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C. elegans Development and Reproduction

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Abstract

Ceanorhabditis elegans is a powerful tool to help understand how organisms develop from a single cell into a vast interconnected array of functioning tissues. Early work in *C. elegans* traced the complete cell lineage and structure at the electron microscopy level, allowing researchers unprecedented insight into the connection between genes, development and disease. Appreciating the stereotyped development and reproductive program of *C. elegans* is essential to using this model organism to its experimental fullest.

This video will give you a peek into the development of a worm from fertilization to hatching, and walk you though the life stages of the newly hatched larvae on its journey to reproductive maturity. The video will detail how the major axes are established, which founder cells give rise to what tissues in the developing embryo and how to discriminate between the four larval stages. Finally, you will learn how to set up a genetic cross and we"ll visit a few applications that manipulate the development and reproduction of *C. elegans* to experimental benefit.

Transcript

Caenorhabditis elegans is an anatomically small and genetically simple multicellular organism with an invariant pattern of development. Despite the fact that other organisms, like vertebrates, have more variable developmental programs, research on worm development and reproduction has yielded important insights into the molecular mechanisms that regulate development in a diverse array of species, including us. A good appreciation of worm development and its life cycle is critical for the success of genetic experiments.

First, let's learn about the key aspects of worm development. Upon fertilization, the first major event is an asymmetrical cell division during which the anterior-posterior axis is established. The dorso-ventral axis is established between the two-cell and the four-cell stage, and the left-right axis is established shortly after the four-cell stage.

Six founder cells appear during the first five rounds of cell division. These are AB, MS, E, C, D and P4. In every worm, these same founder cells will always give rise to the same specific tissues.

Cellular descendants of AB will ultimately give rise to neurons and pharynx tissue. MS gives rise to muscle, pharynx and neurons. Cells derived from E become intestinal tissue. C gives rise to muscle, neurons and skin. Cells from founder D become body wall muscle. And, finally, the P4 cell will give rise to the germline

Cell-cell interactions are critical for determining these ultimate cell fates. For example, the interaction of ABp with P2 is important for giving rise to neurons and epithelial cells. The interaction of ABa with EMS is required for the formation of pharyngeal cells. The interaction between the posterior side of EMS and P2 at the four-cell stage is essential for the E cell that is produced from the EMS cell to differentiate into intestinal cells.

Following the few early divisions, when the embryo reaches approximately the 30-cell stage, the worm egg is laid. Further cell divisions lead to an increase in cell number and formation of organs. Finally, the tiny worm begins to move inside the eggshell, and shortly after its pharynx starts pumping, the egg hatches.

An important aspect of *C. elegans* development is apoptosis, or programmed cell death, that leads to selective removal of certain cells. During the embryonic phase of worm development, 113 cells die as a result of apoptosis.

Having reviewed the embryonic development, let's next learn about the life cycle of a newly hatched worm. The *C. elegans* life cycle comprises of four larval stages — L1, L2, L3, L4 — which are followed by adulthood. Under certain environmental conditions, such as scarcity of food, the late L1 or L2 larvae arrest and enter an alternative developmental program, called the dauer stage. The dauers can stay in this stage for many months, but upon availability of food they re-enter the normal developmental program.

Worms have two sexes — the self-fertilizing hermaphrodites and males. The hermaphrodites have a pointed tail and they are both wider and longer than age matched males. Under a dissecting microscope, the males are easily distinguished by their slim body, but the most profound difference is the distinctive tail of the male worm that bears the copulatory apparatus.

The hermaphrodite germline produces both oocytes and sperm, while the male germline produces only sperm. The germline contains stem cells at the distal tip, which move towards the proximal end to produce mature gametes.

Via self-fertilization, an adult hermaphrodite produces genetically identical hermaphrodite progeny with two sex chromosomes. Occasionally, nondisjunction, which is the failure of the chromosomes to separate properly in the hermaphrodite germline, results in male progeny with only one sex chromosome. High temperature increases the frequency of nondisjunction events

Sexual reproduction is thought to be the driving force for genetic diversity. Even though mating occurs at a low frequency, self-fertilization is the primary mode of reproduction in *C. elegans* in nature. An important unanswered question in worm biology is why males have been preserved through evolution.

Now that you've learned a bit about *C. elegans* development and life cycle, let us see how we can practically apply this knowledge to set up genetic crosses. Before starting, it is important to plan the genetic strategy carefully.

Aseptic technique is important for avoiding bacterial and fungal contamination. Do not let plates dry out, as worm strains may be impossible to recover. On the day of setting up a mating, prepare multiple plates with a concentrated spot of bacteria in the center of the plate. Label the plate with strain names and date. To set up a mating, put three L4 or young adult hermaphrodites and twelve L4 or young adult males on each plate. Incubate at the appropriate temperature and check the plates four days later for cross progeny. The presence of approximately 50% males is the first indication that the cross worked. Pick L4 hermaphrodite cross progeny as these have not mated yet with any males on the plate. Follow them closely to ensure that the observed phenotype matches the expected phenotype.

An understanding of the *C. elegans* life cycle and development has helped to address important fundamental questions in cell biology.

Apoptosis in the germline is an integral part of oogenesis, embryogenesis, and organogenesis in many organisms, including humans. Many regulators of apoptosis are conserved between humans and worms. Therefore, *C. elegans* is a unique system for understanding why so many germ cells die during oogenesis in diverse species.

The only bona fide stem cell lines in *C. elegans* are the germline stem cells at the distal tip. These have been used as a paradigm for understanding how stem cells niches are maintained and how cells commit to differentiation.

Many parasitic nematodes that infect humans go through larval arrest that is similar to the dauer stage in C. elegans. Following infection, they resume development. Many agricultural crops are also invaded by parasitic nematodes that arrest. A better understanding of the dauer mechanisms will lead to better therapies against these nematodes.

You just watched JoVE's introduction to *C. elegans* development and reproduction. In this video, we reviewed embryonic development, cell fate specification, and the life cycle of C. elegans. Research in these areas has yielded important insights into the mechanisms of apoptosis, stem cells and infectious nematodes.

Thanks for watching, and good luck with your *C. elegans* research.