Section B and C

Volume-09

Contents

5. DEVELOPMENTAL BIOLOGY

C. MORPHOGENESIS AND ORGANOGENESIS IN ANIMALS

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Organogenesis in *Caenorhabditis elegans*:

As an adult, *C. elegans* is about 1mm long and consists of only about 1000 somatic cells and 1000-2000 germ cells (exactly 959 somatic cell nuclei plus about 2000 germ cells are counted in one sex; exactly 1031 somatic cell nuclei plus about 1000 germ cells in the other). The anatomy has been reconstructed, cell by cell, by electron microscopy of serial sections. The body plan of this simple worm is fundamentally the same as that of most higher animals in that it has a roughly bilaterally symmetrical, elongate body composed of the same basic tissues (nerve, muscle, gut, skin) organized in the same basic way (mouth and brain at the anterior end, anus at the posterior). The outer body wall is composed of two layers: the protective hypodermis, or "skin," and the underlying muscular layer. A simple tube of endodermal cells forms the intestine.

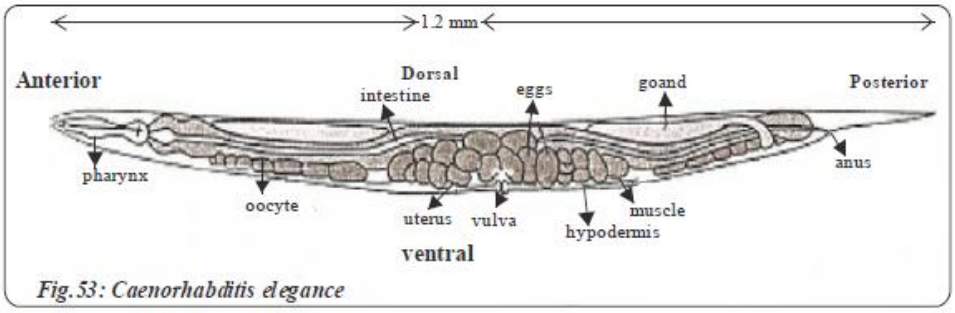

Fig. 53: Caenorhabditis elegance

A second tube, located between the intestine and the body wall, constitutes the gonad; its wall is composed of somatic cells, with the germ cells inside it. *C. elegans* has two sexes- a hermaphrodite and a male. The hermaphrodite can be viewed most simply as a female that produces a limited number of sperm: she can reproduce either by self-fertilization, using her own sperm, or by cross-fertilization after transfer of male sperm by mating. Self-fertilization allows a single heterozygous worm to produce homozygous progeny, a special feature that helps to make *C. elegans* an exceptionally convenient organism for genetic studies. The relative simplicity of *C. elegans* anatomy is reflected in a similar simplicity of its genome. The animal has six homologous pairs of chromosomes, estimated to carry a total of 3000 "essential" genes (that is, genes in which mutations are lethal or have an easily observable effect on the phenotype) and four or five times that number of nonessential genes. The haploid genome consists of approximately 10^8 nucleotide pairs of DNA, which is about 20 times more than *E. coli*, about the same as Drosophila, and 30 times less than humans. Currently, more than 900 essential genes have been identified by mutation. These include genes that influence visible features such as the

shape or behavior of the worm, genes that code for known proteins such as myosin, and genes that control the course of development. Nearly the entire genome has been mapped as a large set of overlapping DNA segments, represented by a library of ordered genomic clones, and a systematic effort has begun to determine the complete DNA sequence of the organism.

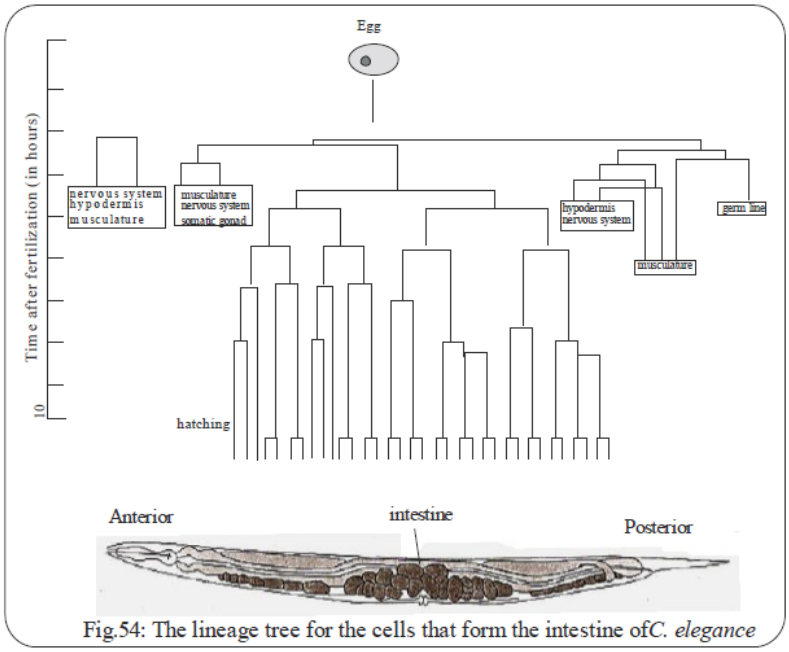

Fig.54: The lineage tree for the cells that form the intestine of*C. elegance*

Nematode Development is almost perfectly invariant

C.elegans begins life as a single cell, the fertilized egg, which gives rise, through repeated cell divisions, to 558 cells that form a small worm inside the egg shell. After hatching, further divisions result in the growth and sexual maturation of the worm as it passes through four successive larval stages separated by molts. After the final molt to the adult stages, the hermaphrodite worm begins to produce its own eggs. The entire developmental sequence, from egg to egg, takes only about three days.

Because *C. elegans* is small and transparent, its individual cells can be followed as they divide, migrate, differentiate, and die in the living embryo, and their pedigree can be traced from egg to adult organism. By this simple technique of direct observation, the behavior and lineage of all of the cells from the single-cell egg to the adult animal have been described. This has made possible a detailed lineage analysis that would be very difficult in larger animals, where individual cells at early stages usually must be specially marked if they and their progeny are to be identified later. Moreover, in larger animals the details of cell lineage show many random variations, even between genetically identical individuals. In the nematode, by contrast, the somatic structures develop by an invariant, predictable cell lineage, and each of the many cell

divisions is precisely timed. This means that a given precursor cell follows the fate of each descendant cell can be predicted from its position in the lineage tree.

The nematode, like most animals, is formed from a relatively large number of cells that can be classified into a much smaller number of differentiated cell types. Given the importance of cell ancestry, one might be tempted to guess that all the cells of given type are descendants of a single "founder cell" committed exclusively to that developmental pathway. Lineage analysis shows, however, that this is not generally true, either for nematodes or for other animals. Thus in *C. elegans* (with a few exceptions such as the intestinal cells and the germ-line cells) each class of differentiated cells-hypodermal, neuronal, muscular, gonadal- is derived from several founder cells originating in separate branches of the lineage tree. Thus cells of similar character need not be close relatives. Conversely, cells of similar character need not be close relatives. Conversely, cells of very different character may be closely related by lineage; for example, some of the neurons in *C. elegans* are sisters of muscle cells.

The problem, then, is to understand the rules that operate in each branch of the lineage tree to generate a specific array of cell types, each in appropriate numbers.

Developmental Control Genes Define the Rules of Cell Behavior that Generate the Body Plan: To explain how the genome specifies the developmental rules, one has to be able to identify the genes that control the cells developmental choices. Mutations in such genes will disturb development, but they are not the only mutations that do so. Some mutations, for example, will cut short all cell lineage and cause premature death of the embryo simply because they disrupt "housekeeping" genes that every cell needs in order to survive and proliferate. Other mutations will affect genes for proteins that particular types of differentiated cells require in order to carry out their specialized function; the body plan will then be essentially normal, but certain cell types, though still identifiable, will malfunction. Mutations in genes that are involved specifically in controlling developmental choices, by contrast, will disturb the body plan: they typically give rise to cells of the normal differentiated types arranged in an abnormal pattern or in abnormal numbers as a result of specific alteration in the lineage tree. Developmental control genes identified in this way can be classified according to the parts of the lineage tree that are affected and, hence, if we know the rules of cell behavior that generate that part of the lineage tree.

Induction of the Vulva Depends on a large Set of Developmental Control Genes: The vulva –the egg-laying orifice in a hermaphrodite- is a ventral opening in the hypodermis formed

by 22 cells that arise by specific lineage from three precursor cell in the hypodermis. A single nondividing cell in the gonad, called the anchor cell, attaches, or "anchors," the developing vulva to the overlying gonad to create a passageway through which the eggs can pass to the outside world. Microsurgical experiments show that the anchor cell is responsible for inducing the three nearest hypodermal cell to form a vulva. If the anchor cell is destroyed by focusing a laser beam on it, these cells, instead of forming a vulva, give rise to ordinary hypodermal cells. And if the anchor cell is shifted relative to the hypodermal cells, there is a corresponding shift in the site at which the vulva lie three others that are also capable of doing so if exposed to the anchor-cell signal. Thus the anchor cell induces vulva differentiation in *C.elegans* just as the vegetal blastomeres induce mesodermal differentiation in the early Xenopus embryo. Only the anchor cell is necessary for this induction: if all the gonadal cells except the anchor cell are destroyed, the vulva still develops normally.

To identify genes involved in a given step of development, one searches for mutations that disrupt the process by screening the progeny of a large population of animals that have been exposed to mutagens. In this way many mutants are found that have a "vulvaless" phenotype, where none of the hypodermal cells behave as though they have received the anchor-cell signal. Another large group of mutants have a converse "multivulva" phenotype, in which all six hypodermal cells capable of responding to the anchor-cell signal behave as thought they have actually received it, so that the worm forms several vulva like structures instead of one. Individual mutations giving a similar phenotype are then tested in pairs to see whether they affect the same or different genes.

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