

ANAL DEPRESSOR MUSCLE REMODELING IN *C. ELEGANS*

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Anal Depressor Muscle Remodeling in *C. elegans*

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Male *Caenorhabditis elegans* undergo a radical remodeling of their anal depressor during late larval stage 4. In both hermaphrodites and males, the larval anal depressor primarily functions as a defecation behavior. However, the muscle's sarcomere reorganizes itself to facilitate copulation only in the adult males (CHEN AND RENÉ GARCÍA 2015). In succinate dehydrogenase (*sdha-1*) mutants, the post embryonic sex muscle has been found to mature inappropriately; this is especially seen in males. In addition to the abnormal sex muscles, we discovered that the sex-common anal depressor muscle also remodels inappropriately in the male. The cause of the abnormal development of these muscles remains unknown; however our findings has led us to hypothesize that the *sdha-1* encoded enzyme is required for cell-cell communication between the developing anal depressor and its neighboring signaling partners. To address which cells are involved in signaling for the developmental muscle remodeling to take place, further study of the *sdha-1* mutation is needed to determine how it may be affecting the Wnt/ β -Catenin Signaling Pathway transduction. In addition, we hope to uncover which cell signals are initiating the abnormal sculpting of the anal depressor during development.

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NOMENCLATURE

ATP	Adenosine Tri-Phosphate
FAD	Flavin Adenine Dinucleotide
FADH ₂	Flavin Adenine Dinucleotide
<i>Sdha-1</i>	Succinate Dehydrogenase complex subunit A
TCA	Tricarboxylic Acid

CHAPTER I

INTRODUCTION

The succinate dehydrogenase enzyme is encoded by the *sdha-1* gene. This enzyme is critical for the TCA cycle and part of the mitochondrial electron transport system. Succinate dehydrogenase catalyzes the oxidation of succinate, to fumarate, and the reduction of FAD to FADH₂. These molecules are key components of the TCA cycle, which eventually forms ATP. Loss of function mutations of the succinate dehydrogenase gene have been found to hinder some aspect of metabolism in *C. elegans*. To better understand how the anal depressor remodeling occurs, mutations that blocks the proper anal depressor development at different points in development have been identified by our lab. One of these mutations has been found to affect the mitochondrial TCA pathway enzyme succinate dehydrogenase.

Mammalian Development

Specification of sexual dimorphic structures in different species evolve due to instructions received from several developmental mechanisms. During early fetal development in mammals, primary sex determination is strictly chromosomal. The undifferentiated tissue result in the development of two different types of gonads (testes or ovaries), which are dependent on the X and Y chromosomes. The male has the ability to generate sperm bearing either the X or Y chromosome, if the X chromosome from the sperm fuses with the egg, the individual will form eggs, and will be phenotypically identified as a female (ARNOLD 2017). Similarly, when an egg receives a Y chromosome from the sperm, the individual becomes XY male, and the testes form. Perturbation of the following pathways have been found to lead to abnormal genitalia

development: hormone independent, hormone dependent, and endocrine (BLASCHKO *et al.* 2012). In rare cases, the formation of atypical genitalia may occur due to problems associated with the SRY gene, adrenal gland abnormality, or high levels of male hormones in the placenta due to a hormone-producing tumor. Several signaling molecules found in the WNT and FGF signaling pathways have been found to be active in the genital tubercle, but their exact function is still not fully understood (CARLSON 2013).

Caenorhabditis *elegans* Development

C. elegans exist as two distinctly different sexes, hermaphrodite and male. Changes in cell lineage patterns and cell differentiation that occurs during the nematodes development results in the sexual dimorphism found between the two (CHEN AND RENÉ GARCÍA 2015). The male and hermaphrodite fate for somatic cells has been found to be determined cell autonomously by the TRA-1 gene. This gene functions as a transcription factor, and thus promotes hermaphrodite development and repression of male development once it has entered the nucleus (HUNTER AND WOOD 1990). If we turn our focus to the tail region of the male and hermaphrodite, similar functions in the tapered like tail is found prior to reaching larval stage 4. However, once larval stage 4 is reached, the tail region begins to undergo a radical remodeling, resulting in the key differences seen in the male and hermaphrodite tail during development. Located in the tail region, a muscle known as the anal depressor is found, and functions as a defecation behavior in larval males and hermaphrodites. The muscle's sarcomere later reorganizes itself to facilitate copulation only in the adult males (CHEN AND RENÉ GARCÍA 2015).

Anal Depressor Remodeling

The anal depressor is a muscle that functions as a defecation behavior in males and hermaphrodites. Once the males become an adult, the muscle facilitates copulation. The anal depressor can be found running vertically between the dorsal hypodermis and the dorsal wall of the rectum, located directly above the anus in the tail region. A single-sarcomere, H-shaped contracting muscle makes up the anal depressor. The contraction of this muscle allows the initial stages of defecation to take place. Once the muscle relaxes, the contents that previously filled the rectum during contraction becomes expelled (THOMAS 1990; ALAN F. BIRD 1991). In normal wild-type worms, during late-larval stage three, initiation of remodeling is believed to be dependent on signaling from the left and right dorsal protractor muscles. Previous findings in our laboratory has led us to suspect that WNT signaling is occurring via two genes: EGL-20 and *lin-44*. Signals sent from these cells are believed to result in the stimulation of the anal depressor, instructing the muscle to undergo the initial stages of development. During late-larval stage 4, a radical remodeling of the anal depressor takes place, resulting in the alteration of the ventral attachment beginning at the rectum to the dorsal spicule protractor muscles (JARRELL 2012). The remodeling of this muscle leads to the development of the copulatory spicule in males, whose function is to lock onto the hermaphrodite's vulva and aid in sperm transfer (GARCIA *et al.* 2001). The goal of this project is to identify whether the *sdha-1* gene is required in the signal transmitting cells, or in the signal receiving cell.

CHAPTER II

METHODS

In order to determine if the *sdha-1* gene is required cell autonomously or non-autonomously in the anal depressor, we will first determine if it is possible to reverse the anal depressor developmental defect site. To determine the cellular role that *sdha-1* has on the remodeling of the anal depressor, the *aex-2* and *unc-103* promoter, along with destination vector pJG30 will be used to drive *sdha-1* rescue. All cloning, ovary injections, and rescue experiment was conducted under the supervision and assistance of Dr. Luis René García and Dr. Brigitte LeBoeuf.

Plasmid Construction

By utilizing the LR reaction protocol, two plasmids (pCK1 and pCK2) were constructed and will later be used to conduct a rescue experiment. The first plasmid contains entry vector pXC108 (*lin-44* promoter) and destination vector pJG30 Gateway recombination site: loxP:*sdha-1 (+)::sl2::CFP::loxp*. To construct our second plasmid, entry vector pBL348 (*aex-2* promoter) and destination vector pJG30 Gateway recombination site: loxP:*sdha-1 (+)::sl2::CFP::loxp* was used.

In order to construct the two plasmids, the following protocol was followed. The pBL348 (*aex-2* promoter) was first diluted by mixing 1 μ L of water to 1 μ L of pBL348 in a microcentrifuge tube. Two microliters of destination vector pJG30 and 5 μ L of sterile water was then added to the microcentrifuge tube and mixed. The LR Clonase II enzyme was then thawed

on ice for approximately one minute and 2 μL was added to the tube to catalyze in *vitro* recombination between the entry and destination vector pJG30. The reaction was then incubated at 25°C for one hour. In order to terminate the reaction, 1 μL of Proteinase K was added and the sample was incubated at 37°C for 10 minutes. The above steps were repeated with pXC108 (lin-44 promoter) allowing for the creation of two plasmids, pCK1 and pCK2. By using the sequence files of pBL348, pXC108, and pJG30, the recombination sequence was constructed and enzymes were selected based on where they would cut the sequence.

A transformation reaction was then conducted with the following protocol. A tube of 50 μL of Transformation Competent DH5alpha was removed from the -80 degree freezer and thawed on ice. Ten microliters of each LR reaction was then added to 50 μL of the Transformation Competent DH5alpha and incubated on ice for 30 minutes. The cells were then heat-shocked by incubating at 42°C on a hot block for a total of one minute. A volume of 500 μL of S.O.C Medium was then added to the cells and the reaction was transferred to a screw top 15 ml conical tube and incubated for one hour with shaking at 37°C. The cells were then transferred to a 1.5 ml microfuge tube and spun down for 2 minutes at maximum speed. A total of 400 μL of liquid was then removed and the remaining volume was suspended. The suspended cells were then added to a 20 ml agar plate containing 100 $\mu\text{g}/\text{ml}$ of ampicillin and incubated over night at 37°C.

Using a sterile pipette tip, a pure colony was picked and transferred into 3 ml of liquid LB + 100 $\mu\text{g}/\text{ml}$ ampicillin and incubated overnight. The Wizard Plus SV Miniprep DNA Purification System was then used to harvest DNA from the bacteria. A volume of 1000 μL of

the sample was added into the microcentrifuge tube and spun down at 14,000 x g for three minutes. The supernatant was then removed and a volume of 250 μ L of cell resuspension solution was added to make the cells more fragile allowing cell lysis to take place. A volume of 250 μ L of cell lysis solution was then added and the tube was inverted five times by hand. Alkaline Protease Solution was then added and the tube was inverted four time and incubated for five minutes. The bacterial lysate was then centrifuged at max speed for 10 minutes at room temperature. The cleared lysate was then transferred to the prepared spin column by decanting. We then centrifuged the supernatant at max speed for one minute and removed the spin column to discard the flow through from the collection tube. A total of 750 μ L of column wash solution was added to the spin column and centrifuged at max speed for one minute; the flow through was discarded once again. The wash procedure was repeated by using 250 μ L of column wash solution and centrifuged at max speed for two minutes. The spin column was then transferred to a 1.5 ml microcentrifuge tube and 50 μ L of nuclease free water was added and spun at 5,000 rpm for three minutes. After the DNA had been eluted, the spin column was removed and discarded and the plasmid was stored at -20°C to prevent them from deteriorating.

By using the sequence files of pBL348, pXC108, and pJG30, the sequence of the recombination after the LR reaction was constructed and enzymes were selected based on their ability to cut the sequence. The NdeI and PstI enzymes were selected based on their ability to digest each plasmid. After running the 20 μ L reaction on a 0.8% 100 ml agarose gel in 1X TAE buffer, it was concluded that the cloning process was done correctly due to the close resemblance between the expected and realistic bands.

We were able to use the protocol provided by the Midi Prep Qiagen kit to obtain a larger supply of pCK1 and pCK2. The following steps were followed for each plasmid. A total volume of 40 mL of bacterial cells was added and centrifuged at 5000 rpm for five minutes. The resuspension buffer was then added (4 ml) to the cells and vortexed. This step was followed by an addition of 4 ml of the lysis buffer and the mixture was inverted five times and incubated at room temperature for five minutes. We then added 4 ml of the chilled neutralization buffer and inverted the mixture 5 times to make sure the contents were mixed. The reaction was then incubated on ice for 15 minutes. After the incubation time was over, the reaction was centrifuged at 10,000 rpm for 10 minutes at 4°C and the supernatant was removed. We then equilibrated a Qiagen tip 100 by adding 4 ml of the Buffer QBT. We then waited for the column to completely empty and then applied the supernatant to the Qiagen tip and allowed it to enter the resin by allowing gravity to take its course. The tip was then washed with the 2X 10 ml wash buffer. A conical tube was then added under the Qiagen tip prior to eluting the DNA with 5 ml of the elution buffer. After the elution buffer was added, the DNA was precipitated by adding 3.5 ml of the isopropanol to the eluted DNA. The contents were then transferred into microcentrifuge tubes and spun at max speed for 10 minutes. The liquid was then removed and the contents were resuspended in 200 μ L of injection water. 20 μ L of sodium acetate and 660 μ L of 200% ethanol was then added and the contents were spun at max speed for 5 minutes. The supernatant was then removed and 200% ethanol was added and later removed and the tubes were left to dry. A total of 100 μ L of injection water was added and each plasmid was mixed prior to checking the concentration of each.

Genetic Crosses

The day before our genetic crosses, six L4 male *pha-1* (e2123); *him-5* (e1490) *rgEx*[Paex-2::YFP] and six L4 hermaphrodite *pha-1* (e2123); *sdha-1* (rg448); *him-5* (e1490) were isolated and plated on separate NGM plates containing a lawn of 10 μ L of *E. coli* OP50. The L4 males and hermaphrodites are then grown at 20°C for 24 hours, allowing the worms to become adults prior to being introduced into new plates. It is important to pick the L4 worms 24 hours prior to conducting the cross to insure that the males are young and able to mate and the hermaphrodites are still virgins. Once the 24 hour time frame has passed and the worms are considered adults, the *pha-1* males and the *sdha-1* hermaphrodites were picked to a new NGM plate containing OP50. Thirty-six hours later, the slow growing *sdha-1* wildtype hermaphrodites were picked and individually plated on a new NGM plate containing 10 μ L of OP50. In the second generation, the slow growing hermaphrodites, *sdha-1*, were picked and moved to a new NGM plate where they could self-fertilize and be maintained. The working stock *sdha-1;pha-1;him-5* *rgEx*[Paex-2::YFP] worms were then starved and the L1-L2 freshly starved worms were frozen and kept at -80°C for future experiments.

Imaging

In order to image the anal depressor, a confocal microscope was used to capture the development during different developmental stages. Agar pads (2%) were first made by adding 1mL of S-basal solution to a small glass tube and 0.02 g of nobal agar. The mixture was then placed on the 100°C plate to melt the mixture. A total of 50 μ L of sodium azide was added to the mixture to anesthetize the worms. Two separate spacers were made using two layers of adhesive tape which were placed on a glass slide. A glass slide was then added in between the two spacers

and 70 μ L of the agar was to the center. An additional glass slide was placed horizontally to flatten the agar into a thin layer for imaging. A small glass pipette was placed under a flame to soften the center of the capillary tube and create two needles. These needles were used as a glass pipette allowing the L4 and adult worms to be transferred with ease. After the worms had been in the agar for 30 seconds, a cover slip was added to prevent the worms from laying its back. The slide was then flipped upside down and a sharpie was used to mark where each worm was located. Before placing the worms under the microscope, oil was added on top of the cover slip for better illusion.

With the assistance of a confocal microscope, the fine details of the anal depressor was captured, allowing the abnormal development of the anal depressor to be further studied. Once the MetaMorph program was running, the devices, configure illumination, and multi dimensional acquisition screens were opened. In the configure illumination screen, the view was set to “observe DIC”, allowing the worms to be found using the 10X and later the 40X objective lens. Once the anal depressor of the worm being imaged was located using the 40X, the view was switched to the confocal DIC and the lever was pulled out to make sure the camera could image the worm. After centering the worm on live view, the view was then switched to the confocal 515 allowing the YFP at the anal depressor to be viewed. To avoid photobleaching, it was important to make sure the laser intensity was at \sim 50 to start with. The intensity was slowly adjusted for each worm if needed. On the main tab of the multi dimensional acquisition screen, Z series, and multi wavelength was selected. The images were saved to the desktop and later quantified. For the wavelength tab, the first wavelength was set to confocal DIC and the second to confocal 515, allowing separate stacks to be taken. To determine where the Z series stacks

would start and end, the fine adjustment knob was used to move from one lateral side of the tail of the worm to the other. Once the boundaries had been set, the acquire button was pressed and approximately 50 stacks was taken.

Quantifying Confocal Images

In order to quantify how phenotypically defective the anal depressor of the *sdha-1* worms were, silhouette images were made. Each 40X confocal image was opened so a 3D reconstruction image could be made. These images were then saved and later opened in Corel Photo Paint where they were converted to RGB. The brightness of each image was then altered as needed and the color was replaced for better visualization. The image was then copied and pasted into Corel Draw where the anal depressor was traced and saved. Measurements of the dorsal width, the connection between the dorsal and ventral sides, and the adult sarcomere was measured along with the dorsal width, H zone, and the ventral zone of the L4 worms using MetaMorph. To quantify our results, Prism 7 was used to plot our data and a Mann-Whitney Test was conducted to calculate the p-value in the adult and L4 worms.

CHAPTER III

RESULTS

After analyzing the *sdha-1; pha-1; him-5* rgEx[Paex:YFP] male worms, it was observed that the tail region looked different from the wild-type *pha-1; him-5* rgEx[Paex:YFP] worms. Due to the difference observed, we wanted to determine what was causing the tail region to look phenotypically different from one another. To do so, different regions of the tail was analyzed and a radical difference in the remodeling of the post embryonic sex muscle called the anal depressor was found. Due to the inappropriate remodeling of the anal depressor, identification of when the abnormal development started to take place was needed. Measurements of the dorsal width, dorsal and ventral connection, H zone, and sarcomere of the *sdha-1 and pha-1* worms was taken during different developmental stages. By doing so, we are better able to determine during which developmental stage the muscle development displayed the largest growth dynamic from that seen in wild-type *pha-1; him-5* rgEx[Paex:YFP].

Silhouette Images of the Adult Worms

In order to determine when the abnormal remodeling of the anal depressor in *sdha-1* mutants was most severe, the confocal microscope was used. The confocal microscope was used to acquire a series of z stacks from one lateral side of the tail of the worm to the other. Silhouette images was then created by outlining the anal depressor in Corel DRAW. The measurement of each anal depressor was then taken using the MetaMorph imaging program. A wide range of phenotypes was discovered when analyzing the anal depressor of the *sdha-1; pha-1; him-5* rgEx[Paex:YFP] adult males.

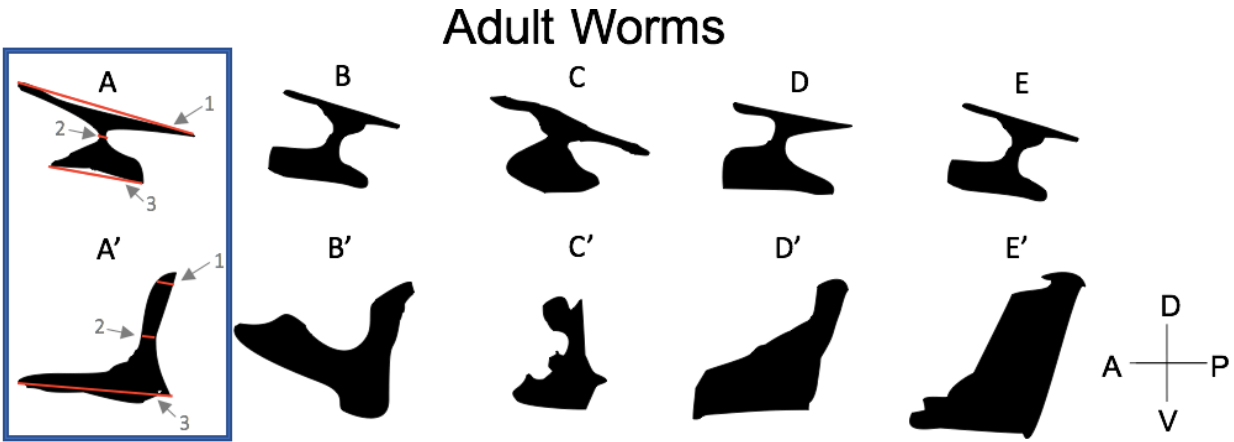


Fig. 1. Silhouette images of the lateral view of the anal depressor in adult worms. Silhouette images A-E are representative of a wild-type anal depressor. The *pha-1:him-5* rgEx[Paex:YFP] worms serve as our control throughout the experiment. A wide range of phenotypes was found when analyzing the *sdha-1:pha-1:him-5* rgEx[Paex:YFP] (A'-E') anal depressor; therefore, measurements of the anal depressor was taken at three different locations (A1-3, A'1-3).

The wild-type anal depressor (Fig. 1A-E) maintained a constant phenotype with very little variation from one another in the 30 worms that were studied. However, when the *sdha-1* (Fig. 1A'-E') worms were analyzed, the anal depressor showed very little resemblance to that previously seen in the wild-type worms. The dorsal width (Fig. 1A'-1), connection between the dorsal and ventral sides (Fig. 1A'-2), and the sarcomere (Fig. 1A'-3) all appeared to substantially deviate from that of the wild-type anal depressor. Due to the large difference in size and shape of the anal depressor of the *sdha-1* in comparison to the *pha-1* worms, measurements of each of the three regions were taken. The measurements were taken of the width of the dorsal region (Fig. 1 Image A1, and A'1), the connection between the dorsal and ventral sides (Fig. 1 Image A2, and A'2), and length of the sarcomere (Fig. 1 Image A3, and A'3) found in the adult worms. These

measurements would allow for the identification of when the abnormal remodeling of the anal depressor starts to take place.

Silhouette Images of the L4 Worms

Due to the inappropriate remodeling found in the adult anal depressor, additional images of the anal depressor was taken during larval stage four to determine when the abnormal remodeling was taking place. The silhouette images were then analyzed and later quantified to determine when and how significant the inappropriate remodeling of the anal depressor was.

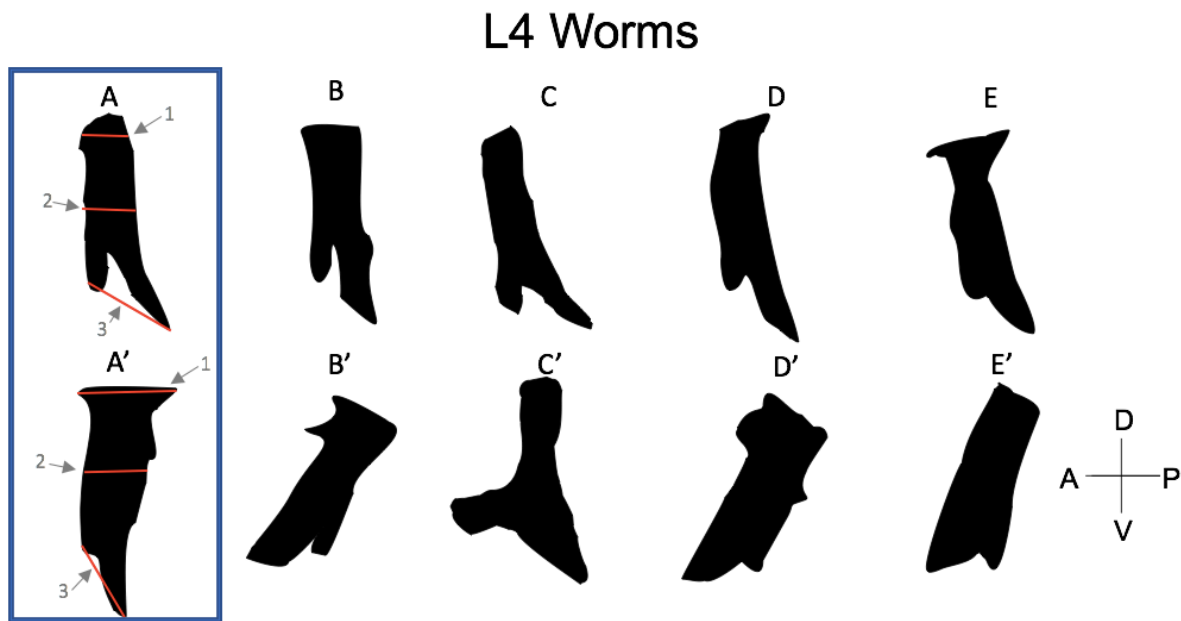


Fig. 2. Silhouettes of the lateral view of the anal depressor in L4 male *pha-1* and *sdha-1* worms. Silhouette images A-E serve as the control *pha-1* wild-type worms and will be quantified for statistical analysis. A narrow range in phenotype was found when analyzing the *sdha-1:pha-1:him-5* rgEx[Paex:YFP] (A'-E') anal depressor. Measurements of the anal depressor were taken in three different locations (A1-3, A'1-3) in each worm that was analyzed.

When comparing the L4 wild-type anal depressor (Fig. 2 Image A1-E) and *sdha-1* mutants (Fig. 2 Image A'1-E) with that of the adult (Fig. 1 Image A1-E, A'1-E), it was found that the slit present in the L4 worms disappeared in the transition from L4 to adults in the *sdha-1* worms. Due to the phenotypic differences found when comparing the *sdha-1* with our control, measurements were taken at three different locations, the width of the dorsal region (Fig. 2 Image A1, and A'1), the H zone (Fig. 2 Image A2, and A'2), and the ventral attachment (Fig. 2 Image A3, and A'3) of the anal depressor. The difference in size and shape discovered when comparing the *sdha-1* silhouettes with that of *pha-1*, indicates that abnormal remodeling of the anal depressor is occurring.

Width of the Dorsal Region in Adult and L4 Male Worms

To determine where abnormal remodeling of the anal depressor was taking place, images of the anal depressor was taken and three different regions was measured. Measurement of the dorsal region of the *sdha-1* worms and the *pha-1* control was taken to determine how significant the abnormal development of the anal depressor in this region was. The measurements (Fig. 1-2) allowed the p-value to be computed to determine if there was a significant difference between the control and *sdha-1* dorsal region.

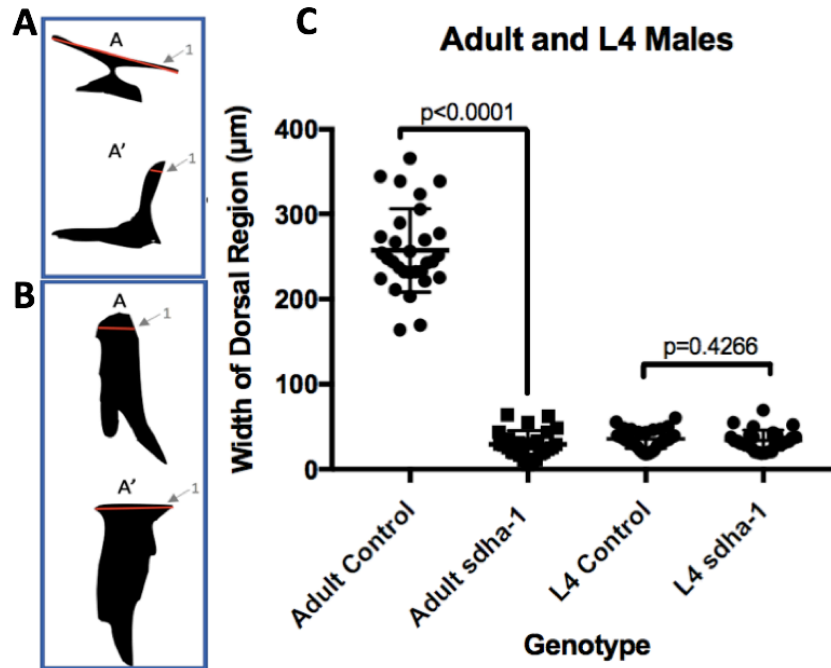


Fig. 3. Width measurements of the dorsal region in the anal depressor of adult and L4 males. An example of how measurements of the dorsal region in adult wild-type and *sdha-1* can be found in Image A (A1-A'1) and L4 males in Image B (A1-A'1). The width of the adult and L4 dorsal region was measured (Image C) and a Mann-Whitney test was used to determine if a significant difference was observed between the control and *sdha-1* males.

The width of the dorsal region of the adult control and *sdha-1*, along with the L4 male worms was taken and the p-value was obtained to determine if a significant difference was found between the *pha-1* and *sdha-1* lines during the remodeling of the anal depressor. When comparing the width of the dorsal region of the anal depressor in adults, a significant difference in length was observed ($p < 0.0001$). Due to the substantial difference found between the width of the adult dorsal region, we decided to also measure the dorsal region of the L4 control and *sdha-1* worms. However, no significant difference ($p = 0.4266$) was found between the two. Thus,

indicating that improper signaling to the dorsal region of the adult anal depressor is resulting in inhibition of the “crawling” of the dorsal region to elongate anteriorly to posteriorly.

Connection between Dorsal and Ventral Sides in Adults and L4 H Zone

After analyzing the significant difference between the remodeling of the dorsal region in the adult control and *sdha-1* anal depressor, we suspected that other parts of the anal depressor are also undergoing the abnormal remodeling. Measurements of the connection between the dorsal and ventral sides of the anal depressor in adults and H zone in L4 males was taken to determine if a significant difference in the control and *sdha-1* was present.

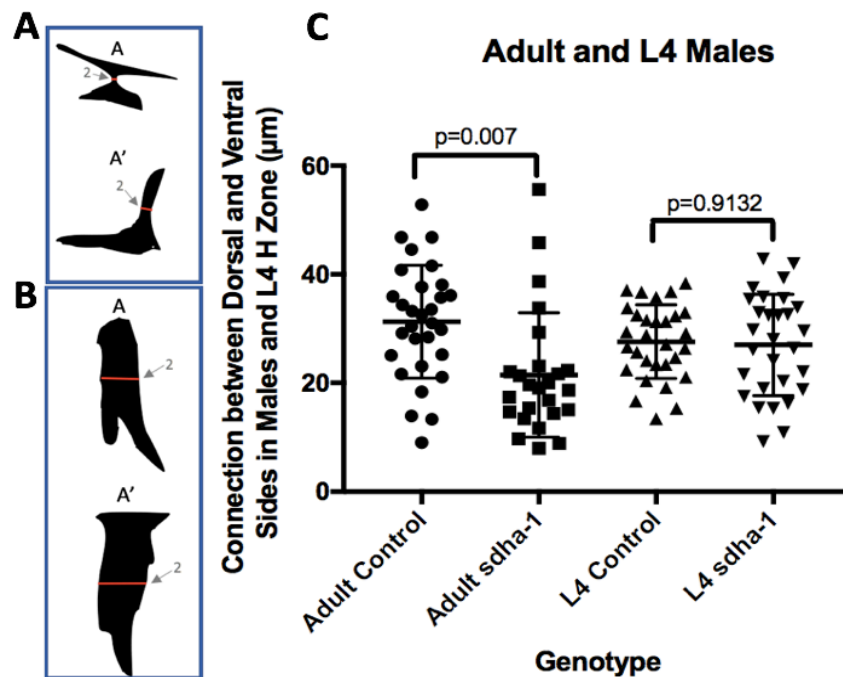


Fig. 4. Width measurements of connection between dorsal and ventral sides in adults and the H zone in L4 male worms. An example of how the connection between the dorsal and ventral sides in adult worms were taken can be found in image A (A2-A'2), and in L4 males in image B (A2-A'2). Measurements (Image C) between the connection of the dorsal and ventral sides and H

zone in L4 worms was taken and a Mann-Whitney test was used to determine if a significant difference was observed between the control and *sdha-1* males.

After computing the p-value by using the Mann Whitney Test, a significant difference ($p=0.007$) was found in the connection between the dorsal and ventral region of the anal depressor of the *sdha-1* and *pha-1* worms. However, no significant difference in the H-zone was found when comparing the wild-type *pha-1* with the *sdha-1* L4 worms. The significant difference found in the connection between the dorsal and ventral region of the anal depressor indicates that a signal is being inhibited, preventing proper remodeling to take place.

Measurements of Adult Sarcomere and L4 Ventral Attachments

A significant difference in the width of the dorsal region and connection between the dorsal and ventral side in the adult worms was found. Due to the significant difference, the adult sarcomere and the width of the L4 ventral attachment was also measured. If improper remodeling was occurring prior to L4, a significant difference should be found in the L4 worms. However, if the improper remodeling does not occur prior to L4 and instead during the transition between L4 and adult, a significant difference will be found in the adult sarcomere.

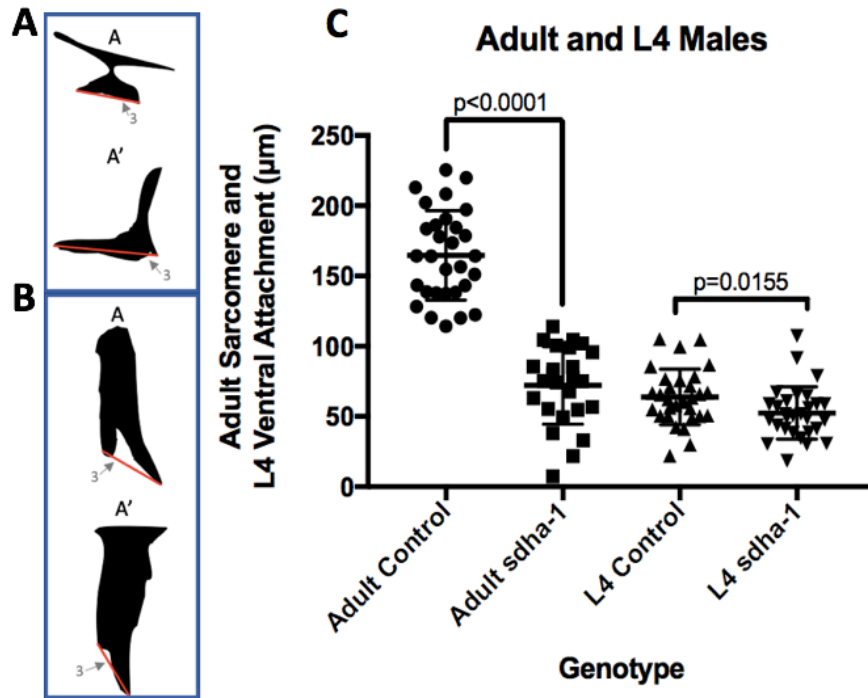


Fig. 5. Measurements of the adult sarcomere and the ventral attachments in L4 male worms. An example of how the adult and sarcomere was measured can be found in Image A (A3-A'3). Image B (A3-A'3) shows an example of how the ventral attachment was measured in L4 worms. Measurements (Image C) of the adult sarcomere and the L4 ventral attachments was taken and a Mann-Whitney test was used allowing us to determine if a significant difference was observed between the control and *sdha-1* males.

When measuring the sarcomere of the control and the *sdha-1* adult worms, the average measurement between the two lines was found to be significantly different from one another ($p < 0.0001$). In addition, when the width of the L4 ventral attachment was measured, a substantial difference ($p = 0.0155$) was found between the control and the *sdha-1* anal depressor. The significant difference found in the adult sarcomere indicates that somewhere along the transition

from L4 to adult, improper signaling is occurring leading to the abnormal development of the adult sarcomere.

CHAPTER IV

CONCLUSION

To address whether the *sdha-1* encoded enzyme is required for cell-cell communication between the developing anal depressor and its neighboring signaling partners, we first crossed *pha-1* (e2123); *him-5* (e1490) rgEx[Paex-2::YFP] males with *pha-1* (e2123); *sdha-1* (rg448); *him-5* (e1490) hermaphrodites. This cross allowed a new line containing *sdha-1* and the Paex-2::YFP transgene to be isolated and further studied to determine how significant the abnormal remodeling of the anal depressor was. After analyzing the abnormal anal depressor in the *sdha-1* mutants, the images were then quantified and the p value was computed in Prism 7 using the Mann-Whitney Test, allowing us to determine which region was most significantly impacted by the abnormal remodeling of the anal depressor in the *sdha-1*.

Abnormal Development of the Dorsal Region

To determine which regions of the anal depressor was not receiving the correct signaling for proper remodeling to occur, the anal depressor images were quantified. When measuring the dorsal region of the anal depressor, a significant difference ($p < 0.0001$) was found between the adult wild-type and *sdha-1* mutants. Because no significant difference was found when measuring the width of the L4 dorsal region, we can confidently conclude that abnormal

signaling is taking place during the transition from L4 to adult in the *sdha-1* worms. It is currently suspected that due to improper signaling, the “crawling” of the dorsal region of the anal depressor elongating in the anterior and posterior direction is inhibited. Therefore, we can conclude that the growth capability of the anal depressor, which allows it to elongate in the anterior and posterior direction, is lost during the transition from L4 to adult. After discovering that the dorsal region was unable to elongate in the *sdha-1* mutants, we wanted to determine if other regions of the anal depressor was also remodeling incorrectly.

Adult Sarcomere and L4 Ventral Attachment

To determine if abnormal development of the adult sarcomere was occurring, measurements were taken and a p-value was computed using the Mann-Whitney Test in Prism 7. A significant difference ($p < 0.0001$) was found in the sarcomere of the adult wild-type and the *sdha-1* worms. It is currently suspected that the filaments contained in the anterior domain is not receiving the proper signals needed for the migration dorsal-anteriorly to take place. In addition to the abnormal development of the adult sarcomere, a significant difference ($p = 0.0155$) was also found when measuring the width of the L4 ventral attachment. The significant difference found in the adult sarcomere indicates that improper signaling is occurring during the transition from L4 to adult, leading to the abnormal development of the adult sarcomere. When analyzing the ventral attachment in the L4 worms, a well-defined slit located midway along the ventral attachment was present. However, when analyzing the adult *sdha-1* adult worms, it appeared that the slit in over half of the worms collapsed during the transition from L4 to adults. It is suspected that the cell is unable to maintain its anchored position during the transition from L4 to adult, resulting in the slit to collapse in the *sdha-1* mutants. Due to the slit collapsing during the

transition from L4 to adult in *sdha-1* worms, the formation of the dorsal and ventral domains is unable to properly develop.

Future Direction

The significant difference found between the control and *sdha-1* worms led to the conclusion that the anal depressor is not receiving proper signaling for the correct remodeling to take place. It was found that somehow the signaling that occur during the L4 to adult transition is being inhibiting, causing the adult anal depressor to be under developed. In hopes of determining whether the *sdha-1*-encoded enzyme is required for cell-cell communication between the developing anal depressor and its neighboring signaling partners, a rescue experiment will be conducted. If the rescue experiment is successful, the anal depressor phenotype will be rescued when *sdha-1* is expressed in the anal depressor muscle. However, if the anal depressor phenotype is not rescued, this will indicate that *sdha-1* is needed in the surrounding tissue for proper remodeling of the anal depressor to take place. If we are unable to rescue the anal depressor phenotype, a new plasmid will be constructed allowing us to express the *sdha-1* gene in the surrounding tissue to determine which tissue needs *sdha-1* for proper remodeling of the anal depressor to take place. In addition, we hope to uncover which cell signals are initiating the abnormal sculpting of the anal depressor, primarily at the dorsal region from L4 to adult and the adult sarcomere. We hope that by studying the *sdha-1* mutation, we can uncover how it is affecting the Wnt/ β -Catenin Signaling Pathway, causing the abnormal development of the anal depressor to take place.

REFERENCES

- Alan F. Bird, J. B., 1991 *The Structure of Nematodes*. Academic Press, San Diego.
- Arnold, A., 2017 A general theory of sexual differentiation. *Journal of neuroscience research* 95: 291-300.
- Blaschko, S. D., G. R. Cunha and L. S. Baskin, 2012 Molecular Mechanisms of External Genitalia Development. *Differentiation; research in biological diversity* 84: 261-268.
- Carlson, B. M., 2013 *Human Embryology and Developmental Biology*. Elsevier/Saunders.
- Chen, X., and L. René García, 2015 Developmental alterations of the *C. elegans* male anal depressor morphology and function require sex-specific cell autonomous and cell non-autonomous interactions. *Developmental Biology* 398: 24-43.
- Garcia, L. R., P. Mehta and P. W. Sternberg, 2001 Regulation of distinct muscle behaviors controls the *C. elegans* male's copulatory spicules during mating. *Cell* 107: 777-788.
- Hunter, C., and W. Wood, 1990 The *tra-1* gene determines sexual phenotype cell-autonomously in *C. elegans*. *Cell* 63: 1193-1204.
- Jarrell, T., 2012 The Connectome of a Decision-Making Neural Network. *Science* 337: 437-444.
- Thomas, J. H., 1990 Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* 124: 855-872.