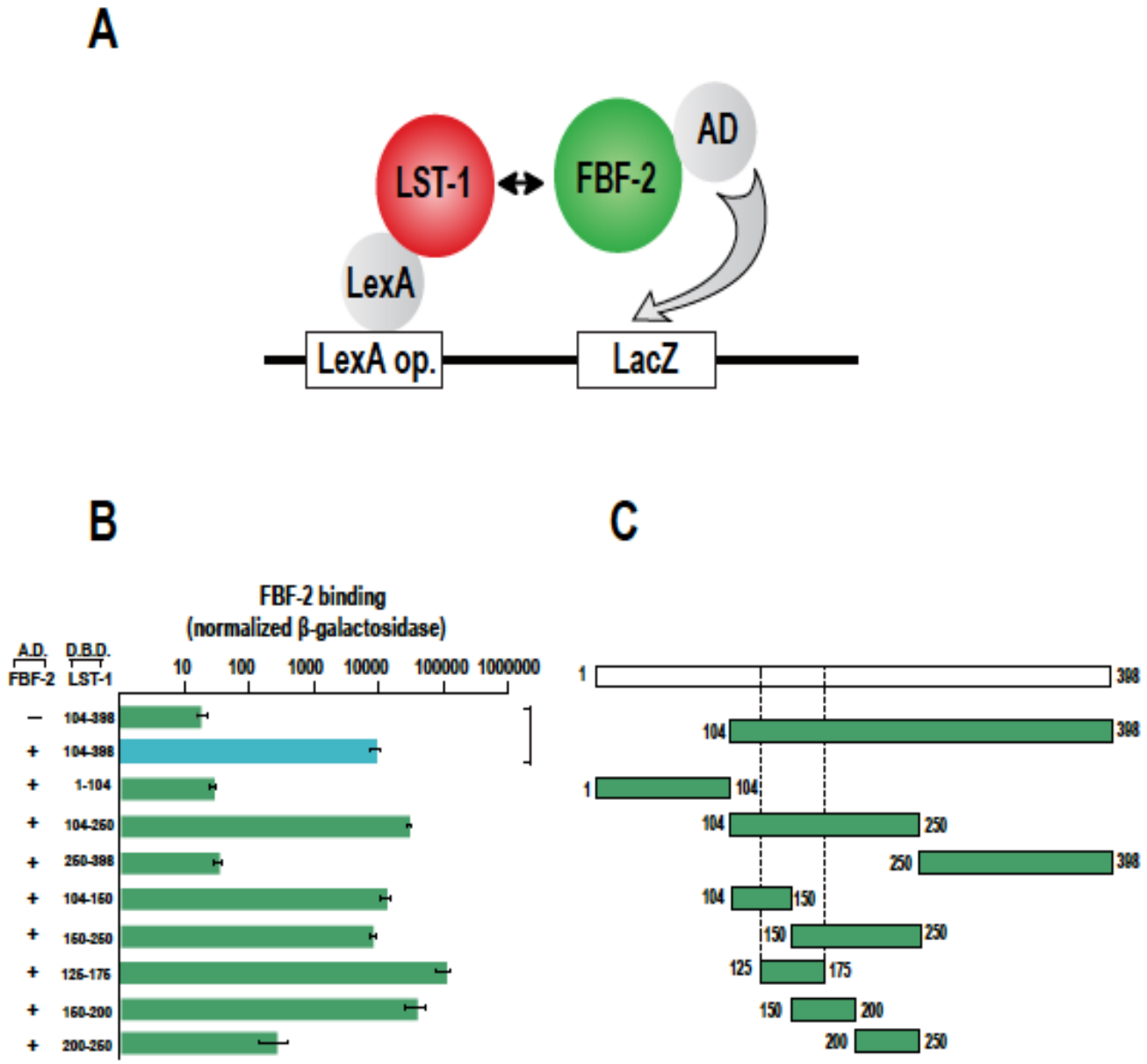


Figure 6: (A) schematic representation of the yeast two-hybrid assay LST-1 fused to the DNA binding domain (B) Binding data of LST-1 truncations and FBF-2 in a yeast two-hybrid assay. LST-1 truncations were fused to the plasmid containing DNA binding domain while FBF-2 was fused to plasmid containing the activation domain (C) Diagrammatic representation of the LST-1 truncations used in the experiment. Error bars indicate standard deviation. Light blur bar indicates positive control.

3.2 Lysine residue at position 153 in LST-1 is necessary for binding to FBF-2

Due to auto activation, and to confirm the result, we fused LST-1 truncations to the Gal4 Activation domain and FBF-2 to the DNA binding domain and repeated yeast two-hybrid assays. We saw similar results when compared to LST-1 in DBD, with LST-1 125-175 showing maximum interaction (fig.7a). We then set out to find specific amino acids on LST-1 that were required to bind FBF-2. To do so, we mutated amino acids along LST-1 from region 125-175, to alanine. Each LST-1 mutant was tested for their binding affinity to FBF-2. Mutation of lysine at position 153 showed most drastic effects of binding to FBF-2 with reduction in β-galactosidase expression by more than 10-fold (fig.7b). We concluded that L153 is required for the interaction of FBF-2 and LST-1. The L153A mutation was modeled into animals, to study the role of LST-1 *in vivo*. We next asked which residues in FBF-2 are crucial for the physical interactions with LST-1.

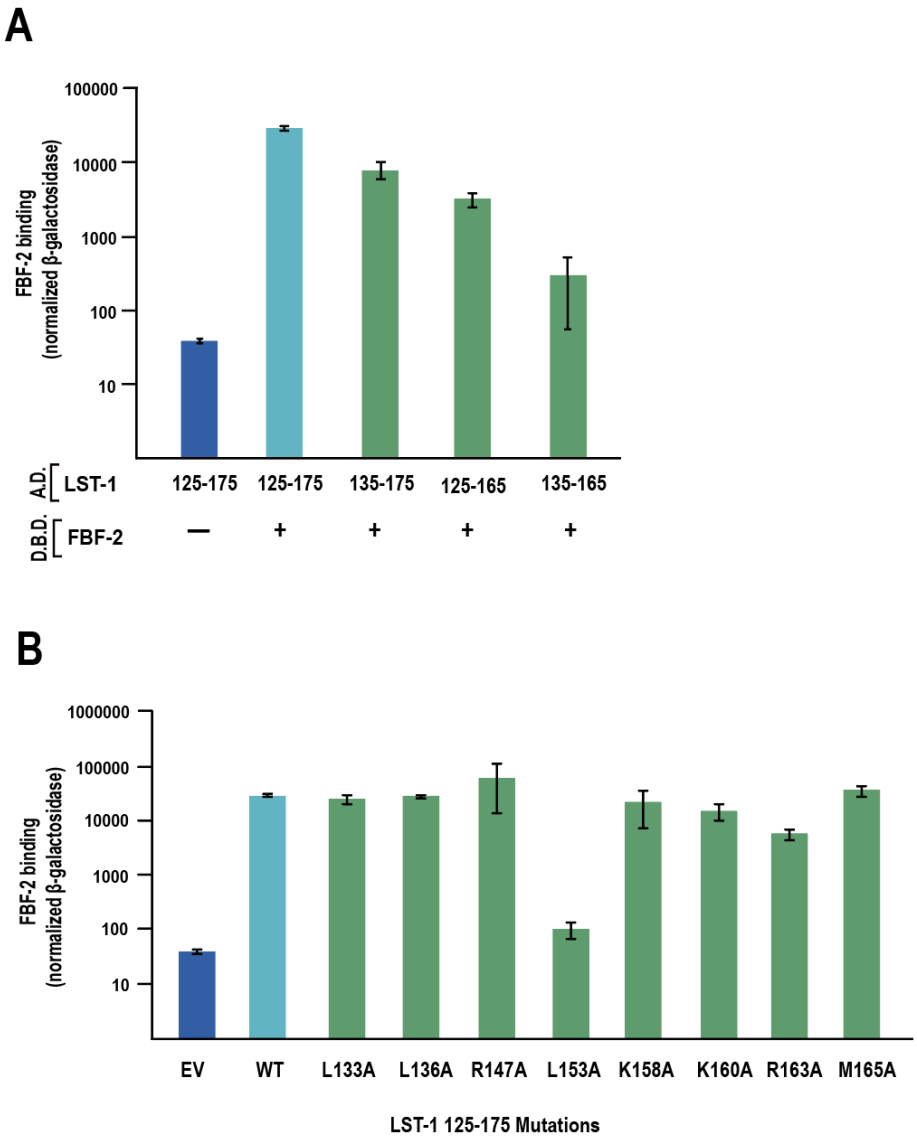


Figure 7: LST-1 fused to the activation domain (A) Binding data of LST-1 truncations and FBF-2 in a yeast two-hybrid assay. LST-1 truncations were fused to the plasmid containing Gal4 while FBF-2 was fused to plasmid containing the lexA DNA binding domain (B) binding affinities of LST-1 point mutants between 125-175 with FBF-2. Error bars indicate standard deviation. Light blue and dark blue indicate positive and negative control respectively. Credits: Alexa Lasley

3.3 FBF-2 requires Y479 and the loop region to bind LST-1

We tested binding of LST-1 to mutant forms of FBF-2. We created several FBF-2 constructs, each containing a mutation of a different amino acid to alanine. We then performed yeast two-hybrid assays to see which mutant form ceased to interact with LST-1. Most of the initial constructs were provided by Dr. Campbell, which he used in his studies for identifying a conserved interface between PUF and CPEB (Campbell, Menichelli, et al., 2012). The others were generated through site-directed mutagenesis. Mutation of aspartic acid 488 or tyrosine 479 reduced binding to LST-1, while all other mutants showed wild-type activity. The tyrosine mutation had greater than a 10-fold reduction in binding activity, therefore this mutation was used in further studies. Subsequent analysis suggests that deletion of the entire loop region, causes loss in binding activity (fig.8a). To test if the entire loop region is necessary or only certain residues in the loop region, we mutated each of the amino acids to alanine and performed yeast two-hybrid assays. We observed that the interaction is stable with mutations at any points except the tyrosine at position 479 (fig.8b). We concluded that the tyrosine at position 479 plays an important role in binding to FBF-2. Next, we were curious if mutating to any other amino acid would restore binding. We substituted the tyrosine at position 479 to several other amino acids such as glycine, alanine, glutamine, valine, phenylalanine and arginine, and performed yeast two-hybrid assays to test their binding affinities. We see that, the position at 479 is very specific to tyrosine. Substitution with any other amino acid fails to regain the interaction (fig.8c). Tyrosine at position 479 on FBF-2 is critical for its interaction with LST-1.

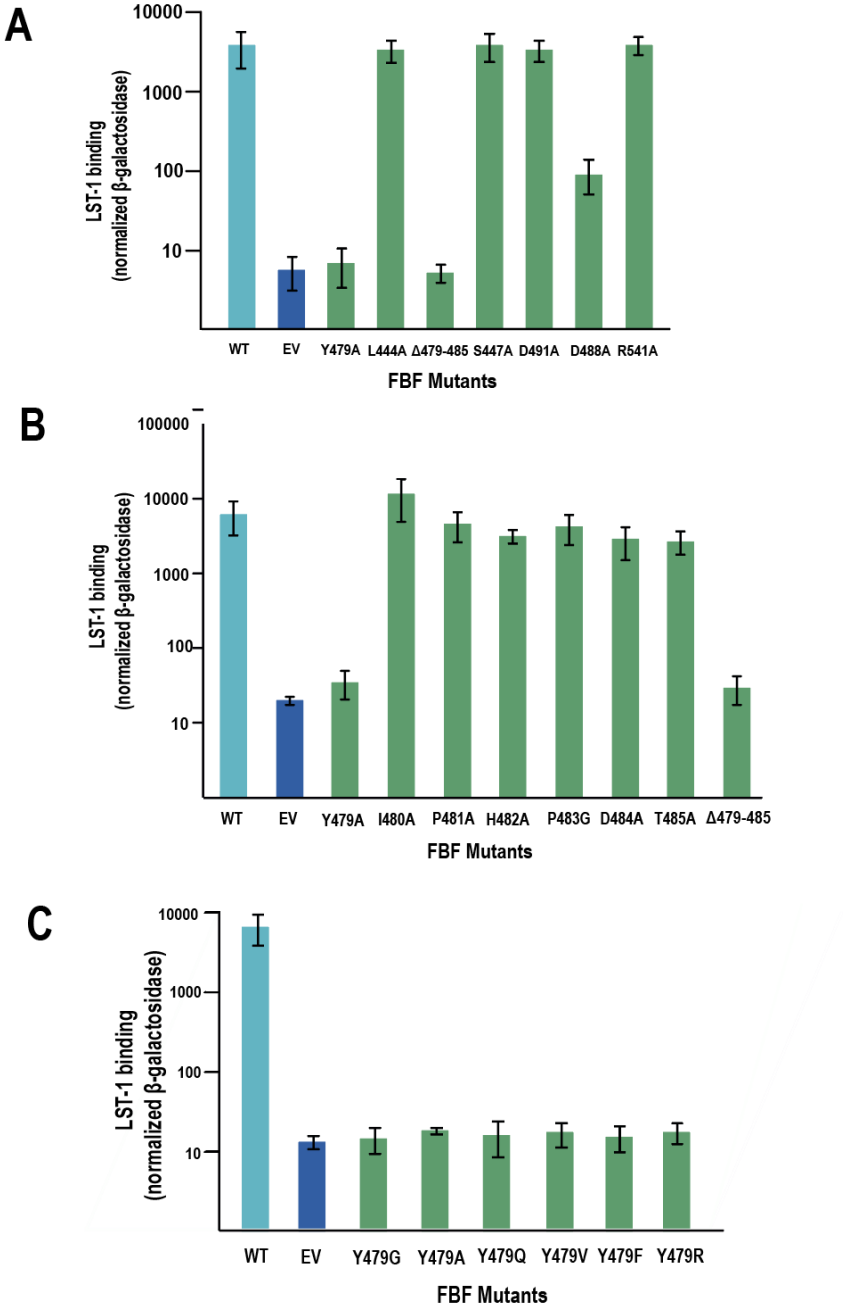


Figure 8: (A) Binding of FBF-2 mutants to LST-1 in a yeast two-hybrid assay (B) binding affinities of FBF-2 mutants along the loop and LST-1 (C) Binding of FBF-2 variants at position 479 with LST-1. Error bars indicate standard deviation. Light blue and dark blue indicate positive and negative control respectively.

3.4 LST-1 binds FBF

To confirm the interaction of LST-1 and FBF-2, we tested the ability of FBF-2 to pull down LST and vice versa. Here we have FBF fused to MBP (maltose binding protein) and LST-1 fused to GST. Two sets of experiment were performed, one where we incubate FBF and LST with GST resin and second with amylose resin. In the GST resin data, when only FBF-MBP is added we don't see a very clear band, when only LST-1 is added we see a clear band at 48kDa, when both FBF and LST-1 are added we see a much thicker band at 98kDa for FBF. Similarly, we performed the experiment using Amylose resin, where we incubated LST-1 tagged with GST, and did not see a band, FBF-MBP showed a thick band and when LST-1 and FBF were incubated together, we see a clear prominent band at 43kDa for LST-1 (fig.9). This data suggests that LST and FBF bind.

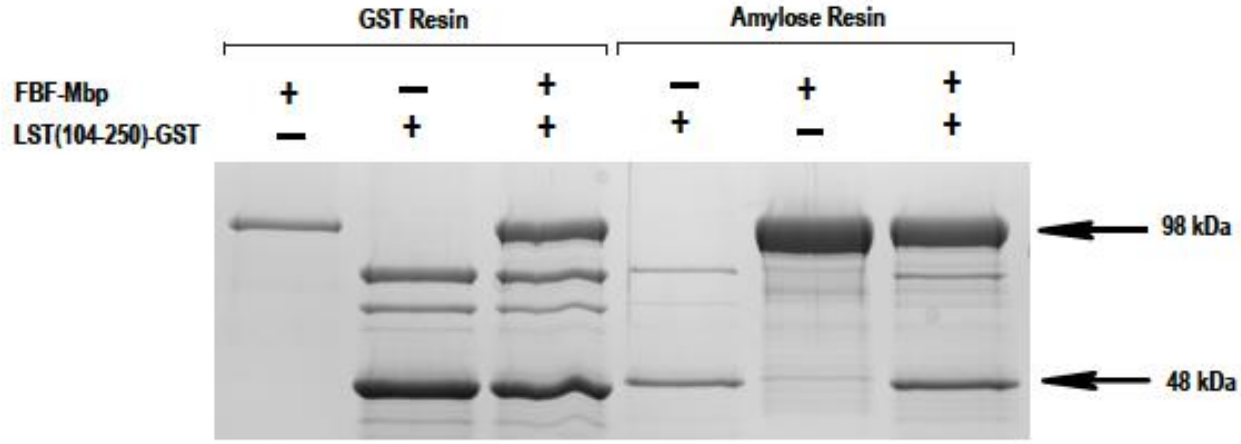


Figure 9: Pull-down assay results of LST (104-250) tagged with GST and FBF-2 tagged with MBP incubated in GST and amylose resin. Credits: Chi Zhang

3.5 The interaction is specific for FBF-2

We examined LST-1 interaction with homologues of FBF. We tested binding of LST-1 to FBF1, FBF2, PUM1, PUM2, PUF8 and dPUM. LST-1 only binds to FBF-1 and FBF-2 (fig.10). We conclude that LST-1 is a specific protein partner of FBF and could play a role in the mechanism of FBF repressing its target mRNA.

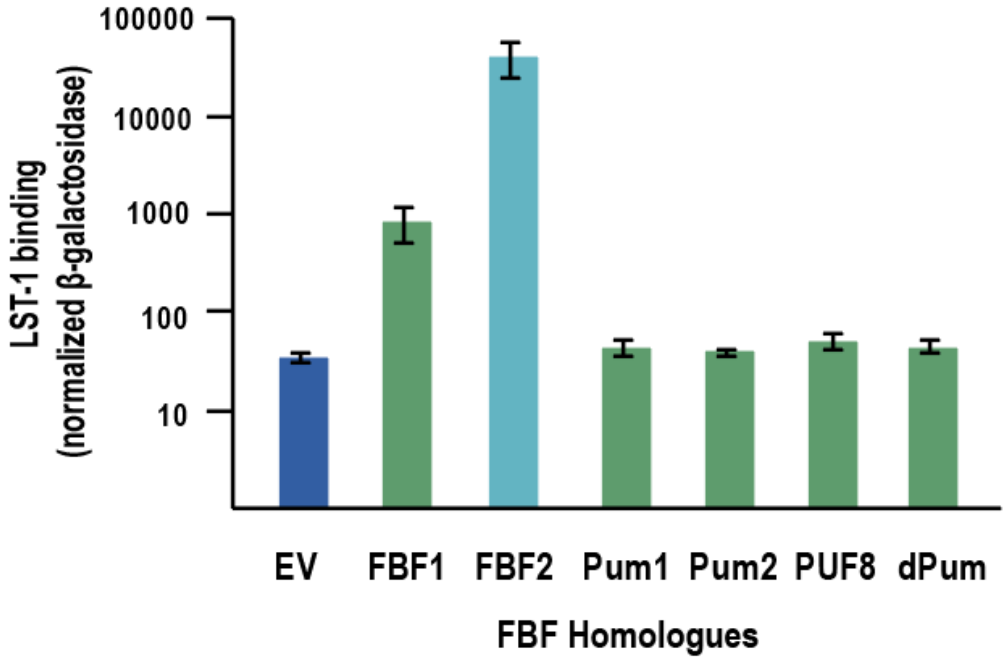
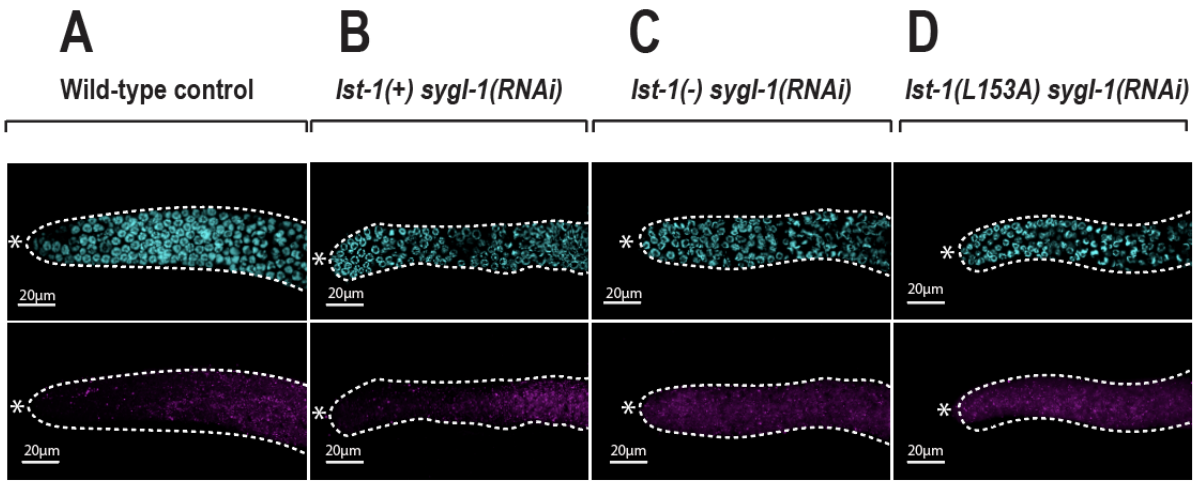


Figure 10: LST-1 fused to DNA binding domain and FBF homologues fused to the activation domain. Graph indicating their interaction. Error bars indicate standard deviation. Light blue and dark blue indicate positive and negative control respectively.

3.6 Gld-1 is highly expressed in the Distal tip of mutant worms

Gld-1 is an mRNA target of FBF. FBF binds to gld-1 mRNA and illicit post transcriptional repression. We hypothesized that LST-1 is required for repression. To test this idea, we performed immunohistochemistry experiments to analyze the expression of GLD-1 in LST-1 mutant worms. LST-1 and SYGL-1 work redundantly (Kershner et al., 2014; Shin et al., 2017). We tested the Gld-1 expression on wild type with normal amount of LST-1 and SYGL-1 and noticed that there is low expression of GLD-1 towards the distal tip which slowly increases towards the proximal end (fig.11a). When only SYGL-1 is deleted we observe the same phenotype with low gld-1 expression (fig.11b). When we delete both LST-1 and SYGL-1 we observe a drastic increase in expression of GLD-1 in the distal tip (fig.11c). Our CRISPR generated mutant would ideally work the same way, due to loss of interaction with FBF-2. Our CRISPR mutant LST-1 L153A displays a phenotype similar to the LST-1 SYGL-1 double knockout, with increased expression of GLD-1 in the distal tip (fig.11d). We measured the intensity of GLD-1 expression in the distal region of the gonad in all four strains and see that there is a significant increase in both the lst-1 sygl-1 double knockout as well as the lst-1 L153A/Sygl-1 deletion. We quantified this using a student's t-test (fig.11e). We conclude that LST-1 likely facilitates FBF-2 repression *in vivo*.



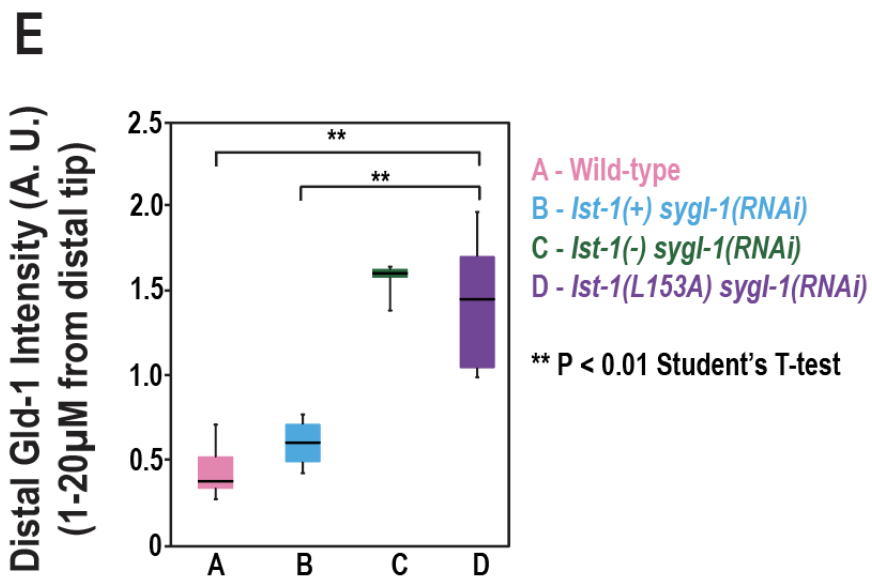


Figure 11: (A-D) GLD-1 protein expression in the distal end of the *C. elegans* gonad, (A) wild type (B) lst-1(+) sygl-1(RNAi) (C) lst-1(-) sygl-1(RNAi) (D) lst-1(L153A) sygl-1(RNAi), stained with α-GLD-1 antibody (pink) and DAPI (blue). (E) Intensity of α-GLD-1 expression across 20µm (1-5 GCD) from the DTC, was averaged and plotted. Student's T-test and tukeyHSD were performed to give a statistical difference of $p<0.01$ and $p<0.001$ denoted by the asterisk. Number of germline tested: Wild-type control, n=30; lst-1(+) sygl-1(RNAi), n=15; lst-1(-) sygl-1(RNAi), n=19; lst-1(L153A) sygl-1(RNAi), n=20.

CHAPTER 4

DISCUSSION

This work clarifies, the role of LST-1 FBF function. FBF-2 and LST-1 bind to each other and are expressed in the same region of *C. elegans (Kershner et al., 2014)*. FBF-2 controls the proliferative cells at the distal end of the germline (Crittenden et al., 2002). But the molecular mechanisms of PUF repression are mysterious. We draw three major conclusions from our study: First, LST-1 binds FBF in the same region as CPB-1, suggesting a shared binding site of FBF-2. Second, the physical interaction of LST-1 and FBF is essential for function of FBF in repressing target mRNA, third, there are no massive defects in the gonad morphology indicating that FBF is dispensable in the function of LST-1. Post transcriptional regulation is a critical factor in the regulation of gene expression. PUF proteins are essential components in this process. They are involved in stem cell maintenance, cell division, neuron function, memory formation and development. PUFs are known to function as posttranscriptional repressors by binding to certain 3' UTR sequences of mRNA or by interacting with other proteins. Once bound to an mRNA, translation is repressed by interacting with other proteins and regulatory machinery involved in translation.

There are at least four possible mechanisms by which this could take place. First, De-capping and/or deadenylation: deadenylases play a role in translational repression (Goldstrohm, Hook, Seay, & Wickens, 2006).They associate with certain PUF proteins as co-repressors. For example, PUMs repress target mRNA by deadenylation, but it is also shown that removal of deadenylases does not completely get rid repression (Van Etten et al., 2012). Another repression mechanism

could be deadenylation independent. The deadenylation independent mechanisms are not as effective in repressing translation as the deadenylation dependent mechanisms. poly(A) tail length also plays a crucial role in this process, in one of the studies done on drosophila PUM, embryos were injected with mRNAs that had a long poly(A) tail and short one, and what they observe was that dPUM represses the mRNA with long poly(A) tail much more efficiently. Other mechanisms include the ccr4p-pop2-not recruitment. Puf5p from yeast is shown to directly bind pop2 of the ccr4-pop2-not complex and recruits deadenylase enzymes. PUFs might activate the process of decapping which mediates mRNA decay. Decapping factors such as dhh1p and Pat1p function as translational repressors. The brat/pumilio/Nanos facilitates translational repression by two mechanisms: either facilitating deadenylation or interrupting interaction with 5' cap. Thus, LST-1 might recruit deadenylation/decapping factors that could facilitate translational repression. We could also analyze the poly(A) tail length of gld-1-1 target mRNA that could provide useful insight into the possible mechanism. Second possibility is localization: Another function of PUF has been localization of mRNA. Puf3 of yeast localizes its target mRNA to mitochondria and puf5 localizes its target mRNA PEX14 to peroxisomes (Quenault, Lithgow, & Traven, 2011). FBF promotes self-renewal of the stem cells in the germline by repressing gld-1 expression, we also see an increase in expression of Gld-1 as we move across the germline proximally. This could mean that FBF-LST protein complex that binds to the 3'UTR of gld-1 mRNA could promote mRNA localization to proximal ends rather than mRNA repression. This in turn increasing expression of Gld-1 proximally and promoting meiosis. Third, specificity: A single protein binds different mRNAs to regulate their expression (Campbell, Bhimsaria, et al., 2012). PUFs require protein partners. These protein partners could affect the RNA binding specificity of the protein. CPB-1 is a known protein

partner of FBF (Campbell, Bhimsaria, et al., 2012). SEQRS experiments have shown that when CPB-1 binds FBF it enhances the binding to certain RNAs (Campbell, Bhimsaria, et al., 2012). They also show that these proteins do not need to bind to the mRNA directly but influence binding of the PUF protein. This is unlikely in the case of LST-1 and FBF as the sequence has high affinity. Perhaps, it is a possibility that additional sites are bound in the presence of LST-1. Fourth, RNP granules: P bodies or processing bodies are conserved granules present in the cytoplasm and involve in mRNA repression and degradation (Parker & Sheth, 2007). In *C. elegans* they are specifically found in the germline (Noble, Allen, Goh, Nordick, & Evans, 2008), and at least three different mRNP granules are present. LST-1 is also a cytoplasmic protein (Shin et al., 2017). All these observations put together suggest that LST-1 FBF complex could bind to the 3'UTR of gld-1 in the distal end of the germline and direct this to one of the RNP granules for degradation. Co-staining LST-1 and PGL-1 would elucidate if this is correct. LST-1 in perinuclear speckles consistent with the approximate location of germline P granules (Shin et al., 2017).

In conclusion, PUFs diversify mRNA function in the germline. My work contributes to an ever-growing body of evidence that protein partners facilitate these extraordinarily important mechanisms in animal stem cells.

CHAPTER 5

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BIOGRAPHICAL SKETCH

Sanjana Rajeev's interest in science was first piqued by her father, who is a scientist. This led her into the magnificent world of science. She learnt to love biology through her high school teacher. Although she began her undergraduate education majoring in Biotechnology (the application of biology), it didn't take her long, after her first encounter with basic science, to realize that she wanted to focus on more basic questions of science, at the cellular and molecular level. The impetus to her already-existing interest in the field was a 2-month summer internship at the 'Centre for Cellular and Molecular Biology', Hyderabad (CSIR-CCMB). She counts this as a life-changing experience which granted her clarity on what she wanted to pursue in her career. From here she went on to perform research in Dr. Campbell's lab at 'The University of Texas at Dallas', which was a significant experience in her research life. She developed skills in planning and troubleshooting of experiments in a more effective way, and her foundation in the basics of molecular biology became stronger. Critical reading of scientific papers has helped shape her into the researcher she is today. She believes that these experiences have prepared her to be a productive member of a research team. While reflecting on her research experience, she realizes that she has gone through important changes in her understanding of scientific research.

She would really like to advance her research career in studying the molecular mechanisms underlying more complex processes taking place in organisms. Treading this path, she hopes to achieve some innovative research results in science.

CURRICULUM VITAE

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EDUCATION

Master of Science (M.S) in Cellular and Molecular Biology *Expected graduation: May 2018*
The University of Texas at Dallas | *Richardson, TX*

Thesis title: "Collaboration between a conserved RNA-binding protein and a specific protein partner restricts stem cell differentiation in the germline of *C. elegans"*
Supervising professor: Dr. Zachary Campbell

Bachelor of engineering (B.E) in Biotechnology *May 2012-May 2016*
B.M.S College of Engineering | *Bangalore, India*

First class with Distinction. GPA: 8.61/10
Dissertation title: "Analysis of hepatocellular carcinoma high throughput data using integrated omics approach."

RESEARCH EXPERIENCE

Graduate Student Researcher *September 2016-Current*
The University of Texas at Dallas | Richardson, TX

- Studied the role of an RNA-binding protein and its protein partner in post transcriptional control of germline stem cells in *C. elegans.*
- Planned and coordinated activities in the laboratory for scientific projects.
- Responsible for collecting and analyzing experimental data and organizing the results into a report or presentation using various scientific, word processing, spreadsheet or statistical software applications or program platforms.

Project Trainee *January 2016 - May 2016*
Geneclat Technologies | Bangalore, INDIA

- Analyzed high throughput data of hepatocellular carcinoma using integrated omics approach.
- Lead a team of four members and was responsible for design of experiment as well as smooth conduct of the research project.
- Coordinated and collected data of hepatocellular patients from public platforms such as a TCGA for analysis.
- Summarized all the results for presentation and thesis to scientific audience as well as public.

Summer Research Intern *May 2015 - July 2015*
CSIR-Centre For Cellular and Molecular Biology | Hyderabad, INDIA

- Purified and characterized Calcium-Binding Proteins: NECAB, NCal, SCGN and NFYA using Biophysical methods such as fluorescence spectroscopy, Isothermal calorimetric titration and circular dichroism.
- Engaged in probing, leading and reflective discussions that helped me easily understand and carry out directions from superiors and peers.
- learnt basic experimental techniques and assisted a graduate student in carrying out experiments.
- Literature survey
- Writing of final report and presentation
- Carrying out day-to-day lab activities in a sterile and appropriate manner.
- Attended lab group meetings every week to discuss research assignments.

Project Trainee *August 2015 - January 2016*

B.M.S. College of Engineering | Bangalore, INDIA

- Constructed a protein-protein interactome in the Akt signaling pathway with the aim of finding novel interacting partners and identifying new drug targets.
- Independent conduct of experiment and analysis of results.
- Presentation of work.

Observer | Manipal Hospitals | Bangalore, INDIA *May 2014 - June 2014*

- Observed various experimental techniques in genetics, molecular biology and microbiology with real patient samples.
- Responsible for producing transcripts of high-level executive meetings and conferences and distributing detailed action briefs after the fact.

ACADEMIC DISSERTATION/THESIS:

"Collaboration between a conserved RNA-binding protein and a specific protein partner restricts stem cell differentiation in the germline of *C. elegans*". *(August 2016-Current)*

An important question in stem cell biology is understanding the molecular factors that maintain stemness. The cells that remain in a proliferative state at the distal tip produce daughters that differentiate into mature gametes as they progress proximally. The stem cell niche is controlled in part by the notch signaling pathway, but the molecular details are opaque. Here we address the role of a recently discovered gene *lst-1* and its interaction with FBF-2. FBF maintains the stem cell pool in *C. elegans*, but we don't understand the mechanisms involved. LST-1 and FBF are downstream targets of Notch signaling. We studied the physical interaction between LST-1 and FBF to identify the amino acid residues that are important for interaction. Leucine at 153 in LST-1 and Tyrosine at 479 in FBF-2 are required for the interaction to take place. The site of interaction on FBF is shared between multiple protein partners. To determine if the interaction between FBF and LST-1 is relevant in animals, we applied CRISPR genome editing to incorporate the L153A mutation. Gld-1 is an mRNA target of FBF in the stem cell region. We measured GLD-1 protein abundance in the absence of either *lst-1* or *sygl-1* or both. We see that there is much higher expression of GLD-1 protein when both *lst-1* and *sygl-1* are absent, suggesting a lack of repression

by FBF. Similar results were observed in the case of our LST L153A mutant worm. This makes us believe that LST-1 facilitated repression by FBF. We envisage that this knowledge provides an essential step towards the discovery of repressive mechanisms through which LST-1 engages mRNA destabilization, decay, or localization machinery.

"Analysis of hepatocellular carcinoma high throughput data using integrated omics approach". (*January 2016 - May 2016)*

Hepatocellular carcinoma is one of the leading forms of cancer. Using Next Generation sequencing, Whole genome analysis was done to identify mutations present in the tumor sample. Transcriptomics analysis was performed for identification of deregulated genes upon mutation. Further, miRNA analysis was performed for identification of microRNAs regulating the mRNA targets. All the above omics were integrated to provide certain specific mutants that could be further studied as a cause of Hepatocellular carcinoma.

"Purification and Biophysical characterization of Calcium binding proteins such as NECAB, Ncal and NFYA." (*May 2015 - July 2015*)

Calcium signaling plays a major role in regulating all aspects of neuronal function. Different types of neurons exhibit characteristic differences in the responses to Calcium signals. This thesis includes the description and purification strategy of few EF hand containing Calcium sensor proteins such as NECAB, NCal, NFYA and their characteristics. In addition, I also explored novel pathways regulated by these proteins and did some preliminary work to find novel interacting partners of NCALD, a well-known Calcium sensor protein present in the retina and neuronal tissues. I purified them and studied their characteristics by using techniques such as ITC, Calorimetry and Circular Dichroism.

RELEVANT SKILLS

Cell Biology:

RNA interference, pull-down assay, yeast two and three hybrid assays, yeast transformation, Immunohistochemistry, fluorescence imaging using confocal microscope and dissection and maintenance of *C. elegans* culture, cell culture.

Biochemistry:

Spectroscopic methods, electrophoretic techniques, chromatographic separations, purification and characterization of DNA and protein, use of the computer for structural information, enzyme kinetics, immunoassay methods, DNA isolation, luciferase assays, sequencing and mapping, and protein purification

Microbiology:

Sterile techniques in isolation and culture of microorganisms, preparation of various media, operation of autoclaves, bright field and confocal microscopes, staining techniques and various biochemical tests

Molecular Biology:

Gel Electrophoresis of DNA, SDS PAGE techniques, PCR techniques, western blotting, restriction Digestion, ligation, cloning and transformation techniques, quantification of DNA and RNA using NanoDrop, basic knowledge in various Bioinformatics tools like NCBI, Swiss PDB, EMBL, RasMol, BLAST, FASTA, CLUSTALW.

ADDITIONAL SKILLS AND ACHIEVEMENT

- Teaching Assistant at 'The University of Texas at Dallas' for the course 'Introduction to biology' for 3 semesters.
- Completed an online certificated course from 'Massachusetts Institute of Technology' through EDX in Introduction to Biology (i.e. genetics, genomics, molecular biology, biochemistry, cancer biology).
- Participated in a One Week Workshop by Aristogene Biosciences Pvt. Ltd. on 'Complete Cloning and Hybridization Techniques'.
- Co-ordinated the Biotechnology department technical fest 'PHASESHIFT' at the institutional level in 2014.
- Member of the Proteomics Society(India) from April 2016-April 2017.
- Hosting of Seminars and technical fests.
- Strong Presentation Skills.
- Handle independent experiments and a team Worker.