Nematode Postembryonic Cell Lineages[1]

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Abstract: The complete postembryonic cell lineages of the free-living nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* are known. Postembryonic cell divisions lead to substantial increases in the number of cells and, in most cases, in the number of types of cells in the neuronal, muscular, hypodermal, and digestive systems. The patterns of postembyronic cell divisions are essentially invariant and generate a fixed number of progeny cells of strictly specified fates. Cell fates depend upon both lineage history and cell-cell interactions: lineage limits the developmental potential of each cell and, for certain cells, cell-cell interactions specify which of a small number of alternative potential fates is acquired. Relatively simple differences in cell lineage account for some of the striking differences in gross morphology both between sexes and between species. Genetic studies indicate that these cell lineage differences reflect one or a few relatively simple mutational events. Interspecific differences in cell lineage are likely to be good indicators of evolutionary distance and may be helpful in defining taxonomic relationships. Both the techniques utilized in, and the information acquired from, studies of cell lineages in *C. elegans* and *P. redivivus* may prove useful to other nematologists. *Key words: Caenorhabditis elegans, Panagrellus redivivus,* anatomy, development, taxonomy, evolution.

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Elucidation of the molecular and cellular mechanisms responsible for animal development and behavior is a challenging goal. One approach to this problem is to study a relatively simple organism. Three assumptions have drawn researchers to the study of simple organisms as biological models: first, simplicity should facilitate understanding; second, many biological mechanisms are likely to be universal, so that what is learned about a simple organism may well provide a relevant framework for thinking about more complex organisms; third, a detailed understanding of any biological phenomenon is likely to be interesting.

The field of molecular genetics provides a compelling example of the utility of biological models. Studies of bacterial viruses have led to an understanding of the chemical basis of heredity that almost certainly is relevant to all organisms on Earth. Having essentially solved the problem of inheritance, a number of molecular biologists decided to explore next the more difficult problems of metazoan development and behavior. These scientists have chosen to work on such diverse organisms as slime molds, fruit flies, leeches, and fish; all hope that at least one of these organisms will prove to be the "bacteriophage of the eukaryotic world," a key to some of the unsolved mysteries of life.

Sydney Brenner, who helped establish many of the fundamental principles of molecular genetics, sought a model metazoan that was cellularly simple (so that its anatomy could be defined completely) and that was amenable to genetic analysis (which had proved so fruitful in molecular biology). Genetic experiments are concerned with rare events and multiple generations and can be done best with small, easily grown organisms that reproduce rapidly. Following the suggestion of Dougherty and Calhoun (11), Brenner (4,5) selected the free-living nematode *Caenorhabditis elegans* as a model organism in which to study the genetics of behavior. Many researchers have joined Brenner, and more than 25 laboratories are currently involved in exploring a variety of aspects of *C. elegans* biology (22, 27). In our laboratory, a major interest has been *C. elegans* development.

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CELL LINEAGE

To understand how a unicellular egg divides to generate the morphologically and functionally diverse cell types of a multicellular organism, three related questions must be answered. First, what is the pattern of cell divisions ("cell lineage") responsible for the development of a particular organism, and how does this lineage vary among individuals of that species? Second, how is the lineage history of a particular cell related to its ultimate differentiated fate? Third, how are the instructions for cell lineage programmed in the genome?

Nematodes have been utilized in studies of cell lineage since the 19th century. Classical observations of early embryonic cell divisions (9,21) indicated that nematode embryogenesis follows a rigid program; i.e., an invariant pattern of divisions produces specific progeny cells that, in turn, generate particular tissues and/or parts of the organism. These studies utilized fixed specimens and were subject to a number of uncertainties, particularly regarding late embryonic cell divisions and specific cell fates. Examination of fixed specimens and, in a few cases, intermittant observation of living specimens also revealed postembryonic increases in both gonadal and nongonadal cell number in a variety of nematode species (see reviews cited above). No detailed postembryonic cell lineages were determined.

Recently, the application of Nomarski optics light microscopy and transmission electron microscopy has allowed a more complete study of nematode cell lineages. Nomarski optics, which results in a very shallow depth of field, permits the visualization and enumeration of all nuclei in living *C. elegans* embryos (10) and larvae (28). Electron microscopy reveals detailed characteristics of specific cells, both during development (19) and in the adult (8,32). The precise knowledge of cell types provided by electron microscopic studies has led to specific hypotheses regarding the relationship between cell lineage and cell fate.

The combination of light and electron microscopy has made possible a complete description of the essentially invariant anatomy of *C. elegans*. The numbers and types of both nongonadal (28) and gonadal (17) cells have been enumerated. Almost the entire circuitry of the 300-cell nervous system has been reconstructed from serial section electron micrographs (2,26,32,33,35; and J. White, E. Southgate, N. Thomson, and S. Brenner, personal communication).

One discovery about nematode anatomy that might prove useful to other nematologists concerns sexual dimorphism, which arises embryonically both in *C. elegans* and in another free-living nematode, *Panagrellus redivivus*. At hatching, males can be readily distinguished from hermaphrodites (*C. elegans*) or females (*P. redivivus*) by a number of criteria, including the presence or absence of a pair of hermaphrodite- or female-specific neurons (HSN's or FSN's) and the size of a particular ectodermal cell (B) in the tail (24,28). The HSN's (or FSN's) can be visualized in the light microscope either using Nomarski optics or after nuclear staining (see below). *C. elegans* larvae can also be sexed by the positions of the four embryonically-derived coelomocytes (28).

Cell lineages have been determined by the direct observation of living individuals in the light microscope using Nomarski optics. Specimens are mounted on a thin block of agar on a microscope slide and, in the case of larvae, provided with bacteria for food. Development proceeds normally, and the migrations, divisions, and deaths of individual cells can be followed by continual observation. Postembryonic cell lineages have proved to be the easier to follow.

Complete postembryonic lineages have been determined for the nongonadal (26, 28) and gonadal (17) tissues of *C. elegans* hermaphrodites and males as well as for the nongonadal (25) and gonadal (24) tissues of *P. redivivus* females and males. The embryonic cell lineage of *C. elegans* to the 182-cell stage has been reported (10), and all of the remaining cell divisions have been determined recently by John Sulston of the Medical Research Council Laboratory of Molecular Biology in Cambridge, England (personal communication). These studies have confirmed the basic conclusions reached earlier from the examination of fixed specimens, although it should be noted that a number of detailed differences have arisen. Further studies of *C. elegans* em-

bryogenesis are discussed in the accompanying manuscript by Wood et al. (36).

POSTEMBRYONIC LINEAGES

Like the pattern of embryonic cell divisions, the patterns of postembryonic cell divisions of both *C. elegans* and *P. redivivus* are essentially invariant; rigidly determined cell lineages generate a fixed number of progeny cells of strictly specified fates. Like the blastomeres that divide embryonically, the blast cells that divide postembryonically generally produce cells of particular tissue types; for example, there are ectoblasts that generate neuronal, glial, and hypodermal cells, and there are mesoblasts that generate muscles and coelomocytes. Postembryonic cell divisions lead to changes in the number of cells and, in most cases, in the types of cells in the neuronal, muscular, hypodermal, and digestive systems. In many ectodermal lineages in *C. elegans,* and in ectodermal, mesodermal, and gonadal lineages in *P. redivivus,* specific progeny cells die soon after their formation. These "programmed cell deaths" can be readily observed as refractile disks under Nomarski optics. In both species, many of the postembryonic cell divisions are involved in sexual maturation. Some sex-specific characteristics arise postembryonically from blast cells with division patterns that are initially identical in the two sexes but later diverge; others arise from cells that divide only in males. Although strikingly invariant, these lineages are not absolutely identical in all individuals; for example, certain cells, particularly in the hypodermis and intestine, divide in only some animals.

Some examples will illustrate the nature of the postembryonic cell lineages of *C. elegans* and *P. redivivus.* All postembryonically generated muscles and coelomocytes are derived from a single mesoblast (M) located on the right side of the young first-stage larva somewhat posterior to the gonadal primordium (25,26,28). M divides dorso-ventrally and then both of its daughters divide transversely, generating a single blast cell in each of the four longitudinal muscle quadrants. These blast cells undergo a series of anterio-posterior divisions and produce body muscles, coelomocytes, and sex mesoblasts. These sex mesoblasts migrate (posteriorly in males; anteriorly in hermaphrodites and females) and then divide during the third larval stage to produce sex-specific muscles. In the hermaphrodite and female, the nuclei of these sex muscles (and their precursors) are located superficially to the gonadal primordium and probably correspond to the "nuclei outside the gonad" noted by Hirschmann and Triantaphyllou in *Helicotylenchus dihystera* (14).

The primordial gonad consists of two germ precursor cells, Z2 and Z3, flanked by two somatic gonadal precursors, Z1, located anteriorly, and Z4, located posteriorly (17, 24). Z1 and Z4 divide during the first two larval stages to generate either five or six descendants each (the number depends on both sex and species; see Fig. 1). Most of these cells are blast cells. Others differentiate and do not divide further: the distal tip cells of the hermaphrodite and female (called "cap" cells by others) (9) function to prevent nearby germ cells from entering meiosis and also control elongation of the gonad (18); the anchor cell of the hermaphrodite and female (also called the "vaginal initial" cell or "I" nucleus) (3,14) acts to connect the gonad to the vulva; the linker cell of males controls elongation of the gonad and acts to connect the gonad to the cloaca. The 10–12 somatic cells of the developing gonad rearrange to form a "somatic primordium" by the end of the second larval stage. During the third and fourth larval stages, these cells divide to generate the somatic structures of the adult gonad, which consists of 56 cells in *C. elegans* males, of 143 cells in *C. elegans* hermaphrodites and *P. redivivus* males, and of about 400 cells in *P. redivivus* females.

Postembryonic ventral cord development involves a set of 12 precursor (P) cells (25,26,28). At hatching, there are six P cell nuclei located subventrally along each side of the animal; the cytoplasm of these cells constitute most of the ventral hypodermis of the young larva. The 12 P cell nuclei (P1–P12) migrate into the ventral cord about midway through the first larval stage and then undergo a characteristic pattern of cell divisions (Fig. 2). Each P cell (collectively known as "Pn") divides to produce a

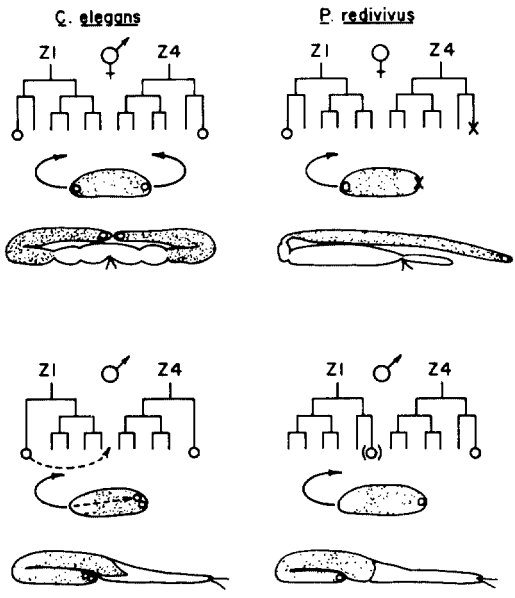

Fig. 1. Gonadal cell lineages (to the second larval molt) and schematic drawings of gonadogenesis in the *C. elegans* hermaphrodite and male and the *P. redivivus* female and male. Two somatic gonadal precursor cells, Z1 and Z4, are present at hatching. Z1 and Z4 divide according to the lineage trees shown; each branch represents a cell division, with anterior ("a") drawn to the left and posterior ("p") drawn to the right. The descendants of Z1 and Z4 that become distal tip cells (dtc's) are indicated with a circle. In the *P. redivivus* female, Z4.pp (the posterior daughter of the posterior daughter of Z4) undergoes programmed cell death (X). In the *P. redivivus* male, only one dtc, Z4.pp, is generated; however, Z1.pp will become a dtc if Z4.pp (or one of its ancestors) has been ablated with a laser microbeam. The dashed arrow indicates the posterior migration of Z1.a in the *C. elegans* male. The general morphologies of the gonads of the second-stage larva (above) and the adult (below) are shown underneath the corresponding lineage; these drawings are not to scale. The positions of each dtc (O) and death (X) are indicated. The solid arrows show the direction of growth of the developing gonad. Stippling denotes the position of germ line nuclei. An inverted "V" indicates the vulva in the hermaphrodite and female and the cloaca in the males. Adapted from Literature Cited 17 and 24.

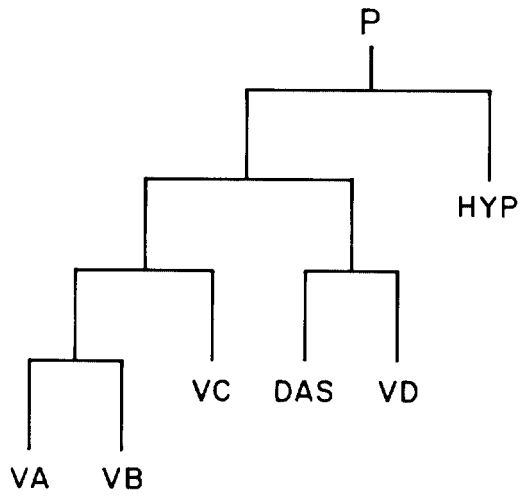

Fig. 2. Cell lineage of the ventral cord precursors P1–P12. In the lineage tree, left branches represent anterior ("a") daughters, and right branches represent posterior ("p") daughters. Each Pn.a neuroblast generates five cells with neuron-like nuclei. Certain Pn.aap and Pn.aaap cells die. Surviving cells have been characterized in *C. elegans* hermaphrodites and (with the exception of P1.aaaa) differentiate into neurons of five distinct classes (VA, VB, VC, DAS, VD), as indicated. Each Pn.p cell (HYP) is a hypodermal cell or generates sex-specific ectodermal cells. Adapted from Literature Cited 28.

larger anterior daughter (Pn.a), which is a neuroblast that generates five neurons, and a smaller posterior daughter (Pn.p). Certain Pn.p cells join the hypodermal syncytium, and others divide and their progeny join the syncytium. Sex and species specific divisions of certain Pn.p cells generate the vulva (in hermaphrodites and females) and tail ectodermal cells (in males). The Pn.p cells have been called "specialized ventral chord cells" by other nematologists (13,14). In *C. elegans,* the fates of the five descendants of each Pn.a neuroblast have been determined by serial section electron microscopy (26, 28,35). In general, cells with an equivalent lineage history differentiate into neurons of a particular morphological class (Fig. 2). For example, all Pn.apa cells (i.e., all cells that are the anterior daughters of the posterior daughters of Pn.a neuroblasts) become "DAS" neurons.

LINEAGE AND FATE

A similar correlation between lineage history and cell fate has been observed in many cell lineages in *C. elegans* and *P. redivivus*. For example, there are 18 rays (bursal papillae) embedded in the copulatory bursa of the *C. elegans* male. Each ray consists of two distinct types of neurons and one associated hypodermal cell (26). The three cells of each ray are derived from a single precursor, and all 18 rays are generated after the same pattern of cell divisions from 18 morphologically similar

precursors (28). As in the ventral cord, all neurons of a particular class have experienced a similar series of cell divisions.

Such repeated patterns of cell division have been termed "sublineages" and are thought to represent modular elements within the developmental program for cell lineage (6,24). Complex cell lineages may be decomposed into simpler sublineages. For example, in the *P. redivivus* female hypodermal blast cells (known as seam cells) undergo a characteristic sublineage during the early second, third and fourth larval stages (25) (Fig. 3). This sublineage generates four hypodermal syncytial nuclei and two seam cells. Each seam cell (except those generated in the fourth larval stage) undergoes the same sublineage one larval period after it is formed.

A sublineage is characterized both by its pattern of cell divisions and by the fates of the progeny cells it generates. In other words, lineally equivalent descendants derived from similar sublineages generally become morphologically and functionally identical cell types. This observation suggested that cell lineage and cell fate might be causally related; i.e., that it might be necessary to execute a particular cell lineage to generate a particular cell type (28). This

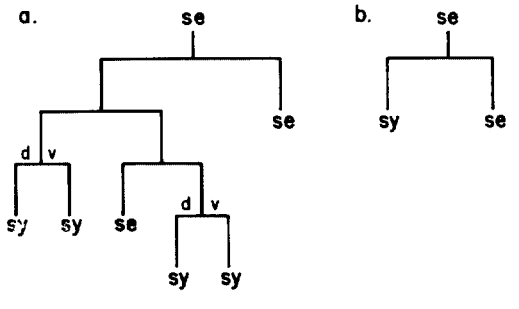

Fig. 3. Lateral hypodermal sublineages. Each seam cell (se) divides during the first through fourth larval periods to generate hypodermal syncytial nuclei (sy) and seam cells. In the lineage trees, left branches represent anterior daughters if unlabeled or dorsal daughters if labeled "d"; right branches represent posterior daughters if unlabeled or ventral daughters if labeled "v." a) The *P. redivivus* seam cell sublineage expressed during the second, third and fourth larval stages. se.aa and se.app divide dorso-ventrally. The seam cells (se.apa and se.p) divide during the next larval period following the same sublineage. b) The *C. elegans* sublineage. This sublineage is also followed in *P. redivivus* by seam cells during the first larval period. Adapted from Literature Cited 24 and 28.

hypothesis has been strongly supported by a variety of further studies (6).

Although a specific lineage history appears to be a necessary condition for the generation of a particular cell type, it is now clear that it is not a sufficient condition: certain cells have multiple developmental potentials. The first evidence that developmental potential is not uniquely defined by cell lineage derived from observations of natural variability during *C. elegans* postembryonic development. For example, the ectoblasts B.alaa and B.araa are lineally equivalent descendants derived from similar sublineages; these cells are generated on the left and right sides, respectively, of the developing male tail (28). During the second larval stage, B.alaa and B.araa centralize. After centralization, either B.alaa or B.araa can be located more anteriorly; their subsequent fates are determined by their relative positions; i.e., the anterior cell follows one lineage and the posterior cell follows another. Both B.alaa and B.araa have both developmental potentials; which potential is expressed depends upon cell position. Similar observations of natural variability have revealed that position also influences cell fate in the development of the vulva (25,28) and the gonad (17).

Even some cells that are invariant in fate during normal development in fact have multiple developmental potentials. Such cryptic potentials have been revealed in *C. elegans* and *P. redivivus* by ablating certain cells and observing that other cells are altered in fate. That these cells have the capacity to express alternative fates implies that they are of multiple developmental potentials. Such ablation experiments are performed using laser microbeam systems to destroy specific cells in living nematodes (24,30,34). The beam from a coumarin-containing dye laser is focussed onto the specimen plane, resulting in a spot smaller than one micron in diameter that can ablate a given cell with no damage to neighboring cells. Nematodes are prepared for laser microsurgery by anaesthetizing them with 1-phenoxy-2-propanol.

In general, laser ablation of one cell has no effect on the fates of other cells (16, 28,30), indicating that in most cases fate is

cell autonomous; however, in a number of instances, cell-cell influences on fate have been revealed. For example, ablation of *C. elegans* vulval precursor cells P5.p, P6.p, and/or P7.p has led to regulation in the ventral hypodermis: other ventral hypodermal cells (P3.p, P4.p, P8.p) can substitute for the ablated vulval precursor(s) (30). Thus, P3.p, P4.p, and P8.p have, but do not normally express, the developmental potential of P5.p, P6.p, and P7.p.

The vulval precursor cells also provide a second example of cell-cell influences on cell fate. Ablation of the gonad in the *C. elegans* hermaphrodite prevents the cell divisions of P5.p, P6.p, and P7.p, which suggests that the gonad plays an inductive role in vulval formation (30). The source of this signal has been identified as the gonadal anchor cell, since ablation of the anchor cell has the same effect (18). Instead of proceeding through the vulval cell lineages, P5.p–P7.p display an alternative developmental potential and divide once to produce two syncytial hypodermal nuclei each; the new fate assumed by these cells is that normally expressed by three other ventral hypodermal cells (P3.p, P4.p, P8.p). Combining this observation with the results described in the previous paragraph, it can be concluded that all six of the cells P3.p–P8.p are of equivalent developmental potential, and which of their alternative potential fates they express depends upon the signals they receive from other cells.

These observations indicate that in nematodes, as in many other organisms, both lineage history and cell-cell interactions determine aspects of cell fate. Cell-cell interactions involving both inductive influences and regulative potential have been identified. Taken together, the studies outlined above suggest that in *C. elegans* and *P. redivivus*, lineage limits the developmental potential of each cell, and, for certain cells, cell-cell interactions specify which of a small number of alternative potential fates is acquired.

COMPARATIVE CELL LINEAGES

The newly hatched larvae of *C. elegans* and *P. redivivus* are very similar. An almost identical set of blast cells divides postembryonically to generate closely related but not identical cell lineages (17,24,25,28). For example, in *C. elegans*, the ectoblast P12.aaa divides and its posterior daughter dies, whereas in *P. redivivus*, P12.aaa does not divide. Similarly, in the *C. elegans* male, M.drpa generates two sex mesoblasts, whereas in the *P. redivivus* male, M.drpa generates a sex mesoblast and a second cell that divides to generate one sex mesoblast and one body muscle. The sublineage used in lateral hypodermal development is considerably more complex in *P. redivivus* than in *C. elegans* (Fig. 3), although the general timing and underlying lineage pattern are similar; this added complexity results in a greater number of hypodermal cells in *P. redivivus*.

The differences between the cell lineages of *C. elegans* and *P. redivivus* fall into four categories:

1) An alteration in the number of rounds of cell division. For example, P10.pa undergoes two rounds of division in the *C. elegans* male to produce four ectodermal cells, one of which forms the hook, a structure associated with the pre-anal sensillum. In the *P. redivivus* male, P10.pa divides only once, which may account for its lack of a hook.

2) A reversal in the polarity of a cell lineage; i.e., the anterior-most descendant derived from a particular precursor cell in one species acquires characteristics associated with the posterior-most descendant in the other species. Gonadal development provides a striking example. As discussed above, the distal tip cells (dtc) indicate gonadal polarity. A dtc is always at the germ cell end of the gonad; i.e., it is vulval or cloacal-distal. In the *C. elegans* hermaphrodite, which is amphidelphic, there are two dtc's, one at the end of each ovary; these dtc's are the anterior-most descendant of Z1 (Z1.aa) and the posterior-most descendant of Z4 (Z4.pp) (Fig. 1). Elongation (and reflexion) of the gonad results in its bipolar adult morphology (Fig. 1). In the *P. redivivus* male, the polarity of the Z1 lineage is reversed, and a gonadal primordium with two adjacent cells with the potential to become dtc's is generated; growth of this gonad leads to a monopolar structure (Fig. 1). It should be noted that the *C. elegans*

male and the *P. redivivus* female generate monopolar gonads via other mechanisms. In the *C. elegans* male, the polarity of Z1 is the same as in the hermaphrodite, but Z1.a migrates posteriorly. In the *P. redivivus* female, the putative posterior dtc, Z4.pp, undergoes programmed cell death; it is likely that the elimination of this cell prevents the elongation of a posterior gonadal arm, which results in the development of a monodelphic gonad (24).

3) A switch in the fate of a cell to a fate normally associated with another cell. For example, in *C. elegans,* P8.p generates nuclei of the hypodermal syncytium and P5.p–P7.p generate cells of the vulva, whereas in *P. redivivus,* P5.p–P8.p generate cells of the vulva.

4) Altered segregation of the potential to generate specific cell types; i.e., a developmental potential associated with one cell becomes associated with its sister. For example, in the *C. elegans* male, only Z4.aa generates ejaculatory duct cells, whereas in the *P. redivivus* male, Z4.ap also generates ejaculatory duct cells. One simple interpretation of "altered segregation" is that precursor cells contain asymmetrically distributed determinants that specify developmental potential; altering the apportionment of such determinants would change the potential of the daughter cells generated.

These differences are likely to represent basic evolutionary modifications of cell lineage. Knowledge of such apparent transformations in cell lineage may be helpful in defining taxonomic relationships. In principle, one could estimate the relatedness of two species by the number of cell lineage transformations that separate them.

For example, considerable variation in the size and morphology of the postvulval sac can occur within a lower taxon; e.g., *Pratylenchus* (23) and *Xiphenema* (20). Studies of the cell lineages of the postvulval sac of the *P. redivivus* female have indicated that suppressed cell divisions and modifications of the general lineage pattern are responsible for decreasing the size and complexity of this vestigial structure (24). A precise knowledge of those cell divisions affected in a variety of related species would probably more accurately reveal taxonomic relationships than would simple anatomical characteristics, such as the length of or the number of cells in a postvulval sac.

The gonad provides another example of why lineage is better than morphology as an indicator of evolutionary distance. As discussed above and in more detail elsewhere (24), the acquisition of a single cell death could transform a didelphic to a monodelphic species. That monodelphy probably has evolved from didelphy repeatedly during evolution and hence is a poor taxonomic indicator has been discussed by Triantaphyllou and Hirschmann (31).

MUTANTS

Mutants may also provide information relevant to the estimation of evolutionary distance. For example, the number of sensory papillae in male tails varies among species (12). In *C. elegans* and *P. redivivus,* these sensory elements are derived from identical sublineages; however, the number of such sublineages differs (18 in *C. elegans;* 14 in *P. redivivus*). That the number of these ray sublineages in *C. elegans* can be altered by mutation in a single gene (W. Fixsen and R. Horvitz, unpublished results) suggests that this morphological feature should be useful in comparing closely related species.

In addition, mutants can help identify cell function (animals missing specific cells would be deficient in the functions of those cells, as in laser ablation experiments; see below) as well as reveal aspects of the genetic programming of cell lineage. Many cell lineage mutants of *C. elegans* have been isolated and characterized (1,6,15,29; and E. Ferguson, V. Ambros, W. Fixsen, H. Ellis, P. Sternberg, and R. Horvitz, unpublished results). In some mutants, certain cells undergo either too few or too many rounds of cell division. In one mutant, there appears to be a reversal in cell polarity. In many mutants, specific cells acquire fates normally associated with other cells.

In these cell lineage mutants, single gene mutations have resulted in the same classes of lineage transformation postulated above based upon the comparative cell lineages of *C. elegans* and *P. redivivus.* Thus, each of these transformations in cell

lineage is likely to involve one or a few relatively simple genetic events.

RELEVANCE TO NEMATOLOGY

Studies of cell lineages in *C. elegans* and *P. redivivus* have employed a variety of techniques that might be of use to other nematologists. Nuclear anatomy can be readily visualized by the staining of fixed specimens by the method of Feulgen (28) or using the fluorescent dyes Hoechst 33258 (1) or DAPI (4′-6-diamidino-2-phenylindole dihydrochloride) (W. Fixsen and R. Horvitz, unpublished results); the protocols employing the latter two stains are particularly easy and rapid. Nomarski optics provides a convenient way to study both anatomy and development. Unfortunately, not all species are as easily visualized as *C. elegans* and *P. redivivus*, mostly because of refractile intestinal granules. Although the direct observation of cell lineages in most parasitic nematodes would not be possible, Nomarski optics could nonetheless provide anatomical data. In addition, parasitic species that can be maintained on appropriately small hosts could be studied. For example, the plant parasite *Aphelencoides blastophthorus* can grow on the fungus *Botrytis cinera;* postembryonic cell divisions similar in timing and positions to those that occur in *C. elegans* and *P. redivivus* have been observed in *A. blastophthorus* (28), and the elucidation of cell lineages should be possible. Another technique that might prove useful in nematology is laser microsurgery (24,30): specific organs or cells can be ablated and the effects on behavior and/or development determined. For example, a set of *C. elegans* neurons with microtubule-rich processes have been demonstrated in laser ablation experiments to be mechanoreceptors that mediate the response to gentle tactile stimulation (7).

Besides techniques, some of the information that has been acquired in our studies of cell lineage should be pertinent to other nematodes. For example, the presence of FSN's (or HSN's), the morphology of the B cell, and the positions of the embryonically generated coelomocytes may provide criteria for sexing nematodes at hatching. Furthermore, as discussed above, both comparative cell lineages and cell lineage mutations have implications for nematode taxonomy. More generally, we hope that a detailed understanding of the anatomy, development, physiology, genetics, and behavior of free-living nematodes will provide fundamental new insights of broad significance in biology; it seems likely that such knowledge will also be relevant to the study and control of other nematode species.

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