LAB ACTIVITY 1

Caenorhabditis elegans

A Tiny Worm That's a Major Model System

INTRODUCTION

In December 1998, researchers announced a milestone in genome analysis. The sequencing of the 97 million base pairs of *Caenorhabditis elegans* was essentially completed. With this achievement, the nematode *C. elegans* became the first multicellular organism for which a complete genome sequence was available.

Since the 1970s, *C. elegans* ("the worm," as it's known to those who study it) has been a favorite model organism of developmental biologists and geneticists. A key characteristic that makes it so attractive for studies of development is the small number of cells that comprise its body — an adult hermaphrodite has exactly 959 somatic cells. Since the worm is transparent, its development can be carefully followed from a single cell to a mature adult. Microscopic studies of this process have traced the exact cell lineage of each of the 959 somatic cells.

Furthermore, mutant *C. elegans* can be isolated with relative ease, and thousands of mutant strains are available for study. This has enabled geneticists to "genetically dissect" the development and physiology of the worm. As a result, *C. elegans* serves as an important model organism for studies involving many complex phenomena associated with multicellular organisms. For example, much of what we now know about the important process of programmed cell death (apoptosis) comes from studies of mutant strains of *C. elegans*.

CHARACTERISTICS OF *C. ELEGANS*

Caenorhabditis elegans is a small soil nematode that eats bacteria and normally reproduces as a hermaphrodite. Morphologically distinct males occur infrequently (< l% of worms in cultures of wild-type strains are males), but they are unnecessary for reproduction. Hermaphrodites have two X chromosomes, and the rare males are produced when a nondisjunction event produces an embryo with only one X.

Hermaphrodites produce offspring by self-fertilization, or a hermaphrodite may be fertilized by a male. It takes only 2 to 3 days for a fertilized egg to develop into an adult. During that time, four larval stages occur, with a molt between each stage. The morphology of the adult is fairly simple (see Figure 1-1). The body of the worm is surrounded by an acellular cuticle. Within the cuticle, the body consists of gonads, an intestine, muscles, nerves, and hypodermal cells that produce the cuticle.

During this laboratory period, you will become familiar with the normal characteristics of typical male and hermaphrodite worms. You will also be introduced to several very strange mutant strains.

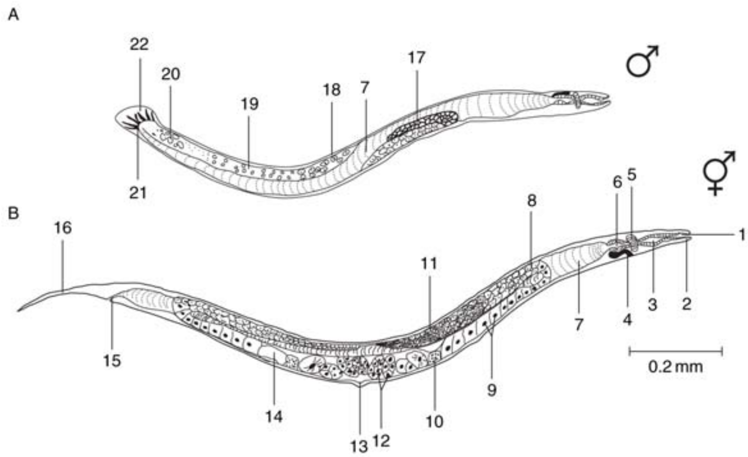

Figure 1.1 – The anatomy of *Caenorhabditis elegans:* (a) an adult male and (b) an adult hermaphrodite. 1. Buccal cavity. 2. One of six lips. 3. Metacropus of the pharynx. 4. Excretory cell. 5. Nerve ring. 6. Terminal bulb of the pharynx. 7. Intestine. 8. Distal arm of the gonad, ovary. 9. Loop of the gonad. 10. Proximal arm of the gonad with maturing oocytes. 11. Spermatheca. 12. Uterus with cleaving eggs. 13. Vulva. 14. Mature oocyte before spermatheca. 15. Anus. 16. Tail. 17. Testis. 18. Spermatocytes. 19. Vas deferens. 20. Mature sperm. 21. Cloaca. 22. Copulatory bursa with rays and copulatory spicules.

PROCEDURE

Microscopic Observations — Living Worms

Obtain a culture of worms labeled "him" (high incidence of males), and view these under the dissecting microscope. This strain of *C. elegans* is morphologically wild-type; however, its cultures yield an abnormally high percentage of males. Observe these living worms and answer the following questions:

Q1 **Can you distinguish the anterior versus the posterior end of the worm? How? (Describe what you see.)**

Q2 **How are males morphologically different from hermaphrodites? (Do not discuss the internal anatomy in your answer to this question.)**

Q3 **How do males and hermaphrodites interact? (Describe what you see.)**

Microscopic Observations — Stained Worms

Prepare a slide with several hermaphrodite and several male worms. Place a drop of dilute methylene blue stain on a glass slide, and transfer the worms into the dilute stain. You will have to work carefully, using the dissecting microscope to pick up the worms (using a probe made with a piece of fine wire). When you place the worms in the stain, they will remain alive for some time. However, within 10 to 15 minutes, they will die or become extremely sluggish so that you can make detailed observations using a compound microscope.

As you work with the slide, make sure that there is enough stain present to keep the coverslip floating above the worms. You may need to add more stain from time to time to prevent the coverslip from crushing the worms.

Draw a sketch of a hermaphroditic worm and another one of a male worm. Make your sketches at least 10 to 15 cm in diameter, and include as much detail as possible. On the appropriate sketch(es), label each of the following items: mouth, vulva, copulatory bursa, ovary, and uterus. You may see additional recognizable features, as well.

Observing Mutant Strains

Several mutant strains are available in separate Petri plates. These plates are labeled with code designations, but not the names of the mutant phenotypes. You should carefully observe these worms using a dissecting microscope and determine which phenotypes are represented by each labeled culture. The following are descriptions of some of the possible phenotypes that may be present in the strains available in your lab. Your instructor may provide names of other mutant phenotypes and descriptions besides those listed here.

dumpy — Dumpy mutants are shorter than the wild-type animals, and, as a result, they certainly do look "dumpy."

him (high incidence of males) — As described earlier, strains with this phenotype look normal, but have many more males than do wild-type strains.

long — These are longer and thinner than the wild-type worms.

roller — Instead of moving forward with an "elegant," sinuous movement, these worms roll sideways, moving by rotating around the long axes of their bodies.

shaker — These worms are somewhat paralyzed.

uncoordinated — There are various kinds of "uncoordinated" mutants. This particular strain is known for not being able to "back up." To see this phenotype, try poking a worm near its head. A normal worm will glide elegantly backwards; an uncoordinated worm will just coil up.

wild-type — One of the coded plates will contain normal worms. These will look like the worms illustrated in Figure 1.1 and the living him worms that you sketched earlier. However, these will have an extremely low frequency of male worms (<1%).

Q4 **List the code designations of each culture that you observed, and indicate the name of the mutant phenotype exhibited in each case.**

NOTES