Developmental Genetics of the Mechanosensory Neurons of C. elegans

MARTIN CHALFIE AND JOHN SULSTON

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England

Received June 16, 1980; accepted August 15, 1980

Touch sensitivity in the nematode C. elegans is mediated by a set of six sensory neurons, the microtubule cells, of well-characterized anatomy and connectivity. The normal touch response is eliminated when these cells are killed by laser microsurgery. The identification of the microtubule cells as the mediators of touch sensitivity allows us to examine the effects of mutations on the development and differentiation of these cells. Forty-two touch-insensitive mutants have been isolated. These fall into 13 complementation groups. Mutations in five of the complementation groups have recognizable effects on the microtubule cells. These phenotypes include alterations of characteristic cellular ultrastructure, absence of neuronal process growth, and the absence of the cell (either by alterations in the patterns of cell division that give rise to the cells or by degeneration or death of existing cells). Because it is likely that we are approaching saturation of genes affecting primarily the microtubule cells, there appear to be relatively few genes that affect the growth and function of this class of cells and no others.

INTRODUCTION

One of the major problems of developmental biology is that of genetic control of neuronal development. To approach this problem we ideally would like to have an organism with a variety of behaviors, a relatively small number of large nerve cells (to facilitate electrical recording) of known anatomy, and sophisticated genetics. As has been remarked before (Quinn and Gould, 1979), no such organism has been found. Yet many mutants with aberrant nervous tissues have been isolated in a wide variety of organisms (for references see Ward, 1977; Breakefield, 1979; Hall and Greenspan, 1979). It has, however, proved difficult to obtain large numbers of mutations affecting the growth and function of specific cells in order to assess the role of gene action in their development. Such a class of mutants could, in principle, be readily isolated and studied by, first, identifying cells mediating a nonessential behavior that can be easily altered by mutation and, second, by examining the mutants for defects in the identified cells. Such mutations should help to identify stages in the development of the cells as well as components necessary for their function. Moreover, analysis of such mutations may indicate which aspects of the development of particular cells are cell specific and which are more general, affecting many classes of nerve cells.

The small free-living nematode C. elegans is an excellent organism in which to look for such neuronal mutants. This animal has been the subject of extensive genetic analysis over the past decade (Brenner, 1974; Herman and Horvitz, 1980). The adult nematode possesses an invariant pattern of cells generated from reproducible patterns of cell division (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979). Most of the anatomy of the 300-cell nervous system has been reconstructed from serial section electron micrographs (Ward et al., 1975; Ware et al., 1975; Albertson and Thomson, 1976; White et al., 1976; Hall, 1977; Sulston et al., 1980; White, Southgate, Thomson, and Brenner, personal communication). These data show that the number, position, morphology, and (where known) cell lineage history of the nerve cells in C. elegans are essentially invariant. This constancy means that developmental defects or alterations can be easily identified.

Many nervous system mutants of C. elegans have been isolated (reviewed by Ward, 1977, 1979). Unfortunately, most of these mutations either have a behavioral phenotype for which the cellular basis is uncertain, e.g., defects in chemotaxis (Dusenbery et al., 1975; Lewis and Hodgkin, 1977) or thermotaxis (Hedgcock and Russell, 1975), or have a cellular phenotype in cells of unknown function, e.g., dopamine deficiency (Sulston et al., 1975).

In this paper we describe a behavior in C. elegans for which the cellular basis is known: touch sensitivity. The touch receptors in C. elegans are a set of six neurons, the microtubule cells (Chalfie and Thomson, 1979). We begin with a description of the cells and their identification as touch receptors by experiments using laser microsurgery. Touch-insensitive mutants can be isolated in C. elegans (Sulston et al., 1975), and we conclude...
with a characterization of such mutants. Many of these mutations have demonstrable effects on the development and differentiation of the microtubule cells.

MATERIALS AND METHODS

Growth and Maintenance of Nematode Strains

For most experiments and for general stock maintenance, wild-type C. elegans var. Briston (N2) and mutant strains were grown at 20°C as described by Brenner (1974). Mutations used in this study are listed below or in Table 1. Unless indicated, genes are defined either in this paper or by Brenner (1974). Mutations are named according to the recently standardized C. elegans notation (Horvitz et al., 1979). Genes are assigned a three-letter prefix and, separated by a hyphen, a number (e.g., met-1); mutations are designated by a one- or two-letter prefix and a number (e.g., e1086).

LG I  
-dpy-5(e61), unc-18(e51,e1091), lev-11(x12)
  (J. Lewis, personal communication), unc-53(e675).

LG III  
-dpy-1(e1), daf-7(e1372), lon-1(e185), sup-5(e1464) (Waterston and Brenner, 1978), dpy-18(e864).

LG IV  
-dpy-13(e184), him-8(e1489) (Hodgkin et al., 1979), unc-30(e191), unc-22(e86, e903, e946, e904, e952).

LG V  
-unc-46(e177), dpy-11(e224), unc-23(e25), him-5(e1490) (Hodgkin et al., 1979), unc-7(e911), EDf1(e1405) (Riddle and Brenner, 1978).

LG X  
-lon-2(e678), unc-6(e78), dpy-7(e88, e1824), dpy-6(e114), him-4(e1287) (Hodgkin et al., 1979), unc-9(e101), unc-7(e93), sup-7(e45) (R. Waterston, personal communication), mndf2, mndf3, and mndf30 (Herman et al., 1979), mndf1, mndf2, mndf3, mndf9, and mndf11 (Meneely and Herman, 1979; P. Meneely, personal communication).

A partial genetic map showing the positions of these genes is given in Fig. 1.

Touch Sensitivity

Nematode touch sensitivity was tested by stroking the tip of an eyebrow hair attached to a toothpick transversely across the anterior or posterior half of an animal. A touch-sensitive animal was one that moved away from the stimulus. To differentiate touch-insensitive animals from those incapable of movement, we also prodded the nematodes with a fine platinum wire. Only those animals that failed to respond to the hair, but moved normally when prodded with the platinum wire, were scored as insensitive to touch. A partial response was recorded when the animal moved after some but not all of the hair touches.

Mutagenesis

Most of the touch-insensitive mutants were obtained by ethylmethane sulfonate mutagenesis of N2 as described by Brenner (1974). A mutagenized parent was allowed to lay 30-50 eggs at 25°C, and the F2 progeny were tested for touch sensitivity as described above. Some mutations, i.e., mec-1(e1737,e1738), mec-2(e75,e1084), mec-8, and unc-86(e1416), were obtained from preexisting stocks. e1737 and e1738 were separated from multiple mutants CB3228 and CB1412, respectively. mec-1(e1292) was isolated after ICR-191 mutagenesis by Don Riddle. mec-6(e1609), a spontaneous mutation, was isolated from a wild-type stock by Barbara Meyer. Jim Lewis, Sydney Brenner, and Bob Horvitz supplied, respectively, mec-1(e1066,e1737), mec-10(e1715), and lev-11(x12); mec-2(e75,e1084) and mec-8; and mec-1(e1738,n174) and unc-86(e1416). We are grateful to all these investigators for drawing our attention to these mutations and making them available to us.
Fig. 1. Partial genetic map of Caenorhabditis elegans. Genes containing alleles that affect touch sensitivity are drawn above the lines representing the C. elegans linkage groups.

Genetics

Complementation tests of the touch-insensitive mutations were done according to Brenner (1974). For X-linked mutations, males carrying one touch sensitivity mutation were mated to hermaphrodites carrying another and lon-2, and the non-Lon hermaphrodite progeny were tested for touch sensitivity. Touch-insensitive mutations that did not fall into any preexisting complementation group were assigned to genes designated as mechanosensory abnormalities or mec. All pairwise combinations within each complementation group yielded touch insensitive heterozygote progeny except in mec-2. Both recessive and dominant alleles of this gene have been obtained, and in trans heterozygotes they show positive and negative intraallelic complementation (Table 2). All genetic experiments were conducted at 20°C. For each gene an unambiguous assignment to a unique linkage group was obtained by two-factor crosses using standard dpy markers (Brenner, 1974). The mec mutations were positioned on the genetic map (Fig. 1) by the following criteria.

mec-2: 6 of 17 Lon recombinants and 10 of 14 Unc recombinants from lon-2 + unc-6/+ mec-2(e75) + gave Met progeny. Similar heterozygotes with mec-2(e1084) yielded 12 of 25 Lon and 10 of 18 Unc recombinants segregating Met progeny. When the dominant allele, mec-2(e1088), was used, 6 of 11 Lon and 3 of 7 Unc recombinants were Met. lon-2 mec-2(e75)/0; mnDp30 males are Lon Met (we have not investigated this anomalous result further).

mec-8: 20 of 26 Dpy and 9 of 24 Unc recombinants from + mec-8(e1612) + +/dpy-13 + him-8 unc-30 segregated Mec progeny. All of the 5 Unc, but none of the 7 Dpy, recombinants from unc-46 dpy-11 + + mec-1(e1066) segregated Mec progeny.

mec-4: lon-2 mec-4(e1497)/0; mnDp2 males are Lon Mec; similar males with mnDp3 are Lon non-Mec. Heterozygote hermaphrodites of the form Df/lon-2 mec-4(e1497) are Mec with mnDf1, mnDf2, and mnDf11, but wild type with mnDf4 and mnDf8. (The data here and for mec-5 for mnDf2 and mnDf11 were kindly provided by Phil Meneely.)
CHALFIE AND SULSTON  Touch-insensitive mutants of C. elegans

TABLE 2

<table>
<thead>
<tr>
<th>Male parental genotype</th>
<th>Hermaphrodite parental genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>e878</td>
</tr>
<tr>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>e75</td>
<td>++++</td>
</tr>
<tr>
<td>e1084</td>
<td>++++</td>
</tr>
<tr>
<td>e1514</td>
<td>++++</td>
</tr>
<tr>
<td>e1523</td>
<td>++++</td>
</tr>
<tr>
<td>e1524</td>
<td>++++</td>
</tr>
<tr>
<td>e1608</td>
<td>++</td>
</tr>
</tbody>
</table>

Note. Hermaphrodites and males of the indicated genotypes were mated and their progeny were tested for touch sensitivity (the lon-2 (e678) marker was used to distinguish cross from self-progeny). Sensitivity was scored on a relative scale from completely touch insensitive (-) to wild-type touch sensitive (+++). Unusual complementation data are those of crosses with the weak allele e1514. Transheterozygotes of e1514 with e1084 and e1524 show negative intraallelic complementation and those of e1514 and e1608 show positive intraallelic complementation.

mec-5: 17 of 24 Lon recombinants and 3 of 24 Mec recombinants of lon-2 + mec-5(e1504)/+ unc-7 + gave Unc progeny. Five unc-7 mec-5(e1504)/+ + heterozygotes segregated 1357 wild type, 357 Unc Mec, 23 Unc, and 16 Mec progeny (P = 2%). lon-2 mec-5(e1504)/0; mnDp2 males are Lon Mec; lon-2 mec-5(e1504)/0; mnDp3 males are Lon non-Mec. Heterozygote hermaphrodites of the form Df/lon-2 mec-5(e1504) are Mec with mnDf1, mnDf2, mnDf4, mnDf8, and mnDf11.

mec-6: 20 of 24 Dpy and 9 of 28 Unc recombinants of dpy-5 + unc-13(e51) + mec-6(e1342) + gave Mec progeny. Two dpy-5 mec-6(e1342)/+ + heterozygotes segregated eight Mec progeny out of 435 non-Dpy progeny (P = 3%).

mec-7: 22 of 24 Lon and 1 of 22 Mec recombinants of lon-2 + mec-7(e1506)/+ dpy-7(e88) + gave Dpy recombinants. All 8 Lon, but none of the 7 Mec, recombinants from lon-2 mec-7(e1506)/f + dpy-7(e88) + gave Dpy progeny. lon-2 mec-7(e1343)/+ + heterozygotes segregated 379 wild type, 81 Lon Mec, 20 Lon, and 22 Mec progeny (P = 9%). It is likely that e1527 is a dominant mec-7 allele since all 4563 progeny of 12 lon-2, e1506/+/ e1527 heterozygotes and all 3830 progeny of 12 lon-2 e1527/+/ e1522 heterozygotes were Mec (P < 0.03%).

mec-8: Six of eight Dpy recombinants of dpy-5 + unc-13(e1091)/+ mec-8 segregated Mec progeny. Two dpy-5 mec-8/+ + animals segregated 503 wild type, 154 Dpy Mec, 14 Mec, and 13 Dpy progeny (P = 4%).

mec-9: mec-9(e1494)/eDf1 males are Mec. Of 20 Dpy recombinants from mec-9(e1494) + + dpy-11 + him-5 unc-76, 8 segregated male non-Mec progeny (the production of males showed they possess the him mutation) and 5 segregated Mec progeny but no males. The remaining segregated neither males nor Mec animals. The Mec phenotype was not scored in the Unc recombinants.

mec-10: 22 of 24 Lon and 4 of 24 Mec recombinants of lon-2 + mec-10(e1515)/+ dpy-7(e88) + gave Dpy progeny. All of the 24 Lon, but none of the 23 Mec, recombinants from lon-2 mec-10(e1515)/+ + dpy-6 segregated Dpy progeny. lon-2 mec-10(e1515)/0; mnDp30 males are Mec.

mec-12: Of the 1086 progeny from four heterozygotes of mec-12(e1605) dpy-1 daf-7/+ + +, there were 37 Dpy Daf, 40 Mec Dpy, 19 Daf, 59 Mec, 1 Dpy, and no Daf animals. These data suggest that mec-12 is to the left of dpy-1. Five mec-12(e1605) dpy-1/+ + heterozygotes produced 845 wild type, 268 Dpy Mec, 48 Dpy, and 78 Mec progeny (P = 10%).

mec-13: All 13 Dpy and 6 Dpy Him recombinants, none of the 9 Him Unc recombinants, and 4 of 17 Unc recombinants of + + mec-13 +/dpy-7(e1324) him-4 + unc-9 segregated Mec progeny.

Genetic Suppression

Double mutants of the mec alleles with either sup-5 or sup-7 were constructed to test suppression at 20°C of the Mec phenotype by either of these allele-specific suppressors thought to suppress null alleles (Waterston and Brenner, 1978; R. Waterston, personal communication). For sup-5, mec males were mated to lon-1 sup-5 hermaphrodites and the mec/+; lon-1 sup-5 progeny allowed to segregate. If the mec allele was not suppressible, Mec Lon progeny were readily obtainable. All sex-linked mec mutations and those autosomal alleles with e numbers below e1526 were tested in this way. Autosomal mec alleles e1526 and above as well as e398 were tested in the following way (R. Waterston, personal communication): dpy-18(e364); sup-7 males were not.
mated to dpy-18(e364); mec hermaphrodites and the progeny were allowed to segregate. Only the progeny of dpy-18; mec/+; sup-7/+ animals were examined. e364 is suppressed by sup-7 to produce wild-type animals and by sup-7/+ to give Dpy-ish animals. These animals were tested for the Mec phenotype. If the allele was suppressed by the homozygote suppressor, then all wild-type animals were non-Mec; if in addition the allele was suppressed by the heterozygote, then all the Dpy-ish were also non-Mec. Complete progeny counts were done on putative suppressible alleles to ensure that the phenotype distribution did not result from a synthetic lethal interaction between the met and sup-7 mutations. Of the 42 alleles tested, only met-8(e398) was suppressed. Both sup-5 and sup-7 suppressed this allele, and suppression was dominant with sup-7 (dominance of sup-5 was not tested): for sup-r, two animals of genotype met-8/+; dpy-18; sup-r+ gave 19 Dpy Met, 179 Dpy, 184 Dpy-ish, and 118 wild types (no Met or Dpy-ish Met animals were observed).

Temperature Sensitivity

Homozygous mutants were tested for the temperature sensitivity of the Mec phenotype after growth for at least two generations at 15 and 25°C. Temperature shift experiments were conducted essentially as described by Hirsh and Vanderslice (1976) for the one mutant, met-5(e1503), displaying temperature sensitivity. Animals were synchronized by having gravid adults lay eggs for 1 hr at either 15 or 25°C. Plates of animals were transferred to the opposite temperature at various times after hatching. All animals were tested for touch sensitivity at transfer and as egg-laying adults (after at least an equivalent of 48 hr posthatching at 25°C).

Microscopy

Live animals were viewed with Nomarski differential interference contrast microscopy as described previously (Sulston and Horvitz, 1977). These animals were photographed on Kodak SO115 film with the aid of a Zeiss photomicroflash. Electron microscopy usually followed the procedure of Ward et al. (1975) except that animals were fixed in 1% rather than 0.5% OsO₄. In some cases animals were fixed in 1% acrolein and 2.5% glutaraldehyde as described previously (Chalfie and Thomson, 1979).

Laser Microsurgery

Identified neuronal nuclei were selectively killed with a laser microbeam developed by John White (Sulston and White, 1980). During these experiments, we found that ablation of a nucleus often did not eliminate touch sensitivity in older larvae (20 hr after hatching). (In one such experiment electron microscopic reconstruction confirmed that a cell with a functional process, but lacking a nucleus, was produced.) Therefore all nuclei were ablated in newly hatched animals.

RESULTS

Touch sensitivity of wild-type C. elegans

Touching an adult C. elegans hermaphrodite on the tail with a fine hair causes the animal to move forward. A similar backward response is elicited if the anterior of the animal is touched. If a stationary animal is stroked near its vulva, i.e., the middle of the animal, it fails to move. Repeated stimulation of either the head or tail causes the animal to become refractory. This adaptation has been previously noted in C. elegans by Hall (1977) and in Rhabditis sp. by Croll and Smith (1970). C. elegans males, dauer larvae, and juveniles of all larval stages (except newly hatched larvae which are too small to be tested adequately) respond identically to the adult hermaphrodites in terms of touch sensitivity.

Description of the Microtubule Cells

Adult animals possess a set of six neurons, the microtubule cells, that can be distinguished from other nerve cells by the possession of a bundle of darkly staining microtubules (Chalfie and Thomson, 1979). In this section we describe the morphology of these cells and in the following section present data from laser ablation studies that suggest that these cells are the principal touch receptors in C. elegans.

The six microtubule cells are diagrammed in Fig. 2a. All the cells possess anteriorly directed processes that run close to the body cuticle; they are separated from the cuticle by a thin layer of hypodermis. In electron micrographs these cells can be identified readily not only by their unusual microtubules, but also by an area of osmiophilic material we call the mantle; the mantle lies along the superficial edge of the cells (Figs. 2b and d). Examination of the mantle in serial section electron micrographs shows that the amount of mantle varies along the length of the process in the case of the ventral cord cells. In addition, periodic darkly staining patches are often seen in the overlying cuticle (Figs. 2b, d). These patches, also seen under the muscle, may represent the attachment of the microtubule cells to the cuticle. Frequently animals with partially detached cuticle are seen in the electron microscope. Although much of the hypodermis has pulled away from the cuticle, that near the microtubule cells (and the muscle) is firmly attached.
Fig. 2. Structure and position of the microtubule cells. The six microtubule cells are ALML, anterior lateral microtubule cell (left); ALMR, anterior lateral microtubule cell (right); PLML, posterior lateral microtubule cell (left); PLMR, posterior lateral microtubule cell (right); AVM, anterior ventral microtubule cell; PVM, posterior ventral microtubule cell. (a) Diagram of the left side of a young adult hermaphrodite indicating the positions of the microtubule cells. Dotted structures represent parts of cells on the right side of the nematode. The right lateral cells (ALMR and PLMR) are not shown but are identical in position and structure to the left lateral cells. Bar, 100 µm. (b) Electron micrograph of the ventral microtubule cells (AVM and PVM) showing the microtubule, mantle (M), and darkly staining portion of the cuticle (D). Fixation here and in (d) was in acrolein and glutaraldehyde. ×54,000. (c) Diagram of an anterior section of an adult approximately one-quarter of the distance from the head. The microtubule cells are embedded in the hypodermis (H) which underlies the cuticle (C). The positions of the posterior lateral microtubule cells (triangles) and the ventral (VC) and dorsal (DC) nerve cords are also indicated. (d) Electron micrograph of a transverse section of the process of ALML. Abbreviations are as in (b). ×54,000. (e) Light micrograph using Nomarski optics showing the position of the ALML process (arrows) in a hermaphrodite during the L4 (final) molt. ×1800. (f) Light micrograph using Nomarski optics of the same cell as in (e) but at a different focal plane showing the ALML cell body. ×1800. (a–d) were taken, with permission, from Chalfie and Thomson, (1979).

Four of the cells are present at hatching: these are the right and left anterior lateral microtubule cells (ALMR and ALML) that lie dorsal to the lateral midline and the right and left posterior lateral microtubule cells (PLMR and PLML) that lie ventral to it. In newly hatched larvae, the processes of these cells lie between the lateral hypodermis and the adjacent muscle quadrant. Beginning at approximately 12 hr after hatching the processes are engulfed by the hypodermis. As a consequence in the adult they lie in grooves in the hypo-
dermis that are some distance from the body wall muscle. The nuclei and (especially in animals entering the final or L4 molt) the processes of these cells can be identified in living animals by Nomarski optics (Figs. 2e and f).

The remaining two microtubule cells arise after hatching from equivalent positions in similar cell lineages (Sulston and Horvitz, 1977; Sulston and Horvitz, unpublished); these are the anterior microtubule cell (AVM; Q2.paa in the notation of Sulston and Horvitz (1977)) and the posterior ventral microtubule cell (PVM; Q1.paa). A single process from each cell enters the ventral cord and runs anteriorly at its ventral edge (Fig. 2b). A branch of the AVM process makes synapses with other neurons in the nerve ring, the major area of neuropil in C. elegans. The PVM process does not extend into the nerve ring.

The complete connectivity of these neurons has been determined from serial section electron micrographs and will be reported elsewhere (White, Southgate, Thomson, and Brenner, in preparation; Chalife, in preparation). We note here a few pertinent items. First, there is no significant synaptic input to the microtubule cells. Second, the branches of ALML and ALMR make gap junctions with the branches of AVM in the nerve ring. PVM and AVM are joined by a gap junction in the ventral cord. Thus, it seems likely that these four cells form a unit. PLMR and PLML are not coupled by gap junctions either to each other or to the other microtubule cells. Third, the major synaptic output of the microtubule cells (except for PVM) consists of apparent chemical and gap junctions synapses with (Y-, β-, γ-, δ-interneurons. These interneurons are the major input to the motor neurons of the ventral cord (White et al., 1976). The pattern of synapses to these interneurons is similar for ALMR, ALML, and AVM but quite different from that of PLMR and PLML. The bilaterally symmetric pair PLMR and PLML each forms similar synaptic connections to the interneurons. (PVM does not make interneuron synapses.) The connectivity thus divides the cells into two groups; an anterior set (ALML, ALMR, AVM, and, possibly, PVM) and a posterior set (PLMR and PLML).

The location of these six cells near to the outer surface of the animal, the division of the cells into two sets (anterior and posterior) on the basis of their synaptic output, the lack of synaptic input, and the synaptic output onto the major interneurons to the ventral cord motorneurons suggest that these cells are mechanosensory receptors.

Identification of the Microtubule Cells as Mechanosensory Cells by Laser Microsurgery

Identified cells can be selectively killed by means of a laser microbeam (Sulston and Horvitz, 1977; Sulston and White, 1980). We have used this technique to examine the role of the microtubule cells in touch sensitivity. Killing a single posterior lateral cell does not detectably affect touch sensitivity, but killing both the posterior lateral cells results in a complete loss of touch sensitivity in the tail (but not in the head). When both cells are ablated, touching the animal at the vulva causes it to back up. If the anterior lateral cells, ALMR and ALML, are killed, the animal is unresponsive when touched in the head until 35-40 hr after hatching; at later times it responds variably. The partial anterior touch sensitivity is lost when AVM as well as ALMR and ALML is killed indicating that AVM becomes detectably functional at 35-40 hr. Under these conditions when the animal is stimulated at the vulva, it moves forward. The response in the tail is unaffected. Partial responses are also registered if AVM and one of the anterior lateral cells or if only one of the three cells is killed. Laser microsurgery of AVM alone diminishes anterior touch sensitivity very slightly. Ablation of PVM has no detectable effect upon touch sensitivity. Killing all the microtubule cells, or all except PVM, results in animals that are touch insensitive over their entire length, except at the very front of the head. There is a residual touch sensitivity at the very front of the animal that does not appear to be mediated by the microtubule cells. This is also seen in the mutants described below.

These experiments suggest that the microtubule cells act as touch receptors in C. elegans. The response in the tail is mediated through PLMR and PLML, and the response in the head through ALMR, ALML, AVM, and, possibly, PVM. The pattern of cell assignments to anterior and posterior sets is consistent with the pattern of anatomical synapses mentioned above.

Mutations Affecting Touch Sensitivity

The identification of the microtubules cells as the touch receptors allows us to analyze the effect of touch-insensitive mutants on the growth and function of these cells. We have identified 42 mutations that render C. elegans touch insensitive. (All of these mutants move normally when prodded with the platinum wire.) Of these mutations, 39 are recessive and define 12 complementation groups (Table 1). Most of these complementation groups were previously undefined and so have been designated mechanosensory abnormality (mec) genes. One gene, unc-66, was characterized previously by criteria other than touch insensitivity (Hodgkin et al., 1979, Sulston and Horvitz, 1981) but a new allele, e1507, was isolated during these studies. The three remaining mutations, all on the X chromosome, are dominant, and so cannot be as confidently assigned to spe-
specific complementation groups. One of these, e1611(mec-13), because of its phenotype and map position is assumed to define an additional gene (see below). e1608 is a mec-2 allele because of its map position and its complementation with mec-2(e1514) (see Materials and Methods), and e1527 is probably a mec-7 allele because it causes the same ultrastructural phenotype as the other alleles of this gene (see below) and is within 0.03 map unit of mec-7(e1506) (Materials and Methods).

Animals with these mutations share a number of common phenotypes. All of them fail to move when gently touched on the head or tail; none of the alleles render only the front or only the back insensitive (with the exception of animals carrying mec-4(e1339) which are sometimes touch sensitive in the tail). Moreover, unlike wild type, they do not respond to vibrations, e.g., tapping of the petri plate on which they are growing. In general, touch-insensitive hermaphrodites do not move as much as wild type on bacterial lawns. Touch-insensitive mutants, however, do move to the same extent as wild type when food is depleted. In addition, adult (but not larval) Mec males move actively in the presence of ample food. Thus, hunger and libido are sufficient to overcome the lethargy seen in these mutants. Mec phenocopies produced by laser ablation of the microtubule cells in the wild-type strain share these characteristics.

The touch-insensitive mutants have been examined by light and electron microscopy for evidence of identifiable lesions in the microtubule cells. Cellular phenotypes have been observed in 5 of the 13 genes. The cellular irregularities range from ultrastructural abnormalities to the complete absence of the cells.

Mutations Affecting the Ultrastructure of the Microtubule Cells

Mutations in two genes (mec-1 and mec-7) lead to the absence of particular ultrastructural features of the microtubule cells. Most striking is the absence of the special microtubules in all five mec-7 alleles (Fig. 3). Only the large microtubules in the microtubule cells are absent; microtubules in other neurons are present in apparently normal numbers. mec-7 alleles fixed in acrolein and glutaraldehyde have microtubule cells containing a few microtubules of similar size to those in other nerve cells (data not shown). Differences in touch sensitivity of heterozygotes are seen in the mec-7 alleles: at 20°C, e1527 is dominant, e1343 and e1505 are semidominant, e1506 and e1522 are recessive.

All other aspects of the microtubule cells in mec-7 animals are indistinguishable from wild type. The positions of the cells and their processes are the same; the processes are clearly visible in the light microscope and can be identified by their position and by the mantle in electron micrographs (Fig. 3). The mec-7 phenotype suggests that the large microtubules are not required for the growth and positioning of the cells, but are necessary for the specific function of the cells, i.e., sensory transduction. Although the molecular nature of the mec-7 mutations is unknown, this may be a defect in a microtubule protein, tubulin, or a microtubule-associated protein, or in an activity required for the assembly of these large microtubules. These mutations will be described in greater detail elsewhere (Chalfie, in preparation).

A second striking feature of these cells, the mantle, is missing in animals carrying mec-1 mutations. The ventral cord cells of homozygotes of six of the nine mec-1 alleles (e1066, e1292, e1336, e1496, e1526, and e1737) were examined and all lacked this structure (Fig. 4). The darkening of the underlying cuticle is also missing in these animals. The mantle is usually missing from the lateral cells as well (see below).

In the light microscope all nine mec-1 alleles show another defect: the cell bodies of the anterior lateral cells are displaced dorsally so that they assume a flattened appearance against the body wall musculature (Fig. 4). Because of their position, the lateral cell processes usually cannot be seen in the living animal. Electron micrographs of adults reveal that the lateral cell processes are not embedded in the hypodermis, but lie between the hypodermis and the muscle, i.e., they appear to retain their embryonic placement (Fig. 4). In a few cases portions of the processes, although closer to the muscle than in wild type, are embedded within the hypodermis. In these instances the mantle is seen. All alleles tested exhibit this variability. The ventral

![Fig. 3. Electron micrograph of a transverse section of the ALMR process in a mec-7(e1506) adult hermaphrodite. Note absence of microtubules, but presence of the mantle (M). ×60,000.](image-url)
Fig. 4. Effect of mec-1(e1066) on ultrastructure and position of microtubule cell processes. (a) Light micrograph of ALMR cell body (arrow) at junction of hypodermis (H) and muscle (M) in a hermaphrodite during the L4 molt. X2000. (b) Electron micrograph of ALMR process in an adult hermaphrodite. Note absence of the mantle. X60,000. (c) Electron micrograph of the ventral cord in an adult. Note that the processes of AVM and PVM are in their usual position (compare with Fig. 2b) but lack the mantle. X60,000.

Microtubule cell processes are not displaced but lie in their correct position at the ventral edge of the ventral cord (Fig. 4). Lewis and Hodgkin (1977) noted the displacement of some amphidial neuronal processes in mec-1 (e1000). We have found similar amphidial defects in two additional alleles (e1737 and n174) but not in the other six mec-1 alleles. Thus, the action of this gene does not appear to be specific to the microtubule cells.

We do not know if the positioning of the lateral processes in mec-1 mutants controls the production of the mantle or if the mantle is responsible for the correct positioning of the lateral processes. In any case, since the ventral processes do not appear to be displaced, these mutations suggest that the mantle or the process that regulates its production is important for the proper functioning of the microtubule cells. Perhaps, it is required for anchoring the microtubule cell to the cuticle. The displacement of the lateral cells in these mutants supports this hypothesis.

Mutations Affecting the Appearance of Nerve Processes

The microtubule cells in mutants of another gene, mec-3, do not appear to mature. In the light microscope, the cell bodies in mec-3 adults are smaller than wild type and often displaced. This is particularly true of the anterior lateral cells (Fig. 5); these cell bodies are more anterior, more lateral, and further from the cuticle than in the wild type. No lateral processes are seen. Only the microtubule cells appear to be affected. The postembryonic lineages that give rise to the ventral microtubule cells are normal, but the ventral cells are often difficult to find in adults. We have not been able to see processes from any of the microtubule cells in electron micrographs of these mutants. Thus it appears that the microtubule cells in mec-3 animals do not have processes. Since such processes are also absent in mutant larvae, it is more likely that processes are never

Fig. 5. Light micrograph of ALMR cell body in a mec-3(e1338) hermaphrodite during the L4 molt. Note the more medial location of the cell and relative sparsity of cytoplasm when compared to the wild-type cell (Fig. 2f).
elaborated in these animals rather than that they degenerate.

Mutations that Prevent the Production of the Microtubule Cells

The *urc-86* mutations produce a complex phenotype (Hodgkin et al., 1979; Sulston and Horvitz, 1981; Chalfie, Horvitz, and Sulston, 1981) but one effect is to prevent the production of the microtubule cells and thus render animals touch insensitive. We have not seen either the cell bodies or their processes in the light or electron microscope. Examination of the cell lineages of the microtubule cell precursors (the Q cells) in these mutants reveals that these patterns of cell division have been altered (Fig. 6). These alterations result in a repetitive pattern of cell division that omits the production of the ventral microtubule cells. It is likely that similar lineage defects also occur in the embryonic lineages that normally give rise to the lateral microtubule cells and thus cause their absence in the mutants. These mutations affect other neuronal lineages and are discussed in more detail elsewhere (Chalfie, Horvitz, and Sulston, 1981).

In addition to their inherent interest, the *urc-86* mutations can be used as tools to elucidate further the other *mec* mutations. For example, because the cells which we believe are the anterior lateral microtubule cells in the *mec-3* mutations are displaced and have no detectable processes, their nature is unclear. However, since the double mutant of *unc-86(e150?)* and *mec-3(e1338)* does not have these cells, it is likely that these displaced cells are microtubule cells that lack processes. A similar use of *unc-86* with *mec-13* is given below.

A Mutation Causing the Degeneration of the Microtubule Cells

Adults carrying the dominant *mec-13* mutation also lack the microtubule cells. Their absence is due to their degeneration after they are produced. When newly hatched larvae are examined in the light microscope, they are found to have up to four very large vacuoles in the positions normally occupied by the lateral microtubule cell bodies (Fig. 7). These vacuoles, which usually possess a granular inclusion often disappear after approximately 10 hr. These vacuoles are the remnants of the lateral microtubule cells. By observing embryos we find that the lateral microtubule cells become vacuolated about 4 hr before hatching. The cell lineages that give rise to the postembryonic ventral microtubule cells are normal, but at a variable time after these cells have been produced (usually not less than 10-12 hr), their cell bodies, too, become vacuolated, and eventually the cells disappear.

This degeneration or cell death is distinctly different in appearance from the programmed cell death usually seen in the postembryonic cell lineages in *C. elegans* in which the nucleus becomes refractile and no vacuoles are seen (Sulston and Horvitz, 1977). (Infrequently a ventral microtubule cell shows a refractile death in *mec-13.*)

The large vacuolated deaths seem to be unique to the microtubule cells. Vacuoles, however, are sometimes seen within the intestine of *mec-13* animals, but they do not result in the loss of any of the intestinal nuclei. Rarely (about 1% of the animals examined) a slightly vacuolated appearance is seen in other neuronal cell
bodies; there is no consistent pattern to which cells are affected. This infrequent vacuolation of other neuronal cells is seen also in the *unc-86; mec-13* double mutant (this mutant does not have the microtubule cells). However, since some vacuoles are seen also in wild-type nematodes (although subjectively at an even lower frequency), we cannot determine whether *mec-13* acts solely on the microtubule cells or whether it acts on many cell types with the microtubule cells being particularly sensitive to its action.

The microtubule cells become vacuolated some time after they are produced. This suggests that *mec-13* may act on the cells after they have begun to differentiate. This conclusion is supported by the double mutant *mec-3* (*e1338*); *mec-13*. These animals lack the large vacuoles, but have the cells in the usual *mec-3* position. Thus it appears that *mec-13* acts upon the microtubule cells after they have matured beyond the point blocked by *mec-3*. (We are currently constructing and examining doubles of *mec-13* and the other *mec* mutations in an attempt to further elucidate the genetic pathway of microtubule cell development.)

**A Temperature-Sensitive **\textit{mec}** Mutant**

Mutants carrying any of the three *mec-5* alleles do not have a detectable phenotype when viewed with Nomarski optics or in electron micrographs. One of the alleles, *e1503*, is temperature sensitive, and the temperature shift response for this mutant is shown in Fig. 8. The temperature-sensitive period is during midlarval growth, indicating that the touch insensitivity is reversible before this time.

**Mutations that May Affect Touch Sensitivity Indirectly**

We have tested animals containing representative alleles of many of the *C. elegans* genes and most of these are either touch sensitive or too paralyzed to test. However, mutants with a twitcher phenotype, i.e., a sporadic and random contraction of the body musculature (Brenner, 1974), are touch insensitive. (During the screening for touch-insensitive mutants, we found a number of touch-insensitive twitcher animals, but they were not kept.) The twitcher phenotype is found in alleles of *unc-22* and *lev-11*, and in one allele of *unc-54* (*e675*; R. P. Anderson, personal communication), and all these alleles produce touch-insensitive animals. (Nontwitcher *unc-54* mutants are touch sensitive.) *unc-54* is the gene for the major myosin heavy chain in the body wall muscle (MacLeod et al., 1977b) and *e675* is an internal deletion within that gene (MacLeod et al., 1977a). Because it is unlikely that *e675* acts directly on the microtubule cells, it is possible that the mutation causes touch insensitivity indirectly. Constant self-stimulation by twitching might permanently habituate the microtubule cells so that they no longer respond to gentle touch.

**DISCUSSION**

The primary goal in identifying the microtubule cells as touch receptors was to enable us to examine the role of gene action on neuronal growth and function at the cellular and, ultimately, the molecular level. Various data including the pattern of synaptic connectivity, the placement of the cell processes, and, more directly, the results of laser ablation of the microtubule cells suggest that these cells mediate touch sensitivity. Additional support comes from the finding that growth of wild-type nematodes in 1 mM colchicine results in touch-insensitive animals whose single cellular defect appears to be the loss of microtubules from only the microtubule cells (Chalfie, in preparation).

The microtubule cells have a number of advantages for the genetic study of neuronal growth and function. First, the behavior is easily scored: fairly large numbers of animals can be screened for touch insensitivity. Second, removal of the cells (either by laser ablation or the action of *mec-13* or *unc-86*) is not lethal to *C. elegans*, i.e., the microtubule cells are not essential for viability. Third, touch receptor activity cannot be assumed by other cells: the laser experiments and the *unc-86* mutants suggest that no postembryonic regulation occurs to replace the microtubule cells. Finally, the microtubule cells can be identified by a number of criteria in the light and electron microscope.

By screening for touch insensitivity, we have isolated 42 independent mutations that fall into 13 complementation groups, five of which result in identifiable cellular phenotypes. Most of the mutations are recessive, and since in some genes many mutations have been isolated,
it is likely that phenotypes expressed in animals carrying these alleles are the result of the loss of wild-type gene activity. In addition, in the case of mec-8(e398), the suppression by sup-5 and sup-7 suggests that we are looking at the null phenotype. The dominant mutations are likely to represent missense mutations. This conclusion is further supported by the intragenic complementation pattern of mec-2 alleles. In other organisms intragenic complementation has been explained as the result of protein–protein interactions in a multimeric gene product (Fincham, 1966) and therefore we suggest that the mec-2 gene product may be a multimeric protein.

Because multiple alleles have been isolated for most of these genes, we feel that we are close to saturating the set of mutations that result principally in touch insensitivity. Our results indicate that the number of genes involved is relatively small. In addition, mutations in a given gene have a unique phenotype (when known); we have not found mutations in a number of genes having the identical phenotype. Thus, for example, only mutations in mec-7 cause the absence of the microtubules, only those in mec-3 block cell maturation.

These mutants display a variety of cellular phenotypes. Mutations in mec-1 and mec-7 result in the loss of the mantle and the microtubules, respectively, yet the growth of the neuronal process in these ultrastructural mutants is essentially normal (the only difference being the displacement of the lateral cells from their normal adult position in the mantleless mutants). These data suggest that neither the large microtubules nor the mantle are essential for the proper outgrowth of the microtubule cell processes. Instead it appears that these structures are important for the final differentiated function of these cells, mechanosensory transduction, and that these mutations have helped to identify functionally important components of the cells. We might hypothesize, for example, that the mantle attaches the cell process to the body wall or cushions the process and that the microtubules add rigidity to the process or are involved in generating a signal in the cell.

Mutations in other genes, however, do affect the development of these cells. unc-86 mutations result in lineage alterations so that the microtubule cells are not produced. mec-3 and mec-13 act after the microtubule cells have been produced: mec-3 early in cell development so that maturation is blocked and mec-13 at some later stage so that the cells die. With these mutations we are beginning to elucidate the genetic pathway involved in the development of the microtubule cells.

We have not detected cellular phenotypes for mutations in most of the mec genes. This is not surprising since many mutations, e.g., those affecting synapse formation or transmitter synthesis, would not have demonstrable phenotypes at this level of analysis. An analysis of the synapse morphology in these mutants is in progress.

In screening for mec mutants, we have restricted ourselves to isolating touch-insensitive mutants that move normally, i.e., mutations that were highly specific to the microtubule cells. In fact, although mutations in some genes (mec-1 and unc-86) have effects on other cells, mutations in other genes (mec-7, mec-3, and, possibly, mec-13) appear to act solely on the microtubule cells. However, in this selection we would not isolate mutations that affect large numbers of neurons and result in paralyzed or severely uncoordinated animals. (Also, we might fail to isolate mutations resulting in an overproduction of microtubule cells or cell processes, because it is likely that such mutations would have no readily detectable phenotype (see Kuffler and Muller, 1974; Treistman and Schwartz, 1976; Goodman, 1977.) Since we feel we are approaching saturation of the class of mutations principally affecting the microtubule cells, it is useful to ask about the types of mutations we have not found in the present screening. Such mutations, if they exist, would either be rare or of a more general or global nature.

One type of mutation that we did not find is one that results in the growth of the microtubule cells in an abnormal direction. Such mutants should have been isolated had they existed. This suggests that the direction of process growth may be under more general genetic control. Interestingly, C. elegans mutants in which the direction of microtubule cell processes have been altered have recently been isolated by Ed Hedgecock (personal communication) in a screen of uncoordinated mutants. These mutants were too uncoordinated to test for touch sensitivity and exhibit abnormal placement of neuronal processes in a number of different cell types.

With the exception of a single allele of one gene, mec-4(e1389), we did not find mutations which resulted in only anterior or posterior touch insensitivity. (e1389 may be a weaker mec-4 allele since posterior touch sensitivity in this mutant is partial and not seen in other mec-4 alleles.) This suggests that the six microtubule cells are fundamentally similar, but display differences as a result of their positioning in the animal and of their production at different times in the life cycle. That unc-86 affects the production of the embryonic as well as the postembryonic microtubule cells suggests a similar cell lineage origin for all six cells.

In summary our ability to collect a variety of mutations all affecting the same cell type has been a unique advantage of using C. elegans. The phenotypes of these mutants lead us to the following hypothesis of genetic involvement in microtubule cell development. Initially
cells that will differentiate into the microtubule cells are produced by specific cell lineages (these can be altered by unc-86 mutations). The cells so produced elaborate a neuronal process, the growth of which requires the activity of the mec-7 gene. The direction that this process grows is under the control of more global, cell-nonspecific genetic instructions. Finally, the expression of a number of genes, some cell specific (e.g., mec-7), some not so cell specific (e.g., mec-1) is required for the proper differentiated functioning of the microtubule cells.

We would like to thank Nichol Thomson for his expert assistance on the electron microscopy and Jonathan Hodgkin, Bob Horvitz, Ed Hedgecock, John White, and Sydney Brenner for their helpful suggestions and discussions. This work was done during the tenure of a British American Research Fellowship of the American Heart Association and British Heart Foundation to M.C.

REFERENCES


