ABSTRACT

The Effects of the egl-13 Transcription Factor is Linked to the pat-3 β integrin

Richa Manglorkar

Faculty Director: Dr. Myeongwoo Lee

The ability to properly reproduce is essential to any organism, including the hermaphroditic model organism *Caenorhabditis elegans*. In *C. elegans* development, the transcription factor *egl-13*, a homolog of human polycomb related transcription factor, is essential for early uterine cells to maintain their function. The *egl-13* transcription factor allows the uterine cells to attach to the inner muscle layer of the body wall and bind to the vulval epidermis. In this study, we utilized RNAi to knock down the expression of *egl-13*. The RNAi worms displayed the Egl phenotype which includes a buildup of eggs within the uterus since the worm is unable to lay eggs and hatched progeny inside the worm abdominal cavity. The next step of the study involved coupling the *egl-13* RNAi with a line of worm containing a *pat-3 β* integrin defect. Integrins are a family of transmembrane receptors that are involved in many cellular processes, including signal transduction. After performing egl-13 RNAi in a *pat-3 β* integrin mutant background, we noted an enhanced phenotype which was quantified through thrashing assays and counting the number of eggs trapped inside the worm. Furthermore, the *egl-13* RNAi

coupled with the integrin defect led to fewer viable progeny, suggesting that a synergistic event between *egl-13* RNAi and *pat-3* mutant defects caused lethality of animals. Therefore, I conclude that *egl-13* transcription factor can be placed in or parallel to integrin signaling for uterus formation.

APPROVED BY DIRECTOR OF HONORS THESIS:

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Dr. Myeongwoo Lee, Department of Biology

APPROVED BY DIRECTOR OF HONORS PROGRAM:

_____

Dr. Andrew Wisely, Director

DATE: _____

THE EFFECTS OF THE EGL-13 TRANSCRIPTION FACTOR IS LINKED TO THE PAT-3 β INTEGRIN

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By

Richa Manglorkar

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CHAPTER ONE

Introduction

C. elegans Background

Caenorhabditis elegans is a nematode worm and a genetic model organism in the scientific field. The use of *C. elegans* as a model organism first started in the early 1970's when Sydney Brenner used the nematode to isolate mutants (Brenner 1974). Because of factors such as a fully sequenced genome, a finite number of cells, and a short life cycle, the nematode quickly became an ideal *in vivo* model organism (Consortium 1998; Sulston 1977)

Reproduction

The ability to properly reproduce is essential to any organism, including the mostly hermaphroditic *Caenorhabditis elegans*. This means that the majority of individuals in a *C. elegans* population will have both sperm and eggs. *C. elegans* that are hermaphrodites have two X chromosomes, while a small percentage of the population are true males with one X chromosome. Only around 0.1% of a *C. elegans* population is male because they arise from a rare meiotic disjunction of the X chromosome, which gives rise to the XX/XO sex-determining system (Sulston 1977)

Hermaphrodites will first produce male gametes, then female gametes, self-fertilize, and lastly deposit the fertilized eggs inside the uterus. The whole process occurs internally within the hermaphrodite. A wild-type hermaphrodite will have between 10-15 fertilized eggs in the uterus at any one time and will heavily depend on several muscles to

lay the eggs, including the uterus and vulva muscles (Eisenmann 2005). Naturally, if the hermaphrodite is incapable of laying eggs, but still capable of self-fertilization, the eggs would accumulate in the uterus. The wild-type arrangement of eggs inside the uterus is a singular line of eggs, the most mature of which are located in middle of the worm right above the vulva (McCarter 1999). After a worm hatches, it goes through four larval (L1-L4) stages before reaching adulthood (Jorgenson 2002).

egl-13 Background

The proper development of a complex structure, such as the uterus, is highly dependent on molecular elements such as transcription factors. Transcription factors are molecules that are able to enter the nucleus and act as a regulator molecule for the production of mRNA, which is the process known as transcription. These transcription factors bind to high-mobility groups (HMGs), which are molecules that directly interact with DNA, such as transcription and gene regulatory proteins. In *C. elegans* development, the transcription factor *egl-13* also known as *cog-2*, a homolog of human polycomb related transcription factor, plays several roles and has even been linked in partial parallel pathways for neuronal cell fate (Peterson et al. 2013 and Feng et al. 2013). The EGL-13 protein encodes a SOX box domain transcription factor.

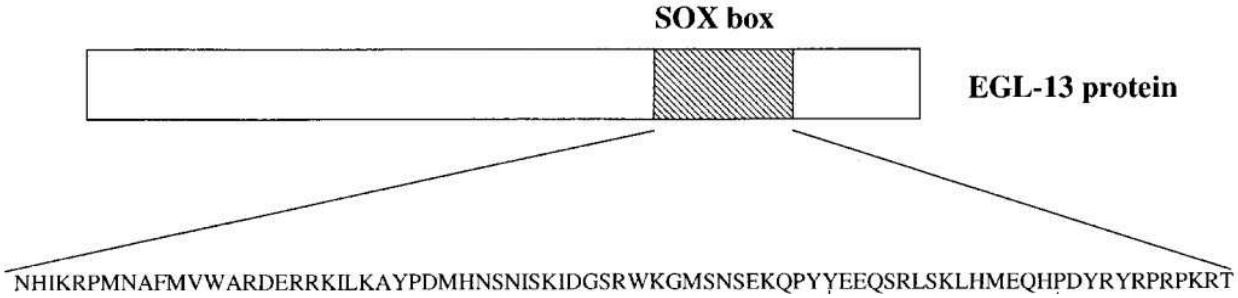

Figure 1—The amino acid sequence for the EGL-13 protein within the SOX box domain. (Cinar et al. 2003)

The SOX domain is found in a group of transcription factors that are at least 50% identical to the HMG box of the Sex-determining Region Y (SRY) gene (Denny et al. 1992). In humans, the SRY gene is located on the Y chromosome and is essential to proper human sex-determination. Although the *egl-13* transcription factor has been linked with neuronal pathway regulation, it was initially discovered through mutant screening for worms with egg-laying defects; hence the transcription factor was given the 'egl' name (Desai 1989). In conjunction with egg-laying defects, *egl-13* has also proven to be essential for precursor uterine cells to maintain their structure and function.

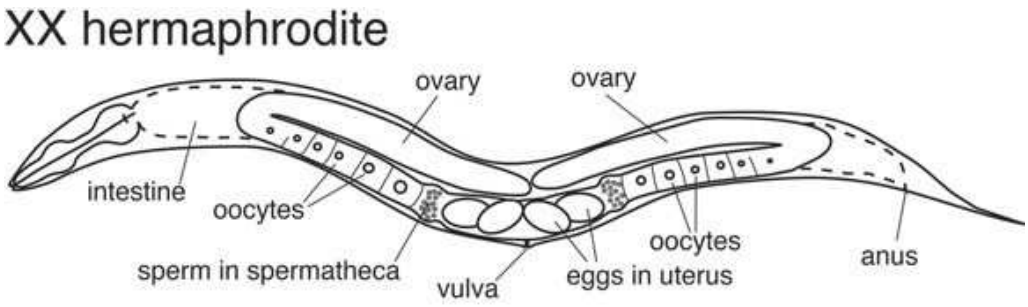

Figure 2—The general reproductive anatomy of a hermaphroditic *C. elegans*. (Zarkower et al. 2006)

Reproductive Anatomy and Development

During early development, the uterus forms inside the nematode, specifically in the hermaphrodite. The uterus must then properly link to the inner body wall and ultimately to the external vulva. In *C. elegans,* the vulva is a structure found on the ventral surface of the animal, half way between the rostral and caudal ends as shown in Figure 2. The vulva is a multifunctional structure and is responsible for enabling tasks such as mating and egg-laying. Therefore correct uterine and vulva development must occur for successful reproduction since the hermaphrodite lays eggs through the vulva opening.

In the developing larva, a group of cells guides the developing uterus to the vulva, which acts as a nexus from the inner body cavity to the external environment. The groups of cells that guide this process have been identified as uterine anchor cells (AC), π cells, and ρ cells (Hanna-Rose 1998). These three groups of cells are precursor cells that give rise to other cell lineages through various processes such as cell-signaling and cell induction.

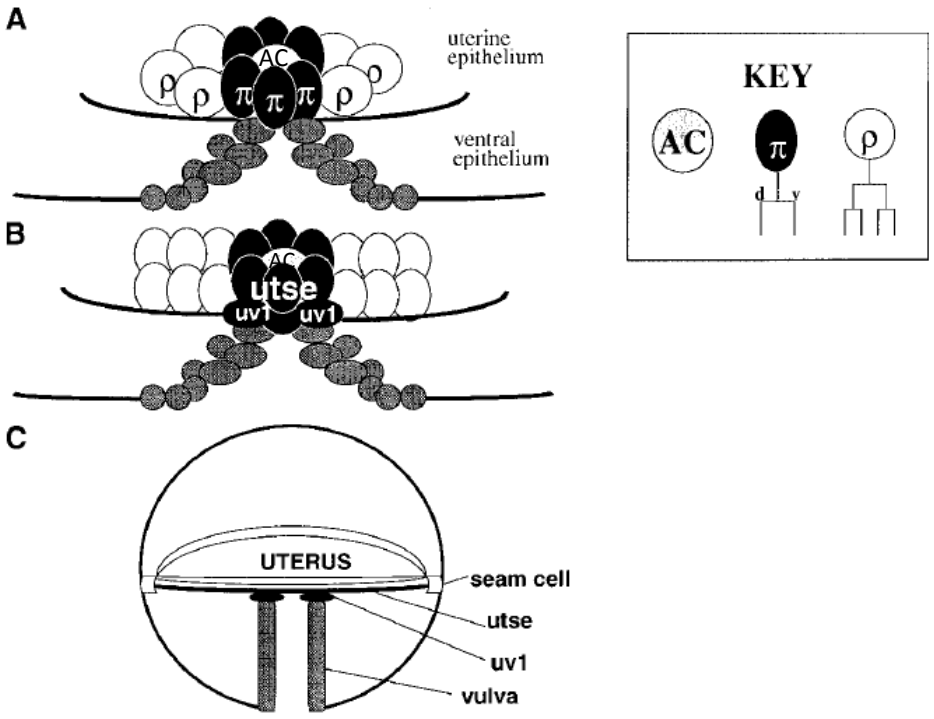

Figure 3—(A) Differentiated π and ρ cells on the uterine epithelium surrounding the anchor cell while touching the ventral epithelium. (B) *utse* and *uv1* cells that have differentiated from their π cell lineage and established a dorsal-ventral axis. (C) End result of developing uterus and vulva in a wild-type worm. π = π cells, ρ = ρ cells, *utse* = uterine seam cell, *uvl* = neurosecretory cell (Cinar 2003 et al.)

Figure 3 above illustrates the location and function of these important precursor cells. Part A of Figure 3 shows that the uterine epithelium is the surface of the uterus that is located inside the abdominal cavity of the nematode. The ventral epithelium is the internal lining of the abdominal cavity. The anchor cell (AC), which is shown surrounded by π cells above, is located on the uterine epithelium. The role of the AC is to initiate cell division in the surrounding π cells through cell induction.

After one round of cell division, the π cells form a dorsal and ventral axis. This first round of division leads to two distinct lines of cells that originated from the π cells

and are composed of large dorsal cells and smaller ventral cells. These smaller ventral cells are labeled *uv1* and are neurosecretory cells between the uterus and the vulva, which can be seen in Part B of Figure 3 (Cinar 2003 et al.) The *uv1* neurosecretory cells are actually neurons that convert electrical neuron impulses into chemical responses. The larger dorsal cells that also arise from the π cell lineage are the *utse* cells, which stand for uterine seam cells. The importance of the anchor cell (AC) lies in the fact that the AC induced the surrounding π cells to undergo mitotic division and initiated differentiation into *uv1* and *utse* cells. Equally important is the fact that after the first round of mitotic division occurs, the AC migrates down, towards the ventral epithelium. The migration of the AC is what stops the *uv1* and *utse* cells from undergoing an addition round of cell division. Therefore, the AC acts as an important regulator that induces π cells to divide and halts cell division after one round of mitosis (Hanna-Rose 1999).

The ρ cells undergo two rounds of cell division unlike the π cells which undergo one. The ρ cells remain an important part of the uterine epithelium after these two rounds of division. Ultimately, the *uv1* cells then interact with the ventral epithelium cells, as depicted in Part B of Figure 3, in order to begin vulva formation (Cinar et al. 2003). This process occurs during the Larval 4 (L4) stage of the worm development, which is the last larval stage before the *C. elegans* reaches adulthood. In adulthood, the uterus has fully developed and attached to the ventral epithelium and vulva as shown in Part C of Figure 3.

Another study observed the uterine-seam formation process in an *egl-13* mutant and compared it to the established wild-type process described above. A study from the Hanna-Rose lab discovered that although the π cells do undergo a normal round of cell

division that establishes a dorsal-ventral axis, in the *egl-13* mutant they undergo an additional round of division that is abnormal compared to the wild-type worm (Hanna-Rose 1999). The additional round of π cell division means that in the *egl-13* mutant, there are twice as many *uv1* and *utse* cells as compared to the wild-type worm as seen in Figure 4. Having twice as many cells also means that the establishment of a dorsal-ventral axis is also impaired. The doubling of the *uv1* and *utse* cell lines and the loss of the dorsal-ventral axis cause the uterus to remain unconnected to the vulva. The vulva structure is still present in *egl-13* mutants; however the uterus was not attached to it. Because of the abnormal division of π cells in the *egl-13* mutant, they determined that proper uterine-vulva development depends on the *egl-13* transcription factor.

A. Wild-Type Uterus Development

B. *egl-13* Mutant Uterus Development

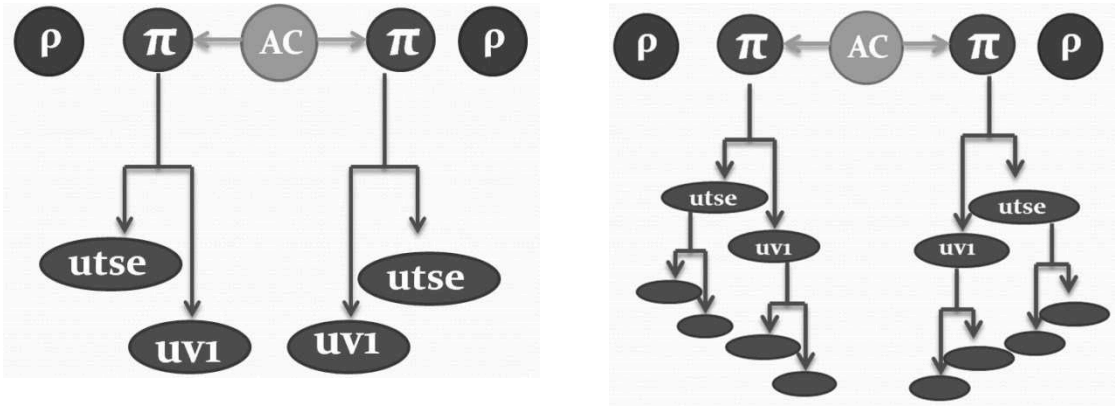

Figure 4—(A) Schematic representation of cell divisions in the wild-type uterus development. (B) Schematic representation of cell divisions in the *egl-13* mutant uterus development

Furthermore, *egl-13* affects only π cells, since the ρ cells divided normally in the *egl-13* mutant. Therefore, it was determined that *egl-13* is necessary specifically for the π cells to fuse with the internal structure of the vulva (Cinar et al. 2003).

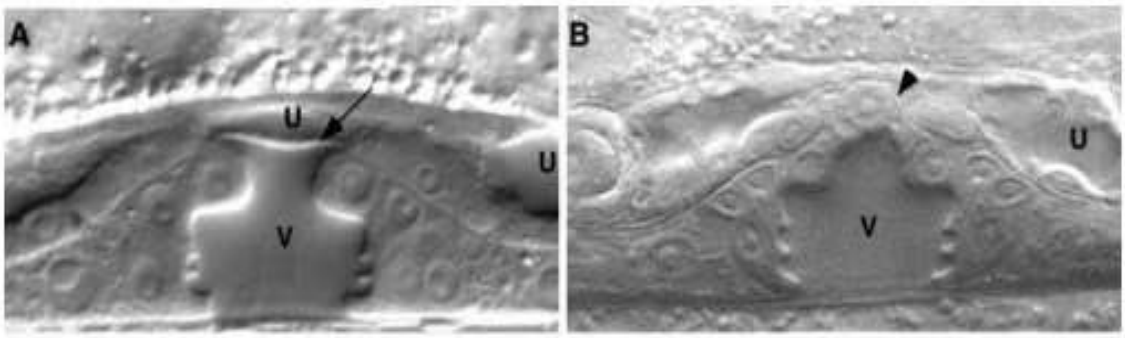

Figure 5—(A) Developing uterus right before the anchor cell becomes visible. (B) Developing uterus when the anchor cell is visible. U = uterine lumen, V = vulva lumen, arrow = vulval apex, arrowhead = anchor cell failing to infiltrate the vulval apex. (Hanna-Rose 1999)

Another study revealed that the AC, indicated above by the arrow in Figure 5, does not fuse with the *utse* cells in *egl-13* mutants (Hanna-Rose 1999). As stated previously, the AC migrates ventrally in order to stop extraneous cell division in the π cell lineage. Therefore, when the AC does not migrate ventrally and fuse with the utse cells, the mitotic cell division for the π cell line no longer has an off switch. Therefore, the lack of AC fusion leads to the improper *utse* cell division which means that the uterus never attaches to the vulva, as it occurs in the *egl-13* mutant. In Figure 5 above, U labels the uterine lumen and V labels the vulva lumen which will never properly connect in a worm displaying the Egl phenotype. The arrowhead above indicates where the AC failed to properly infiltrate the vulval apex in the mutant worm as compared to the wild-type (Hanna-Rose 1999). This study indicated that without the *egl-13* gene present, the AC

does its initial job of inducing the π cells to divide, but after that division, it cannot migrate and fuse with the daughter cells of the π lineage to make the *utse* cells as previously described. The study ultimately concluded that *egl-13* is essential to the final cell fate of the *utse* cells. Also, the study indicated that the *egl-13* gene product is essential not only for the π cells but also for the AC.

Since the *uv1* neurosecretory cells are initially affected by the lack of *egl-13*, this corresponds to the role of *egl-13* in other neurobiological processes (Desai 1989). The SOX box domain is very active neurologically in other animals and as previously mentioned has been shown to play a role in *C. elegans* neuron cell fate development (Wegner 1999).

RNAi

RNAi stands for ribonucleic acid interference. The central dogma of genetics states that DNA gives rise to RNA through the process of transcription and RNA gives rise to protein through the process of translation. The goal of RNAi is to very specifically interrupt this process for a particular gene or protein. In order for RNAi to work, specific RNA molecules will bind to mRNA that expresses the target gene. This binding inhibits the mRNA from translating a protein and knocks down the protein expression in the organism. This method is very useful, because by inhibiting a protein, an individual can then observe any deficiencies the animal may have and therefore learn *in vivo*, the functional role of the gene product. The reason RNAi knocks down genes and does not knock them out, is because RNAi does not fully inhibit all targeted mRNA. Therefore, after performing RNAi, some cells in an organism may express the gene but the majority

of the cells, if RNAi was done correctly, would no longer express the target gene. The specifics of the RNAi protocol is described the methods section.

Integrins

Integrins are receptor molecules located on the surface of the cell and like many proteins, are made up of subunits, specifically named α and β. (Humphries 2000). Integrins structurally contain a ligand-binding site located on the extracellular surface and a cytosolic domain located on the intracellular surface. Integrins are frequently involved in cell signaling via mechanisms such as signal transduction pathways.

Signal transduction pathways are a mechanism of cell-signaling that involves a signaling molecule from the outside of the cell. The signaling molecule, also known as a ligand, must bind to a receptor on the surface of the cell that is specific to a signaling molecule. Once this binding has occurred, in response the receptor begins a cascade of events on the inside of the cell. These transduction cascades can include a large variety of molecules including G-proteins, phospholipases, and kinases. The ultimate goal of signal transduction pathways is to cause an intracellular response to an extracellular signal. One of those responses can be transcription through activation or upregulation of transcription factors.

This study particularly utilizes an integrin mutant called *pat-3 (sp)*. PAT-3 is the β subunit of the integrin receptor that has been altered in this mutant line, specifically through a frameshift mutation that makes the *pat-3 (sp)* line a splice mutant. Compared to an intact β *pat-3* integrin subunit, like the one found in *pat-3 (+), pat-3 (sp)* has an extra 19 amino acids because it has retained part of an intron (Kihira 2012). In *pat-3 (+),* the

intron was properly spliced out of the sequence and therefore does not have extra amino acids in the primary amino acid sequence. A change in the primary amino acid sequence of a protein can greatly change the functionality of the protein.

Study Rationale

As previously discussed above, the *egl-13* transcription factor has been linked to parallel pathways involving neuronal cell fate and is therefore associated with signal transduction pathways. Although the *egl-13* transcription factor has been linked to many other elements, it has never before been associated with *pat-3* β integrin as a potential receptor molecule in a larger signal transduction pathway.

CHAPTER TWO

Materials and Methods

C. elegans

All *C. elegans* were grown on NGM agar plates that were kept at room temperature. The plates were seeded with OP50 *Escherichia coli* as the bacterial lawn on the NGM plates. The *C. elegans* consumed the *E. coli* as their food source. The following strains of *C. elegans* were used in this study:

Name of *C. elegans* Line	Description of *C. elegans* Line
pat-3 (+)	Rescued worm line with properly functioning β subunit in the integrin receptor.
pat-3 (sp)	Splice mutant worm line that contains 19 extra amino acids in the β subunit of the integrin receptor.
N2	Wild-type

Figure 6—Names and descriptions of all the worm lines used in this study.

NGM/RNAi plates

In order to make 1L of solution of NGM add 1L of H_2O, 3.0 grams of NaCl, 17.0 grams of agar, 2.5 grams of peptone by weight to a 2L flask and cover the flask with aluminum foil. Prepare a solution of 1M KH_2PO_4 into a 100ml bottle by adding 13.2ml of 1M KH_2PO_4 solution to 86.6ml of 1M KH_2PO_4 solution. Autoclave both bottles for approximately 60 minutes. Cool the media to 55ºC and add 1.0 ml of 5% cholesterol, 1.0 ml of 1M $CaCl_2$, 1.0 ml of 1M $MgSO_4$, and 25mL of the autoclaved 1M KH_2PO_4

solution. For RNAi plates add 1.0 ml of ampicillin from a 40mg/ml stock and 1.0ml of β-lactose from a 20% stock solution. Pour solution into plates.

Agarose for slide mounts

Dissolve 2.0 grams of agarose by weight into 100ml of DI water. Place the bottle in the microwave until the agarose melts and then store solution at room temperature.

RNAi Protocol

RNA interference was performed using the bacterial feeding method (Fire 1998). RNAi bacteria, specifically *E. coli*, were grown in LB broth medium with ampicillin and incubated (Kamath et al. 2001). The RNAi bacteria were then aliquoted onto an agarose plate to form a bacterial lawn. Around five worms are then transferred onto the plate containing the RNAi bacteria. They were left for 72 hours to lay eggs. At the end of the 72 hours they are taken off the plates, leaving only eggs. The eggs left on the plate hatched and consumed the RNAi bacteria, which knocked out the target gene in the organism.

Microscopy

All worms were observed on a Nikon TE2000-U Diaphot microscope using a slide with an agarose pad and 0.5mM levamisole. All images were taken at a 100 ms time exposure setting and were analyzed using the Metavue imaging software from the Molecular Devices Co.

CHAPTER THREE

Egl Phenotype Characterization and Results

The first stage of this study was to establish baselines for the Egl-13 phenotype. All worms in this study were observed during the same point in time during their adult development.

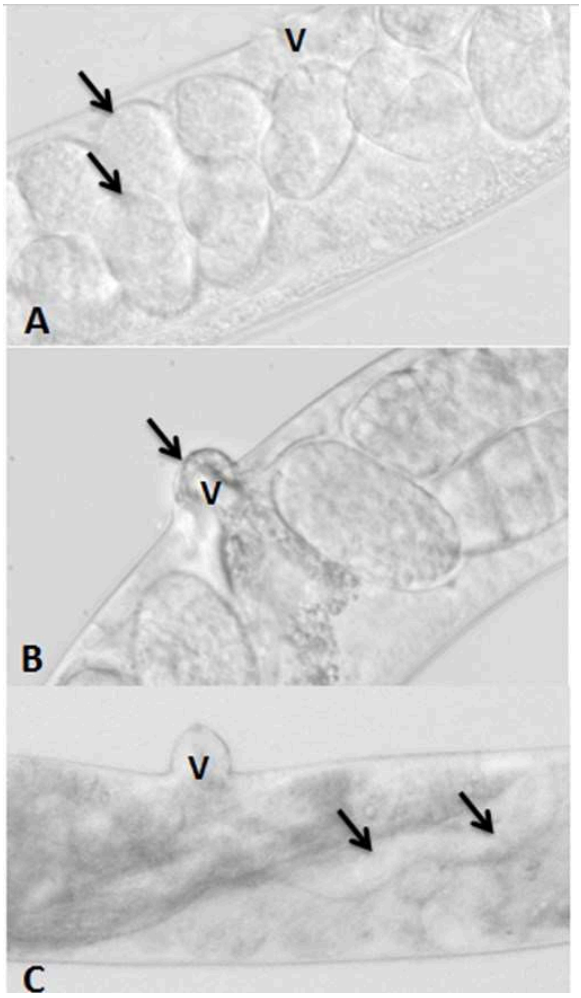

Figure 7—Characterization of Egl-13 phenotype. (A) non-linear egg arrangement and more than 10-15 eggs in the uterus (B) protruding vulva (C) hatched progeny inside uterus. V = vulva, arrows = indicate described phenotype

Figure 7 above provides an overview of the visual characteristics of the Egl-13 phenotype. Throughout the figure above, V, stands as a reference for the position of the vulva and sets up a dorsal-ventral axis for viewing the worm. The arrows in Figure 7A shows two characteristics of the Egl-13 phenotype which are a disorganized, non-linear arrangement of eggs and more than 10-15 eggs in the uterus. In a wild-type worm, fertilized eggs should be positioned linearly above the vulva and only 10-15 eggs should be present in the uterus at any one time.

Another main characteristic of the Egl-13 phenotype is a protruding vulva as can be seen in Figures 7B and 7C. A wild-type vulva is shown in Figure 7A. In order for a worm to be considered to have the Egl-13 phenotype, it does not necessarily need to have the protruding vulva phenotype. For example, the worm shown in Figure 7A is still a worm with the Egl-13 phenotype because of its disorganized egg pattern, even though it does not have a protruding vulva.

The final characteristic shown in Figure 7C is progeny hatching inside the uterus. The arrows in Figure 7C are pointing to the body a hatched worm. This characteristic directly relates to the inability of the hermaphrodite to lay eggs. In a wild-type that is able to properly lay eggs, progeny always hatch outside the uterus. The offspring in an Egl-13 worm continue to grow inside the hermaphrodite until their size and number eventually lead to the death of the hermaphrodite as shown in Figure 8A below. The arrows indicate the offspring that were trapped inside the worm before the vulva ruptured and leaked out the inner contents of the hermaphrodite. Figure 8 below is a representation of the fate for the majority of Egl-13 worms.

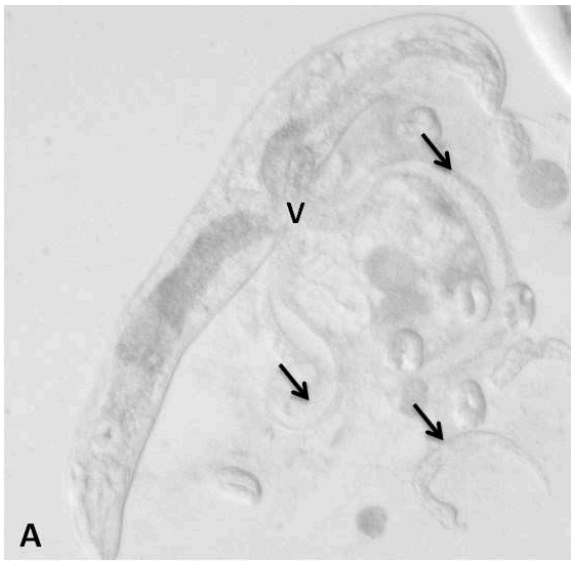

Figure 8—Representation of end-stage result of the Egl-13 phenotype. V = vulva, arrows = progeny that have forcefully exited the uterus.

Other characteristics of the Egl-13 phenotype include bloated bodies and sluggish motor behavior, both of which are direct manifestations of egg retention. All of these characteristics are the baseline phenotypes that were used for the rest of the study.

In Figure 9A below, the column labeled β *pat-3 (+)* represents a line of worm that has an intact β integrin and therefore serves as a wild-type for comparison. As seen in the data, when sixteen worms from a population of β *pat-3 (+)* worms were observed under a light microscope, none exhibit the Egl-13 phenotype. The second column labeled β *pat-3 (+)* with *egl-13* RNAi indicates that this worm line has the same intact β integrin background, but in this group of worms *egl-13* has been knocked down via RNAi. After RNAi has been performed and *egl-13* is no longer expressed, 7/17 *pat-3(+)* animals displayed the Egl-13 Phenotype. These preliminary results help to establish a baseline for comparison to other worm line experiments.

Part A	β *pat-3 (+)*	*egl-13* β *pat-3 (+)*
Number of animals with Egl- phenotype/Randomly selected animals	0/16	7/17

Part B	β *pat-3 (sp)*	*egl-13* β *pat-3 (sp)*
Number of animals with Egl- phenotype/Randomly selected animals	1/16	26/38

Figure 9—(A) Data for the *pat-3 (+)* background with and without *egl-13* RNAi. (B) Data for the *pat-3 (sp)* background with and without *egl-13* RNAi.

The figure below contains Normarski microscope images of two sets of worm populations. Figure 10A is from the *pat-3 (+)* line with no *egl-13* RNAi and therefore represents the wild-type phenotype. The wild-type phenotype can be described in every way opposite to the Egl-13 phenotype: linear egg arrangement, 10-15 eggs in the uterus, no protruding vulva, and there are no progeny hatched inside the uterus. Figure 10B is the *pat-3 (+)* with *egl-13* RNAi. This worm phenotypically looks very different from Figure A and represents all the characteristics of the Egl-13 phenotype. Because both of these worm lines have the *pat-3 (+)* background, they do not have an integrin mutation.

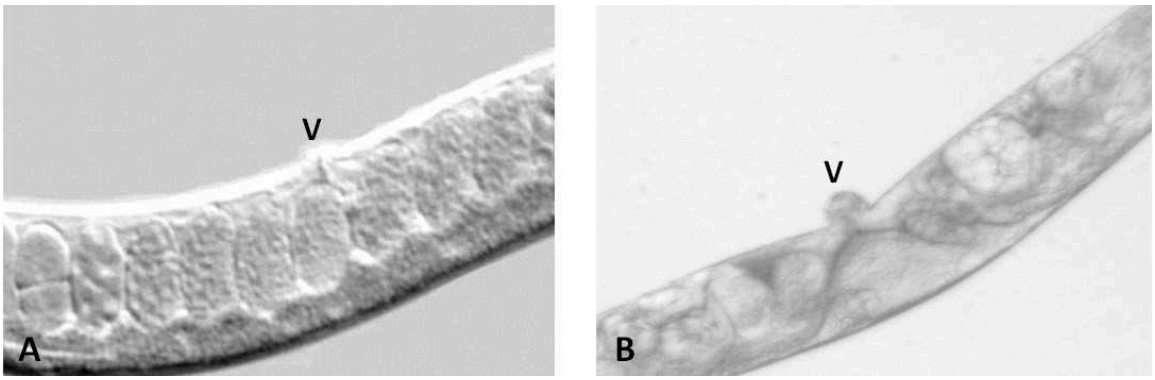

Figure 10—(A) *pat-3 (+)* background without RNAi. (B) *pat-3 (+)* background with *egl-13* RNAi. V = vulva.

The next phase of the study was to compare RNAi results in a different worm line background, specifically a background that did not have a normal β integrin. The cytoplasmic tail sequence region of the β integrin has proven to be important in cell migration. Therefore, altering the functional region of the β integrin would give insight into the function of the integrin. As stated previously, the *'sp'* background is a splice mutant in the integrin tail sequence; it has a blocked integrin splice and has a longer integrin tail. The same analysis was done with the *sp* background.

In Figure 9B above the β *pat-3 (sp)* population of worms represents the mutant background that does not have the wild-type β integrin. Out of sixteen randomly chosen worms, one displayed the Egl-13 phenotype. The one worm out sixteen that did have the Egl-13 phenotype represents an anomaly, since this population of worms had no RNAi and the phenotype could only presumably be accounted for because of a spontaneous mutation. Figure 11A below is an image of the one *pat-3 (sp)* worm out of sixteen *pat-3 (sp)* worms that did not receive RNAi and displayed an idiopathic Egl-13 phenotype.

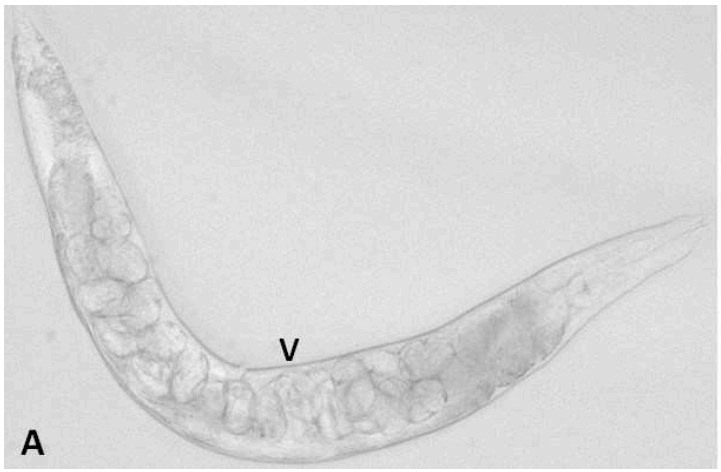

Figure 11—(A) Worm with *pat-3 (sp)* background that expressed the Egl-13 phenotype without *egl-13* RNAi.

Also included Figure 9B is data for the β *pat-3 (sp)* with *egl-13* RNAi population of worms, which means all of these worms have had a knock down of *egl-13*. Out of thirty-eight worms that were randomly selected, twenty-six had the Egl-13 phenotype. Of course, compare to the β *pat-3 (sp)* worms, this increase in Egl-13 phenotype was expected since they had undergone RNAi.

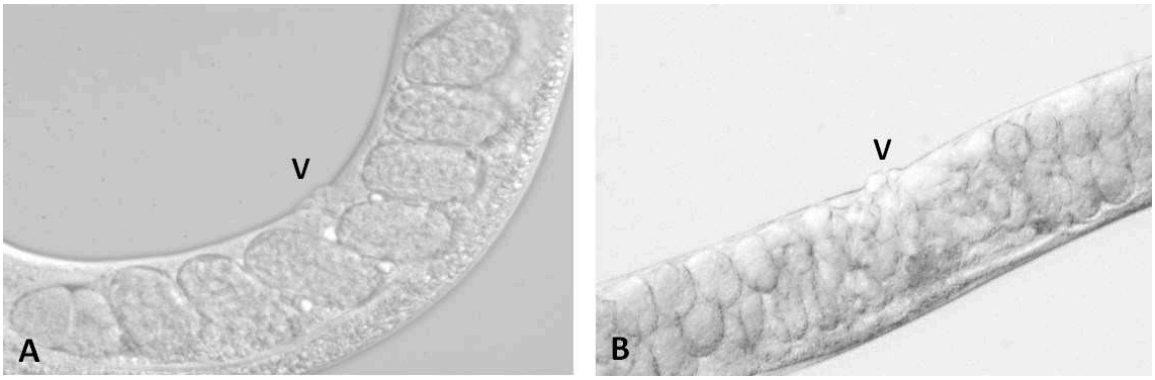

Figure 12—(A) Worm with the *pat-3 (sp)* background without *egl-13* RNAi. (B) Worm with the *pat-3 (sp)* background with *egl-13* RNAi.

Figure 12 above contains Normarski microscope images of two sets of worm populations. There is very clearly a difference between Figure 12A without RNAi and Figure 12B with RNAi both the in *pat-3 (sp)* background. Figure 12A is the β *pat-3 (sp)* that has the normal linear egg arrangement and Figure 12B has the non-linear egg arrangement.

When observed under a light microscope, worms with *egl-13* RNAi appear to be more sluggish and have decreased motor mobility. In order to test this, worms were placed on an agar plate containing water. When a *C. elegans* is placed in liquid, its immediate response is to quickly bend its whole body back and forth. Therefore, the rationale for the thrashing assay was that if worms with *egl-13* RNAi had fewer bends in a given time period when compared to a worm without *egl-13* RNAi, it would signify that *egl-13* RNAi decreases motor capability. Figure 13 shows the results of all the thrashing assays. The n value is the number of animals that were tested in each category and the numbers recorded in each column are the number of bends an organism made in a set 20 second interval.

	N2 n = 11	***egl-13* N2*** n = 14	***pat-3 (+)*** n = 12	***egl-13 pat-3 (+)**** n = 11	***pat-3 (sp)*** n = 5	***egl -13 pat-3 (sp)**** n = 7
	65	18	16	14	52	5
	51	23	64	28	38	14
	50	11	62	36	37	6
	70	25	40	15	29	16
	44	21	36	24	41	8
	62	39	78	16		9
	60	29	76	29		19
	62	31	86	37		
	70	24	56	28		
	72	20	64	26		
	72	32	100	20		
		26	26			
		32				
		40				
Avg.	**61.6**	**26.5**	**58.7**	**24.8**	**39.4**	**11**

Figure 13—Thrashing assays results in each worm background with and without *egl-13* RNAi. The averages of the results are in the bottom row. * indicates statistical significance at a p-value of < 0.001 in the *egl-13* RNAi groups in comparison to their background counterparts.

RNAi results were also quantitatively assessed by counting the number of eggs trapped inside the uterus *egl-13 pat -3 (+)* and *egl-13 pat-3 (sp)* worms. These counts were done under the same conditions temperature conditions and time restraints after hatching. The results of the egg count are shown in Figure 14 below.

egl-13 pat-3 (+)* n = 10	***egl -13 pat-3 (sp)**** n = 10
13	44
15	38
25	37
28	26
26	17
19	47
15	34
20	38
24	19
20	49
20.5	**34.9**

Figure 14—Results from egg counts taken in both the *egl-13 pat-3 (+)* and *egl-13 pat-3 (sp)* backgrounds. Averages of the results are in the bottom rows. * indicates statistical significance in the different values between the two groups at a p-value of < 0.01.

Based on the results of the egg count, the *(sp)* background worms retain more eggs than the *(+)* background worms. This may also support the results of the trashing assay that indicate that the *(sp)* splice mutant background may have an enhanced Egl-13 phenotype than its *(+)* background counterparts.

CHAPTER FOUR

Discussion

An important conclusion drawn from this study is that there is a statistical difference between the quantified results of *egl-13 pat-3 (+)* and *egl-13 pat-3 (sp)*. In Figure 9A, the *egl-13 pat-3 (+)* had seven out of seventeen worms, or about 41% of the worms, with the Egl-13 phenotype. In Figure 9B the *egl-13 pat-3 (sp)* worms, on the other hand, showed that twenty-six out of thirty-eight, or about 68% of the worms, had the Egl-13 phenotype. The increase from 41% to 68% suggests a relationship between the *egl-13* transcription factor and the β integrin. Because the two populations of worm being compared have both had RNAi, the single contributing factor that accounts for the increase in Egl-13 phenotype is therefore a change in the β integrin background. An association between a mutant β integrin and *egl-13* knockdown indicates a synergistic effect since the Egl-13 phenotype becomes more enhanced after both of these factors are combined in a worm population. This would also indicate a signal transduction pathway relationship between an integrin receptor that interacts cytoplasmically in order to signal the downstream *egl-13* transcription factor inside the nucleus of the cell.

Furthermore, the thrashing assays support that the *(sp)* background worms had an enhanced Egl-13 phenotype in comparison to the *(+)* and N2 background. In Figure 13 in an N2 background without *egl-13* RNAi, the worms had an average 61.6 bends per 20 seconds of observation which dropped to 26.5 with RNAi. The decrease in mobility can be seen in each RNAi worm as compared to their respective non-RNAi backgrounds. In

pat-3 (+) the number of bends dropped from 58.7 and to 24.8 and in *pat-3 (sp)* from 39.4 and to 11. Decreased mobility in the *egl-13* RNAi worms can be associated with severity of the Egl-13 phenotype. Overall, the N2 and *(+)* backgrounds had a 57% and 57.8% decrease in mobility respectively. The *(sp)* background had a 72% decrease in mobility which is indicative of a more enhanced Egl-13 phenotype in the integrin splice mutant background. Using an unpaired t-test the N2, *pat-3 (+),* and *pat-3 (sp)* backgrounds all showed a statistically significant decrease in mobility after RNAi at a p-value of < 0.001.

As state previously, a wild-type worm has between 10-15 eggs in the uterus at any given time. The results in Figure 14 indicate a 70% higher egg retention rate in the *egl-13 pat-3 (sp)* worms in comparison to the *egl-13 pat-3 (+)* worms. Again, these results suggest an enhanced Egl-13 phenotype in the *pat-3 (sp)* background. Using an unpaired t-test, a comparison between the egg counts in the *egl-13 pat-3 (+)* and *egl-13 pat-3 (sp)* groups indicates statistically significance in the different values between the two groups at a p-value of < 0.01.

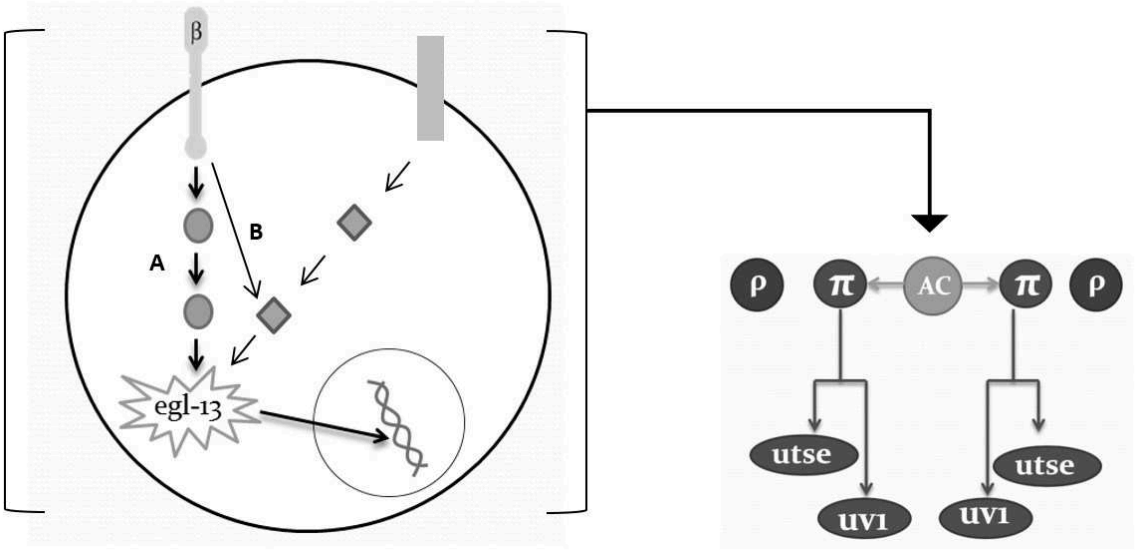

Figure 15—Summary figure of conclusion in this study.

Figure 15 is a visual representation of the results of this study. The β PAT-3 integrin, which is imbedded in the cell membrane is the receptor for a signal transduction pathway that includes the transcription factor *egl-13*. In Figure 15 '**A**' represents the possibility of a direct signal transduction pathway between the β PAT-3 and the *egl-13* transcription factor and '**B**' represents a parallel pathway the involves the β integrin. In both scenarios, once *egl-13* is activated, it enters the nucleus and promotes the transcription of mRNA that will turn into a protein product. The protein products associated with the *egl-13* transcription factor are then essential to the proper cell development of the anchor cell and π cell lineage for uterus-vulva development. In the absence of the *egl-13* transcription factor as well as a mutation of the β PAT-3 integrin, there is a synergistc negative effect on proper uterine cell differentiation. Therefore, I conclude that *egl-13* transcription factor can be placed in or parallel to integrin signaling for uterus formation.

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