ABSTRACT

Yu-Chien Cheng

Towards generating a *C. elegans* cell line: increasing seam cell divisions

(Under the Direction of Dr. Edward T. Kipreos)

The nematode *C. elegans* is an important genetic model organism. In the past six years, three noble prizes have been awarded to scientists working with this organism. The use of *C. elegans* as a biomedical model, however, has been held back by the absence of a *C. elegans* cell line. A cell line allows the growth of a particular type of cell in tissue culture and the study of specific cells of interest with greater efficiency. To generate a cell line in *C. elegans*, we focus on the adult stem cells called seam cells, which have the potential to be manipulated into generating a cell line. A cell line requires continuous proliferation of seam cells, so I tested whether the inactivation of genes known to regulate normal cell differentiation would lead to continuous seam cell proliferation. These genes are the negative cell-cycle regulators *cul-1*, *lin-35*; the stem cell division regulators *rnt-1/bro-1* and *pop-1*; seam cell-fate specification transcription factor *elt-5;* epidermal differentiation factor *lin-26*. I inactivated these genes with RNA-mediated interference (RNAi). Seam cells were tagged with a vector that expresses Green Fluorescence Protein (GFP) for their visualization with a florescence microscope. The combination of *pop-1* and overexpressed *rnt-1/bro-1* produced the highest seam cell counts, followed by *cul-1*. The inactivation of the combination of these genes will be used as a starting point for making a *C. elegans* seam cell line whose presence will greatly benefit the research communities.

INDEX WORDS: *C. elegans*, Cell Line, Seam Cell Proliferation, *pop-1*, *rnt-1/bro-1*, *lin-26*, *lin-35*, *cul-1*, *elt-5*.

TOWARDS GENERATING A *C. ELEGANS* CELL LINE: INCREASING SEAM CELL DIVISIONS

by

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DEDICATION

I would like to dedicate this thesis to my family, particularly my parents who have given my abundant opportunities to learn and grow. My aunt and uncle in Americus, Georgia also have been so gracious and accommodating to my needs. Thank you, Mom and Dad, for being there for me even when I'm thousands of miles away. Without the whole-hearted support of you guys, I would not have been able to persevere through my entire college endeavor. Thank you, Aunt Judy and Uncle Cheng, without you guys, I would not have been where I am now. You guys have made me who I am today.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1
INTRODUCTION

C. elegans is a small nematode that, as an adult, is only about 1 mm long (Kipreos, 2005). It has been used as an important model organism since Sydney Brenner introduced it in 1963 (Riddle et al., 1997). Its importance in research can be highlighted by three Nobel prizes won by the scientists who study *C. elegans* in the past six years. The apoptosis pathway and RNA interference (RNAi) were first discovered in *C. elegans* (Hall and Altun 2008). Green Fluorescence Protein (GFP), isolated from the jellyfish, was first used transgenically to study cells in *C. elegans*. These discoveries highlight that *C. elegans* is a powerful model system.

The advantages *C. elegans* offers include the ease of maintenance, the simplicity of the body structure, and the powerful genetic tools developed in this system. *C. elegans* has a short life cycle of 3 days; post-embryonically, it develops through four larvae stages (L1~L4) before it reaches adult. It has two sexes, hermaphrodite and male. Hermaphrodites produce about 300 offspring, and 10,000 worms can be easily grown on Petri dish. Hermaphrodites self-fertilize; homozygous mutations can be generated phenotypically and studied in the offspring. In addition, *C. elegans* is the first animal to have its entire genome sequenced. With RNAi, scientists can study a myriad of gene functions (Riddle et al., 1997).

Despite the advances and progress made in research in *C. elegans*, there is currently no *C. elegans* cell line available. A cell line is a group of continuously dividing cells derived from a specific type cell. Model organisms such as *Drosophila melanogaster* and mouse have developed cell lines that allow researchers to perform biochemical studies to gain insight into cells'

properties (Clemens 2000). This lack of a *C. elegans* cell line has held back biomedical research.

The difficulty of generating a *C. elegans* cell line stems from the fact that all somatic cells in *C. elegans* have an invariant cell lineage. "Cell lineage" describes the pattern of cell division throughout development. An invariant cell lineage means that cell divisions are constant from one individual animal to another in temporal and spatial manners. This mode of determinate development is characterized by precise and reproducible cell fates described by the cell lineage map (Figure 1). Why the cells divide in exactly the same ways can be attributed to the small number of cells involved in creating *C. elegans*. Unlike in *Drosophila melanogaster,* where massive number of cells are produced first and specified later by morphogen gradients, with only 550 cells in the embryo, each cell in *C. elegans* follows a strict lineage. *In vitro,* cell divisions are stopped at different developmental stages, and the cells become terminally differentiated (Schnabel 1997).

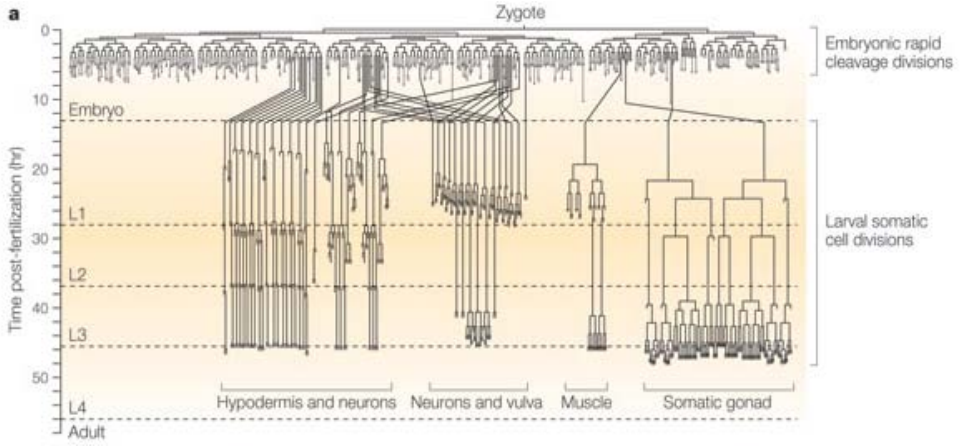

Figure 1: *C. elegan*'s invariant cell lineage (Kipreos, 2005).

The precise pattern of division and terminal differentiation creates major hurdles for generating cell lines, which are the products of continuous cell proliferation. Nevertheless, one

type of somatic cells seems promising for generating cell lines: adult stem cells. Adult stem cells are capable of undergoing asymmetric divisions that each time gives rise to a stem cell daughter and a non-stem cell daughter. This allows the adult stem cells to propagate themselves. Adult stem cells also go through symmetrical divisions that give rise to two stem cell daughters. Symmetrical divisions serve as a means to make up for the occasional loss of stem cells and as a reservoir for generating different types of tissues including blood cells, epithelia and bones (Kagoshima et al 2007).

In *C. elegans*, there is only one type of adult stem cell, called seam cells. They have a critical role in the morphogenesis of the worm. Seam cells allow for the development and growth of the embryo and larvae by giving rise to epidermal and neuronal tissues (Koh and Rothman 2001). The seam cells, shown in figure 2, are arranged in a single row on both of the lateral

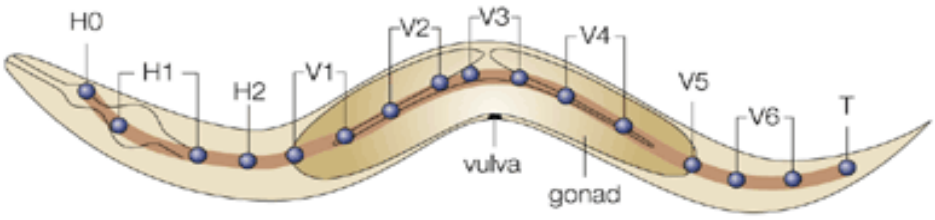

Figure 2: seam cells in an adult worm. Blue nuclei represent the seam cells. The labels above the blue nuclei indicate from which the blastomeres are the seam cells derived (Rougvie, 2001).

surfaces of the epidermis, referred to as the hypodermis in *C. elegans* (Herman, 2006). Most of the epidermis is derived from the AB 'founder cell', and seam cells are generated from H, V, and T cells (Koh and Rothman 2001). Throughout post-embryonic development, seam cells undergo asymmetrical division similar to that of mammalian adult stem cells, giving rise to an anterior seam cell and a posterior

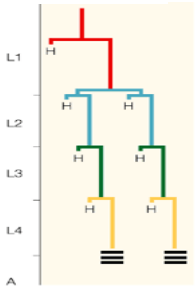

Figure 3: seam cell lineage for seam cells V1-V4 and V6 (Rougvie, 2001).

hypodermal cell, which is a terminally differentiated cell that fuses with the epidermis (Figure 3) (Sulston et al., 1980). Additionally, at the L2 stage, the seam cells undergo proliferative stem cell divisions, in which two seam cells are generated (Figure 3). In a wild type animal, seam cells divide and increase in number from 10 to 16 from hatching to the late L4 stage (Figure 3). Seam cells divisions are limited, and they eventually fuse together and then secrete the alae, a cuticle that runs the length of the adult body (Herman, 2006).

Despite the fact that seam cells divide extensively until differentiation, the number of seam cells generated at the end of L4 stage is less than twofold the number of the seams cells present before the division starts. Generating a seam cell line would require much more cell proliferation. To increase seam cell proliferation, the seam cell division pattern would have to be altered. Genes that are known to be involved in the seam cell division or cell proliferation were knocked down with RNAi. These include different categories of genes: negative cell cycle regulators *cul-1, lin-35*; seam cell division regulators *pop-1, rnt-1/ bro-1*; seam cell-fate specification transcription factor *elt-5*; and epidermal differentiation factor *lin-26*. The *rrf-3* mutation causes the loss of an RNA-directed RNA polymerase and has been shown to be sensitive to RNAi. *rrf-3* was incorporated as a preexisting genetic background to increase the gene knockdown efficacy (Simmer, 2002).

Cell cycle regulators control the progression of the eukaryotic cell cycle by various mechanisms. A cell cycle consists of four stages gap 1(G1), synthesis (S), gap 2 (G2), and mitosis(M). Carefully regulated cell cycle divisions are required for proper cell growth and differentiation (Boxem and Heuvel 2001)**.** Cyclin Dependent Kinases (CDKs) play an important role in the cell cycle progression. CDKs are composed of an activating subunit, cyclin, and a catalytic kinase subunit, CDK. There are various cyclins and CDKs that are required for the

progression through different stages of the cell cycle. Throughout the cell cycle, the level of the cyclin fluctuates while CDKs remain relatively constant. Cell cycle progression is achieved by the presence of specific cyclins along with the catalytic CDK. Conversely, cell cycle progression is stopped by Cyclin-dependent Kinase inhibitors (CKIs) which negatively regulate the level of different cyclins and CDKs. Proteasomal degradation of CKI often serves to release the cyclins from inhibition and initiate the progression through a specific cell cycle stage. The balance between the levels of cyclins and CKI, therefore, is critical in driving the cell cycle progression (John et al., 2001).

Cullin 1 (*cul-1*), a cell cycle regulator that is required for exiting the cell cycle from G1 to G0 (Kipreos, 1996). *cul-1* is involved in the most common protein degradation pathway in the cells, ubiquitin-mediated proteolytic pathway. Ubiquitin is a conserved amino acid polypeptide that is covalently attached to proteins targeted for degradation by proteasomes. This process is mediated by ubiquitin-ligases. In *C. elegans*, such ubiquitin ligase is called the SCF complex, and *cul-1* serves as the backbone of the SCF complex (Basu and Kipreos 2008). The SCF complex regulates the levels of proteins that act to inhibit or promote the transition from G1 to S phase. Cyclin E, a G1 cyclin required for G1/S transition, is a target of the SCF complex. *cul-1* mutant accumulates cyclin E and exhibits hyperplasia of diverse tissues (Dealy et al., 1999). This abundance of cyclin E probably contributes to the inability of *cul-1* mutants to exit the cell cycle from G1, leading to continuous progression through the cell cycle. Since the loss of *cul-1* leads to somatic cell proliferation in numerous tissues, it is worth investigating if cul-1 is also involved in regulating seam cell proliferation.

lin-35, another negative cell cycle regulator in *C. elegans*, is the ortholog of human Retinoblastoma (Rb), protein that is a tumor suppressor (Sherr, 1996). Rb was found to be

commonly mutated in rare heritable retinoblastomas (Lee, 1987). In *C. elegans*, *lin-35* negatively regulates progression from G1 to the S phase by sequestering the transcription factor E2F, which is required to turn on genes for initiating DNA synthesis. Sequential phosphorylation by the G1 CDKs at multiple sites on *lin-35* relieves E2F's inhibition by LIN-35, allowing E2F activation, and thus promoting the transition from G1 to S. G1 phase is a particularly important cell cycle phase for controlling cell growth and differentiation, for it is when the cells decide to either withdraw from or commit to cell cycle division. Uncontrollable cell cycle division and proliferation, characteristics of tumor cells, are commonly the result of defects in the genes controlling G1 progression (Pardee, 1989). Even though *lin-35* mutants do not exhibit any cell proliferation or identifiable mutant phenotypes by themselves (Fay et al. 2002), it is still worthwhile investigating its possible interaction with other genes.

rnt-1, a stem cell proliferation and differentiation gene, is a *C. elegans* ortholog of the RUNX family of transcription factors, which can act as repressors and activators depending on the DNA sequence bound (Canon and Banerjee, 2003; Stein et al., 2004). Studies have shown that *rnt-1* coordinates proliferation and differentiation of the seam cells in *C. elegans* (Ji et al., 2004; Lee et al., 2004; Kagoshima et al., 2005; Nimmo et al., 2005). *bro-1*, *C. elegans* CBFß homologue, forms a heterodimeric DNA binding complex with *rnt-1* and increases not only the affinity of *rnt-1* for DNA, but also the specificity of *rnt-1* DNA binding **(**Kagoshima et al., 2007). The *rnt-1/bro-1* complex promotes the proliferative seam cell division pattern at the L2 stage, in which two seam cells are generated from each division (figure 4). *rnt-1/bro-1* acts to promote symmetrical stem cell divisions at the expense of creating differentiated, hypodermal fate (Kagoshima, 2007). *rnt-1/bro-1* also promotes the progression from G1 to S phase in seam cell division (Xia et al, 2007). In addition, *rnt-1/bro-1* functions to specify seam cell fate by

interacting with *C. elegans'* Groucho homolog, UNC-37/TLE, a transcription co-repressor to repress targeted gene transcription. The loss-of function mutation in *rnt-1/bro-1* and *unc-37* results in a more severe decrease in seam cell number than in a single mutant alone. In other words, *rnt-/bro-1*'s transcription repression activity is required for seam cell proliferation (Xia et al., 2007). Over expression of RNT-1 and BRO-1 is reported to lead to massive seam cell proliferation (Kagoshima et al., 2007).

POP-1 is a TCF/LEF-1 transcription factor that functions downstream of the Wnt signaling pathway. The Wnt pathway is highly conserved and controls the processes of body-axis formation, organogenesis, and binary cell fate decisions associated with asymmetric cell division, cell polarity, cell migration, and stem cell proliferation (Bowerman, 2005). In the Wnt signaling pathway, beta-catenin is the effector molecule, which, when the pathway is not activated, is bound by a destruction complex that destabilizes and targets beta-catenin for proteasomal degradation. In this inactive state, TCF/ LEF transcription factors in the nucleus act as repressors through binding with the co-repressor Groucho. However, when the cell surface receptor is bound to the Wnt ligand, the destruction complex is inhibited and blocked. This leads to the production of free beta-catenin molecules that can enter the nucleus and displace Groucho from TCF/LEF-1, activating the transcription of targeted genes. How *pop-1* regulates stem cell proliferation is still unknown. Nevertheless, *pop-1* is known to play a pivotal role in establishing polarity in EMS blastomere divisions. The asymmetric distribution of *pop-1*, higher in anterior daughter cell and lower in the posterior, leads to the generation of E and MS from EMS' division (Lin et al., 2002).

The epidermal differentiation gene, *lin-26*, encodes a zinc-finger transcription factor and is expressed in all epithelial cells of the ectoderm and somatic gonad (Labouesse et al. 1994). *lin-*

26 mutants produce epidermal cells that quickly degenerate. This observation implicates its requirement in epithelial differentiation (Labouesse et al., 1996). The study led by Quintin et al (2001) later found that *lin-26* acts as a major control gene and can induce the expression of different markers to promote epithelial cell fate (Quintin et al., 2001). Since seam cells are one of the main cell types in the epithelium in *C. elegans* (Koh and Rothmanm 2001), inactivating *lin-26* is likely to block the daughter cells from differentiating into hypodermal cells. Blocking the differentiation of hypodermal cell might lead to an increase in proliferating cells.

ELT-5 is a GATA transcription factor expressed in seam cells. GATA transcription factors are a family of proteins that contain two related zinc-fingers that mediate DNA binding (Yu et al., 2002). ELT-5, along with a set of factors, directs the specification of seam cell fate from the epidermis. Mutants of *elt-5* exhibit defects in seam cells, such that seam cells are inappropriately fused with the epidermis (Koh and Rothman 2001).

Cell cycle regulators, cell specification genes, and stem cell division regulators, as described above, are involved either directly or indirectly in the seam cell divisions. The implication of their involvement is that when mutating these genes, seam cell numbers are likely to increase or decrease according to the regulatory nature of these genes. Our goal is to identify genes whose mutation would be most beneficial to seam cell proliferation.

This study shows that changing the levels of stem cell division regulators is most effective in achieving the cell proliferation required for generating seam cell lines.

CHAPTER 2
MATERIALS AND METHODS

C. elegans strains used/ generated

The strains used or generated were as follows: N2, MT10430, *lin-35*(*n745*); ET396, *him-5*(*e14667*)V; wIs51[*scm::GFP*; *unc-119*(+)], NL2099, *rrf-3* (*pk1426*); ET396, *him-5*(*e14667*)V; wIs51[*scm::GFP*; *unc-119*(+)], and NL2099, *rrf-3* (*pk1426*); msIs114[*rnt-1::GFP + rol-6*]; mIs344[*bro-1::GFP + rol-6*]; wIs51[*scm::GFP + unc-119*+].

RNAi

RNAi bacteria were taken from the RNAi library, streaked out on carbenicillin and tetracycline plates, and then single colonies were grown in the incubator overnight. The bacteria cultures were induced in 2XYT liquid medium for three hours with 1mM IPTG. The bacteria were then seeded on Petri dishes. Gravid adult hermaphrodites were transferred onto the RNAi bacteria until their progeny reached the L4 stage.

Fluorescence microscopy

L4 and adult hermaphrodites tagged with the seam cell-specific marker SCM::GFP were analyzed for their seam cell numbers using the fluorescence microscope.

Statistical analysis

Student's 2-sample t-test statistical analysis was used to test for the significance of the seam cell number among worms with different genetic backgrounds and RNAi treatment.

CHAPTER 3
RESULTS

Inactivation of cell cycle regulator *cul-1* led to moderate seam cell proliferation.

The increase in seam cell number in *cul-1* RNAi was significant. Seam cell number in *cul-1* RNAi increased 14.5 %, 18%, and 14.4 % for *rrf-3,SCM::GFP*, *lin-35, SCM::GFP*, and *rrf-3,rnt-1/bro-1* respectively when comparing with the parent mutants (Table 1).

Inactivation of cell specification gene *elt-5* resulted in a decrease in seam cell number

elt-5 RNAi had a negative effect on the seam cell number for all three double mutants. The decrease in seam cell number was 10.7 %, 15.6 %, and 30.6 % for *rrf-3, SCM::GFP*, *lin-35, SCM::GFP*, and *rrf-3, rnt-1/ bro-1* respectively (Table 1). Particularly, the decrease in seam cell for *rrf-3 ,rnt-1/bro-1* was about twofold of that other two double mutants. The seam cell number of *rrf-3, rnt-1/bro-1* in *elt-5* RNAi was no different than that of the control *rrf-3, SCM::GFP*. This shows that *elt-5* RNAi totally eliminates the effect of RNT-1/BRO-1 on seam cell proliferation. Together with the finding that *elt-5* RNAi decreases most seam cells in *rrf-3, rnt-1/bro-1*, it is most plausible that *elt-5* had a specific negative interaction with *rnt-1/bro-1*.

***pop-1* RNAi gave most seam cells among RNAis**

From all the genes investigated, *pop-1* RNAi gave the highest seam cell count. *rnt-1/bro-1* alone gives a seam cell count that is 40% more than the control. *pop-1* RNAi, however, produced more than 40% of increase in seam cell count: 54.7 % for *rrf-3, SCM::GFP*, 99.0% for *lin-35,SCM::GFP* and 108 % percent of increase for *rrf-3,rnt-1/bro-1* when comparing to respective

parent strains. This increase in seam cell number upon pop-1 RNAi is shown in specific micrographs of the same segment of the worm in Figure 5 and 6.

lin-26 RNAi yielded mixed result in seam cell count

The effect of *lin-26* RNAi was significant for *lin-35, SCM::GFP*, which exhibits 10. 5 % of seam cell increase in *lin-26* RNAi but was insignificant for *rrf-3, SCM::GFP* and *rrf-3,rnt-1/bro-1*. Since *lin-35* by itself does not have an effect on seam cell proliferation, this increase is possibly due to a specific interaction between *lin-35* and *lin-26*.

lin-35 alone does not lead to seam cell increases, but *lin-35* mutation with *lin-26, cul-1*, and *pop-1* RNAi all led to larger seam cell increases

Seam cell numbers in *lin-35* mutants were not statistically different from the control *rrf-3;SCM::GFP*. This lack of an effect on seam cells can be explained by the non-essentiality of *lin-35*'s role in cell cycle regulation (Boxem and Heuvel 2001). In *lin-26, cul-1*, and *pop-1* RNAis, *lin-35* had more seam cell increase that the *rrf-3* control (Table 1). This can be attributed to *lin-35*'s possible interaction with these genes or *lin-35*'s elevated sensitivity to RNAi. A study led by Ben Lehner et al (2006) described *lin-35* as a mutation that has the most sensitivity to RNAi; it increases the efficacy of RNAi even more than *rrf-3* mutants. *lin-35* mutant exhibits more severe phenotypes in RNAi experiments (Lehner et al., 2006). Therefore, the increase in seam cell number observed in *lin-35* could be due to the fact that a greater RNAi effect was observed. Nevertheless, among all the RNAi, *pop-1* has the largest synergistic interaction with *lin-35*, demonstrated by the observation that *lin-35* in *pop-1* RNAi had 44.3 % (99 % - 54.7%) more seam cell number increase than the control in *pop-1* RNAi (Table 1).

pop-1 RNAi and overexpressed *rnt-1/bro-1* gave the most seam cell counts

rnt-/bro-1 in *pop-1* RNAi were the experimental combination that gave the most seam cell counts, averaging 45.8, an 183% increase from the average 15.9 obtained for the control (Figure 4, Table 1). In addition, the standard deviation (±20.3) for overexpressed *rnt-1/bro-1* alone was the highest (Table 1). High standard of deviation indicates that the *pop-1* RNAi effect was variable, and therefore the exact number of seam cell increase cannot be predicted. Nevertheless, from the percent increase (183%), which is bigger than the sum of the percent increases from respective *pop-1* RNAi (54.7%) and *rnt-1/bro-1* (99%) alone, it can be extrapolated that mutating *pop-1* and *rnt-1/bro-1* led to a synergistic effect on seam cell proliferation (Figure 6).

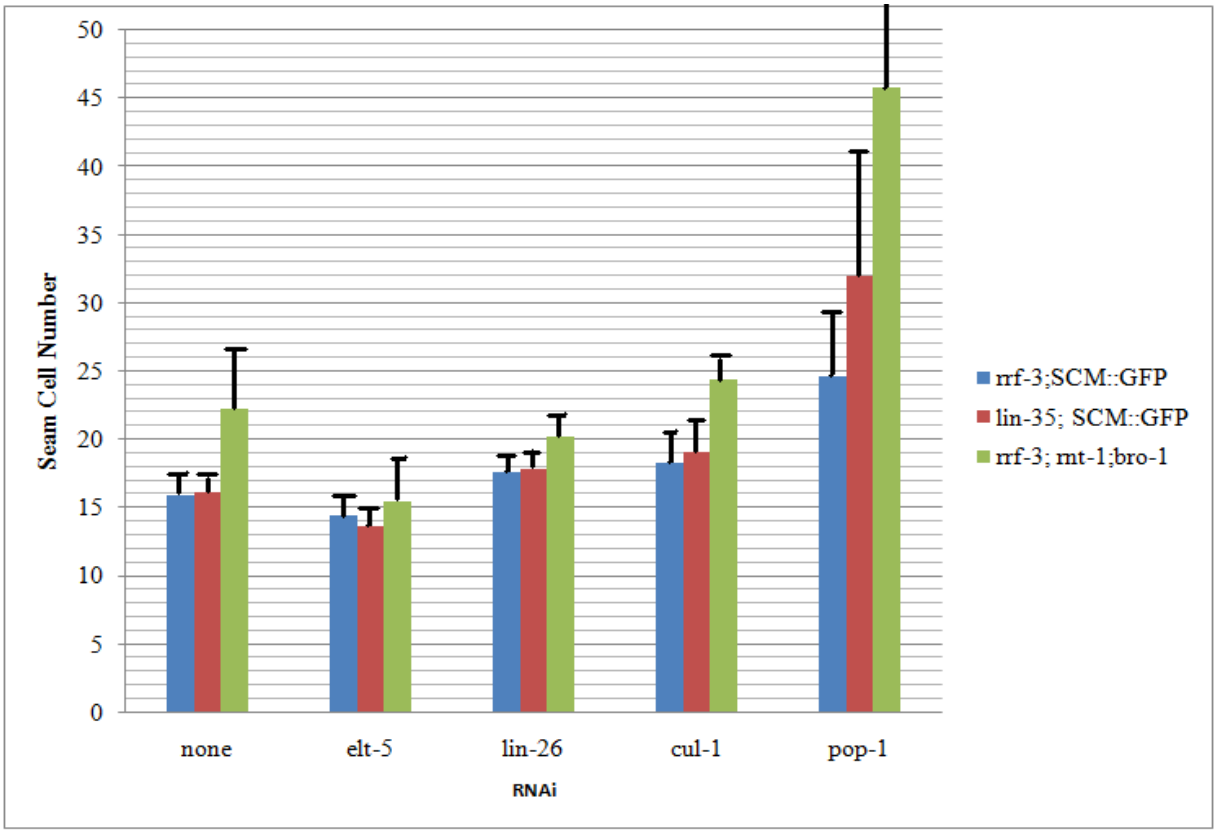

Figure 4. Seam cell number obtained in *rrf-3, SCM::GFP*, *lin-35, SCM::GFP*, *rrf-3*, *rnt-1/bro-1* genetic background and in no RNAi, *elt-5*, *lin-26*, *cul-1*, and *pop-1* RNAi.

Table 1: Descriptive statistics of the seam cell number with gene background in *rrf-3, SCM::GFP, lin-35, SCM::GFP, rrf-3, rnt-1/ bro-1* and in no RNAi, *elt-5, lin-26, cul-1,* and *pop-1* RNAi.

Gene background	RNAi	Mean seam cell number	Percent increase compared to the parent strain	Statistical significance compared to the parent strain	Percent increase compared to control	Significance compared to control
rrf-3, SCM::GFP	*None*	15.9±1.31	Parent	Parent	Control	Control
rrf-3, SCM::GFP	*elt-5*	14.2±1.52	-10.7 %	P=0.038	-10.7%	P=0.038
rrf-3, SCM::GFP	*lin-26*	17.0±1.16	No	No	No	No
rrf-3, SCM::GFP	*cul-1*	18.2±2.20	+ 14.5 %	P=0.014	+ 14.5 %	P=0.014
rrf-3, SCM::GFP	*pop-1*	24.6±4.13	+ 54.7 %	P=0.000	+ 54.7 %	P=0.000
lin-35, SCM::GFP	None	16.1±1.45	Parent	Parent	No	No
lin-35, SCM::GFP	*elt-5*	13.6±1.48	-15.6 %	P=0.003	-14.5%	P=0.006
lin-35, SCM::GFP	*lin-26*	17.8±1.23	+10.5 %	P=0.024	+12.0%	P=0.009
lin-35, SCM::GFP	*cul-1*	19.0±2.25	+18.0%	P=0.003	+19.5%	P=0.002
lin-35, SCM::GFP	*pop-1*	32.0±8.31	+99.0%	P=0.000	+100.1%	P=0.000
rrf-3, rnt-1/bro-1	None	22.2±4.60	Parent	Parent	+40.0 %	P=0.001
rrf-3, rnt-1/bro-1	*elt-5*	15.4±3.24	-30.6 %	P=0.000	No	No
rrf-3, rnt-1/bro-1	*lin-26*	20.1±2.00	No	No	+ 26.4%	P=0.001
rrf-3, rnt-1/bro-1	*cul-1*	25.4±2.09	+ 14.4 %	P = 0.004	+50.0 %	P=0.000
rrf-3, rnt-1/bro-1	*pop-1*	45.8±20.3	+ 108 %	P=0.000	+183 %	P=0.000

*The significance test and percent increase of the seam cell number of each row was done twice, by comparing with the respective parental strains (*rrf-3, SCM::GFP*, *lin-35, SCM:GFP*, and *rrf-3, rnt-1/bro-1*) in no RNAi and with *rrf-3, SCM::GFP* (control).

** In *rrf-3, rnt-1/bro-1* mutant, *rnt-1/bro-1* was overexpressed.

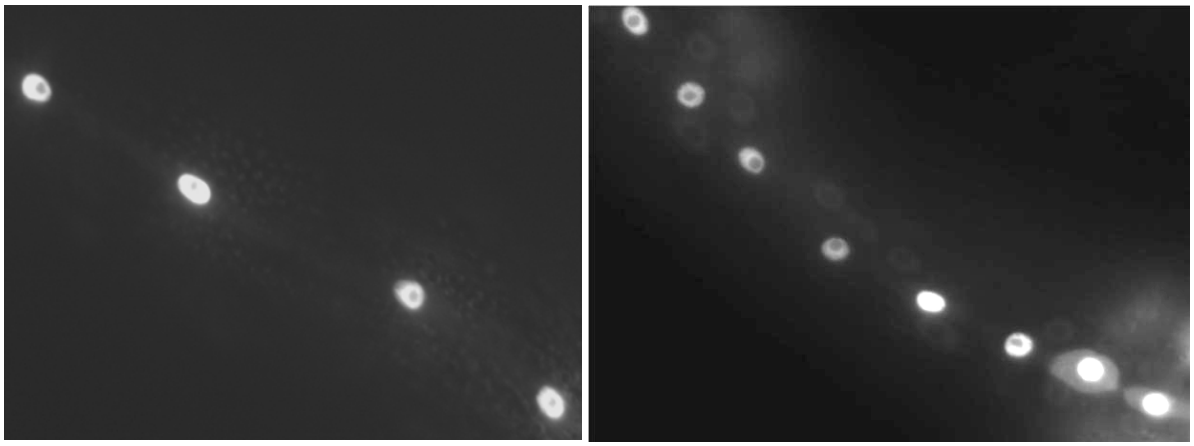

Figure 5: (Left panel) seam cells in L4 *rrf-3, SCM::GFP* animal (control) . (Right panel) seam cells in L4 *rrf-3, SCM::GFP* animal with *pop-1* RNAi.

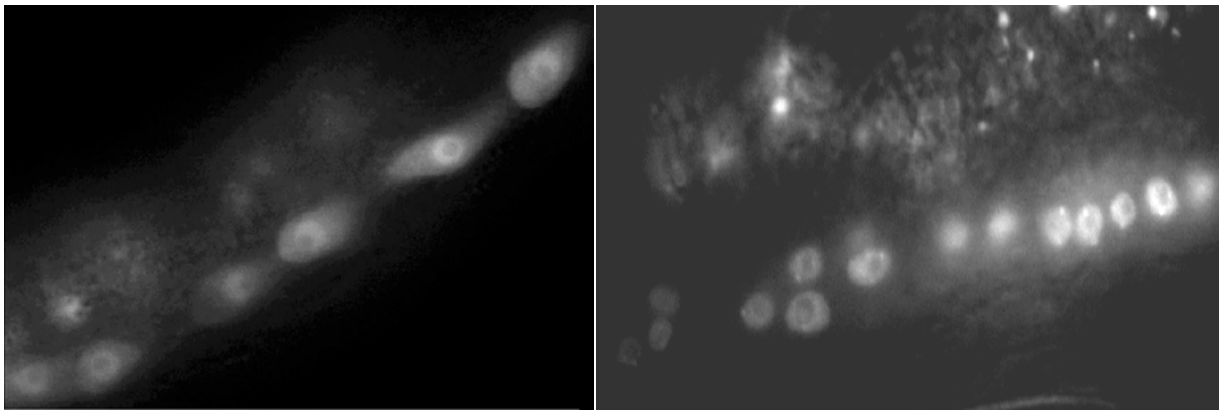

Figure 6: (Left panel) seam cells in a L4 *rrf-3,rnt-1/bro-1* animal. The GFP marker is stained in the nuclei of the seam cells of overexpressed *rnt-1/bro-1*. (Right panel) seam cells in a L4 *rrf-3, rnt-1/bro-1* animal with *pop-1* RNAi

CHAPTER 4
DISCUSSIONS

Mutating seam cell division regulator genes is most effective in achieving seam cell proliferation

pop-1 and *rnt-1/bro-1*, both of which are involved in cell fate determination, have the most positive impact on seam cell proliferation. *pop-1* knockdown had a bigger seam cell increase than overexpressed *rnt-1/bro-1*. When the gene expressions of these two genes were changed together, synergistic seam cell hyperplasia resulted. The discrepancy in the increase in seam cell number reflects their respective regulatory roles in seam cell proliferation. While *rnt-1/bro-1*'s role in promoting seam cell regulation has been described, *pop-1* regulatory mechanism on seam cell division is still largely elusive. Their proposed mechanisms are described in Figure 7. *rnt-1/bro-1* is known to promote the proliferative stem cell divisions. Overexpressed *bro-1* mutants repeated the L2 proliferative stem cell divisions in L3 stage, when the seam cell division in wild type is asymmetric (Kagoshima et al., 2007). It is very likely that proliferative stem divisions continue until the seam cells become terminally differentiated and contributed to the massive seam cell increase. *pop-1* seems to regulate seam cell division differently. Differential localization of pop-1 is required in producing E and MS fates from EMS blastomere divisions (Lin et al., 2002). Therefore, it is plausible to conclude that the loss of *pop-1* protein led to a loss of asymmetry, resulting in both anterior and posterior daughter cells' adoption of symmetrical seam cell fates (Mizumoto and Sawa 2007). This conversion of hypodermal cell into seam cell fate can happen as early as the first seam divisions and can

potentially explain why *pop-1* mutants had more severe seam cell hyperplasia than *rnt-1/bro-1*. The results show that both *pop-1* and *rnt-1/bro-1* are key seam cell regulators; *pop-1*ensures asymmetrical seam cell divisions while *rnt-1/bro-1* promotes proliferative seam cell divisions. Since *pop-1* and *rnt-1/bro-1* seem to have opposite roles in seam cell regulation, they most likely cooperate to balance the processes of seam cell proliferation and differentiation. Research shows that *rnt-1*collaborates with *pop-1* to regulate the division or the polarity of T cells, the most posterior seam cells, in the Wnt signaling pathway (Kagoshima 2007). *pop-1* mutant and overexpressed *rnt-1/bro-1*, therefore, were expected to exhibit largest seam cells increase.

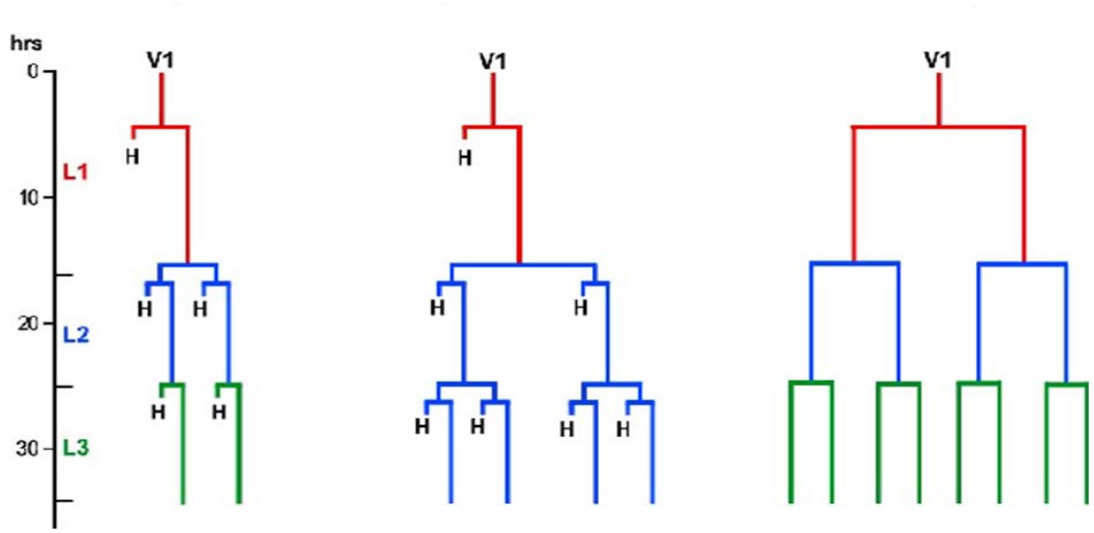

Figure 7: seam cell division patterns. From left to right, wild type, *rnt-1/bro-1* overexpression, *pop-1* RNAi. The divisions for *rnt-1/bro-1* and *pop-1* are proposed.

Negative cell cycle regulators knockdown has modest or no impact on seam cell proliferation

Cell cycle regulators' impact on seam cell proliferation did not show a general and consistent trend in this study. *cul-1* RNAi had a moderate effect on seam cell proliferation, while *lin-35* mutant did not exhibit seam cell increase. Nevertheless, *lin-35* combined with other

RNAis did show more changes in seam cell number than control in RNAis. Two possibilities of this occurrence are: *lin-35* mutant increased RNAi sensitivity; *lin-35* may work with other genes to compound the change in seam cell number. My current data is not sufficient to draw a definite conclusion on which possibility is bona fide or if both possibilities are true.

elt-5 knockdown has detrimental impact on seam cell number

elt-5 RNAi led to a universal decrease in seam cell number; this result is consistent with the role *elt-5* plays in specifying seam cell fate. This result indicates that seam cell specification gene is critical for the generation of seam cells (Hall and Altun 2008).

Knocking down epithelial differentiation factor *lin-26* has no effect on seam cell proliferation.

lin-26 RNAi did not impact seam cell number. Although *lin-26* is required for the differentiation of epithelium, research showed that it does not have the ability to determine cell fate (Quintin et al., 2001). Epidermis tissue identity is specified by *elt-1*, another GATA factor that acts upstream of *lin-26*. Seam cell specification gene *elt-5*, most likely induced from elt-1as well, confers seam cell their identities (Quintin et al., 2001). Therefore, even though the differentiation of hypodermal and seam cells was blocked, their respective cell fates would have become specified or determined regardless of their differentiation states. In other words, even though the hypodermal cells were not properly differentiated in *lin-26* mutants, their fates would remain those of hypodermis. The study led by Labouesse et al. (1996) reported that mutation in the weakest allele of *lin26* (*n156*) resulted in poorly formed hypodermal cells whose cell fates remained the same. Since *lin-26* is unable to specify seam cell fate, the result that *lin-26* knockdown did not exhibit decreased seam cell number was not unexpected. Even though a

seam cell increase was not observed, hypodermal cells might have increased, for *lin-26* knockdown can cause the dedifferentiation of the hypodermal cells. Using only seam cell specific GFP reporter, therefore, is limited in that proliferation of other types of cells cannot be observed. Since cell proliferation is essential for the maintenance of any tissue culture, proliferation of any type of cells can be useful for generating *C. elegans* cell lines.

CHAPTER 5
CONCLUDING REMARKS

Stem cell regulators were found to have the most effect on seam cell proliferation, followed by negative cell cycle regulator gene. *pop-1* knockdown and *rnt-1/bro*-1overexpression in particular offer most potential in seam cell proliferation, due to their ability to convert the fate of hypodermal cells into seam cells. *cul-1* mutants gave a moderate increase in seam cell number. *lin-35* was found to enhance the RNAi effect of above genes. In all, *pop-1*, *cul-1* and *lin-35* can be inactivated together with overexpressed *rnt-1/bro-1* to produce compounded seam cell proliferation beneficial to the maintenance of seam cell cultures. The presence of a *C. elegans* cell line would benefit the research communities tremendously.

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