The Nematode *Caenorhabditis elegans*: A New Organism for Intensive Biological Study

At a recent conference (*1*) in Woods Hole, Massachusetts, investigators (*2*) met to discuss the nematode *Caenorhabditis elegans*. This free-living worm may, according to some workers, become the *Escherichia coli* or at least the bacteriophage T4 of the animal world. Small (about 1 mm in length) and semitransparent, *C. elegans* provides for research the advantages of a short life cycle (3 days) and a simple anatomy—it contains about 810 nongonadal nuclei. It is both easy to cultivate, on *E. coli* as a food source, and convenient for genetic analysis. Its genes are carried on five autosomes and a sex chromosome (X), and it has a genome size about 20 times that of *E. coli*. It generally reproduces as a self-fertilizing hermaphrodite (XX), but occasional males (XO), which arise by nondisjunction, permit sexual reproduction as well.

Some of the pioneering work on *C. elegans* was done by Dougherty and coworkers (*3*), Nigon (*4*), Dion and Brun (*5*), and especially Brenner (*6*), who has made detailed studies of the animal's genetics. Investigators are also getting close to achieving a complete anatomical description of the animal's nervous system at the electron microscope level; *C. elegans* contains only about 300 neurons, and already available is a detailed reconstruction of the anterior sensory neuroanatomy, the ventral nerve cord, and the associated dorsal cord which is composed of processes from cells in the ventral cord (*7*). At the meeting, White described the reconstruction of the complex nerve ring that surrounds the pharynx and is thought to play a major role in processing sensory inputs to produce motor outputs, and Hall described the tail ganglia. Because of its small size, the nervous system cannot be studied directly by standard electrophysiological techniques and must await technical advances in electrode technology or in optical methods in which potential-sensitive dyes are used. Stretton, however, showed that the anatomy of the related nematode *Ascaris*, which is sufficiently large for conventional electrophysiological studies, is virtually identical to that of *C. elegans* at least in the ventral cord. Walrond described experiments indicating which of the seven types of motor neurons in the ventral cord are inhibitory and which stimulatory, and Russell presented a model to account for control of movement in *C. elegans* based on its ventral cord circuitry.

Work is also being conducted on nematode neurotransmitters. Sulston *et al.* (*8*) previously localized eight catecholamine-containing neurons, but found that mutants lacking catecholamines showed no demonstrable alteration in behavior. At the meeting Johnson reported that in *Ascaris* acetylcholine appears to function as an excitatory transmitter because it is synthesized in excitatory but not in inhibitory motor neurons. He also reported on the identification of a *C. elegans* mutant that is defective in acetylcholinesterase and another that is defective in choline acetyltransferase (the latter was isolated by its resistance to the drug trichlorfon). Lewis described the properties of putative acetylcholine receptor mutants isolated by their resistance to the acetylcholine analog levamisole. Horvitz reported on the behavior of serotonin-deficient mutants. It seems likely that the combination of biochemical, genetic, anatomical, and physiological approaches being taken by these researchers will provide insight into the processing of neuronal signals in *C. elegans* and eventually lead to an understanding of the animal's behavior at the level of its neuroanatomy and neurophysiology.

A major portion of the meeting was devoted to description of the worm's development at the cellular level and to studies of mutations that perturb it. The adult animal has only about 950 somatic nuclei in five major cell types (hypodermis, muscle, neurons, gut, structural gonad), and its anatomy at the cellular level is virtually invariant. Upon hatching from the egg, the juvenile or first-stage larva has only 550 somatic cells. During development, about 300 postembryonic cells arise from the division of blast cells present at hatching. In previous studies, Sulston and Horvitz (*9*) traced in detail the origins of these postembryonic cells and found that there is a remarkable reproducibility from one animal to another in times of cell division, paths of cell migrations, cell deaths, and ultimate differentiated cell fates. Schierenberg and von Ehrenstein have now established cell lineage in the egg up to the 182-cell embryo. Using the light microscrope with differential interference contrast optics and video recording, these workers have found that characteristic rates of cell division are maintained in different subclones, regardless of cell migrations. They have also made detailed reconstructions of a 294-cell and 540-cell embryo from electron micrographs of serial sections. The 540-cell stage is reached somewhat early in embryogenesis, about midway between fertilization and hatching and prior to most of the cell growth and differentiation that takes place before hatching, indicating that cell division and migration precede differentiation per se. Nevertheless, from the correspondence between the positions of cells in the 540-cell embryo and first-stage larva, von Ehrenstein has identified most of the embryonic cells with regard to their future fates. There is optimism that a complete description at the cellular level of the development of *C. elegans* from the egg to adulthood will soon be available.

The postembryonic lineage of the gonad somatic cells has been determined by Kimble. The gonad arises during larval development from a four-celled precursor structure in both the hermaphrodite and the male. In the hermaphrodite the two somatic cells of the precursor give rise to a total of 142 cells which form the gonadal sheath, spermatheca, and uterus. In the male a similar lineage produces about 50 cells to form the spermatheca and vas deferens. As in other tissues, the lineages are invariant in their significant features.

A potentially useful genetic technique for lineage studies is being developed by Babu, who previously isolated mutants in which the gut cells fluoresce because of a defect in tryptophan catabolism (*10*). Siddiqui (from Babu's laboratory) reported that x-irradiation of embryos heterozygous for the fluorescence mutation gives rise to adults with fluorescent patches in the gut, thus suggesting an approach to the study of somatic segregation in *C. elegans*.

The factors responsible for lineage patterns are being investigated by means of a laser, which is used for the selective destruction of certain cells, and by inducing mutations that alter lineage. Although there appear to be some inductive or positional effects of cell differentiation, much of the developmental process appears to be cell-autonomous. Two examples of nonautonomy were reported by White: (i) the ultimate fate of some ventral cord cells depends in part on their positions and (ii) if gonad development is prevented by laser ablation of the precursor cells, then the hypodermal cells that normally proliferate to form the vulva fail to do so. Albertson reported on the behavior of certain blast cells that normally undergo a migration that is followed by a pattern of division that results in the formation of five neurons and a hypodermal cell. In a particular mutant

strain the blast cells undergo up to three abortive attempts at division to yield a polyploid cell; this cell differentiates to display cellular features of either hypodermal or nerve cells. Horvitz has isolated a large number of mutants that are defective in vulva formation and hence cannot lay eggs. These mutants have been used to define nine different genes in which defects appear to affect the vulval lineage pattern directly, by preventing either divisions or normal migrations of precursor cells.

Development of the male is virtually identical to that of the hermaphrodite until some hours after hatching, when a few of the postembryonic cell lines display a male-specific pattern of division, migration, and differentiation. Intersex and transformer mutants defective in genes that control these processes were described by Klass, Ward, and Brun. Such mutants should prove useful in determining how the male developmental pattern is superimposed upon that of the hermaphrodite.

A large number of temperature-sensitive, sperm-defective mutants have been isolated by Hirsh and Vanderslice (*11*) and by Ward. At nonpermissive temperatures, some mutants appear to be blocked early in spermatogenesis and form no sperm, whereas others produce normal numbers of inactive sperm. All the mutants can lay viable eggs when mated with normal males. Ward reported that male sperm, which are amoeboid in *C. elegans*, migrate rapidly to the hermaphrodite spermatheca after copulation and effectively supplant the endogenous sperm. Sperm may be required for some step in oogenesis, because some sperm-defective mutants fail to produce oocytes alone but will do so when mated to males. The abundance of mutants and the prospects for isolating sperm in quantity make this system promising for studying the genetic control of a cellular differentiation pathway.

A special feature of the *C. elegans* life cycle has been studied by Riddle. Normally, the larva progresses through a series of four molts to adulthood. In the absence of food, however, the second molt produces a dauerlarva, which has an altered cuticle and can withstand adverse conditions (for example, 4 percent sodium dodecyl sulfate) and no food for periods up to 60 days. When presented with food, the dauerlarva molts and continues the normal progress toward adulthood. Riddle has identified seven genes whose functions are involved in the choice between the regular and the dauer developmental pathways. Mutants defective in these genes are constitutive dauer formers which enter the dauer pathway even in the presence of food. Some of these mutants have defects in sensory neuronal anatomy. Mutant analysis indicates that a larger number of genes probably is essential for recovery of the dauerlarva and return to the standard developmental pathway.

Brenner (*6*) estimated that *C. elegans* carries about 2000 genes (*5*), somewhat fewer than *Drosophila*. If this is the case, then more than 10 percent of them have already been identified. Horvitz, who has undertaken the task of collating mapping data from different laboratories, has reported that over 200 genes have been identified and mapped with various degrees of precision.

Many mutants defective in myosin, paramyosin (a protein of the thick filaments), and other muscle components have been reported by Waterston and Epstein and co-workers [see (*12*)]. At the meeting, Epstein reported that 12 genes have been identified as being involved in muscle development. Of these, two appear to be structural genes, one of them for a myosin heavy chain that is unique to the body wall and the other for paramyosin that is shared by the body wall and the pharynx. At least two different myosins exist in the nematode body wall muscles (*13*), and these two myosins are differentially synthesized during larval development, correlating with increases in the number of sarcomeres per muscle cell and the length and depth of each sarcomere. The identified myosin gene controls the structure of the body wall muscle myosin. Some 30 alleles of this gene have been found. MacCleod reported on biochemical mapping of the alteration in one mutant myosin by chemical cleavage and peptide analysis, demonstrating a small internal deletion.

Myosin and paramyosin mutations may also provide genetic access to the translational apparatus of *C. elegans*. Waterston showed that a revertant of a certain paramyosin mutation carries a second site suppressor affecting certain null mutations in the myosin and paramyosin genes and certain alleles in genes with other diverse functions. This mutation may be a promising candidate for the first transfer RNA nonsense suppressor to be described in a metazoan organism.

Caenorhabditis elegans also is well suited to studies on the phenomenon of aging, in view of its short life cycle and the existence of a non-aging developmental variant, the dauerlarva (*14*). Klass reported on the effects of nutrition and other influences on life-span, and Mitchell presented evidence for entrainment of increased life-span by prolonged cultivation under semistarved conditions. However, attempts to find mutations that directly alter the life-span have failed thus far.

Many other studies were presented in addition to those mentioned, all of them indicating that *C. elegans* may become an important model organism for intensive biological study during the next few years.

R. S. EDGAR
Division of Natural Sciences, Thimann Laboratories, University of California, Santa Cruz 95064

W. B. WOOD
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder 80309

References and Notes

1. The conference took place from 13 to 16 April 1977 and was supported in part by the National Institute on Aging.
2. Among the participants in the conference were D. Hall (Albert Einstein College of Medicine); D. Mitchell (Boston Research Institute); J. Lewis (Columbia University); S. Ward (Harvard Medical School); D. Albertson, R. Horvitz, S. MacCleod, J. Sulston, and J. White (Medical Research Council Molecular Biology Laboratory, Cambridge, England); P. Babu and P. Siddiqui (Tata Institute, Bombay); D. Hirsch, J. Kimble, and M. Klass (University of Colorado); G. von Ehrenstein and E. Schierenberg (Max Planck Institute, Göttingen); J. Brun (University of Lyon, France); D. Riddle (University of Missouri); R. Russell (University of Pittsburgh); T. Stretton and J. Walrond (University of Wisconsin); and R. Waterson (Washington University).
3. E. C. Dougherty and H. G. Calhoun, *Nature (London)* **161**, 29 (1948); E. S. Dougherty, E. L. Hansen, W. L. Nicholas, J. H. Mollett, E. A. Yarwood, *Ann. N.Y. Acad. Sci.* **77**, 176 (1959).
4. V. Nigon, *Ann. Sci. Nat. Zool.* **11**, 1 (1949).
5. M. Dion and J. L. Brun, *Mol. Gen. Genet.* **112**, 133 (1971).
6. S. Brenner, *Genetics* **77**, 71 (1974).
7. S. Ward, J. N. Thomson, J. G. White, S. Brenner, *J. Comp. Neurol.* **160**, 313 (1975); R. W. Ware, D. Clark, K. Crossland, R. L. Russell, *ibid.* **162**, 71 (1975); J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Phil. Trans. R. Soc. London* **275**, 327 (1976).
8. J. Sulston, M. Dew, S. Brenner, *J. Comp. Neurol.* **163**, 215 (1975).
9. J. E. Sulston and H. R. Horvitz, *Dev. Biol.* **56**, 110 (1977).
10. P. Babu, *Mol. Gen. Genet.* **135**, 39 (1975).
11. D. Hirsh and R. Vanderslice, *Dev. Biol.* **49**, 220 (1976).
12. R. H. Waterston, H. F. Epstein, S. Brenner, *J. Mol. Biol.* **90**, 285 (1974); H. F. Epstein, R. H. Waterston, S. Brenner, *ibid.*, p. 291.
13. F. H. Schachat, H. E. Harris, H. F. Epstein, *Cell* **10**, 721 (1977).
14. M. Klass and D. Hirsh, *Nature (London)* **260**, 523 (1976).

26 July 1977