The Impact of Formin Homology Genes on Development in *C. elegans*

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S-CELL-003

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Abstract:

C.elegans is a model that can be used for studying cell migration, a process in all animals. Currently it is believed that for cells to migrate rearrangement of the cytoskeleton is required. As a result genes that code for actin polymerization like Formins are likely significant to cell migration. In *C.elegans* one example of cell migration that occurs during the larval and development stages is the SM migration. SM cells are precursor muscle cells that migrate towards the gonad of hermaphrodite worms where they divide and differentiate into egg laying muscles. Failure of SM cells to migrate results in phenotypes that prevent egg laying. In *C.elegans* RNA interference (RNAi) can be used to silence genes and determine their importance. In *C.elegans* RNAi is systemic throughout the worm's body; the next generation inherits the RNAi expression the same as the one before. Sets of *C.elegans* that had Formin genes downregulated via RNAi were scored for wt/phenotype and embryo to worm ratios. I predicted that out of the experimental genes I worked with *fozi-1,* a gene helping to decide cell fate in coelomycetes, cells related to SM cells would impact larval development the most. In an ANOVA, Only the systemic RNAi expression of the experimental genes was allowed to reject the Null hypothesis that there was no difference between ratios from the control or the experimental genes. However in a Tukey HSD test the adjusted p values showed the experimental genes had little difference from the control.

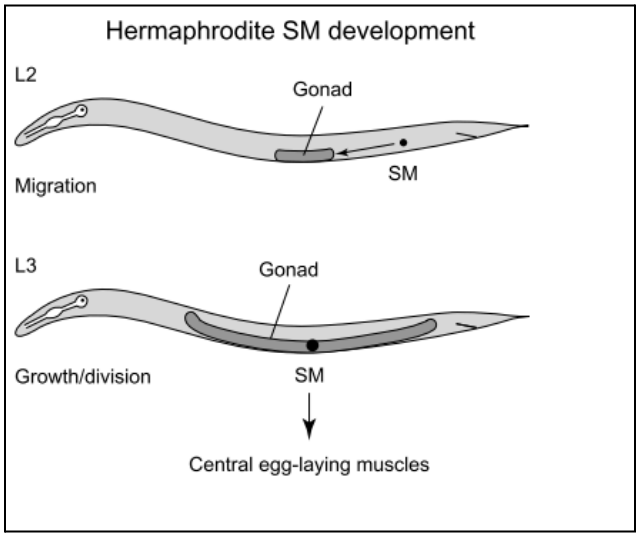
Introduction:

C.elegans is a common model organism and the first organism to have its genome fully sequenced. *C.elegans* is unique for a multitude of reasons but one of the most interesting is that the majority of all specimens are hermaphrodites. *C. elegans* are either born as hermaphrodites or as males. Males make up an extremely small ratio of the *C. elegans* population but are beneficial in the wild and labs because they create genetic variation. Males and hermaphrodites are sexually dimorphic and can be distinguished by the tapered tails present in hermaphrodites and the thicker fanned tails present in males. (Corsi et al, 2015). Adult *C.elegans* have around 1000 somatic cells (non sex cells) allowing in-depth analysis which would be much harder to achieve on a larger organism (Yochem et al, 2012)

The *C.elegans* life cycle includes four larval stages and adulthood, the end of each stage is marked by a molt. Newly hatched L1 worms generally only take three days to reach maturity and are then capable of egg laying. This quick development allows for researchers to rear multiple generations of *C.elegans* in as short of a time as a couple weeks. (Yochem et al, 2012) (Corsi et al, 2015). *C.elegans* are generally kept on agar or in liquid suspension, where they can be fed on *E.coli* bacteria.

The ease of culturing *C.elegans* in labs along with its comparatively simple biology make it an ideal organism for tracking genes, development, and other physiological studies. *C.elegans* is a great model for studies involving cell migration. Because *C.elegans* have a rather small genome it is easier for researchers to track the effects of specific genes on cell migration. As mentioned before *C.elegans* have around 1000 somatic cells as adults (Chen and Stern, 1998). In hermaphrodites there are many well studied types of cell migrations, one particularly important migration is the migration of the Sex Myoblasts (SM) (Sherwood, 2017). The SM migration is

responsible for the development of muscle cells surrounding the vulva of hermaphrodite worms. SM cells are precursors to muscle cells known as myocytes. Hermaphrodites have two SM cells that migrate anteriorly from the posterior of the worm beginning in L1. Successful migration results in wildtype worms. Failure to migrate can result in egg laying defective phenotypes, along with other developmental mutants. Because of *C.elegans* transparent nature it makes it easy for researchers to note different phenotypes and changes in morphology. Some of the phenotypes that result from the failure of the SM pathway are; bag (bag of worms), pvl (protruding vulva), explv (exploded pvl), dev (developmental delay), and stl (sterile). Bag prevents worms from laying eggs however, the worms still produce embryos and the embryos eventually hatch inside of them leading to their deaths. Pvl appears as a bump along the worm's side and may progress into a bag or explv. Pvl, explv and bag limit egg laying. A pvl animal may become a bag if the phenotype is extreme enough. Other phenotypes like dev and stl may not kill the worms directly but can limit fertility. (Chen and Stern, 1998).



Cell migration is not only important to study in *C.elegans* as it is responsible for many vital systems in humans as well. One example is the immune system which is made up of many cell types which migrate to different parts of the body and counter invading bacteria. Cell migration can also play a role in cancer. When cellular division is uncontrolled there is a chance that the cells can become cancerous. Cancers will sometimes metastasize or migrate to a different part of the body in order to get better access to resources. This is a form of unregulated cell

migration and can drain energy and resources from an animal's body (WANG et al, 2015). Finally, cell migration occurs during gastrulation, the stage where the embryo goes from being one dimensional to three dimensional. (Sherwood and Plastino, 2017) Cell division is necessary for the body to function and for embryonic development in the first place therefore it's necessary to study the effects of cell migration even in non-human models.

While many people have evaluated the factors behind cell migration in full body systems there is less knowledge about the intracellular factors that contribute to the internal aspect of migration. Cells migrate by using various parts of their cytoskeleton made up of proteins including actin to move. Actin specifically forms both the lamellipodia and the filopodia, two structures that are responsible for stretching the cell and resulting in cellular crawling. After actin is polymerized it's condensed forming into lamellipodia that extend in the direction of cell movement. Second, filopodia, which are akin to a second stage of actin build off of the lamellipodia and continue to extend the cell in the direction of motion. Finally an integrin, a type of adhesion protein, binds the cell to the extracellular membrane of the nearby cells. Myosin, a molecular motor, powers contractile motion causing the cell to crawl. The cycle repeats when the adhesion releases from the extracellular matrix (Kuszak, 2022).

Experimental and Control Genes:

In the experiment the three independent genes that were chosen for this research are all part of the Formin Homology domain. Formins are a highly conserved group of genes with homologs in humans and *C.elegans,* Formin's main role is in aiding actin polymerization. However, since being discovered they have been found responsible for defects in cytokinesis, cell polarization and defects in morphogenesis in flies, mice and yeast. Formins have also been found to be responsible for the formation of filopodia. (Breitsprecher et al, 2013). Fomins

specifically remove the end cap proteins on the end of Actin allowing actin monomers to become polymers. When Formins are absent, actin polymerization occurs at a slower rate than when in the presence of Formins. There is also a current theory that Formins may contribute to the protrusive forces that help lead cell migration (Mechanobiology Institute Singapore, 2014).

Of the genes chosen Gene one, *fozi-1* is currently thought to be responsible for actin binding and fate specification of M lineage cells. Specifically the fate of coelomocytes which are also partially differentiated during the L1 larval phase similar to the SM cells. However, the coelomocytes occur in three pairs of two and only one of those pairs differentiates during L1. Another difference between the SM cells and coelomocytes is that while the SM cells are migratory the coelomocytes are not and are fixed in place (Tahseen, 2009) (Kishore et al, 2023). I choose to work with this gene because of the general ties that Formins have to actin polymerization and by proxy cell migration. *fozi-1* was also a prominent choice because it has been seen to affect cell fate specification in other M lineage cells. The SM cells as precursor muscle cells need to differentiate and divide and could possibly be affected similarly to the coelomocytes by *fozi-1*.

The second gene that was chosen for this research is *fhod-1* which was used twice in the experiment because at the time there were two isoforms available in the gene bank, *fhod-1(a)* and *fhod-1(b)*. This means that these genes could code for almost identical proteins, however because they have different exons they can result in some differences that could be significant. These genes like *fozi-1* are responsible for actin filament binding activity (Kishore et al, 2023). Like in *fozi-1* actin binding plays a role in cell movement and the overall stability of the cell as actin makes up part of the cytoskeleton (Pollard, 2009). These genes also had a human homolog FHOD1 which is a current suspect for human hypertrophic cardiomyopathy. While the exact role

that these genes may play in *c.elegans* could be very different it is important to be aware of the human impacts of certain genes.

Outside of the experimental genes two control genes were used in this experiment. *l4440* and *egl-15*. *l4440* was an experimental control to make sure that RNAi interference by itself had no adverse effect. The plasmid for *l4440* results in the creation of double stranded RNA (dsRNA) but not dsRNA that targets a gene within *C.elegans* (Adikes et al, 2022). *egl-15* is a positive control, *C.elegans* with this gene downregulated are likely to have more mutant phenotypes, and lay less eggs because *egl-15* plays a role in the SM pathway. *egl-15* has been studied to be directly involved in *C.elegans* egg laying behavior (Kishore et al, 2022).

Strains:

Two strains were used in this experiment. These strains are not out publicly and were given to us by the Matus lab for use in this research. The first strain (LP756) used in these experiments was modified to express a membrane targeting protein tagged with the fluorescent protein, mKate, in the cells of the M lineage. In this first strain RNAi works in the whole animal. The second strain (APL67) also contained the M lineage specific fluorescent marker and was modified to allow RNAi to only work in the M lineage cells. This decreased the overall amount of mutations, because only a smaller portion of the worm could be affected. However, it allowed us to determine the effects on the M lineage specifically and therefore also the SM pathway. (Adikes et al, 2022).

RNAi interference:

To study genes related to SM cell development I used RNA interference (RNAi) . This is a method to silence genes using double stranded RNA (dsRNA). RNAi is an alternative to CRISPR that doesn't completely "knockout" genes but it does significantly reduce their expression. RNAi works by using dsRNA also known as Double Stranded RNA Viruses to introduce the double stranded RNA required into a specimen inorder to knockdown the expression of specific targeted genes. There are multiple ways to introduce the dsRNA into C. elegans. Worms can be fed bacteria containing the dsRNA, injected with samples of the dsRNA and also simply soaked in dsRNA. My lab prefers the method of feeding the worms RNAi through target plasmid producing E.coli. All of these methods have been shown to be effective at producing mutants with the targeted genes knocked down. Something else important about RNAi is that it is inheritable within C. elegans and the knocked down gene expression can be passed on to the next generation. This phenomenon has not been studied in other organisms (Grishok, 2005).

In this research I targeted my genes of interest with RNAi, creating worms that were deficient in those genes. I then scored the worms for specific phenotypes indicating that the SM pathway was inhibited. I also created embryo plates where I could compare the ratio of embryos to worms in normal worms to RNAi worms to see if the ratios were abnormal in comparison. Significant amounts of phenotypes like bag, pvl or explv indicate that SM migration might be impacted by the down regulation of the gene and the gene is essential for the pathway(Chen and Stern, 1998).

Research objective:

My objective was to find genes involved in the SM pathway in C. elegans. I did this by observing RNAi treated animals and noting phenotypes that are indicative of SM pathway failure such as bag, pvl, or expvl. If a deficiency of a gene results in high amounts of bag or other egg laying defective phenotypes then there is a likelihood that the gene is important to the SM pathway.

Hypothesis:

If *C.elegans* experience the down regulation of Formin homology genes such as *fozi-1*, *fhod-1(a)*, and *fhod-1(b)* either systemically (LP756) or limited to cells part of the M lineage (APL67), then animals with *fozi-1* down regulated will have the highest number of phenotype defects and the lowest embryo to worm ratio. Downregulated *fozi-1* will result in the most adverse effects because it helps determine cell fate in M lineage cells which the SM are descended from and therefore may play a role in determining the fate of other cells.

Null hypothesis:

There is no difference between each gene group in both the normalized embryo and phenotype ANOVAs. The ratios of wt/phenotype are the same regardless of gene downregulated. And, or The embryo/worm ratios of *C.elegans* are consistent regardless of gene downregulation.

Alternative hypothesis:

There is a significant difference between the ratios of phenotypes and embryos depending on the gene downregulated in both the normalized embryo/worm and phenotype/wt ANOVAs.

Methods:

***C.elegans* care:**

C. elegans were kept on Normal Growth Medium (NGM) Agar plates with OP50 E.coli. Seeded plates, or those with E. coli, are created by taking 200 ul (Microliters) of E. coli and spreading them across the surface of an agar plate. Then the plate has to sit overnight before the E.coli have grown enough to result in a large enough lawn for the *C.elegans*. The plates with C. elegans will be kept in incubators at 22 C unless they are a temperature sensitive strain(in which case then they will be kept in an incubator at or below 18 C) . All worms in this study were maintained at 16 C. Worms will be relocated to new agar plates via methods such as chunking or picking.

Creating RNAi Plates:

After choosing a target gene, grab a pair of gloves, pipette tips and agar plates. Go to the Morrel basement and retrieve your bacteria/plasmid from the appropriate box. All plasmids are sorted in the RNAi screen clones sheet. Whilst wearing gloves remove the PCR tube and scrape along the edge of the PCR tube with a pipette tip. Very little is required to start a colony. Hold the pipette tip at an angle and swipe back and forth across the top of the agar plate containing ampicillin and tetracycline. If you are growing multiple strains at once, mark the bottom of the dish with a sharpie then cover that area of the plate with your desired strain repeating the previous steps. After seeding your plates recap and replace the PCR tube in the correct slot and box so other labs can find it. Make sure to label plates with correct strain names and put them in

the 37 C incubator. The following day take a pipette tip and dab one of the colonies on the plate, so that it is on the pipette tip.

Immerse the pipette tip in 5 ml of LB broth containing cabinicilin then place the tube in the 37 C incubator before replacing it on the incubator shaking test tube rack at (225 rpm) for 16-18 hrs. The following day induce RNA production by adding 10µl IPTG (Isopropyl ß-D-1-thiogalactopyranoside). Let the test tube shaker sit at 37 C for another hour. After another hour remove the test tubes from the incubator.

Prep all RNAi plates for bacteria by pipetting a ratio of 2.5µL carb (carbenicillin) and 10µl IPTG onto each plate. Make 10% more than necessary (Medwig-Kinney, 2022). Dip a glass rod in ethanol and flame it and let it cool for 3 seconds. Use this to spread the IPTG and Carb. Remove test tubes from the rack. If the IPTG and Carb is dry plate 125-250µl of bacteria onto each plate. The more that's added the longer it will take to dry but the more worms the plate will feed. Plates should take roughly a day to dry before they are ready for worms.

Creating Fluorescence Microscope Slides:

First lay duct tape out onto the lab bench in two parallel rows. Then lay a non-well slide across the two strips of duct tape. Pipette a drop of agar at 80 C onto the slide. The second slide is quickly dropped on top of the first slide in a cross formation. Forming a plus sign, push down on both sides opposite the agar. The drop should expand and flatten but if it cracks it likely means that you waited too long to push down and the agar dried. Alternatively the agar may not be as hot as you may need it to be. After the agar is flattened, take the microscope slide and wiggle the top slide to detach it from the bottom. This should leave the agar circle on the bottom slide. Set the top slide aside. Now set your slide on a microscope stage and pipette 4 µL of M9

media onto the slide. Your slide is now worm ready, however when picking place worms into the M9 not the agar otherwise you will have difficulties imaging your worms. (Medwig-Kinney, 2022) Once ten worms of your desired age and type are on the slide, take a cover slip and place it on top of the slide. Then apply a small amount of wax at the edges of the cover slide to ensure it stays. Repeat the process however many times as necessary.

Picking / Chunking:

When you want to move a small amount of worms from one plate to another Picking is the most efficient method. If you are attempting to move a lot of worms at once however chunking is a far better option and is often performed to prevent starvation on overcrowded plates. The methods for both are fairly similar. To pick, take a wire tool and flame in an ethanol flame then using a microscope identify the worm that you want to pick up. Scoop under the midsection of the *C.elegans* and pull up. Switch to your second plate and hold the wire against the plate until you see the worm crawl off. After, stick the wire back in the flame to sterilize and repeat as necessary.(Medwig-Kinney, 2022)

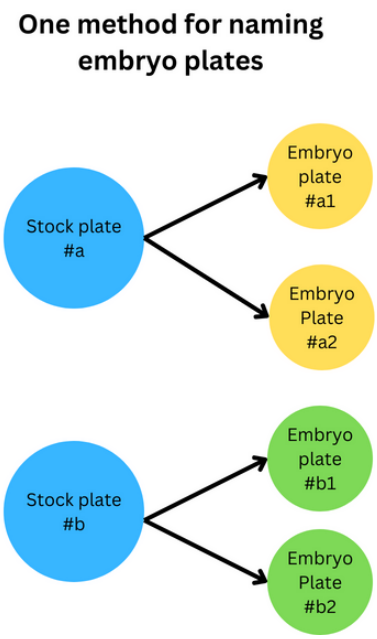
To chunk plates take a cutting tool (it doesn't have to be sharp) and dip it in bleach. Remove the cutting tool and flame for a few seconds in an ethanol burner. Take the plate that you wish to chunk and cut through to the bottom of the plate. Try to keep the chunk roughly the same width and length. After cutting, stick the cutting tool underneath the chunk and slide the chunk off the plate. The side without worms should be the side touching your spatula. Then flip the chunk over and press the worm side into the agar plate that you wish to transfer to. This way the worms will be able to crawl out from under the chunk. (Medwig-Kinney, 2022)

Bleaching procedure:

To create RNAi plates, synchronized worms must be pipetted onto the plate. To do this batches of worms can be bleached, resulting in many L1 worms. Gather your plates that you would like to bleach and add 1 ml of distilled water to each plate. Tilt the plates back and forth to dislodge the worms from the plate. Dispose of your last pipette tip and transfer your worms to a 15ml conical tube. Keep strains/genes separate as needed. Add more water to each of your conical tubes so that the final volume is 8 ml. Pipette 600 µl of 5M potassium Hydroxide (KOH) to each test tube. Now add 1200 µl of bleach to the test tube and cap the test tube and let it sit on the test tube rocker in the 18 °C incubator for 5 minutes. After this time period when you observe the test tube in the light you should see no or very few adult bodies left. If there is a significant amount, replace it in the incubator and leave for another 5 minutes. Now that the adults are dissolved you can centrifuge the test tubes to create an "egg pellet". During this time if you do not have an even number of test tubes or have a blank test tube ready, prepare a separate conical tube with approximately the same volume to balance out the centrifuge. Place the tubes diagonal to each other to balance the centrifuge. Set the centrifuge to 400g for 2 minutes. After the two minutes take your test tubes out one at a time and dump the supernatant in one swift flowing motion to avoid disrupting the pellet. Then refill the tube up to the 15 ml mark with water. Flick or shake the tube to resuspend the pellet. Repeat with the remaining tubes. Once more, centrifuge all your tubes and then pour out the water again, except this time resuspend in M9. perform two more washes with the M9, then after the final wash suspend in 2 to 3 ml of M9 and put all your test tubes on a rocking incubator. The eggs will hatch and will be able to plate between 1-2 days after bleaching.

Scoring and Creating Embryo Plates:

To create an embryo plate start with one of your original RNAi plates. For each full experiment you should have two RNAi plates for each gene and strain. Get a blank agar plate and picking tools. Pick 3 adult hermaphrodite worms to the new plate. Do not be picky or precise with the worms that you pick; this introduces error. After adding the worms mark the date and time to the exact minutes that you finished creating the plate. Repeat the process again, taking three more worms from the same plate as before and placing them on a new plate. Repeat the process with each gene. Make sure that you name your plates consistently otherwise you will have trouble distinguishing later.



When you are finished you must wait roughly 24 hours to score. However if you are consistently an hour early scoring for embryos it doesn't make much difference. To draw straight lines along the bottom of the plate using a sharpie. Then take a tally counter count embryos as you see them. For the best efficiency use the counter and go up and down in rows. After counting all the embryos on the E. coli check the surrounding agar for any extras. (Medwig-Kinney, 2022)

Scoring Phenotypes and Percent bag:

Score phenotype plates by counting the number of total worms and then counting the number of each individual phenotype. Mark worms that have multiple phenotypes as part of two categories not a separate category. You can calculate the percent bag of a plate by taking the total number of wt + other phenotype worms and the number of bags and dividing the bag by the total number of wt + other phenotypes. This gives you a rough idea how many worms per plate are bags and how that compares to other plates.

Data and Data analysis:

To interpret both my embryo ratios and my phenotype data I created normalized data. The purpose of creating this data was to see if some of my C. elegans strains were closer to wildtype ratios or more abnormal. For example in both the APL67 strain I collected data for and two controls and in fozi-1 while in the LP756 strain data was collected for all three experimental genes. *l4440* is the regular control gene that we used to reference the average numbers of embryos and wt worms that should be seen on average. In comparison *egl-15* is our positive control plate. egl-15's were expected to have more bags than average and less eggs than normal.

After creating the normalized data, all sets of both APL67 and LP756 data were typed onto a separate google sheet in order to be imported into R studio- a coding software that is useful for creating graphics and interpreting data. Once imported the following scripts were used to perform an ANOVA and create boxplots representing the normalized data.

R-scripts for ANOVA and Box Plots:

Anova interpretation R-studio script

```
#LP756- phenotype- data
library(ggplot2)
library(readxl)
library(tidyverse)

View(LP756_to_plot_nomalized_phenotypes)

LP756_to_plot_nomalized_phenotypes %>%
  group_by(RNAi) %>%
  summarize(var(Egl, na.rm = TRUE))
```

```
testmodel<- LP756_to_plot_nomalized_phenotypes %>%
   group_by(RNAi) %>%
   summarize(var(Egl, na.rm = TRUE))

view(testmodel)
#anova
ANOVALP756phenomodel<- aov(Egl~ RNAi, data=
LP756_to_plot_nomalized_phenotypes)
summary(model)

# ANOVA data below
#call models APL756phenomodel variations
summary.aov(ANOVALP756phenomodel)

#addhoc
TukeyHSD(ANOVALP756phenomodel)
```

The script above was used to create Anova and Tukey HSD data in R studio.

Every spot that has a hashtag in front of it means that that line is silenced and is not actually part of the code and is just a note I inserted to help keep track of my work.

The first lines of code Library; ggplot, readxl and tidyverse are all data packs that tell the computer how to interpret the data and the code.

Below the #Anova there is a line that tells the computer to analyze the data, ANOVALP756phenomodel (Anova model for the LP756 phenotype data) and to then give a summary of that model below in the coding softwares output box.

After that there is a note, #addhoc telling the computer to analyze the following data, ANOVALP756phenomodel in a TukeyHSD test and then provide the summary in the output. I used this same code for analyzing the data for APL67 and also the data I obtained from the embryo/worm ratios. The only part that was changed was the ANOVALP756phenomodel

which was replaced with the respective model datasets for APL67 embryo ANOVA, LP756 phenotype ANOVA or the LP756 embryo ANOVA.

Box Plot R-studio script

```
library(readxl)
library(ggplot2)
library(tidyverse)
# start

ggplot(APL67_to_plot, aes(y=RNAi,
                          x=Egl,
                          color=RNAi)) +

  geom_boxplot()+
  labs(y="Rnai Treatment",
       x="Normalized Embryo/Worm Ratio",
       title ="APL67 Normalised Embryos")+

#modifications

theme_minimal()
```

The script above was used to create boxplots in R studio

Any line with a hashtag in front of it indicates that text is silenced and does not contribute to the code.

The following lines Library; readxl, ggplot, and tidyverse are all separate data packs that tell the coding software how to analyze the data and code.

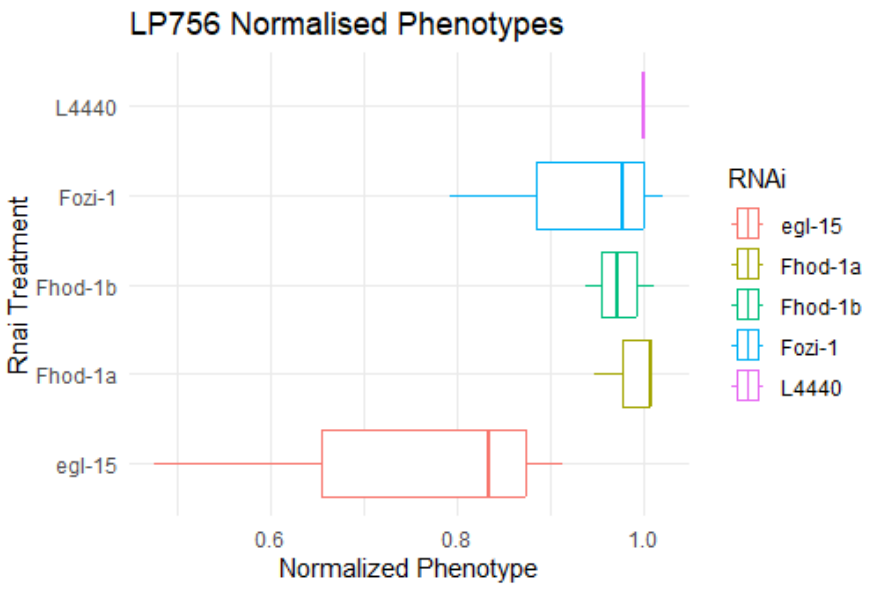
Ggplot tells the computer to create a plot using the APL67 data. Aes, shorthand for aesthetic,tells the computer to set the y axis to RNAi data and the X axis to *egl-15* data. Rnai data was the heading of one column and *egl-15* was the heading of another. The plus sign connects this statement with the following that says to take the APL67 data and display it in a box plot with

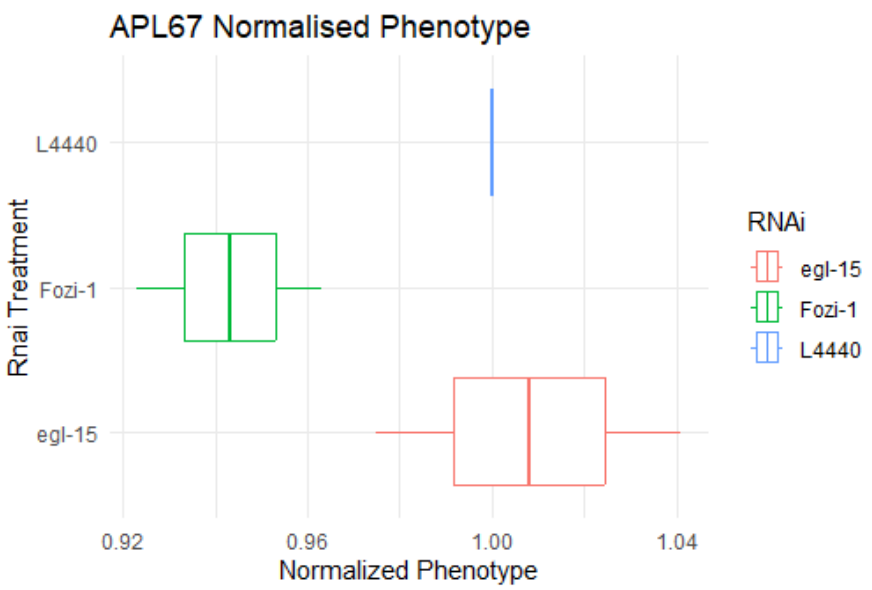
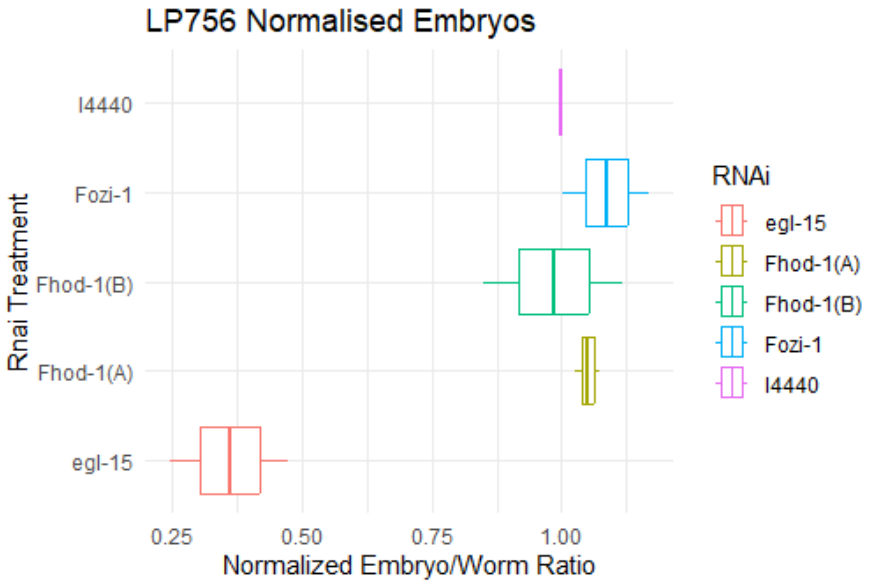
RNAi treatment as its y axis title and Normalized embryo/worm ratio as its x axis title.

The final piece of code is to set the box plot theme to minimal.

Box plots:

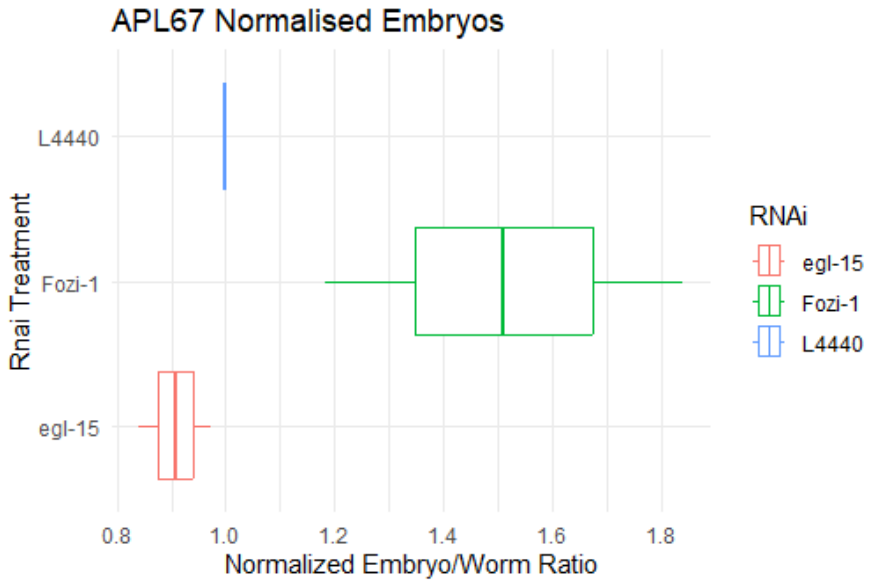
After Creating box plots with R studio the overall trends were interpreted based on how close or far away they were from the control gene *l4440*. Any number of < .75 or less was considered an abnormal ratio of wt/phenotype. Or any number of < .75 or less was also considered an abnormal ratio of embryos/worms. Any number > 1 was considered extremely normal because it was higher than *l4440* which should have represented the wildtype frequency ratios. It could be hypothesized that the genes with higher than one ranges may have improved fertility and a higher chance of being wt in comparison to *l4440*. There is no current explanation for this phenomenon.

LP756 normalized phenotype	
None of the independent genes are < . 75 and therefore all the genes present are likely to have little to no effect on the SM pathway.	

APL67 normalized phenotype Because of the reduced RNAi expression the range of values is reduced and none of the experimental genes have a minimum < .75. Therefore it can be assumed that *fozi-1* has little effect on the SM pathway.	
LP756 normalized embryo None of the independent genes are < . 75 and therefore all the genes present are likely to have little to no effect on the SM pathway.	

APL67 normalized embryo

Because of the reduced RNAi expression the range of values is reduced and none of the experimental genes have a minimum < .75. Therefore it can be assumed that *fozi-1* has little effect on the SM pathway.



Anova and Tukey HSD:

LP756 Embryo ANOVA

The Anova for LP756's normalized embryo count is the only one that came back with significance and therefore the Null hypothesis could be rejected. Because the P value (highlighted in red) of the Anova is less than the .05 alpha value the resulting data can be assumed to be significant. As a result a Tukey ad hoc test was performed to analyze where the difference in means was located.

LP756 normalized embryo ANOVA

```
 Df Sum Sq Mean Sq F value  Pr(>F)
RNAi          4 0.7335 0.18336   11.85 0.00917 **
Residuals     5 0.0774 0.01547
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.'
0.1 ' ' 1
```

Tukey HSD

```
Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = egl ~ RNAi, data =
LP756_to_plot_normalized_embryo)

$RNAi
                              diff        lwr        upr
p adj
```

Tukey HSD

The Tukey test shows that the groups with significant differences between the adjusted p values are

- *Fhod-1(A)-egl-15*
- *Fhod-1(B)-egl-15*
- *fozi-1-egl-15*
- *l4440-egl-15*

From this data there are no experimental genes that have a significant difference with *l4440*.

```
Fhod-1(A)-egl-15      0.69086936  0.1918909 1.1898478
0.0135014
Fhod-1(B)-egl-15      0.62523085  0.1262524 1.1242093
0.0205336
fozi-1-egl-15         0.72686654  0.2278881 1.2258450
0.0108534
l4440-egl-15          0.64116565  0.1421872 1.1401441
0.0184982
Fhod-1(B)-Fhod-1(A) -0.06563851 -0.5646170 0.4333400
0.9803264
fozi-1-Fhod-1(A)      0.03599718 -0.4629813 0.5349757
0.9979243
l4440-Fhod-1(A)      -0.04970371 -0.5486822 0.4492748
0.9929029
fozi-1-Fhod-1(B)      0.10163569 -0.3973428 0.6006142
0.9146235
l4440-Fhod-1(B)       0.01593480 -0.4830437 0.5149133
0.9999157
l4440-fozi-1         -0.08570089 -0.5846794 0.4132776
0.9505852
```

Discussion:

After analyzing APL67 and LP756 data no experimental gene observed in this research had a significantly adverse effect on the *C.elegans* larval development or SM pathway.

The LP756 data did not show any significant differences between the control genes and the experimental genes in either set of normalized data. However, there were instances of the data from LP756 having ranges above one meaning that they had higher embryo/worm ratios or wt/phenotype ratios than the control gene. This is likely due to how that data was calculated because the *l4440* data was used as the divisor to find the ranges of the other genes for the box plot that made the *l4440* data point default to a

range of one. In reality this range is likely much larger making the experimental genes that have ranges above one less significant in context. Furthermore when an ANOVA was performed on the data used to create the box plots only the data for the LP756 embryo ratio came back with a p value under .05 (the alpha value).

When interpreting this data with a Tukey HSD (Honestly significantly different), a test used to determine the exact locations of significant differences, the test showed little significance. Tukey tests work by showing the adjusted p values between the specific parts of an experiment For example the Tukey test showed that *fhod-1(a)* and *egl-15* had significant differences between their adjusted p values, meaning that there was variance between those two groups specifically. In this case the Tukey was performed only on the embryo data for LP756 because that was the only significant data found, the Tukey showed that the experimental genes and *l4440* had the most significant differences between themselves and *egl-15* (the positive control). This can be inferred as further support for Formin down regulation resulting in mostly wild type worms.

The APL67 data shows no conclusive results for the impact of down regulating *fozi-1* on the SM pathway. *fozi-1* similar to the LP756 data, shows embryo to worm ratios that are greater than *l4440* which are less significant in context because of how they were calculated. However, it is interesting to note that the *egl-15* phenotype and embryo data is higher than expected and could be a possible indicator of a decline in effectiveness in the strain.

The phenotype data collected from both strains is not as reliable as initially intended. Mainly because there are a multitude of alternate reasons for mutations to occur in *c.elegans* there is more than one reason for worms to be non wild type other than the

effectiveness of Formins in larval development. Because the mutations can occur from various sources any mutation in *C.elegans* can not be automatically attributed to defects in larval development or SM migration.

Limitations:

While pursuing this research I did encounter some limitations. One of the major limitations I encountered during this experiment was I did not have the ability to test all of my experimental genes in the APL67 strain and therefore cannot fully present any conclusions on the effects of other Formins in the SM pathway at this time. This was partially to do with the limited time frame that this experiment took place in and the limited amount of results that I encountered while observing the strain which caused me to narrow the scope of my research to just the LP756 strain.

Something else that could have also affected the results of APL67 specifically is that the strain could have possibly lost its effectiveness. Over time it is possible that the worms used for APL67 data became less effective, resulting in the abnormally high range for *egl-15* in the embryo/worm ratio data.

Future studies:

In the future I would like to continue looking at *fozi-1* and repeat the current experiment with *fhod-1(a)* and *fhod-1(b)*. Specifically since *fozi-1* is important in coelomocyte differentiation, I want to use fluorescence microscopy to analyze the similarities and differences between *fozi-1* worms and *l4440* worms. The strains we worked with in this research project all are modified to express mkate, a fluorescence marker, in the M lineage cells. So if I were to

image *fozi-1* worms there should be defects in coelomocyte differentiation. Currently I have imaged some *l4440* and *fozi-1* however of the worms that I images appeared to all be wt individuals. I would like to further investigate the morphological differences between *fozi-1* and *l4440* in the future.

Some other avenues of future research that would be interesting to explore is using crispr to knockout these same genes from the genome of the *C.elegans* that are being worked with. It is possible that many of the genes that I have been working with, especially the isoforms *fhod-a(1)* and *fhod-1(b)* are compensating for the loss of the other when one is down regulated. Or alternatively the RNAi downregulation is not as complete as it needs to be. If crispr were used instead there would be less uncertainty that the expression had been removed completely.

Something that I would choose to exclude in future research is the Phenotype to wildtype ratios. I initially thought that they would prove helpful because while I did not observe many bags (indicators of an SM defect) I did notice that there seemed to be higher numbers of mutations in the experimental genes than in the control gene. I hypothesized that I could use the ratio of wiltype worm to mutant worm as a point of comparison for further analyzing how similar or different the effect of the experimental genes was on the larval development. However I initially neglected to include that this allows for many other sources of error to be included in the data. Mainly that the effects of the experimental genes did not have to be the key reason behind a mutation occurring.

Acknowledgements:

I would like to thank my fellow lab members Alyssa Lunman, Edna Karel Binga Leboundou for their help, and especially Dr. Rebecca Adikes for her guidance in completing this project.

I would also like to thank the Matus Lab members Ononnah B. Ahmed and Nicholas Plamisano and the Pani Lab, especially Theresa V. Gibney, for generating the strains used in this project.

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