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The Genetics of Development and Behavior in Caenorhabditis elegans[1]

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Abstract: The current genetic research on *Caenorhabditis elegans* and the application of genetic techniques to the analysis of development and behavior in this animal are reviewed. Some aspects of the work are emphasized more than others and this inevitably reflects the author's own interests and prejudices. An effort was made to point out the adavntages that *C. elegans* offers for certain types of investigations and to point out, in general terms, the relevance of this work to other areas of biological research. *Key Words:* neurobiology, sexuality, muscle.

There are now nearly 20 laboratories working on the genetics, development, neurobiology, biochemistry, or behavior of *Caenorhabditis elegans*. A recent *C. elegans* workshop (supported by a grant from the National Institute on Aging) was held at Wood's Hole, Massachusetts. The conference was attended by 75 participants from the United States and Europe, including many students and postdoctoral researchers. Why is there so much scientific interest in *C. elegans*? Exactly what are all these people doing? This review answers these questions in the hope of serving two different types of readers: *(i)* nematologists interested in the application of genetic studies to nematodes, and *(ii)* geneticists or cell biologists interested in using nematodes as model organisms in genetic and cellular research.

Much of the published work on *C. elegans* is in the very recent literature. Indeed, I must frequently refer to unpublished results since the published papers do not yet reflect the scope of current research on this organism. Within the past 2 years, communication between research groups has been greatly facilitated by the distribution of a *C. elegans* newsletter, edited by R. S. Edgar and organized largely through his efforts. The newsletter carries contributed notes on experimental techniques and recent research results, informing subscribers of research progress long before publication.

Most investigators working on *C. elegans* are the professional children or grandchildren of Sydney Brenner, a molecular biologist and geneticist who began working on *C. elegans* in 1965 after an extensive search for a model organism to study the genetic specification of development and behavior (5). Brenner is well known for his work on *Escherichia coli* and bacteriophage T4. Perhaps, it is not surprising that many of us who have been trained in Dr. Brenner's laboratory at Cambridge, England, come from similar research backgrounds.

A) *The experimental organism and its anatomy:* The possible use of free-living nematodes in genetic research was first suggested by Dougherty and Calhoun (18), in 1948. Early work on *C. elegans* concerned its cultivation (19), sexual cycle (44) and some genetic differences between varieties (25). Brenner initially chose *C. elegans* for study because it is well suited for genetic analysis and because the structure of the animal is simple enough that its complete neuroanatomy can be determined by electron microscopy. Thus, behavioral mutants can be carefully analyzed to determine what changes have been produced in nerve or muscle tissue. Because the wild-type organism must be characterized before mutants can be properly studied, much of the published work on *C. elegans* concerns normal development, anatomy, and behavior.

The worms are easily and inexpensively

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grown on *E. coli* in liquid medium or on the surface of agar-filled petri dishes. Although *C. elegans* has most major types of differentiated tissue (nerve, muscle, hypodermis, intestine, and gonad), an adult contains only about 800 somatic cells (54).

The nervous system of *C. elegans* consists of about 300 neurons, including a circumpharyngeal nerve ring, dorsal and ventral nerve cords, and a variety of sensory receptors and ganglia (62). About 70% of the neurons are in the head. Sense organs (sensilla) are arranged in two concentric rings around the mouth and include both chemoreceptors and mechanoreceptors (57, 58). *C. elegans* exhibits chemotaxis to a number of compounds (56). It responds to a tap on the head by moving backward and then turning to move forward in a different direction. The nerve ring contains the endings of the sensory receptors in the head plus interneurons, motor neurons, and processes which come from the tail and form axonal connections (62). The ventral nerve cord, posterior to the pharynx, is made up of interneurons from the ring and tail as well as motor neurons whose cell bodies are linearly positioned along the cord (63). The dorsal nerve cord innervates the subdorsal muscles and is made up only of cell processes which are connected to their respective cell bodies in the ventral cord by commissures.

Caenorhabditis elegans generally reproduces as a self-fertilizing protandric hermaphrodite. Two reflexed gonadal arms, each containing an ovary, oviduct, spermatheca, and uterus, terminate at the vulva, located midway along the ventral side. The mature hermaphrodite is structurally a female in which the gonad produces and stores sperm prior to the production of oocytes. Eggs are fertilized by endogenous sperm and undergo some of their development inside the parental hermaphrodite. The entire life cycle takes about 3 1/2 days at 20 C (7), with each adult hermaphrodite producing 250-350 progeny.

B) *Hermaphrodite genetics:* Self-fertilization drives populations to homozygosity so that it is easy to isolate isogenic clones of animals. This character greatly facilitates the detection of mutants in a diploid organism. Because chemically induced mutations appear first in the heterozygous condition, recessive mutations cannot be recognized in the F_1 progeny of mutagenized animals. Heterozygous hermaphrodites automatically segregate homozygous mutants as one-fourth of their progeny. In contrast, segregation of homozygous mutants in amphimictic organisms requires manual cloning (brother-sister matings) and homozygotes appear only in the third generation after mutagenesis. Many individual hermaphrodites can be grown together and screened for mutants since there is no danger of losing the homozygous form of a mutant by cross-fertilization. Once mutants are obtained, even the severely uncoordinated or deformed ones can be propagated by self fertilization. Thus, many mutants, which would be lethal in *Drosophila* or the mouse, are viable in *C. elegans*. This fact not only simplifies maintenance of genetic stocks but makes possible the growth of large populations of homozygous mutants for biochemical analysis.

Reproduction by self-fertilization, however, does not provide a means to recombine independently isolated mutations. Genetic analysis, therefore, depends on the existence of males, which are produced spontaneously in hermaphrodite populations by meiotic nondisjunction at a frequency of 0.1% (33). Males possess five pairs of autosomes and one X chromosome, whereas hermaphrodites possess two X chromosomes in addition to the autosomal complement (29, 44). Mutants have been found which have an increased frequency of nondisjunction. These mutants, called *him,* produce a high incidence of males (33). Males are distinguished from hermaphrodites by their smaller size and by the copulatory bursa at the tip of the male tail. A male culture can be propagated by mating males with hermaphrodites. Half the progeny produced by such cross-fertilization are male. In practice, a culture of wild-type males is constantly maintained and these males are used for mating with mutant hermaphrodites. Such crosses produce heterozygous males which are then used to transfer the mutant marker to other hermaphrodites, so that genetic mapping and complementation tests are possible.

Heat shock has been used by several

workers as a method of increasing the spontaneous frequency of males in hermaphrodite populations. This approach is sometimes desirable for facilitating the isolation of males homozygous for a particular marker of interest. A method used by Meneely and Herman (unpublished) involves shifting a few young hermaphrodites for 36 h from their normal growth temperature of 20 C to 27.5 C. After return to 20 C incubation, the heat-treated animals produce about 5% male progeny.

The small size of the animal (1 mm long), its short generation time (3 1/2 days at 20 C), and the large number of progeny per animal (250-350) are important factors in genetic analysis. Because genetic experiments frequently require the detection of rare events, it is an advantage to grow a large number of individuals in the small space. A petri plate seeded with a single hermaphrodite will contain nearly 10^5 individuals after one week. Confining the animals to the 2-dimensional agar surface permits rare individuals in large populations to be observed with a dissecting microscope. Methods for the isolation, complementation, and mapping of mutants of differing morphology or behavior have been described by Brenner (6). A large library of mutants, induced by a variety of mutagenic agents (ethyl methanesulfonate, diethyl sulfate, X-rays, phosphorus-32), is available for study. Mutations are induced by ethyl methanesulfonate (EMS) at a high frequency, averaging 5×10^{-4} per gene (6). This frequency means that, on average, one in every 2,000 F_1 progeny of mutagenized animals will be heterozygous for a mutation in a given gene. Nearly 200 loci have now been placed on the genetic map. Most genes are represented by more than one independently isolated allele.

The majority of the mutants characterized thus far are nonlethal and display a clearly visible phenotype. A large number of mutants are "uncoordinated" (now representing nearly 100 genes). Uncoordinated phenotypes range from small aberrations in movement to nearly complete paralysis. Morphological mutants include dumpy, small, long, and blistered animals. Dumpy mutants are shorter than wild-type animals and correspond to 20 different genes dispersed over the 6 linkage groups. Dumpy mutations are particularly useful markers for mapping most other classes of mutants, since the double mutants are easily distinguished. Both morphological and uncoordinated mutants can be used for mapping many other types of developmental mutants (Fig. 1).

An increasing variety of mutations are being studied, including those affecting drug-resistance (6), sensory behavior such as chemotaxis (22, 41), thermotaxis (28) and male sexual behavior (33), catabolic pathways (3), dopamine biosynthesis (53), muscle assembly (23, 24), sex determination (37), development of the ventral nerve cord (51), and temperature-sensitive lethal mutants affecting embryogenesis (55) and gonadogenesis (1, 31). A series of translocations and duplications have recently been characterized as a first step in assembling a collection of "balancers" for recessive lethal mutations (29).

Maintaining genetic stocks is less difficult with *C. elegans* than with some other organisms used in developmental or behavioral studies, such as *Drosophila* or the mouse. Stocks of *C. elegans* remain viable when frozen and stored in liquid nitrogen. Freezing is more reliable than repeated subculturing because there is less opportunity for human error, and frozen stocks do not revert or pick up modifiers. Stocks are mailed between laboratories by a method analogous to that used for bacteriophage (small aluminum-foil packets are enclosed in a letter).

The chromosome mechanics are not nearly as sophisticated for *C. elegans* as for *Drosophila,* an organism with a long history of genetic research. Even so, at least two laboratories are actively involved in correcting this situation. Baillie and co-workers (unpublished) at Simon Fraser University have used some of the point mutations induced by Brenner to isolate and balance several types of lethal mutations, including gamma-ray-induced deficiencies. Such studies are potentially useful for elucidating the fine structure of gene organization in a small region of a chromosome. Herman and co-workers (unpublished) at the University of Minnesota have genetically characterized X-ray-induced chromosome rearrangements which prevent genetic recombination on the affected chromosomes.

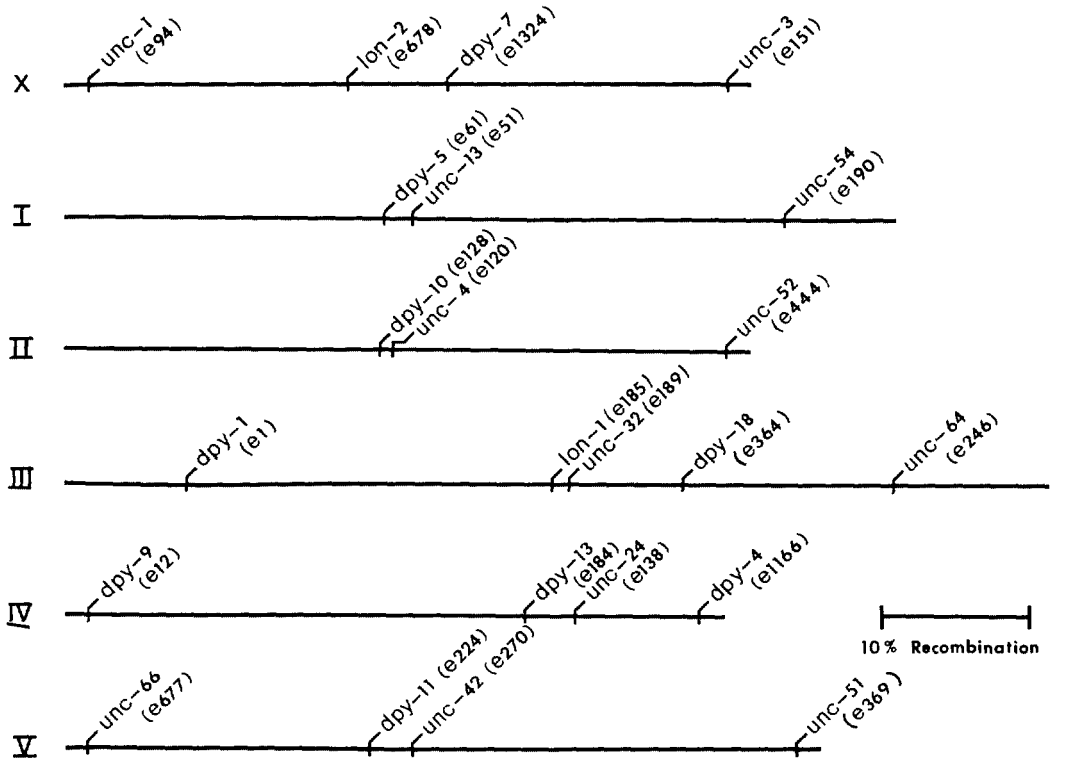

FIG. 1. Genetic map of *Caenorhabditis elegans* showing the locations of 23 reference markers commonly used in genetic mapping of newly isolated mutants. Gene designations are *unc* (uncoordinated), *dpy* (dumpy) and *lon* (long). All loci were mapped by Brenner (6) with the exception of *dpy-4* IV (Riddle, unpublished). The standard reference alleles are given in parentheses.

These crossover suppressors are being used to maintain lethal mutations in balanced heterozygotes. Thus far, a variety of developmental lethals have been found, including egg-lethal and larval-lethal mutants as well as steriles. Herman (unpublished) has mapped a number of such lethal mutations on linkage group II, and their map locations cluster in the same pattern as the visible mutants previously mapped by Brenner (6).

Tetraploid variants of *C. elegans* have been characterized by Herman and Madl (unpublished). The tetraploids can be induced by heat shock and are genetically stable. Triploids can be produced by crossing tetraploid males with diploid hermaphrodites, or vice versa. The triploid hermaphrodites have small brood sizes and lay many eggs which do not hatch. In agreement with Nigon's earlier work (44), it has been observed that tetraploid hermaphrodites are either high-frequency male producers (giving about 45% male progeny) or low-frequency (less than 1%) male producers. The results from Herman's laboratory suggest the following genotypic assignments for the observed classes of animals (where A denotes one set of autosomes): 4X/4A, low-frequency male producer; 3X/4A, high-frequency male producer; 2X/4A, male; and 2X/3A, male.

Most of the genetic work on *C. elegans* has been done with the Bristol variety chosen by Brenner (6). However, *C. elegans* var. Bergerac, which has been studied by J. Brun for more than 20 years, has been the subject of some genetic experiments (17, 45).

C) *The simplicity of Caenorhabditis elegans:* This nematode possesses few cells and few genes. From the frequency of recessive lethal mutations, Brenner has estimated that *C. elegans* has only about 2,000 genes with indispensable functions (6). The haploid DNA content is 8×10^7 base pairs (about 20 times the genome of *E. coli*) and 83% of the DNA consists of

unique sequences (52). This amount is the smallest DNA content reported for an animal. Nevertheless, only one twenty-fifth of the DNA would be required to code for 2,000 average polypeptide chains. This same dilemma exists in *Drosophila melanogaster,* where genetic experiments (35) estimate the total number of genes at 5,000, while the haploid DNA content is about 2×10^8 base pairs (38). This number corresponds to about 40,000 base pairs per gene or about 40 times the amount of DNA necessary to code for an average polypeptide. A variety of theories have been advanced to explain why the genetic units in eukaryotic cells are so large (46). Regardless of that problem, the point to be emphasized here is that although *C. elegans* shares the mysteries of eukaryotic gene organization the total number of genes is small.

The genetic and anatomical simplicity of *C. elegans* increases the possibility of understanding the development of the whole organism. Although some aspects of development seen in more complex organisms (regulation, positional information) may not easily be studied in *C. elegans,* differential gene expression, the determination of cell lineages, cell movement, differentiation, and morphogenesis can be investigated. Nematode development is characterized by the early determination of cell fates. This system simplifies the interpretation of the effects of genetic alteration upon development, since tissue in which the mutant gene is not expressed is likely to develop normally and independently of neighboring tissue that expresses a mutant phenotype. The cell number, and the relative positions of cells with respect to one another are essentially invariant among isogenic wild-type individuals of *C. elegans* (54). This relationship simplifies comparison of a mutant's anatomy with the wild type. The phenotypes of isogenic mutant animals are generally quite uniform.

D) *Embryonic development:* A reliable method for fixing and embedding the relatively impermeable eggs of *C. elegans* involves prolonged osmium or glutaraldehyde plus osmium fixation at elevated temperatures (Günter von Ehrenstein, pers. comm.). A characterization of the entire embryonic development of *C. elegans* at the ultrastructural level using serial sections has been initiated. In complementary studies, cell lineages in cleaving embryos are observed by interference-contrast microscopy. So far cell divisions and migrations have been followed up to the 182-cell stage (16). In the fertilized egg, the sperm and oocyte pronuclei meet in a region destined to become the posterior of the embryo. After the first cell division, the posterior cell is smaller, allowing early recognition of the axis of the egg. The length of the cell cycle in the early cleavage stages is as short as 15 to 20 min. Gastrulation, which starts after the 24-cell stage with the invagination of two endodermal precursors into the center of the embryo, is complete at the 51-cell stage.

Lew and Ward (40), who determined the embryonic cell lineage which gives rise to the intestine, found that the entire organ is derived from one of the posterior blastomeres of the 8-cell embryo. Developing intestinal cells undergo both symmetrical and asymmetrical (stem-cell) lineages. Biochemical differentiation was followed by observing development of a high-gut-fluorescence mutant isolated by Babu (3). Such mutants are defective in enzymes of tryptophan catabolism and accumulate fluorescent intermediate compounds. The intestinal cell lineage of the mutant is normal, and fluorescence begins to appear in embryos having as few as eight gut cells.

Hirsh and co-workers (32) have begun a genetic study of embryogenesis with temperature-sensitive lethal mutants which fail to hatch if incubated at a restrictive temperature. The majority of the mutants studied thus far exhibit maternal effects; i. e., the genes represented by some of these mutants are normally expressed in the hermaphrodite prior to fertilization. Such genes may specify aspects of oocyte structure or may determine the supply of nutrients necessary for survival of the embryo. An advantage of temperature-sensitive mutants is that incubation temperatures can be changed at various times during development so that "temperature-critical" periods can be determined for each mutant. The simplest interpretation of the temperature-critical period (t-crit) is that it corresponds to the time when normal gene product is

required to permit survival. Interestingly, maternal mutants are found to exhibit early t-crits, around the time of fertilization. Mutants lacking the maternal effect or mutants dependent on zygotic gene expression for normal embryogenesis exhibit t-crits at later stages. The high frequency of maternal-effect mutants suggests that the maternal genome plays the major role in the early development of *C. elegans*.

E) *Postembryonic development:* A newly hatched larva resembles an adult in general proportions and body movement but is only about one-sixth as long. The postembryonic development requires about 40 h at 20 C with molts occurring at 14, 22, 30, and 40 h after hatching. Egg laying by the adult commences at about 50 h (9).

Observation of living *C. elegans* under Nomarski differential interference-contrast optics allowed Sulston and Horvitz (54) to directly follow all nongonadal postembryonic cell divisions, migrations, and cell deaths. In the case of the ventral nerve cord, the correlation of cell type with lineage history has been determined directly by analysis of serial-section electron micrographs of individuals of known lineage (51).

During larval development, the number of nongonadal nuclei increases from about 550 in the newly hatched L1 to about 810 in the mature hermaphrodite and to about 970 in the mature male. The pattern of cell divisions is essentially invariant between individuals. Rigidly determined cell lineages generate a fixed number of progeny cells of strictly specified fates. These lineages range in length from one to eight sequential divisions and lead to significant developmental changes in neuronal, muscular, hypodermal, and digestive systems (54). For example, just before the L1 molt the ventral nerve cord increases in cell number from 15 to 57 cells as neuroblasts migrate into the cord from lateral positions and divide. The retro-vesicular ganglion at the anterior end of the ventral nerve cord increases from 12 to 20 cells, and the intestine increases from 20 cells to 30 cells. Until recently, it had been supposed that only gonadal cells underwent proliferation after hatching.

Proliferation and differentiation of gonadal tissues continues throughout the L3 and L4 stages. During the L3 stage, hypodermal nuclei in close proximity to the developing gonad begin to divide, producing 22 cells which form the vulva by the L4 molt. The vaginal and uterine muscles are derived from a single posterior mesoblast which begins dividing about midway through the L1 stage, eventually producing 16 daughter cells, including body wall muscle cells and two coelomocytes (10) which migrate anteriorly. The muscle nuclei of the gonad do not assume their final positions until the L4 stage. In the adult these muscles are used for laying eggs. Although newly hatched male and hermaphrodite larvae are quite similar anatomically, male-specific lineages produce a variety of tissues in late development not found in the hermaphrodite. During the L3 stage, cell divisions occur in the male ventral nerve cord and pre-anal ganglion. Certain hypodermal and neuroblast lineages produce the genital papillae of the caudal alae. The genital papillae probably have a mechanosensory function during copulation. Other male-specific cell lineages generate additional neurons, the vas deferens, the cloaca, and the copulatory spicules.

At hatching the L1 has only four gonadal cells; the outer two constitute somatic cells, the inner two germinal cells. These cells proliferate, forming the adult reproductive system of about 2,500 nuclei. Most of these nuclei are contained in the adult ovaries in a common core of cytoplasm, and some are packaged into oocytes during the reproductive period (31). Divergence of the sperm and egg lines apparently occurs after the L2 stage, since it is possible to isolate homozygous-recessive mutants among F_1 progeny of L1 and L2 hermaphrodites (33).

Hirsh et al. (31) have begun a genetic study of gonadogenesis in conjunction with their work on embryogenesis. Some of the mutants exhibiting t-crits during embryogenesis also have t-crits during gonadogenesis, implying that these genes are required in both phases of development.

Much of the development of *C. elegans* seems to be based on a lineal determination of cell function (54). The high degree of invariance of the cell lineages suggests such a mechanism, but evidence is stronger from experiments with a laser microbeam system

(developed by John White at Cambridge). The laser beam is used in conjunction with Nomarski optics to destroy individual nuclei without damaging surrounding cells. The experiments suggest that the fates of cells in a variety of tissues are determined autonomously. Cell fates remain invariant even though undamaged cells sometimes differentiate in positions which would normally be occupied by cells with different fates. The study of mutants with abnormal cell lineages supports those observations (54). For example, a genetic block in one branch of the cell lineages of the posterior lateral ganglia or the ventral nerve cord does not alter the developmental fates of cells generated by another branch of the lineage.

There are some notable exceptions to the rule of cell autonomy in development. The vulval structures fail to form if gonadal tissue is absent. The lineages of the ventral hypodermal cells are arranged symmetrically around the midpoint of the developing gonad, as if that point were a source of a positional determinant. Positional information also seems to play a role at the ends of the ventral nerve cord and in development of the male tail (54). Although positional influences may affect cell lineages in specific instances, there is no evidence that *C. elegans* is capable of regulative development *per se*. Structures normally derived from a cell which has been eliminated, either by mutation or by the laser, are invariably absent in the adult. No regulatory processes produce extra divisions to compensate for genetic or laser-induced deficiencies.

Mutants affected in postembryonic cell lineages have been grouped into several catagories by Horvitz and Sulston (unpublished). These include mutants blocked in the hypodermal lineages that generate the vulva, mutants blocked in the migrations of the ventral cord precursor cells (which generate much of the ventral nervous system as well as the vulva), and mutants with generalized blocks in all postembryonic cell divisions. Two classes of mutants exhibit excess cell divisions. Multivulva mutants (representing 3 genes) suffer from overproliferation of ventral hypodermal cells. Another mutant overproduces deirid and postdeirid neurons.

The relation of DNA replication to cell division in cell-lineage mutants has been studied by D. G. Albertson, J. G. White, and J. E. Sulston (unpublished). The mutant E1348, *lin-5* II, is defective in postembryonic nuclear and cell division. At mitosis, abnormal metaphase plates form in this mutant. Neuroblasts which would normally generate ventral-cord neurons in the late L1 stage accumulate about six times the diploid quantity of DNA within a single nucleus as determined by quantitative fluorescence measurements. Interestingly, normal cell division would produce six cells (five neurons and one hypodermal cell) from each precursor cell. Precursors for lineages normally producing a greater number of daughters display higher DNA contents in the mutant. The precise control of chromosome replication that is observed in *C. elegans* is apparently independent of cytokinesis, but the mechanism by which chromosomes become destined for a fixed number of replications remains an intriguing question.

It is interesting to ask what effect the block in cell division has on cell differentiation. The polyploid cells produced in E1348, in fact, display characteristics of the progeny cells they should have produced and frequently resemble motor neurons. In another mutant, similar to E1348 but blocked only in the last rounds of division, the undivided cells take on the differentiated characteristics their posterior daughters would have assumed (H. R. Horvitz, unpublished).

F) *Sex Mutants:* Reproduction in *C. elegans* provides many research possibilities for the scientist interested in sex. To date, research has concentrated on: *(i)* the process of fertilization using fertilization-defective mutants; *(ii)* sexual transformation (transformer mutants develop into males in spite of an XX genetic complement); and *(iii)* intersex mutants which display some characteristics of both the hermaphrodite and the male. Not included in this category are the *him* mutants, which produce a high incidence of normal XO males in hermaphrodite populations because of an increased frequency of chromosome nondisjunction.

S. Ward and co-workers (unpublished) are using fertilization as a model system to study the genetic control of cell surface structure and cell-cell surface interactions

by light and electron microscopy. When the oocyte is mature, contractions of the oviduct wall push the cell into the spermatheca, where it contacts many sperm. The egg then is squeezed into the uterus through a constriction. Extra sperm are normally carried into the uterus along with the egg, but they migrate back through the constriction to the spermatheca so that the efficiency of sperm utilization approaches 100%. The sperm are ameboid cells with a specialized pseudopodial region which is extended from the cell during migration. When males mate with hermaphrodites, they deposit their sperm in the region of the vulva and these sperm migrate past the embryos in the uterus to the spermatheca. After mating, male sperm preferentially fertilize oocytes even though hermaphrodite sperm are still present (the hermaphrodite returns to use of its own sperm only when the male's sperm are exhausted). The switch from self-cross to out-cross and back to self-cross progeny can be observed when genetically-marked hermaphrodites are mated with wild-type males.

The observations suggest that sperm recognize a chemical signal which guides them to the spermatheca. Also, male sperm must be different from hermaphrodite sperm in some way. Perhaps, they produce a substance which reversibly inactivates hermaphrodite sperm.

Argon and Ward (unpublished) have isolated a series of temperature-sensitive mutants which are sterile at the restrictive temperature (25 C) and lay unfertilized eggs. The mutants can be rescued by mating with wild-type males. The mutant males are sterile, although they do transfer sperm to the uteri of hermaphrodites. These observations suggest that the sperm are defective, and one goal is to analyze biochemical defects in the sperm of these mutants. Isotopically labeled sperm can be purified by insemination of unlabeled hermaphrodites.

In Hirsh's laboratory, Lois Edgar (unpublished) has found temperature-sensitive sterile mutants exhibiting a variety of sperm defects. Some mutants produce no sperm, some produce sperm with abnormal morphology, whereas others produce normal-looking but nonfunctional sperm. The temperature-critical periods for all these mutants generally coincide with the time of spermatogenesis. Nonconditional sterile mutants of the Bergarac strain of *C. elegans* have been isolated (Nicole Mounier, unpublished) and classified cytologically as affecting either gonadal differentiation, sperm maturation, oogenesis or oocyte maturation.

Sex-transformer mutants were first described by Hodgkin (33). Autosomal recessive mutants representing two *tra* genes were found which develop into "pseudomales." Since all homozygous individuals are male, stocks must be propagated as heterozygotes. Mutations in at least one transformer gene produce functional males. When these pseudomales mate with hermaphrodites, all the progeny are hermaphrodites (since both parents are XX) and they are heterozygous for the *tra* mutation. The heterozygotes then segregate pseudomales among their progeny in the Mendelian ratio of one in four. Klass et al. (37) recently isolated a temperature-sensitive transformer mutant that can be propagated in the homozygous form at 15 C but develops into males at 25 C. Temperature shifts during development cause the production of intersexes. The gonads of the intersex forms have characteristics of both male and hermaphrodite gonads, with the balance depending on the time of the temperature shift. Pseudomales are very useful in developmental studies on sexual differentiation, and they are potentially useful in routine genetic analyses as well. For example, complementation of X-linked behavioral or morphological mutants is frequently difficult because hemizygous (XO) males express the mutation and cannot mate. In contrast, pseudomales can be used to carry X-linked markers in the heterozygous state where recessive mutations are not expressed.

An intersex mutant studied by G. A. Nelson, K. K. Lew and S. Ward (unpublished) is, in a sense, a reverse transformer. At 25 C, XX hermaphrodites produce no sperm, whereas XO animals are intersexes rather than males. The intersex animals have normal male somatic structures but their gonads contain oocytes or abortive gametes rather than sperm. Some intersex animals have a vulva. The appearance of a vulva correlates with the degree of sim-

ilarity of the underlying gonad to the hermaphrodite. Successful matings between intersex animals and normal males have not been observed.

G) *Molting and cuticle formation: Caenorhabditis elegans* molts four times in its life cycle, as with most other nematode species. Each molt is preceded by a period of lethargus during which pharyngeal pumping and body movement are suppressed (9). Prior to lethargus, the hypodermis begins secretory activity necessary for the formation of a new cuticle beneath the old cuticle. When groups of lateral hypodermal "seam" cells are killed with a laser microbeam, or if they are absent in a mutant such as E1348 (described in the previous section), the overlying lateral alae (lateral field) do not form (50).

It has been proposed that in some nematode species the excretory duct releases fluid which weakens the old cuticle (4, 15). *C. elegans* is apparently different, since laser experiments indicate that the excretory system is not essential for molting (50). Furthermore, the loosening of the old cuticle, which is correlated with pharyngeal gland secretion, begins at the tip of the head, rather than at the excretory duct (50). At ecdysis the cuticular lining of the pharynx breaks, and the posterior piece is passed into the intestine. The old cuticle is inflated around the tip of the head, and the nematode pulls back from it until the remainder of the pharyngeal lining is expelled. Once the mouth is free, the animal pushes against the old cuticle with its head until a cap breaks away, or a hole is made, and the nematode crawls out of its old cuticle.

Studies on the structure and genetic specification of the cuticle (39, 65) by J. Laufer, M. Kusch, and R. S. Edgar (unpublished) are already producing interesting results. Cuticles are purified by sonication of worms followed by washing with salt or lauryl sulfate (SDS) solution. Cuticle material is obtained complete with pharyngeal and anal linings and the basement membrane which separates the pharnyx from the other organs. The cuticle is composed of two layers which are held together by "struts," seen as refractile dots in phase-contrast microscopy. Mild treatment of cuticle preparations with pronase or collagenase breaks the struts, whereas prolonged treatment dissolves the inner cuticle layer. The outer layer is resistant to enzymatic digestion as well as a variety of chemical treatments which break weak bonds or disulfide bridges. This response indicates that it is the outer layer, which is composed of the most highly modified and cross-linked forms of collagen (39). Cuticles are not visibly affected by chitinase or lipase treatment.

Some of the mutants described by Brenner (6), such as the dumpy, roller, and blistered mutants, have long been suspected of having cuticle defects. Roller mutants have been characterized in both the Bristol (30) and Bergerac (Ouazana and Brun, unpublished) strains of *C. elegans*. Edgar and co-workers (unpublished) have observed that roller mutants and some dumpies have aberrant lateral alae displaying multiple loops or breaks. The cuticles removed from roller mutants are helically twisted. In blistered mutants, the blisters form between the two cuticle layers, with struts apparently broken or missing. Further biochemical studies should establish the specific nature of the defects in these mutants more clearly. Particularly with dumpy and roller mutants, it is recognized that cuticle aberrations could be a secondary consequence of a defect in another tissue.

H) *Dauer larvae:* At the second molt, *C. elegans* can either continue development on to sexual maturity or, if environmental conditions are unsuitable, arrest development and enter a morphologically recognizable nongrowing state called the dauer larva. The dauer larva may survive for months in the absence of food (36), but if it does encounter food, it begins to eat and then molts to resume normal development. The duration of the dauer state has no effect on post-dauer life span (36).

Dauer larvae, which differ structurally from all other stages of the same species, were first identified as a special larval stage of insect-parasitic nematodes. These larvae were termed "dauerlarven" by Fuchs (21) in 1915. This German term can be translated into English as "everlasting larvae." Dauer larvae, characteristic of many nematode species, represent an obligate stage in

the life cycle of some parasites. In *C. elegans,* dauer larva formation is facultative. A similar stage has been observed for the closely related nematode *Caenorhabditis briggsae* (64) and other members of Rhabditidae.

In *C. elegans,* the dauer larvae are relatively thin with an axial ratio nearly double that of other larvae (9). They have a greater specific gravity as measured in density gradients, and an altered cuticle as seen in electron micrographs. The outer cortex is thicker, and there is an additional striated layer not seen in the cuticles of other juvenile stages. Because of the cuticular structure, dauer larvae resist inactivation by detergents, anesthetics and a variety of other chemical agents (8). Dauer larvae exhibit characteristic behavior patterns. Pharyngeal pumping is completely suppressed. They usually lie motionless, but respond negatively to mechanical stimuli. If they encounter projections on the agar surface, dauer larvae may attach themselves with their tails and wave their bodies in the air. In the soil, such behavior may allow them to attach to passing vectors and be carried to new locations. Dauer larvae are thermotactically opposite to nondauer larvae (28). Postembryonic cell lineages are arrested when dauer larvae are formed. Primarily, these lineages involve proliferation and differentiation of gonadal tissues and the formation of sex organs.

The developmental sequence leading to the formation of dauer larvae is particularly advantageous for genetic study since the sequence is not essential for survival of the nematode in the laboratory. Thus, it should be possible to isolate mutations in all genes which play a role specifically in the pathway. Secondly, various mutant types are easy to select. The resistance of dauer larvae to detergents such as sodium dodecyl sulfate (9) provides an effective means of selecting mutants which form dauer larvae abnormally in the presence of food. Detergent resistance permits selection of mutants which recover slowly from the dauer state, or mutants whose recovery is temperature-dependent.

Two types of mutant have been isolated and partially characterized: constitutive mutants which produce dauer larvae even when food is abundant, and defective mutants which do not produce dauer larvae under any conditions (47). The constitutive mutants represent six genes, whereas the defective mutants isolated thus far have been assigned to an independent set of 12 complementation groups. Although the genetic evidence suggests that there may not be many more than six genes of the constitutive class, it is estimated that there are 30 to 60 genes of the defective type.

Some of the dauer-defective mutants exhibit sensory defects. They lack the normal chemotactic response to salt gradients as measured in the "orientation assay" developed by Ward (56), and electron micrographs of some mutants have revealed morphological abnormalities in a specific class of sensory neurons comprising the amphids. These neurons had previously been implicated in the chemosensory response to salts (41).

The amphids are a pair of prominent sensilla, each consisting of 12 neurons, 8 of which extend processes from cell bodies near the nerve ring anteriorly where they terminate in amphidial channels on the lateral sides of the stoma. The ends of the neurons are exposed to the outside environment. The amphidial neurons are normally ciliated, i.e., neuronal processes contain microtubules arranged in the typical "9+2" configuration. Ultrastructurally, the amphidial neurons of the nonchemotactic, dauer-defective mutants examined are packed with electron-dense material in the space occupied by ciliated processes in the wild type. In the mutants, normally cylindrical processes are enlarged and irregular, and fail to reach their normal length (47).

These results suggest a sensory signal for dauer larva formation, mediated by the amphids. Any nonlethal mutation which results in the failure of these neurons to develop properly might be detected as a dauer-defective mutant, since the animal's central nervous system cannot receive the starvation signal from the environment.

There is a relationship between the constitutive and defective mutants in the pathway of dauer larva formation. Epistatic relations between genes were determined by constructing double mutants, homozygous for both a "constitutive" and a "defective" mutation. In certain such pairs, the con-

stitutive phenotype is suppressed. On the basis of the suppression patterns, a genetic pathway has been constructed with individual genes assigned to the control of various steps (47). Interestingly, the mutants exhibiting abnormal chemotaxis could be assigned to an early step in the developmental sequence. It seems plausible, therefore, that the genetic pathway represents a pathway of neurotransmission involving reception of an environmental signal, and the conversion of that signal into a neuro-endocrine response (15). Further ultrastructural studies may reveal cells other than amphidial neurons which participate in signal processing. If biochemical experiments reveal a specific chemical signal for dauer larva formation (or a compound which prevents dauer larva recovery), it would be interesting to test its effect on the growth of other nematode species.

I) *Nematode neurobiology:* Historically, neurobiology has occupied a position of prominence in nematological research, as exemplified by the classical work of Goldschmidt on *Ascaris* neuroanatomy (reviewed in ref. 10). The combined genetic and ultrastructural approach which is possible in *C. elegans* is now bringing the study of neural structure and function to a new level of resolution (62). Ultrastructural analysis of behavioral or uncoordinated mutants can implicate specific cells in the specification of particular neural functions. This approach has its limitations, however. Although electron microscopy can unambiguously establish the "wiring diagram" of neural connections it cannot determine, for example, which circuits are inhibitory and which are excitatory. Physiological studies are necessary for complete understanding of how the nervous system works, but electrophysiology on *C. elegans* is beyond current technology. There is the attractive possibility, however, of reaching a synthesis between genetic and ultrastructural results obtained from *C. elegans* and electrophysiological data from larger nematodes such as *Ascaris lumbricoides* (recently reviewed by Jarman in ref. 13). Such a synthesis depends on a high degree of functional similarity between neurons in the two nervous systems.

Much of the work on *C. elegans* has concentrated thus far on normal wild-type neuroanatomy (2, 33, 54, 57, 58, 62, 63), and these studies have generated many specific ideas about the function of the nervous system. Characterization of mutants has centered on the sensory nervous system and on various aspects of neurotransmission. The first sensory mutants isolated were defective in chemotaxis (22, 41). A large collection of chemotaxis-defective mutants is being studied by Dusenbery (20), who has expanded the repertoire of known attractants and repellants established by Ward (56). *C. elegans* exhibits chemotactic responses to CO_2, various ions, amino acids, pH, cyclic AMP, and even pyridine. The worms avoid D-tryptophan (21) and several sugars. Some of the mutants simply fail to respond to stimulants, whereas other mutants avoid substances which normally serve as attractants. Most mutants examined thus far exhibit pleiotropic effects on chemotaxis; they are defective in response to more than a single stimulant. Ultrastructural studies of chemotaxis-defective mutants have implicated a variety of the anterior sensory neurons in chemotaxis to salts, including the amphids, amphidial "finger cells" and the inner labial sensilla (41).

The diversity of nematode behavior has been pointed out by Croll (11, 14). Hedgecock and Russell (28) showed that wild-type *C. elegans* will follow isothermal lines in a thermal gradient, whereas thermotactic mutants wander randomly over the gradient. Culotti and Russell (unpublished) have isolated mutants representing six genes which lack the normal ability to avoid conditions of high molarity. The ciliated sensory endings of the cephalic (lateral submedial) sensilla are disorganized in one such mutant. The cephalic neurons in the head are among the eight neurons in the *C. elegans* hermaphrodite which contain catecholamines (53) (the male has six additional dopaminergic neurons in the tail). Dopamine or its related compounds presumably are transmitters in these cells. Catecholamines are detected microscopically by formaldehyde-induced fluorescence. Mutants selected for their lack of fluorescence (called *cat* mutants) are either blocked in catecholamine biosynthesis or blocked in release of catecholamines from vescicles located in the cell bodies (53). Upon first

examination, it was thought that the *cat* mutants exhibited normal behavior in spite of the absence or near absence of catecholamines. However, it now seems that "osmotic avoidance" behavior is at least one identifiable function of dopaminergic neurons.

Certain pharyngeal neurosecretory cells in *C. elegans* produce a yellow formaldehyde-induced fluorescence characteristic of serotoninergic neurons. These neurons also accumulate exogenously supplied serotonin. R. H. Horvitz and J. E. Sulston (unpublished) found that added exogenous serotonin (a catecholamine) stimulates the rate of egg-laying by normal adult hermaphrodites, and they found two egg-laying-deficient mutants (*unc-86* III and *unc-91* I) which are induced to lay eggs by addition of serotonin. These mutants exhibit reduced levels of formaldehyde-induced fluorescence in the pharyngeal neurons. Horvitz and Sulston suggest that the serotoninergic neurons induce egg-laying in wild-type *C. elegans,* and that the mutants are defective in egg-laying because of their reduced levels of serotonin. This hypothesis is in agreement with previous work by Croll (12) which showed that serotonin affects vulval contractions and oviposition.

An intriguing class of sensory mutants (representing eight genes) has been characterized by Sulston (unpublished). These touch-insensitive *(tin)* mutants fail to move away when tickled gently along the body with an eyelash mounted on a stick. At least some of these mutants have been shown to be defective in a pair of extremely long microtubule-containing neurons which extend down the lateral sides of the body. Observation of the mutants with Nomarski optics shows that the nerve cell bodies are absent or displaced. Destruction of these cells with a laser beam also results in touch-insensitivity.

One attractive means of isolating neurological mutants does not depend on behavioral phenotypes but relies on resistance to neurotoxins. In such selections, only particular mutants are able to grow and reproduce, since wild-type growth and movement is inhibited by the presence of a nematicide in the medium. The first drug-resistant mutants of *C. elegans* were isolated by Brenner (6). He obtained mutants resistant to lannate (similar to aldicarb) and a separate set of mutants resistant to tetramisole (and Levamisole). Lewis and co-workers (unpublished) at Columbia University have been pursuing the genetics and pharmacology of Levamisole resistance. About 12 genes have been identified which convey resistance to this drug, which is an acetylcholine agonist. Resistant mutants are uncoordinated in varying degrees, and the evidence suggests that they are deficient in acetylcholine receptors.

The enzyme which synthesizes the neurotransmitter acetylcholine is choline acetyltransferase (CAT). Johnson and Russell (34) have developed an assay for this enzyme in *C. elegans* and have found two types of enzyme which differ from one another in sensitivity to inactivation and substrate preference. Mutants have been found that are deficient in one type of CAT. One such mutant was selected in Hirsh's laboratory (unpublished) as being resistant to trichlorfon (a CAT inhibitor) and is severely uncoordinated. In general, the chemically induced mutants which are resistant to nematicides are uncoordinated and slow growing. Although such mutants can be maintained under the ideal growth conditions provided in the laboratory, they would not be likely to survive in a natural environment. These drug-resistant mutants offer the neurobiologist a genetic handle for studying the protein components involved in neurotransmission.

J) *Molecular genetics of muscle: Caenorhabditis elegans* is currently the best system available for studying the genetic basis of muscle development, structure, and function. Mutations in at least 12 genes are known to affect body musculature. Severely defective mutants are paralyzed or dystrophic, but the paralyzed mutants are viable as homozygotes because active males are not required for the production of offspring. Also, it is possible to obtain mutants paralyzed in the body which are not affected in pharyngeal function, so that feeding and growth are possible. In this respect, it is fortunate that body wall and pharyngeal muscle are composed of at least some distinct proteins. The body muscle proteins are present in large amounts relative to most other proteins in *C. elegans,*

and their unique solubility properties permit their rapid purification. The body musculature can be observed, using polarized light microscopy to visualize birefringence produced by the ordered lattice of normal filaments (24). This approach is a sensitive technique for the detection of disorganized muscle in mutant animals. Electron microscopy and extensive biochemical analyses can also be applied to the study of muscle mutants (24, 27, 42, 60).

There are two characteristics of this system which make it particularly useful: *(i)* It is a model system in cytodifferentiation. Very little is known about the genetic specification of the very complex structures found in differentiated eukaryotic cells. Elucidating the program for muscle assembly during development could make a major contribution toward the solution of the problem, and may even shed some light on muscular dystrophies in higher organisms. *(ii)* Molecular genetics is feasible in this system because both the genes and their protein products are amenable to analysis. In the case of most mutants of *C. elegans,* as well as other eukaryotes, the molecular defects which underlie the observed phenotypes are completely unknown. Only in rare instances have mutant genes been correlated with specific protein products. Identification of the structural gene for a myosin heavy chain (43) and the gene for paramyosin (61) in *C. elegans* has provided the basis for studying the fine structure of these genes and the mechanisms by which expression of these genes is controlled.

At least two myosins in the body muscle of *C. elegans* (49) contain heavy chains encoded by distinct genes. The gene *unc-54* I is the structural gene for the major myosin heavy chain (43). Gel electrophoresis of muscle proteins from one *unc-54* mutant, E675, showed that it contains a shortened form of the 210,000 dalton heavy chain found in wild-type animals (24). Peptide analysis of the E675 heavy chain has shown that the structural alteration is an internal deletion near the C-terminus of the protein (42). Many alleles of the *unc-54* gene have been characterized, although in less detail than for *e675.* Some mutants apparently produce no *unc-54* myosin, whereas other mutants produce altered proteins, some of which assemble into abnormal filaments. These latter mutants are dominant, and one allele is temperature-sensitive.

A set of four mutants with abnormal muscle structure comprises the *unc-15* locus. In one of the mutants, E1214, paramyosin is completely absent from both body wall and pharyngeal muscle. In the other three mutants, paramyosin is present but does not assemble into thick filaments (paramyosin forms the core of the thick filaments in normal invertebrate muscle). In these mutants, myosin filaments lacking paramyosin cores are present, but these filaments fail to integrate stably into the myofilament lattice. Perhaps, the most striking feature of the mutants is that paramyosin paracrystals are formed in the muscle cells, producing bright patches of birefringence in polarized-light microscopy (61).

The muscle mutants are very amenable to reversion experiments since the animals are paralyzed. Revertants with restored or partially restored movement can be selected readily (48). Reversion studies have led to isolation and identification of the suppressor mutants. Suppressor mutations compensate for the original mutation but are located elsewhere in the genome. A suppressor mutant, then, is actually a double mutant which carries the original muscle mutation plus a compensating suppressor mutation in a different gene. One such suppressor of muscle defects, called *sup-3,* compensates for mutations in *unc-54* or in *unc-15* and may be specific for thick filament defects. Most of the mutant alleles of these two genes are suppressed, indicating that the suppressor replaces the function of these genes or circumvents the need for these genes in some indirect way (48). One hypothesis is that the suppressor mutation increases the level of myosins other than *unc-54* myosin in muscle cells.

A second type of suppressor was found among revertants of E1214, the *unc-15* mutant which produces no paramyosin. The suppressor restores paramyosin in this mutant. The suppressor of *e1214* also is able to suppress certain alleles of several other genes, some of which do not involve muscle (59). Such allele-specific, but gene nonspecific suppression has been called informational suppression in bacterial systems. This type of suppression generally involves alteration of the protein synthesiz-

ing system of the cells, so that certain mutant genes can be read as though they were normal. The most common mechanism involves alteration of transfer-RNA structure as is the case in nonsense suppression. If it can be shown that informational suppression in *C. elegans* results from altered tRNA species, as it does in microbial systems, it would be the first demonstration of this phenomenon in a multicellular animal.

K) *Conclusion:* The genetic work on *Caenorhabditis elegans* should have an important impact on nematology as well as biology in general. At this early stage, however, this view must be taken largely on faith. I have not attempted to assess what specific applications this work may have in nematology. Indeed, it is for nematologists to make such judgments, and I believe it is proper to leave speculation on these matters to the reader.

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