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Chapter 1

Using the worm, *Caenorhabditis elegans*, in undergraduate genetics and developmental biology laboratories

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Introduction

The first part of this lab (Introduction to *C. elegans*) can be used by itself at the introductory level or in combination with further experiments. In a genetics class, crosses to demonstrate dominant vs. recessive mutations, complementation, or epistasis, can be done easily as an extension to the introduction. In an upper level class, the included Sample Experiment can be used to allow the students to investigate the cause of a mutant phenotype using crosses and GFP transgenes. The Introduction introduces the major phenotypes of the worms, and shows students how to pick the worms, and recognize males, hermaphrodites, and various mutants. This lab will take most students about 1-2 hours. The best resource for background material for this introduction is “*C. elegans* II” (Riddle et al., 1997). The Sample Experiment is more involved and more difficult, and is best done over a two-week period with about 4-5 hours of total time for the students. Preparation time for the Instructor requires maintenance of worms, pouring and spotting plates, all of which should be started about 1 week prior to start of the lab (to allow for worms to grow to adult stages).

Student Outline

Introduction to the worm, *C. elegans*

This lab introduces one of the most well known invertebrate organisms for developmental genetics studies (the other being the fruit fly, *Drosophila melanogaster*). The ability to conduct genetics investigations on these organisms has been extremely valuable in determining the functions, molecular identities, and pathways of many genes involved in the development of invertebrates and vertebrates alike (including humans). These 1 mm long soil worms can be cultured easily in the laboratory, and have a relatively small number of cells (about 1000). Furthermore, the lineage (cell ancestry) of each of these cells is known, and the genome of the worm has been fully sequenced.

The genetic approach to developmental biology generally involves the use of mutant animals containing a specific mutation in a gene of interest. If a gene malfunctions or completely fails to function, or if it is expressed in the wrong place, at the wrong time, or in the wrong amounts, the resulting phenotype may indicate something about the gene's function in normal development.

In this lab, you will explore the basics of *C. elegans* as a model system. You will learn how to make a “pick” for moving worms around, become familiar with the basic life cycle and development of the worm, and learn some basic techniques for manipulating the worm and setting up genetic crosses. Two different experiments are presented, of which both use GFP transgenes and straightforward genetic techniques to answer questions about developmental processes.

I. Background

A. Culturing techniques

C. elegans worms are raised in the laboratory in covered Petri plates containing agar medium overlaid with a “lawn” of *E. coli* that the worms eat. Overgrowth of the bacteria is controlled by limiting the amount of uracil in the medium and by seeding the plates with a mutant strain of *E. coli* bacteria (OP-50) deficient in uracil production. The bacteria grow until the uracil is used up and then stop, so a fine lawn is produced.

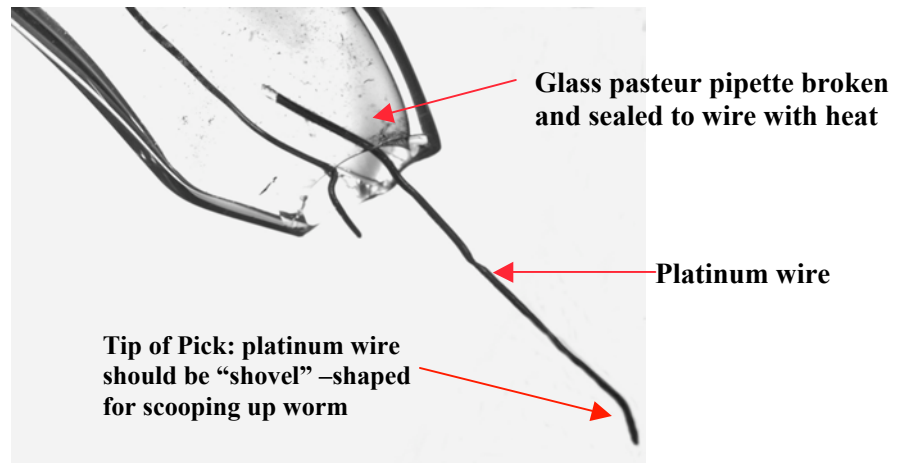
Since wild bacteria and molds can also grow on the culture plates, sterile techniques must be used. Keep the plates covered as much as possible. Flame your “pick” (a piece of platinum wire

attached to a glass pipette, Figure 1) in the alcohol lamp before and after you touch it to the plate. Do not touch the surface of the medium with your fingers.

View the worms on the plate with a dissecting scope illuminated from underneath, with the mirror covered by a white index card or a frosted mirror. For most work, particularly transferring worms, a low objective magnification of 12 to 25X is best. Higher magnifications may be needed for studying morphology and identifying males.

Worms are moved by “picking” them up with the pick. This is the trickiest part! The wires can bend and break easily, so be careful to store them upright in the holder supplied. You may need to adjust the angle of the wire, or flatten the wire itself in order to improve its performance.

Figure 1. The “pick”



The procedure for picking is the following:

- 1) Sterilize the pick by flaming it in an alcohol lamp until it is red hot. It cools very rapidly, so you can immediately move your pick onto the plate to pick up a worm.
- 2) While viewing the worms with the dissecting scope, attempt to pick up one (or more if you're good at catching worms) by going under the worm with the flat part of the pick. Try not to poke holes in the agar with the pick since the worms can use holes to burrow into the agar. Another approach that some worm pickers use is to scoop some *E. coli* onto the pick and then use this blob to help scoop up the worm (think of an ice cream scoop). If the blob is sufficiently sticky, the worm will stick to the pick and can be easily transferred to another plate.
- 3) Transfer the worm on the pick to a new plate by placing the pick gently on the surface of the bacterial medium -- again without poking holes -- and wait for the worm to crawl off the pick onto the medium. You can also gently brush the pick across the bacteria, thus dislodging the worm. The receiving plate should be at room temperature.

Repeat these steps for each transfer. It is important when you are setting up a cross that you do not transfer any eggs or larvae over to your mating plate. If you think you might have done this, you can either remove the worms to a new plate, or try to pick off and burn the offending larvae or eggs.

B. Life cycle

Reproduction.

In *C. elegans* there are two sexes, males and hermaphrodites. The major mode of reproduction is by self-fertilization in the hermaphrodite. The sperm are produced in the last larval stage, L4, and

stored in the hermaphrodite's spermatheca. The adult hermaphrodite then switches to producing eggs, which are fertilized by the stored sperm, and laid. Since the hermaphrodite has two X-chromosomes, as well as 5 pairs of autosomes, its offspring are mostly XX hermaphrodites. However, X-chromosome non-disjunction can occur and when it does, XO individuals result. These are the males. Frequency of males in self-fertilizing hermaphrodite populations is about 1 in 700.

When males mate with hermaphrodites, half of the outcross progeny will be XO males. There will always be some self-progeny (XX) despite mating. However, there is some preference for outcrossing (using sperm from the male), and stocks containing males in reasonable numbers can be maintained by mating hermaphrodites with males. There are also mutant lines (e.g., *him-5*) in which the frequency of non-disjunction, and therefore of males, among the hermaphrodite self-progeny is increased.

The general criteria for distinguishing males from hermaphrodites include:

- 1) Males are thin and move faster
- 2) The fan shaped tail makes it appear that the tail is folded back on itself, instead of being a long thin spike as in the hermaphrodite.

Life stages.

The life cycle consists of the embryo, 4 larval stages and the adult. The life cycle length is highly dependent on temperature. At 20 °C, one life cycle takes about 3 days, while at 16 °C one life cycle takes almost a week. The larval stages, L1 through L3 look about the same, but differ in size and are separated by molts in which a new cuticle is secreted under the old cuticle, which is then shed. Growth occurs between the molts. L4 worms are distinguishable from the other earlier larval stages in that the developing vulva can be seen as a small clear spot on the ventral side of the worm, near the middle of the body (Figure 2).

C. Mutations and marker genes in worms

Nomenclature for mutations in C. elegans

Mutations in worms are always given designations consisting of three letters, written in lower case, followed by a dash and a number, e.g., *unc-4* or *him-5*. The letters are abbreviations for a brief description of the mutant phenotype. For example, “*unc*” stands for “UNCoordinated,” “*lin*” for “abnormal LINEage,” and “*him*” for “High Incidence of Males” (for more information see Riddle et al., 1997).

You will note that these are relatively general phenotypes.

Mutations at any of several loci might be expected to give the phenotypes. Therefore, numbers are used to indicate mutant genes at different loci, e.g., *him-4* and *him-5* both produce high incidence of males when mutated, but are different genes on different chromosomes. The numbers have no intrinsic meaning, but are generally related to the order of discovery of the mutations. When one wishes to refer to a phenotype, rather than a specific mutant, the name is capitalized, e.g, Unc or Lin. There are usually various alleles associated with each mutant locus.



Figure 2. Embryonic to larval stages

Marker mutations

Mutations that result in viable, easily observed phenotypes are always useful for doing genetic experiments. They are called markers, and they make it possible to identify individuals that carry, or do not carry, chromosomes containing the gene(s) of interest. Since worms are small and do not have as many distinctive features as other animals, the markers used by *C. elegans* investigators involve general shapes, such as short, fat body (*dpy*), or behaviors such as movement by rolling (*rol*) or poorly coordinated body movements (*unc*).

GFP Transgenic animals

Transgenic *C. elegans* are generated by injecting DNA into the germ line of adult hermaphrodites. The DNA is incorporated into the oocytes, and forms “arrays,” unstable tandem repeats of the DNA, which remain extrachromosomal. The array can be integrated onto a chromosome (randomly) via gamma-irradiation. Transgenic animals can be used to assess gene function and expression. GFP, originally isolated from the jellyfish *Aequorea victoria*, is now a commonly used marker for expression. GFP fluoresces bright green when illuminated with the correct wavelength of light, and thus can be visualized in live animals. Using standard subcloning techniques, the DNA sequences for GFP can be hooked up to any known promoter sequence, and introduced into the worm by microinjection into the germ line. A “GFP line” is then established in which this GFP + promoter sequence is carried from generation to generation, and can be integrated as above. Once such a line is established, the specific cells that are transcribing DNA from the promoter used in the fusion will also express GFP, thus essentially marking those cells. This is a similar technique to making a lac-Z expressing transgenic line, as is done in many model organisms, but the overwhelming advantage is that the GFP can be visualized in the live animal. Also, since GFP gets made into a protein only if it is “in frame,” one is actually visualizing the location of a protein of interest.

II. What to do in the lab

Make a Pick. Cut a piece of platinum wire about 0.5-1" long with a razor blade. Holding the wire with a pair of forceps in one hand, affix the wire onto the tip of a glass Pasteur pipette by holding both in the flame of a Bunsen burner. Once the wire sticks inside the glass, use the forceps to squeeze the glass down onto the wire, thus cementing it into place. Now look at the wire under the microscope. If the tip is jagged, cut it off with a razor blade. You can also flatten the tip a little by pressing down on the wire with something round. The pick works best when the wire is bent to look a little like a shovel (Figure 1).

Look at the different mutants. Start by looking at the wild type worms (called N2; Figure 3a). Identify embryos, larvae, and adults. How do the worms move? What do they look like? Compare what you see in the microscope to the figures showing male and hermaphrodite worms. Now look at the mutants, one at a time (included here are *Dpy*, *Lon*, and *Egl*; Fig 3 b-d). What is the mutant called? Can you tell why it is called this? How do the worms move and what do they look like? Think about what kinds of genes might cause these mutant phenotypes.

Practice identifying and picking males and hermaphrodites. Return to the plate of N2 worms. Scan the plate to see if you can find any males. Males move faster than hermaphrodites, are thinner, and have a flat, fan shaped tail. Get a clean plate with no worms on it. Now attempt to “pick” some males and hermaphrodites from the N2 plate onto the new plate. Pick the worms into the spot of bacteria--they will come off the pick more easily if they are placed into the bacteria. Remember,

you do not want to poke holes in the plate you are transferring to. Holes in the original plate are not as critical. Note: this is difficult at first! There is a steep learning curve, but once you figure out how to do it, it will be relatively easy.

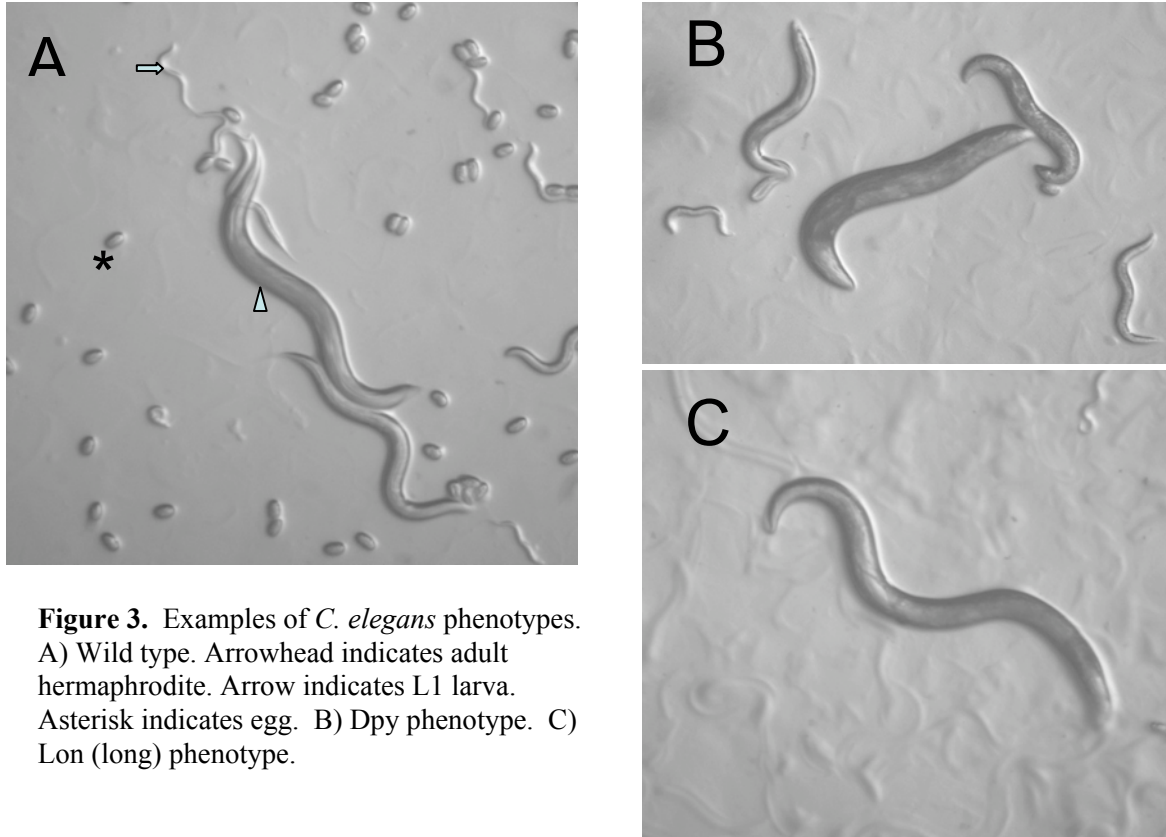
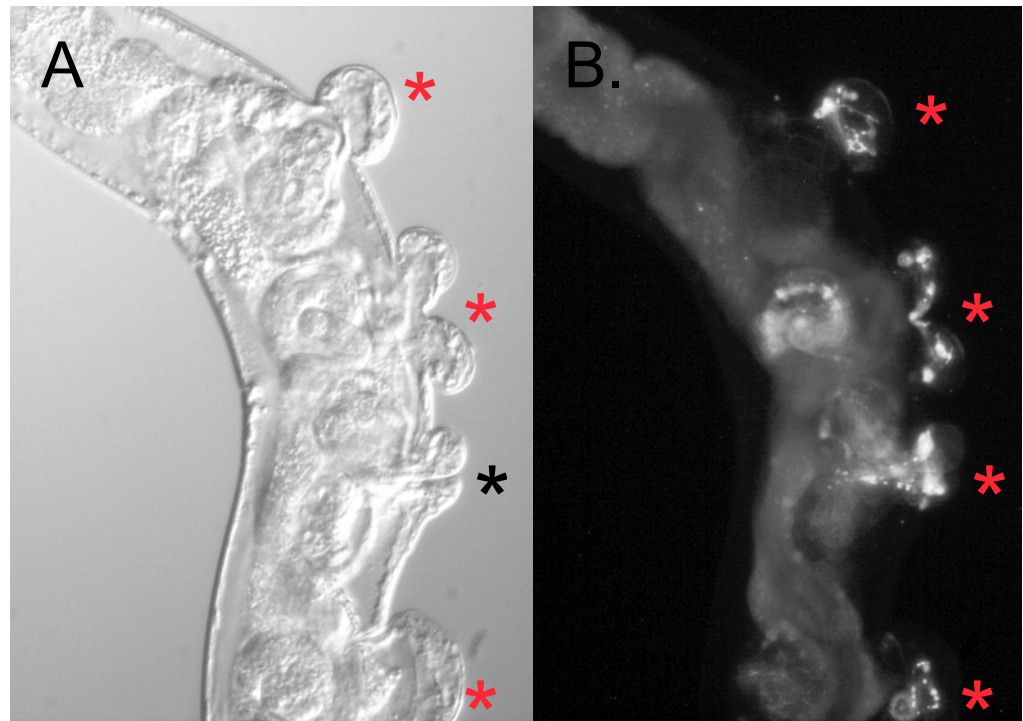


Figure 3. Examples of *C. elegans* phenotypes. A) Wild type. Arrowhead indicates adult hermaphrodite. Arrow indicates L1 larva. Asterisk indicates egg. B) Dpy phenotype. C) Lon (long) phenotype.

Looking at GFP transgenic worms under the fluorescent microscope (Figure 4). To look at the worms under high magnification, you have to mount them onto a slide with a small amount of agarose on it to cushion the worm. You need: 1% Agarose, glass slides and 18 mm square glass coverslips. To paralyze the worm, use sodium Azide (toxic! Wear gloves). An alternative to Sodium Azide is to cool the worms in the refrigerator after mounting them.

1. Melt the agarose for about 1 min in the microwave (or until it is completely in solution).
2. With a pasteur pipette, put a small drop of agarose on top of a glass slide. Immediately cover the agarose with a coverslip and press down gently. After about a minute, the agarose will be gelled and you can remove the coverslip, leaving a square of agarose on the slide. You can make this square smaller to make it easier to find the worm later.
3. Now, put a few μ l of water onto the agarose square to keep it moist.
4. Work quickly so that the agarose pad does not dry out.
5. Pick a few worms that you want to look at from the plate of worms into the solution on top of the agarose. Add an about 5 μ l of Sodium Azide.
6. Put a coverslip on top of the worms (gently).
7. Look at them under the microscope.

Figure 4. *let-60; ajm-1::GFP*. Muv worms. Muv worms have multiple vulvae, indicated in A and B with asterisks. A) DIC image. Functional vulva indicated with black asterisk. B) GFP fluorescence. This transgenic strain expresses GFP in adherens junctions, present in the vulva.



III. Sample Experiment:

Investigating the cause of a phenotype using GFP transgenic *C. elegans*

(This lab is designed to take two ~2-3 hour lab periods, one week apart. An additional hour or so is required between the two lab periods).

In this lab, you will investigate the defect responsible for an egg-laying defective (*Egl*) mutant. To understand what makes these worms unable to lay eggs, you will use two different GFP transgenic strains to help mark sets of cells that could be involved in causing such a defect. The experiment will consist of crossing each of two different GFP transgenic strains into the starting egg laying defective strain, and then looking at if and how the GFP expression changes in the *egl* mutant background. The phenotype of the resulting mutants will allow you to figure out whether the *egl* mutant you are studying is *Egl* because of defective neurons, or because of a defective vulva.

The two GFP strains we will use allow the visualization of two specialized structures necessary for egg laying: Hermaphrodite Specific Neurons, called HSNs, and the vulva. The HSNs synapse directly onto the uterine and vulval muscles, and thus are required for the expulsion of eggs. The HSN neurons are present only in hermaphrodites; in male *C. elegans*, these neurons die by programmed cell death (apoptosis) during embryonic development. The vulva is the connection between the uterus and the outside world: if the vulva does not form correctly, eggs cannot be laid. This specialized egg-laying organ is comprised of 22 cells. These cells derive from 3 cells that are initially situated along the ventral midline of the L1 larva. The 3 cells are part of an equivalence group of 6 cells that are all capable of forming part of the vulva. Defects in the specification of these cells can lead to an incompletely formed vulva, resulting in worms that cannot lay their eggs.

Background information on the sex specific neurons: CEMs and HSNs

In *C. elegans* hermaphrodites, 131 cells die by programmed cell death (apoptosis). Apoptosis is different from necrosis, in which cells explode and induce death in nearby cells. In apoptosis, cells exhibit shrinkage, chromatin condensation, and specific cleavage of DNA. In fact, apoptosis is simply another programmed cell fate. To further understand how cell death is working in the development of *C. elegans*, researchers continue to isolate mutants that display defects in the cell death pathway, specifically looking for the maintenance of cells that normally die, or for the death of cells that normally live (Ledwich et al, 2000; Xue et al, 2002).

One example of cell death that is useful to us in this lab is a set of sex-specific neuronal cell deaths. In males, there are four neurons called cephalic companion cells (CEMs) present in the head, near the pharynx. These cells probably mediate specific chemotaxis of males towards hermaphrodites, although mating can occur without the CEMs. In hermaphrodites, these cells die by programmed cell death. In hermaphrodites, the HSNs are neurons required for egg laying. In males, these cells die by programmed cell death (Desai et al, 1988).

The GFP strain we will use to see if the HSNs are responsible for the egg-laying defect actually has two different GFP constructs integrated -- one that drives expression in the CEMs and male tail, and one that drives expression in the HSNs. The CEM and male tail neuronal GFP expression is driven by a promoter for the *pkd-2* gene -- a gene that has homology to a human gene involved in polycystic kidney disease. The other promoter is for *tph-1*, tryptophan hydroxylase, a key enzyme for serotonin biosynthesis and is thus expressed in serotonergic neurons including the HSNs. The strain that carries both of these transgenes is called *smIs26* (Is stands for integrated strain; Ding Xue, personal communication).

Background information on *C. elegans* vulval determination

Vulval development depends on signaling events among the precursor cells from which the vulva eventually forms, as well as signaling from cells of the somatic gonad that are dorsal to the future vulva. The vulva develops from an equivalence group of cells called the vulval precursor cells (VPCs). There are 6 cells in the VPC group (P3p-P8p), all of which are capable of making the cells of the vulva. Normally, only P5p, P6p, and P7p make up the vulva, while P3p, P4p, P8p make part of the epidermis. The middle three P cells are signaled to become vulval cells by a cell called the anchor cell, just dorsal to the vulva. If the signal from the anchor cell is blocked, the vulva cannot form.

In the 1980s and '90s, huge mutant screens were done to identify mutants that had defective vulval formation. These mutations generally resulted in one of two phenotypes: Vulvaless (Vul), in which the normal VPC's (P5-7p) do not form a vulva, and Multivulva (Muv), in which all cells of the equivalence group (P3-8p) behave as VPC's so that multiple (nonfunctional) structures are formed. Subsequently, the genes causing these phenotypes were cloned, and found to be members of the Ras and Notch/Delta signaling pathways. The various members of the Ras pathway discovered in *C. elegans* have homologs in mammals, making this system an attractive one for studying cell fate (Ferguson et al, 1985).

The GFP marker we will use to look at the vulva is expressed in several tissues including the vulva in adult hermaphrodites. These transgenic worms contain the promoter for a gene called *ajm-1*, which is a component of adherens junctions hooked to the GFP cDNA. In the adult worm, adherens junctions are most visible in the pharynx of the head, and between the cells that make up the vulva (Koppen et al, 2001). Thus, this construct can be used to visualize the vulva itself.

Setting up the experiment

The alleles you will be working with:

smIs26 – this is an integrated GFP line (Chromosome IV). In wild type males, the GFP marker is expressed in male-specific neurons in the head (CEMs) and tail. In hermaphrodites, the GFP marker is expressed in the HSNs, and in one neuron in the head. An important component of this strain is that it also carries an extra wild type copy of the *unc-76* gene, which one has to remember when looking at phenotypes later.

ajm-1 – this is an integrated GFP line (Chromosome X). In wild type animals, this GFP marker is expressed in the pharynx and in the vulva.

him-5 – a mutation that increases the incidence of males (Chromosome V).

unc-76 – a mutation that causes the worms to have severe problems moving. They tend to kink or ben, and also tend to be a little smaller than a wild type worm (Chromosome V).

egl-41 – the mutation you are trying to characterize. This mutation prevents the hermaphrodites from laying eggs (although it is not 100% penetrant). Because of this, the worms fill up with eggs (since they cannot lay them); eventually the eggs hatch within the mother and crawl out (Chromosome V).

The strains you are using carry multiple mutations or markers. Each strain is homozygous for each of the mutations.

smIs26::GFP/smIs26::GFP; him-5/him-5: (Figure 5) These worms appear wild type. Remember the GFP transgene is not a mutation, just an extra piece of DNA integrated onto chromosome IV. There are males on these plates because of the *him-5* mutation.

ajm-1::GFP/ajm-1::GFP: (Figure 7) These worms appear wild-type. Remember the GFP transgene is not a mutation, just an extra piece of DNA integrated onto chromosome X. There are also males on these plates.

egl-41/egl-41; unc-76/unc-76: (Figure 8) The most obvious phenotype for these worms is that they are Unc. They are also Egl, which you will only notice in the adults that are full of eggs.

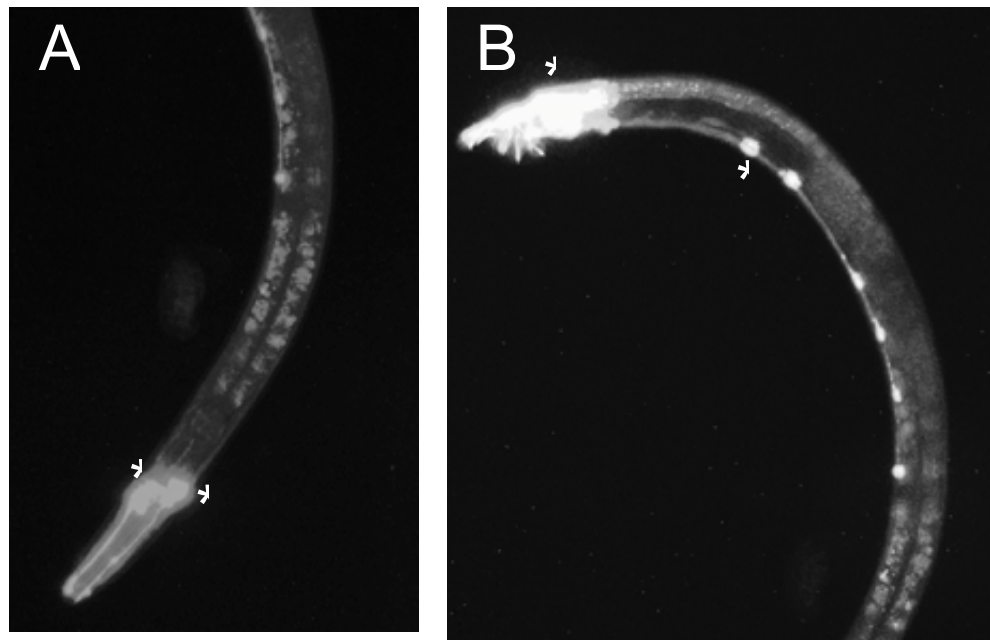


Figure 5. *smIs26* GFP male. Asterisks indicate CEMs in A., and ventral neurons and tail in B.

Figure 6. *smIs26::GFP* line. GFP is expressed in the HSN neurons. In both panels, asterisk indicates the vulva. A. GFP image, showing a single HSN (arrow). B. DIC image of same worm.

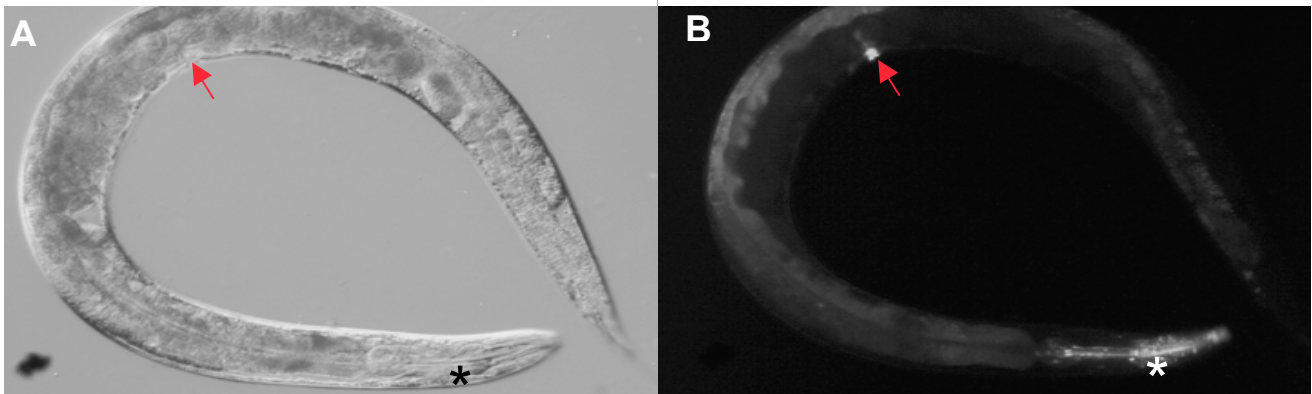
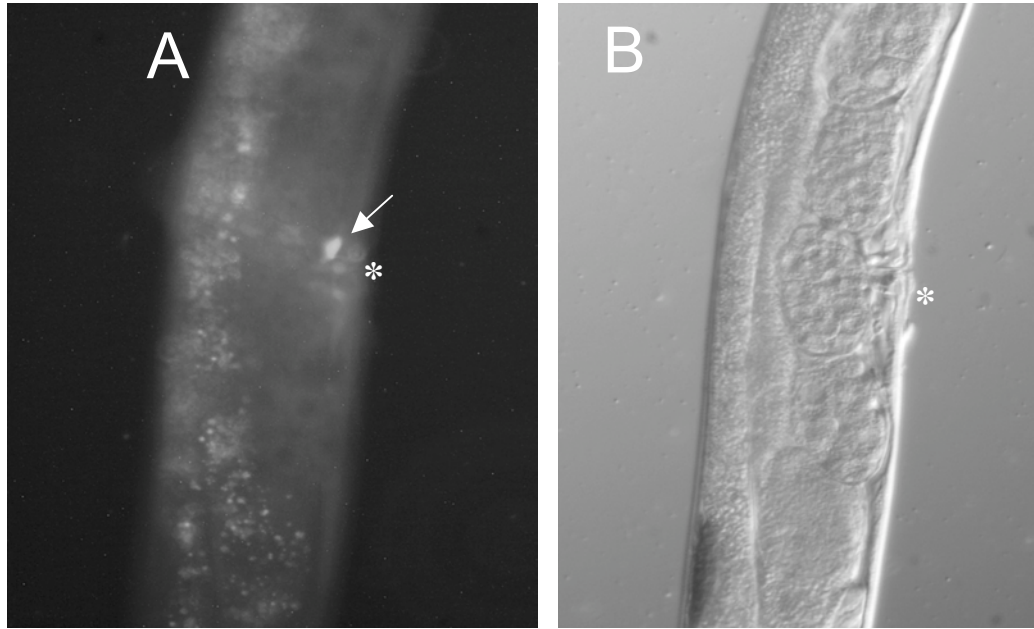
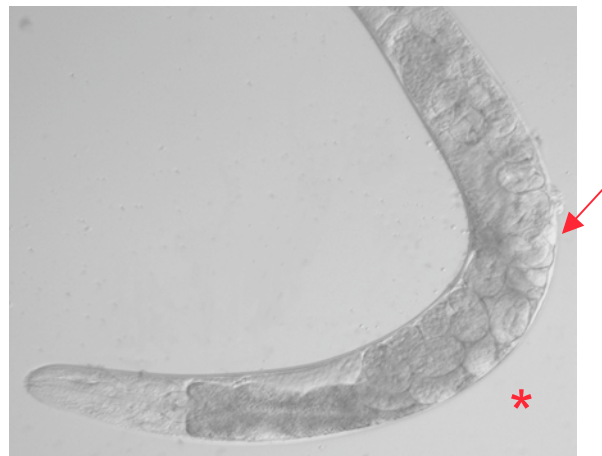


Figure 7. A. DIC image of adult *ajm-1::GFP* hermaphrodite. Asterisk indicates pharynx. Arrow indicates vulva. B. GFP image of same hermaphrodite.

Figure 8. *egl-41* worm. Egl worms are egg laying defective; as a result, “old” eggs (hatching stage) can be seen still inside the hermaphrodite (asterisk). Arrow indicates the vulva.



Details:

1. Set up crosses that will help you figure out what is wrong with the *egl-41* worms. Set up two plates of EACH kind of cross with 2-3 hermaphrodites and 6-8 males on each plate. You should use each of the GFP strains in your analysis.
2. When you set up your crosses, be sure to write your name or group number on the bottom of the plates, put a rubber band around them and put them in a box with a cover (this keeps them from drying out).
3. Decide at what temperature to grow your worms. At 16 °C, it will take them about 6 days to go through one life cycle; at 20 °C, about 4 days, and at 25 °C, 2-3 days. IMPORTANT: come back frequently to check on your worms. If there are too many worms on your plates (no *E. coli* left for them to eat), move them to 16 °C. If you don't do this, your plate could be overgrown by the next lab period, and you will have to start over!!! If there aren't many worms when you check, put your plate at 25 °C to speed things up.
4. Take pictures of your starting strains so you have something to compare your end results with.
5. Make sure you understand the crosses and what you are looking for.
6. *When you have F1 cross progeny (ie, non unc-worms!),* pick each worm to its own plate to make sure the worm and its progeny will have enough food. It is a good idea to pick at least 3 F1s from each of your crosses. *It is also a good idea to set up another cross (repeat step 1) at this time so that if something goes wrong, you don't have to start over.*
7. *When you have F2 progeny:*
 - Check the F1 plates for the F2 progeny. Your goal is to pick F2 worms that will help answer your question. For the *ajm:GFP* cross, these worms will be Unc and Egl. For the *smIs:26GFP* cross, these worms will be just Egl (realize that some of the F2 worms will be just Egl, and not contain the *smIs26:GFP* at all. How will you tell them apart from the *egl-41; smIs26GFP* worms?)
 - Ideally, you will want to look at *older adult hermaphrodites* so you can see the best GFP expression and the best “Egl”ness.
 - Once you have picked the right worms, put them on an agarose slide and take pictures of them on the microscope, using BOTH DIC optics and fluorescence. If you have not yet done so, you need to also take pictures of each of the starting strains of worms.
 - Collect and interpret your data.

***C. elegans* Lab Questions**

1. Briefly describe how genetics can be done using the “forward” vs. “reverse” approach. What are the start and end points of each approach?
2. How would you create a GFP-expressing transgenic line of *C. elegans* that specifically expressed GFP in muscle cells only?
3. In this, you will try to characterize what prevents *egl-41* mutant worms from laying their eggs. Illustrate the crosses you will do, and the genotypes and phenotypes of the F1 and F2 progeny for each cross. Which worms are you picking in each generation, and why?

4. Generate a **hypothesis** for the experiment you are about to do (or have just done). What are your **predictions** for the outcome of the experiment (ie, describe what you think will happen and why)?
5. Describe a different experiment that you could do that would further help you to characterize the *egl-41* phenotype (there are many possibilities—just describe one possible approach).

Notes for the Instructor

Background info on the Sample Experiment

The goal of this lab is two-fold:

1. Remind (or introduce to) students how to do genetic crosses
2. Develop a hypothesis about what is wrong with a mutant called *egl-41*, and test their hypothesis using two different GFP marker strains.

In order for students to understand the processes behind the experiment, a brief primer on vulval development and sex-specific neurons is useful.

Vulval development

The vulva develops from 3 cells (P5p, P6p and P7p). These three cells are part of an equivalence group actually comprised of 6 cells, referred to as the VPCs. VPCs are signaled by the anchor cell, which lies dorsal to the VPCs (Ferguson and Horvitz, 1985; Wang and Sternberg, 2001).

Sex-specific neurons

HSNs (Hermaphroditic specific neurons) aid in egg laying. They are serotonergic cells (they use the neurotransmitter serotonin). Without them, worm cannot lay eggs. Males also have sex-specific neurons, located in their head and tail. The tail is full of neurons not present in the hermaphrodite. In the head, 4 CEMs (cephalic companion neurons) probably help in mating behavior.

egl-41 is an egg-laying defective mutant because the HSNs (2 hermaphrodite specific neurons responsible for egg laying) are missing. These neurons die by cell death in this mutant. The other reason it might not lay its eggs properly would be if it didn't have a vulva. Thus, students can test why the worms are Egl by crossing two different GFP strains that allow them to visualize the HSNs or the structural components of the vulva, into the *egl-41* strain.

egl-41 is also known as *sel-10*. It has been cloned and encodes a member of the ubiquitination pathway. *egl-41* is probably also involved in the sex determination pathway in *C. elegans* (Ding Xue, personal communication). One observation that has been made is that in *egl-41* worms, the HSNs are missing, but the CEMS, which are normally male-specific neurons, are actually present (Jager et al., 2004). Thus, *egl-41* mutants have male-specific neurons but not hermaphrodite-specific neurons – this difference in sex-specific cell death should be visible in the *egl-41; smIs26* worms. They should have neurons in their head that glow, but no HSNs glowing around the vulva.

The HSNs are difficult to see—even under the best circumstances, the GFP expression is not extremely bright. However, if students know where to look (either side of the vulva), and know they should be looking in the hermaphrodite head for GFP expression (because the NSMs, present in the

head, are also illuminated by this GFP construct), they should be able to come to the conclusion that the HSNs are missing in the *egl-41; smIs26* worms.

For background information, it is probably necessary to introduce the students to most if not all of this information. Depending on the level of the class, details can be added or left out. The lab works well when the students are left to “discover” on their own what causes the Egl phenotype, using their results and reading the literature to understand the data. However, in a more beginning level class, or in a class more focused on genetics and less on developmental biology, more information could be given and the students can be asked to replicate the known results.

Additional Tips

- Students should remember to flame their picks, or worms will become contaminated.
- If worms do become contaminated, you can “clean” them by placing a drop of bleach onto a clean plate and picking hermaphrodites full of eggs into the bleach. The hermaphrodite will dissolve in the bleach, but the embryos still inside the hermaphrodite will survive. When they hatch and crawl out, they should be clean.
- If you want a strain to have a higher proportion of males (and the strain does not contain a Him mutation already), you can create males by placing L4 worms at 30 °C for 4-6 hours. The high temperature will induce non-disjunction, so you should see some males in the progeny. You can then cross these males back to hermaphrodites, and you will get about 50% males in the next generation.
- To maintain worms from week to week, the strains need to be passaged about once a week. This means they need to be placed on a fresh plate with OP50 bacteria. There are two ways to do this: 1) Pick a few gravid hermaphrodites to the clean plate. Within 3-4 days at room temperature, the 100 or more progeny from these hermaphrodites will range from embryos to adults and will provide students with plenty of material to look at, or 2) “chunk” the plates. This means cut a small agarose chunk containing worms from the old plate and invert it onto the new plate. If you want a lot of worms fast, this is a quicker way to passage the plates. If your plates “starve” (no food), simply chunk the worms to a new food source – the worms will come out of starvation phase and develop normally.
- *C. elegans* go through an entire life cycle (adult in P0 to adult of F1) in about 3 days at room temperature. At the higher temperature of 25 °C, the life cycles takes only 2 days; at 16 °C it takes about 6 days. Worms can also go into a “dauer” or dormant state when food supply runs out, and can live for a very long time in this state. Normal life span of the worm is 2-3 weeks.

Materials

General Supplies

- | | |
|---|--|
| • Bunsen burners (1 for every two people in workshop) | • Glass slides (1-2 boxes) |
| • Forceps (1 for every person in workshop) | • Glass coverslips 18 mm square (1 box) |
| • Alcohol lamps (1 for every two people) | • 1 microwave |
| • Glass Pasteur pipettes and bulbs | • Gloves (all sizes, for handling of Sodium Azide) |

Platinum wire for “picks” (VWR cat # 66260-068). This comes in a 30 inch length, so a quantity of 1 is sufficient for making about 15-20 picks.

Microscopes

1 dissecting microscope for every person, or 1 for every two people if there are not enough scopes to go around. In order to get the best possible view of worms, the light should come up from below the stage. This can be easily done by using a fiber optic light source and reflecting the light off the mirror.

1 compound microscope (for whole group) with fluorescence and DIC optics; must have a GFP cube for best visualization of GFP constructs. In a pinch, an FITC cube will also work.

Digital camera hooked to computer to visualize what is on fluorescence microscope (or a standard camera hooked to the microscope).

Solutions/Reagents for participants

25 mM Sodium Azide (10 ml, aliquoted into 0.5 ml volumes: 1 per two people)

d H₂O (0.5 ml aliquots: 1 per two people)

1% agarose (100 ml, in microwaveable bottle; 1 for whole class)

NGM plates spotted with OP50 *E. coli* (recipes in Appendix).

Worm strains

Strains are available from the *C. elegans* strain consortium (CGC), or directly from labs that use them. Below is a list of the strains used in this lab, with their strain names, which usually need to be used for ordering. Not all strains have been submitted to the CGC, and so it is often best to request the strains from the lab in which they were made. If anyone would like the strains used in this lab, I have access to them, since they were all made at the University of Colorado, in the labs of Ding Xue and Min Han.

Strains used in this lab

- ***smIs26; him-5***: GFP strain expressed in male tail, CEMS, NSMs and HSNs
- ***ajm-1::GFP***: GFP strain expressed in vulva and head (adherens junctions)
- ***ajm-1::GFP; let-60 (n1046 gf)***: same strain as above, with another mutation that makes the worms have multiple vulvae (Muv).
- ***egl-41 (n1074) unc-76***: egg laying defective strain, also Unc
- ***smIs26; him-5; egl-41 (n1074)***: the strain resulting from one of the crosses described in Experiment 1 above. This strain shows that *egl-41* is lacking its HSNs.

The following strains were used as examples of common phenotypes like Unc, Dpy and Lon

- ***unc-26***: These worms are Unc (uncoordinated).
- ***lon-2***: These worms are Lon (long).
- ***dpy-3***: These worms appear Dpy (short and fat, dumpy).
- ***let-60 (n1046)***: gain of function Ras mutation that leads to multiple vulvae (Muv).

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Ding Xue and Min Han (and members of their labs): worm strains

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Literature Cited

- Desai C, Garriga G, McIntire SL, Horvitz HR (1988). A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. *Nature* 336, 638-646.
- Ferguson, E.L., and Horvitz, H.R. (1985). Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. *Genetics*, 110, 17-72.
- Koppen M, Simske JS, Sims PA, Firestein BL, Hall DH, Radice AD, Rongo C, Hardin JD (2001). Cooperative regulation of AJM-1 controls junctional integrity in *Caenorhabditis elegans* epithelia. *Nature Cell Biology* 3, 983-991.
- Ledwich D, Wu YC, Driscoll M, Xue D (2001). Analysis of programmed cell death in the nematode *Caenorhabditis elegans*. *Apoptosis* 322, 76-88.
- Riddle, D.L. Blumenthal, T., Meyer, B.J., Priess, J.R., editors. (1997). *C. elegans II*. Plainview (NY). Cold Spring Harbor Laboratory Press.
- Wang, M. and Sternberg, P.W. (2001). Pattern formation during *C. elegans* vulval induction. *Current topics in Developmental Biology* 51, 189-220.
- Xue D, Wu YC, Shah MS (2002). Programmed cell death in *C. elegans*: the genetic framework. in *Frontiers in Molecular Biology*. Apoptosis. MD Jacobson and N McCarthy (eds). 40: 23-55.

Appendix A: Protocols

OP50 culture and seeding procedure

- Pick a single colony of OP50 *E. coli* (grown on LB plates) into 100 ml L-Broth
- Grow overnight at 37 degrees C, shaking
- Store at 4 degrees (good for about 1 month).

“Spot” OP50 onto plates (a nickel-sized spot is good) using sterile technique

Dry plates (with lids on) for about 2 days at room temperature

Plates can be stored in sealed container for about 1 month at 4 degrees C.

L-Broth

- dH₂O 950 ml
- Bacto tryptone 10 g
- Bacto yeast extract 5 g
- NaCl 10 g
- Stir until dissolved, pH to 7, adjust volume to 1 L, autoclave on liquid cycle.

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NGM plates (2 L batch makes about 135 60x15mm plates) – this is enough for whole class:

- Sigma Agar 35 g
- NaCl 6 g
- Bacto Peptone 5 g
- ddH₂O 1.95 L
- Autoclave 45 min on liquid cycle. Allow to cool 2 hr. Then add:
 - 10 mg/ml cholesterol 1 ml
 - 1M CaCl₂ (filter sterilized) 2 ml
 - 1 M MgSO₄ (filter sterilized) 2 ml
 - PPB 50 ml
- While still liquid, dispense into 60 x 15 mm plates (for large batches, an automated plate pourer can be used. For small batches, a 25 ml plastic pipette with motorized bulb works ok).
 - 10 mg/ml Cholesterol
 - 2 g cholesterol in 200 ml 95% EtOH; Filter sterilize
 - PPB
 - Potassium phosphate monobasic 108.3 g
 - Potassium phosphate dibasic 46.6 g
 - dd H₂O 1 L
 - pH to 6.0 with H₃PO₄ or KOH
 - Autoclave 35 min

Useful Web sites:

<http://wormbase.org/>

<http://elegans.swmed.edu/>

If you wish to order a worm strain from the CGC, contact:

Theresa Stiernagle
University of Minnesota
6-160 Jackson Hall
321 Church Street S.E.
Minneapolis, MN 55455
USA

Phone: (612) 625-2265

Fax: (612) 625-4648

email: stier@biosci.cbs.umn.edu. Worms can also be requested directly from Jennifer Knight.

Appendix B. Pictures showing expected results.

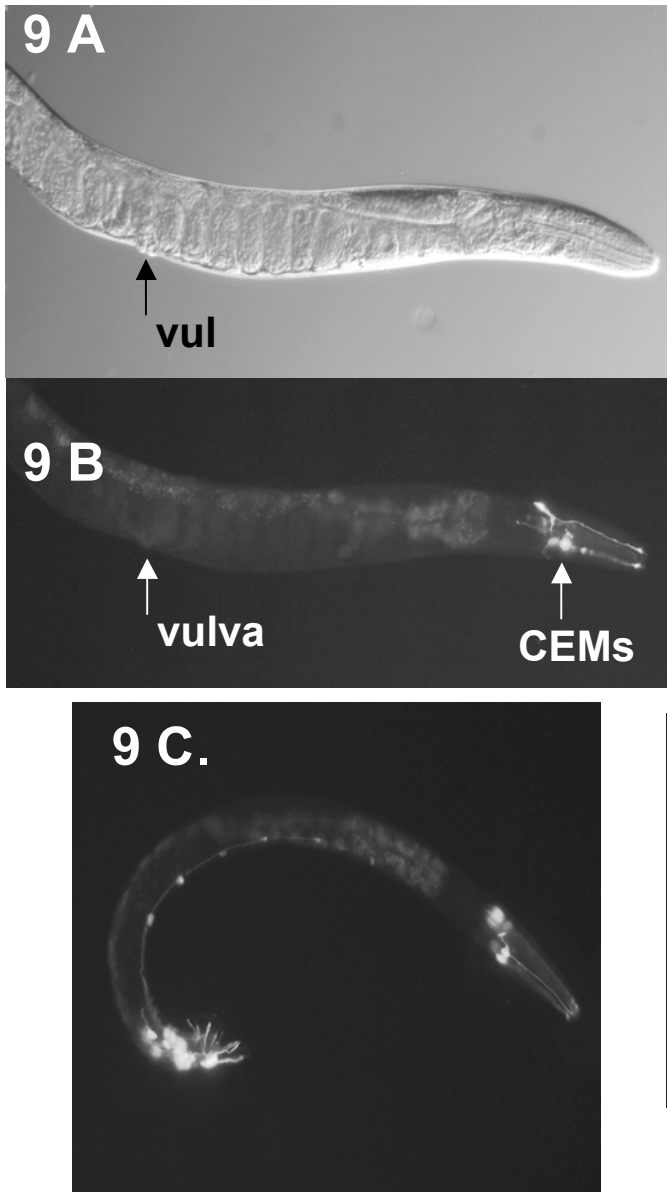


Figure 9. A. DIC image, adult *smIs26; egl-41* hermaphrodite. B. GFP image, same worm (hermaphrodite). Note inappropriate CEMs. C. Male *smIs26*, showing CEMs and other expressing neurons.

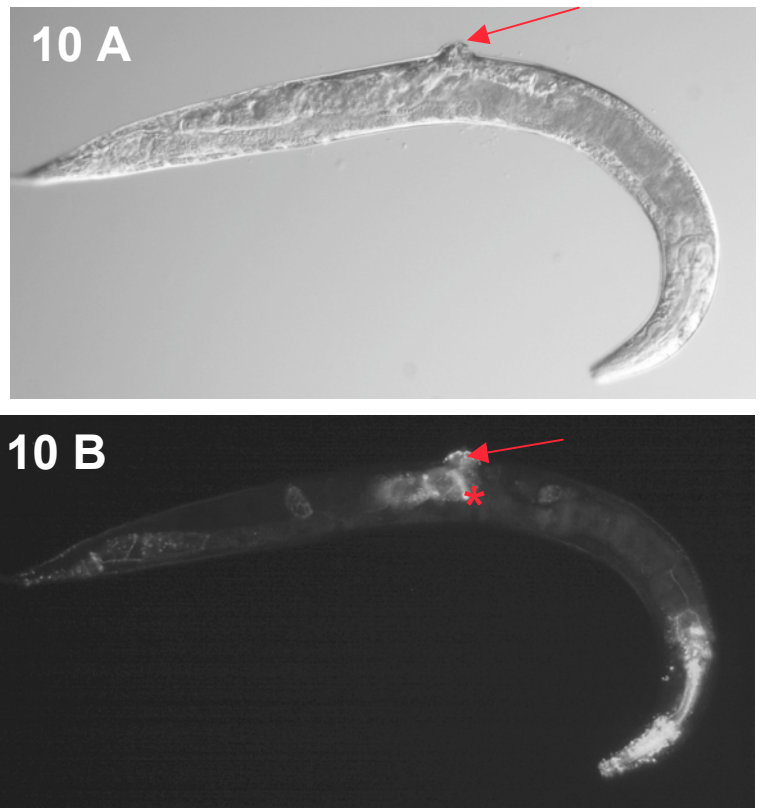


Figure 10. *ajm-1* GFP; *egl-41*. A. DIC image. B. GFP image. Arrow indicates vulva. Asterisk indicates hatched L1 within the hermaphrodite, showing normal GFP expression.