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Using GFP Transgenic *C. elegans* to Demonstrate Gene Expression

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Gene expression is the process by which a gene is used as the instructions to synthesize a protein. Proteins affect an organism's phenotype, which can be characteristics (such as eye color) or behaviors (such as movement). It is often difficult for students to grasp the concept of gene expression. To address this specific topic, I introduced a laboratory exercise in which students observe gene expression by using a *myo-3::gfp C.elegans* strain to determine the cells/tissues that express the *myo-3* gene/protein. This laboratory was implemented in a first year general biology course for biology majors. This exercise gave students the ability to make a connection between gene and protein and to see gene expression.

Keywords: gene expression, *C. elegans*, GFP, genotype, phenotype

Introduction

The objective of this exercise is to use an animal model, *C. elegans*, to allow students to visualize gene expression. This exercise gives students practice in using a compound microscope, using a fluorescent microscope, making a modified version of a wet mount, and micropipetting. The level of difficulty for the lab procedure is easy and appropriate for first year undergraduates. The lab could also be modified for upper level courses in Genetics or Molecular/Cellular Biology. In addition, to looking at gene expression mutant worms can be utilized investigate a behavioral phenotype. For example, chemotaxis experiments test the ability of worms to detect chemicals. Several mutants exist that have deficiencies in detecting chemicals (ie. mutations in *che-2*, *odr-10* and *tax-4* genes).

C. elegans as an Animal Model

C. elegans is a great animal model to use in undergraduate labs because they are transparent, enabling visualization of the general anatomy under a compound microscope. In addition, many transgenic *C. elegans* strains in which GFP is used as a reporter for a specific gene already exist. For example, the myo-3::gfp strain contains a transgene in which the gene that encodes GFP is under the control of the regulatory elements for the myo-3 gene, which is expressed in the muscles including the body wall muscles, vulva, and anus (see Fig. 1 in Appendix A). This transgenic strain will express GFP in the muscle tissues. Thus, muscle cells will glow green when excited by ultraviolet light. I do not go over the details of making the transgenic animals or how the reporter works, but that could be appropriate for a genetics course.

Student Outline

Objectives

- Use compound microscopes
- Use a common model organism, *C. elegans*
- Observe gene expression using a transgenic *C. elegans* with a GFP reporter
- Understand the difference between gene, protein and gene expression

Introduction

Gene expression is the process by which a gene (a section of DNA) is used as the instructions to synthesize a protein. This process consists of two major steps: transcription and translation. Transcription is the process by which the DNA sequence of a gene is copied to produce an mRNA transcript. During translation, the mRNA sequence is used to direct protein synthesis. Proteins affect an organism's phenotype, which can be characteristics (such as eye color) or behaviors (such as movement). So, an organism's genotype (all of its genes) influence its phenotype (characteristics/behaviors) via proteins, which are synthesized based on the DNA instructions in each cell. You can observe the location of proteins in an organism or cell to get a better understanding of what its function is or might be. In addition, characteristics and/or behaviors of an organism can give us a better understanding of the function of individual genes/proteins. In this laboratory exercise, you will become familiar with the basic anatomy of a *C. elegans* so you are able to identify some basic tissue/cell types. Next, you will look at a specific transgenic C. *elegans* to determine the tissues/cells that express a specific protein (gene). GFP reporter transgenes are valuable tools for observing gene expression in live organisms, such as *C. elegans*. The gene encoding the green fluorescent protein (GFP) is a commonly used reporter gene derived from jellyfish. Cells that express GFP glow green when using a fluorescent microscope. GFP reporter transgenes have become the primary tool for gene expression analysis in *C. elegans* (looking at where a specific gene is expressed by using GFP as a marker). You will look at the GFP reporter transgene, *myo-3::gfp*, a transgenic *C. elegans* strain, to determine where GFP (thus, the protein/gene *myo-3)* is normally expressed.

Methods and Data Collection

Part A: Observation of C. elegans and Identification of Basic Anatomy

In the first week of this exercise, you will observe wild-type *C. elegans* and become familiar with their basic anatomy. Working in a group of two, you will observe *C. elegans* (that have been mounted for you) under a compound light microscope. You will use your cell phone to take an image through the ocular of the compound light microscope and open the image on your computer. You must be able to identify the following anatomical structures of the *C. elegans* in your image: nerve ring, body wall muscle, dorsal nerve cord (DNC), ventral nerve cord (VNC), spermatheca, vulva, oocytes, gonad, embyros, intestine, pharynx, tail and head. Use a schematic from the wormbook as a guide to help you identify these structures. Once your instructor is satisfied that you are familiar with the anatomy, you may proceed to your next task.

Part B: Identification of Tissues/Cells That Express Myo-3 in C. elegans

In week 2 your group (2-4 students) will collect *myo-3::gfp C. elegans* from an agar plate and mount them on a microscope slide. To collect the worms, add 1 ml of sterile water to a 35mm petri dish containing *myo-3:gfp C. elegans*, using a plastic transfer pipette. Rotate the plate to cover the entire surface with water and let it sit for 1 minute. Tilt the plate to 45-degree angle and use a P1000 set at 1000µl to suck up the worms and transfer them into a 1.5ml microcentrifuge tube labeled with your initials. Allow the worms to settle for 2 minutes. The worms will sink to the bottom and form a worm pellet. Using a P1000 micropipette, remove the liquid from the 1.5ml microcentrifuge tube without disturbing the pellet of worms and dispose of the liquid in the designated container. Add 500ul of sterile water to the microcentrifuge tube with the worms. Cap the tube and gently invert 5 times to mix. Allow the worms to settle for 2 minutes and remove the liquid without disturbing the pellet of worms. Dispose of the liquid in the designated container. Add 100µl of sterile water to the microcentrifuge tube with the worms. Your worms are now ready to be mounted. Use a razor blade to cut the end of a 10µl micropipette tip off, to widen the opening. You will use this cut micropipette tip to transfer your worms onto an agarose pad. Before mounting your worms you must make an agarose pad on a microscope slide. Use a plastic transfer pipette to quickly pipette 1 drop of liquid 2% agarose onto a microscope slide. Immediately place a microscope slide on top of the first microscope slide making a sandwich with the agarose in the middle of the microscope slides. Wiggle the slides apart leaving the agarose pad on one of the microscope slides. Do not take the slides apart until you are ready to mount your worms or the agarose pad with dry out. Transfer 3µl of worms onto the agarose pad using your cut micropipette tip. Add 3µl of TRI/TET anesthetic on top of the worms using an uncut 10µl micropipette tip. Place a coverslip on top of the agarose pad with the worms. View the *myo-3::gfp* worms using a fluorescent microscope. You will first visualize the worms under white light, the image will be projected onto the computer screen so that you can take an image with your cell phone (white light image). If the worm is moving allow 10

minutes for the anesthetic to work. It will be difficult to get a clear picture if the worm is moving. Next, the ultraviolet light will be turned on, the image will be projected onto the computer screen, and you will capture an image using your cell phone (GFP image).

Data Analysis

You will open both images (white light and GFP) on your computer. You will use the white light image to identify general anatomical structures as you did in part A. You will use the GFP image to determine which tissues/cells express the *myo-3* gene (are fluorescent green) by comparing it to the white light image. Once you have determined which tissues/cells express *myo-3*, write your answer on a sheet of paper and give it to your instructor. Your instructor will let you know if you are correct or need to try again.

Discussion

This laboratory exercise will introduce you to the concept of gene expression, using a common animal model, *C. elegans*. As we discussed in Foundations of Molecular Biology, gene expression is the process of using the instructions encoded in our genes (segments of DNA) to build proteins. When a gene is expressed, a gene (segment of DNA) is copied to make an mRNA transcript, this process is called transcription. This mRNA transcript is then used to synthesize a protein, this process is called translation. We often use the terms turned on or turned off to indicate whether a gene is expressed (turned on) or not expressed (turned off). When a gene is expressed (turned on), the processes of transcription and translation take place in order to build a protein based on that specific DNA sequence of the gene. When a gene is not expressed (turned off) these processes do not occur. Every cell in an organism contains all of the same genes (DNA instructions to make proteins). Even though all of our cells have the exact same DNA, different cells express different genes (synthesize different proteins) based on their function. For example, red blood cells express specific genes that allow them to transport oxygen throughout the body. Red blood cells express the genes HBA and HBB, which provide the instructions for making the proteins alpha-globin and beta-globin. Together the proteins alpha-globin and beta-globin form hemoglobin, which binds to oxygen molecules and transports them to all of the tissues in the body. Other cells in the body such as skin cells do not express HBA or HBB because they do not need the hemoglobin protein for their function. We can use molecular/genetic tools such as reporter genes to visualize gene expression. When a gene is turned on in a cell the protein GFP will be transcribed and translated so we can visualize it under a microscope. If a cell is glowing green then you know that the gene is turned on, the gene is being expressed. However, if the cell is not glowing green then you know that the gene is turned off, the gene is not being expressed. Over the next two weeks you will work with a laboratory partner to observe a myo-3::gfp *C. elegans* strain. Any cell in this *C. elegans* strain that expresses the *myo-3* gene will glow green due to GFP, while cells that do not express *myo-3* will not have GFP. You and your lab partner will be asked to identify which tissues/cells express the *myo-3* gene.

Materials

A compound light microscope is required for each student group. One fluorescent compound microscope is required for observing the *myo-3::gfp C. elegans*. The fluorescent microscope is best if it has a camera attached to project the image onto a computer screen, but you can take a picture through the ocular if you do not have a camera. You will also need a hot plate and P1000 and P10 micropipettes.

The following materials are needed for this lab exercise. *C. elegans* strains wild-type (N2) and myo-3::gfp (RW1596) available from the CGC, Caenorhabditis Genetics Center, (www.cgc.umn.edu) for $7 per strain. You may also order OP50 E. coli for $7 per plate from the CGC, which is the food source for *C. elegans*. Nematode Growth Medium, NGM (item #173520) available from Carolina Biologicals. Microscope slides, square glass coverslips #1.5, plastic transfer pipettes and razor blades available from Fisher Scientific. TRI/TET anesthetic is a 10X solution of 1% tricaine (item # AC118000100) and 0.1% tertamisole (item # #ICN15211905) available from Fisher Scientific prepared in M9 buffer, PBS or dH_2O. You can use the 10X concentration for anesthetizing the worms or dilute to a 2X concentration. P1000 micropipette tips, P10 micropipette tips and 35mm petri dish available from USA Scientific. A 2% agarose gel can be made from any agarose and TAE or TBE buffer.

Notes for the Instructor

I use this lab for an introductory Molecular Biology course for 96 students (6 lab sections of 16 students each). The expense and preparation time is manageable when they are working in groups of 2-4 students. However, This could present challenges when trying to accommodate larger lab groups.

This lab exercise is completed over 2 weeks. Part A will require ~60 minutes and is completed in week 1. Part B will require ~90 minutes and is completed in week 2. The objective for part A is to become familiar with the anatomy of the *C. elegans*, using a compound light microscope. For part A, I collect and mount the worms for the students. You could extend part A and require the students to collect and mount the worms. The objective for part B, is for the students to collect, mount and view *myo-3::gfp C. elegans* with fluorescence to identify the cells/tissues that express *myo-3* (GFP positive cells/tissues). I have the students take a picture of the worms under ultraviolet light. They use this image to identify which cell/tissues express *myo-3* (are GFP positive). This requires either a camera mounted on the microscope or a hand-held camera positioned over the ocular.

Several design elements of the exercise were specifically chosen to reduce cost and preparation time. For part A, I collect and mount the worms for the students so that only one plate of worms is needed for each lab section. The protocol for collecting and mounting the worms is described in part B. If you only want to buy one strain of worm, you could use the *myo-3::gfp* worms for part A and part B. The students then work in groups of two to identify the worm anatomy in part A. For part B, I have the students work in groups of 4 so that each lab section needs only 4 worm plates (one plate of worms per group). You could also combine these labs into 1 week to reduce the number of worm plates that are needed.

Some additional *C. elegans* strains that I have used include: tax-4:gfp (BR5602), GFP is expressed in sensory neurons, and unc-119::gfp (DP132), GFP is expressed in all neurons. One additional strains that I have visualized but have not incorporated into a lab is *tbg-1*::GFP and *pie-1*::GFP::H2B (TH30) labels gamma tubulin (centrosomes) and histones (chromosomes). This could be used to watch mitosis in real time. However, the TH30 strain fluorescence is difficult to visualize unless you use a 60x or 100x objective. These strains are available from the CGC (www..cgc.umn.edu) for $7 each.

One of the most difficult challenges for implementing this exercise can be the *C. elegans* husbandry. There are a number of resources available for learning about *C. elegans*: www.wormbook.org; www.wormatlas.org; www.wormbase.org. Worms are grown on NGM agar that is seeded with OP50 *E. coli*. When seeding NGM agar plates, an *E. coli* culture must be grown up overnight and then spread onto the NGM agar plate. The *E. coli* must grow on the NGM agar plate overnight before adding worms onto the plate. Once the plates are seeded with *E. coli* and grown overnight they can be used to add new worms or stored in a sealed container for several weeks. The simplest method to maintain worm cultures is called chunking. Chunking worms is cutting a chunk of agar from an existing plate of worms and transferring that chunk to a newly seeded agar plate. A chunk of NGM agar and worms from a current worm plate is placed into a newly seeded NGM agar plate, using a metal spatula using sterile technique. General maintenance of the worms requires chunking every five to six days. When preparing worms for the laboratory days, worms must be chunked two to three days prior to lab. If you prepare the plates too far in advance they will be overgrown and the worms may burrow into the agar, which will make it difficult to collect them. If you prepare the plates too late, they will be mostly juvenile worms, which are smaller and more difficult to see anatomical structures.

When making the agarose pads, I keep the 2% agar on a hot plate so that it stays liquid during the lab. I recommend having the students practice making an agarose pad, while they are collecting the worms. It may take them a few tries to get it right. If the agarose pad has

a lot of bubbles or tears, they can simply wipe it off of the slide, rinse the slide with water, dry the slide and reuse the same slides to try again.

These exercises are done in the last two weeks of the semester. Because the observation of the *myo-3::gfp C. elegans* is the last lab meeting, the assignment is very simple. The students are only asked to identify the tissues/cells that express myo-3, because the assignment must be completed during the lab meeting. This could easily be modified to include an assignment on generating a labeled figure with a legend.

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About the Author

Jen Rowsell has been an Instructor at Saint Mary's College since 2016, where she teaches courses in introductory Biology, Anatomy and Physiology, and Neuroscience, primarily for first and second year students.

Appendix A
Visualization of *C. elegans* with a GFP Reporter Gene

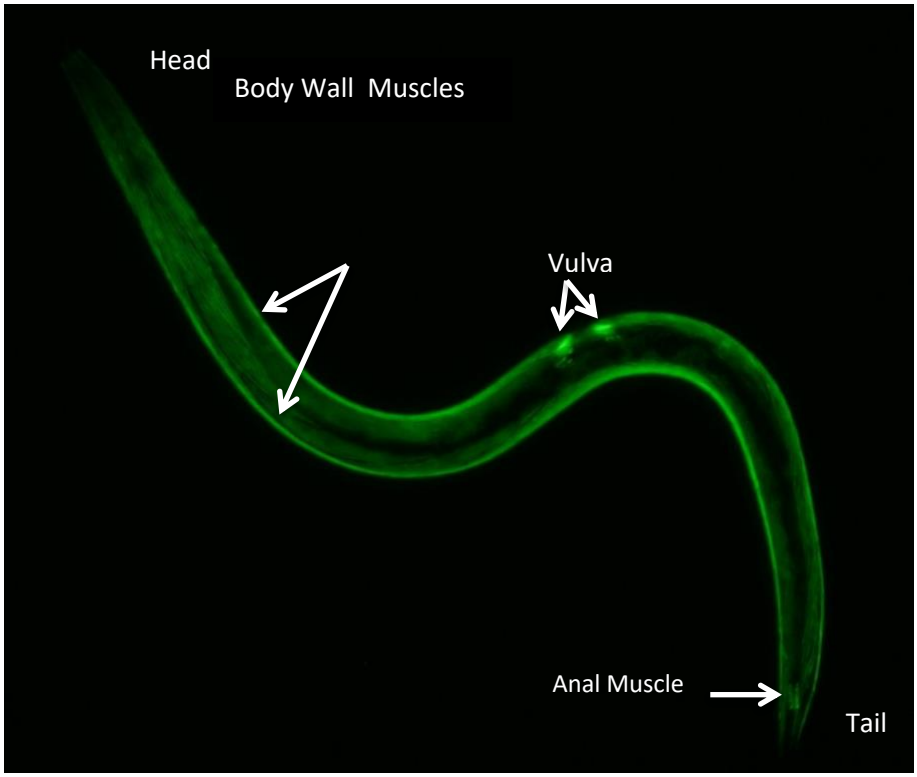

Figure 1. Adult transgenic *C. elegans* strain (*myo-3::gfp*) expressing GFP in the muscle tissues: body wall muscles, vulva and anal muscle. Photo credit: Jennifer Rowsell.

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