Structural and Functional Diversity in the Neuronal Microtubules of Caenorhabditis elegans

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ABSTRACT Tannic acid fixation reveals differences in the number of protofilaments between microtubules (MTs) in the nematode Caenorhabditis elegans. Most cells have MTs with 11 protofilaments but the six touch receptor neurons (the microtubule cells) have MTs with 15 protofilaments. No 13-protofilament (13-p) MT has been seen. The modified cilia of sensory neurons also possess unusual structures. The cilia contain nine outer doublets with A subfibers of 13 protofilaments and B subfibers of 11 protofilaments and a variable number of inner singlet MTs containing 11 protofilaments. The 15-p MTs but not the 11-p MTs are eliminated by colchicine-treatment or by mutation of the gene mec-7. Concomitantly, touch sensitivity is also lost. However, whereas colchicine treatment leads to the loss of all MTs from the microtubule cells, mutations in mec-7 result in the partial replacement of the 15-p MTs with 11-p MTs. Benzimidazoles (benomyl and nocodazole) have more general effects on C. elegans (slow growth, severe uncoordination, and loss of processes from the ventral cord) but do not affect the 15-p MTs. Benomyl will, however, disrupt the replacement 11-p MTs found in the microtubule cells of mec-7 mutants. The 11-p and 15-p MTs also respond differently to temperature and fixation conditions. It is likely that either type of MT will suffice for the proper outgrowth of the microtubule cell process, but only the 15-p MT can function in the specialized role of sensory transduction of the microtubule cells.

MATERIALS AND METHODS

Growth and Maintenance of Nematode Strains

For most experiments, wild-type C. elegans var. Bristol (N2) and mutant strains were maintained at 20°C as described by Brenner (6). The C. elegans mutations used in this study, all of which are on the X chromosome, are dpy-7(e88), mec-7(e1343, e1505, e1506, e1522, e1527, n434), and szT1(1;X), a translocation that balances the X chromosome (P. Deak and A. Fodor, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary, personal communication). (These mutations are named according to standard C. elegans nomenclature [16; genes are designated by a three letter code and, separated by a hyphen, a number [e.g., mec-7], and alleles of a gene are given a one- or two-letter prefix and a number [e.g., el345].) The dominant n434 mutation, isolated by Chip Ferguson (Massachusetts Institute of Technology, Cambridge, MA), was assigned to mec-7 because it lacks the large MTs from the microtubule cells, and because it maps within 0.05% of mec-7(e1506) (12 heterozygotes of genotype n434/dpy-7e1506 segregated 2793 Mec, 955 Dpy Mec, and no wild-type animals).

Strains of genotype dpy-7mec-7/szT1 were constructed by standard genetic procedures. (These strains are designated by the mec-7 allele they contain. For example, el345+/ has the genotype dpy-7mec-7(e1345)/szT1.) Since only the
mec-7/+ heterozygotes are wild type, they are easily identified. These heterozygotes show the same touch sensitivity as those without the dpy or balancer (M. Chalfie, unpublished data).

**Touch Sensitivity**

Touch sensitivity was tested by stroking animals transversely with a thin hair as described previously (10). Touch-insensitive animals (e.g., those produced by the mec-7 mutations) are those that fail to respond to the hair stimulus but will move when prodded with a fine platinum wire. A partial response is one in which the animal moves after some but not all stimuli.

**Temperature Sensitivity**

Temperature sensitivity of the mec-7/+ heterozygotes was tested after growth of the heterozygote strains at 15°, 20°, and 25°C. Because of the substantial dominant lethality conferred by mec-7 at 25°C, progeny of late larval animals shifted from 20°C to 25°C were tested at this temperature. Strains at other temperatures were tested after growth for at least two generations at the appropriate temperature. Temperature shift experiments were conducted as described by Hirsh and Vanderslice (15). Animals were synchronized at hatching (±1 h) at either 15° or 25°C. Plates of animals were transferred to the other temperature at various times after hatching. All animals were tested for touch sensitivity at transfer and as egg-laying adults (after an equivalent of at least 48 h post-hatching at 25°C).

**Drug Experiments**

Effects of various drugs on the touch sensitivity of wild-type nematodes were tested on animals grown on agar plates in which the drugs had been previously dissolved. Colchicine (Sigma Chemical Co., Ltd., London) and vinblastine (Eli Lilly and Co., Ltd., Basingstoke, England) were added to the hot agar as dry powder. Podophyllotoxin (a gift of Dr. G. Cole of ICI Pharmaceuticals, Macclesfield, England), nocodazole (a gift of Dr. J. G. Adams of Janssen Pharmaceuticals Ltd., Harlow, England), and benomyl (a gift of Dr. C. Scazzocchio, Chalfre, unpublished data) were added in absolute ethanol (final ethanol concentration, 2% by volume). Luminicolchicine was prepared from colchicine using the method of Mizel and Wilson (20) except that higher initial concentrations of colchicine and longer UV exposure times were used. After separation on preparative silica gel-60 plates with indicator (E. Merck, Darmstadt, FRG) using CHCl₃/Methanol (95:5, vol/vol) (28), the major UV product was eluted with absolute ethanol and added to the agar.

Drug shift experiments were performed at 20°C. Wild-type animals synchronized at hatching (1 h) on a drug-containing or control plate were shifted at various times to the other type of plate. Touch sensitivity was tested at transfer and when the animals became egg-laying adults.

**Microscopy**

The microtubule cell bodies and the lateral microtubule cell processes were examined in living animals using Nomarski differential contrast interference microscopy as described previously (10). The length of the cell process was estimated as the distance from the center of the cell body to the middle of the first pharyngeal bulb in animals anesthetized in 1% 1-phenoxypyrol-2-propanol.

For most preparations, animals were fixed for electron microscopy either in 1% OsO₄ in 100 mM sodium phosphate buffer, pH 7.4, for 1 h (OsO₄ fixation; Atlan 31) or were prefixed in 2.5% glutaraldehyde (TAAB Laboratories, Reading, England), 1% acrolein (TAAB Laboratories), 1% 1-phenoxypyrol-2-propanol, 2 mM MgCl₂, and 100 mM sodium phosphate buffer, pH 7.4, and then fixed in glutaraldehyde followed by OsO₄ (G+A fixation; 11). Fixed animals were embedded and sectioned as described by Ward et al. (31). The microtubule cells can be identified in electron micrographs by their position within the animal, by the possession of the large microtubules, or by the extracellular mantle that covers the cells (see below and reference 10).

Average MT lengths and numbers were estimated from serial sections of G+A-fixed animals of various ages as before (11).

The protofilament substructure of MTs was examined after tannic acid fixation by the procedure of Kim et al. (17) except that the animals were cut with a scalpel to facilitate fixation and that the samples were embedded as before (31) after en bloc staining with 1% uranyl acetate.

**RESULTS**

**Protopilament Structure of C. elegans Neurotubules**

*C. elegans* neurons contain two types of free cytoplasmic MTs (11). One type of MT has an average outer diameter of ~24 nm and is found in the majority of nerve processes. The second type is larger (30 nm) and is only found in the six touch receptor cells (the microtubule cells) of the animal. (Of ~800 G+A-fixed MTs in lateral touch cells, only two had the smaller diameter. A few very short (<1 μm) smaller-diameter MTs were seen in newly formed cell processes of ventral touch cells.)

The difference in size of these MTs can be accounted for by the number of protofilaments they possess: the smaller, more common MTs have 11 protofilaments, whereas the larger ones in the microtubule cells have 15 protofilaments (Fig. 1). (These will be referred to as 11-p and 15-p MTs, respectively.) The protofilaments of 56 MTs in ventral cord neurons could be
FIGURE 2 Tannic acid fixation of a lateral microtubule cell with a single 12-p MT (white arrow). Note also that some of the MTs (black arrowheads) have internal structures revealed by the fixation. These counted without ambiguity; all 36 had 11 protofilaments. 136 countable MTs were examined in the microtubule cell; 133 of these MTs had 15 protofilaments, two had 16, and one had 12 (Fig. 2).

In addition to being found in all neurons except the microtubule cells, the 11-p structure is invariably observed in MTs in C. elegans muscle, hypodermis, and intestine (data not shown).

Structure of Modified Cilia in Sensory Neurons

Ciliated neurons in C. elegans show an unusual arrangement of MTs; in addition to the ring of nine outer doublet MTs, they possess a variable number (one to six) of inner singlet MTs (31, 32). The outer doublet MTs are similar to those found in other organisms (e.g., 3, 21, 30), i.e., they have an A subfiber with 13 protofilaments and a B subfiber with 11 protofilaments (Fig. 3). The inner singlet MTs, however, contain 11 protofilaments. Sections of these cells distal to the ends of the B subfibers thus show nine 13-p MTs intermixed with a smaller inclusions may relate to the dots sometimes seen within G + A fixed MTs in these cells (see Fig. 6 of reference 11) or to the "adluminal component" described by Linck and Langevin (18). × 300,000. 

FIGURE 3 Tannic acid fixation of the ciliated endings of amphidial neurons. The doublets (d) contain A subfibers (a) with 13 protofilaments and B subfibers with 11 protofilaments. The inner singlet MTs (s) have 11 protofilaments. Note the hooks (h) as the B subfibers end. × 300,000.
number of 11-p ones (see also Fig. 27 in reference 31, which shows ciliated neurons with the two different-size MTs).

At the point where the B subfiber ends, its remnant appears as a hook in transverse sections (Fig. 3). The handedness of these hooks is exactly as described by Heidemann and McIntosh (14), i.e., 95% (42 of 44) are clockwise when viewed in the direction of the basal body.

Effects of Fixation Conditions and Temperature on 15-p and 11-p MTs

A number of investigators have remarked on the differential sensitivity of populations of MTs to various experimental conditions (e.g., 2, 7). As seen in Fig. 4, the 15-p and 11-p MTs respond differently to osmium tetroxide fixation and low temperature. The 15-p MTs are readily seen with osmium tetroxide fixation alone; the 11-p MTs are better visualized with glutaraldehyde followed by osmium tetroxide. The 15-p MTs are also more stable to cold treatment than the 11-p ones.

Differential Effect of Mutations on C. elegans MTs

Mutations in the gene mec-7 produce animals that are touch insensitive from hatching and in which the 15-p MTs have been eliminated from the microtubule cells (10). The MTs of other neurons are unaffected in the mutants. Usually, the microtubule cell processes are present in these mutants, but sometimes, particularly in homozygotes of the dominant alleles (see below), processes are not found. Although the mutant microtubule cell processes lack 15-p MTs, they do contain 11-p MTs (Fig. 5). These 11-p MTs do not form a bundle as the 15-p MTs do in the wild type. (Moreover, the array of MTs is not continuous; some sections show no MTs.) The number and length of the 11-p MTs in mec-7 homozygotes (e1343 and e1506) have been determined (Table I). There are ~100 11-p MTs with average lengths of ~10 μm in each cell. (This length is closer to that of the 11-p MTs in the wild-type ventral cord.

![Figure 4](image1.png)

**Figure 4**: Effect of temperature and fixation conditions on ventral cord MTs. (a) G + A fixation at 20°C; (b) OsO₄ at 20°C; (c) G + A fixation at 4°C (after incubation overnight at 4°C). Note that the larger MTs in the microtubule cells (arrows) are relatively unaffected by these treatments as compared with the smaller MTs in other cells. × 60,000.

![Figure 5](image2.png)

**Figure 5**: Ventral microtubule cells (AVM and PVM) in mec-7(e1343). Note that the MTs are the same size as those in the other ventral cord neurons. The arrow indicates the cell-specific mantle that characterizes the microtubule cells (10). × 60,000. The inset shows a tannic acid-fixed lateral microtubule cell from mec-7(e1506). The MTs have 11 protofilaments. × 300,000.
hatching to -30 h (at 25°C). These data suggest that the mec-7 temperature-sensitive period is very broad, extending from pronounced temperature effects (Fig. 6). As seen in Fig. 7, the temperature shift experiments because they show the most product. e1343/+ and e1522/+ heterozygotes were used for be exploited to examine the time of action of the mec-7 gene touch insensitive at 25°C. touch sensitive at 20°C but have few or no large MTs and are p MTs, e.g., e1522/+ animals have the large MTs and are corresponding of touch sensitivity and the presence of the 15- mutant, i.e., touch insensitive, within this range. 'There is a temperatures between 15° and 25°C ton434/+ heterozygotes that are e1506/+ heterozygotes that are virtually wild type at temper-

neurons [7 μm; 11] than to that of 15-p MTs in the wild-type microtubule cells [20 μm; Table I and reference 11]. The wild-type microtubule cells have 450 MTs and, given the rarity of the 11-p MTs in these cells (see above), there are, therefore, at most one or two 11-p MTs in a touch receptor process. These data suggest that mec-7 acts selectively on the 15-p MTs and results in their partial replacement by 11-p MTs.

Some of the mec-7 mutants are temperature sensitive as heterozygotes (these mutants are not temperature sensitive as homozygotes [10]). There is considerable allelic variation in this temperature response (Fig. 6); the mutants range from e1506/+ heterozygotes that are virtually wild type at temperatures between 15° and 25°C to n434/+ heterozygotes that are mutant, i.e., touch insensitive, within this range.1 There is a correspondence of touch sensitivity and the presence of the 15-p MTs, e.g., e1522/+ animals have the large MTs and are touch sensitive at 20°C but have few or no large MTs and are touch insensitive at 25°C.

The temperature sensitivity of the mec-7 heterozygotes can be exploited to examine the time of action of the mec-7 gene product. e1343/+ and e1522/+ heterozygotes were used for temperature shift experiments because they show the most pronounced temperature effects (Fig. 6). As seen in Fig. 7, the temperature-sensitive period is very broad, extending from hatching to ~30 h (at 25°C). These data suggest that the mec-7 gene is expressed throughout this time.

The broad temperature-sensitive period can be correlated with the growth of the 15-p MTs during C. elegans development. At hatching, the lateral microtubule cells contain very few MTs, which are quite short (~2.5 μm) compared with those in the adult (Table II). In addition, the array of MTs is not continuous (i.e., series of sections contain no MTs), yet these young larvae are touch sensitive (an indication that functional synapses have formed). Three MTs in newly hatched larvae have been examined after tannic acid fixation; all were 15-p MTs. By 12 h, the MTs have increased in both number and length. From 12 to 36 h, there is an increase in the number of MTs per section but the length of these MTs remains constant.1

1 The temperature sensitivity of e1527/+ animals has been used to demonstrate that it is an allele of mec-7. e1527/lon-2 e1506 animals are completely touch insensitive at 15°C, whereas e1527/lon-2 animals are partially touch insensitive.

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**TABLE I**

| MT Lengths and Numbers in Microtubule Cells from Wild Type and mec-7 |
|-----------------|-----------------|-----------------|
|                 | Wild type       | mec-7(e1343)    | mec-7(e1506)    |
| **N**<sub>av</sub> | 29.7 ± 0.8      | 4.0 ± 0.4      | 2.8 ± 0.5      |
| **l</sub>av | 20.2 ± 4.2      | 8.9            | 8.9            |
| **L**<sub>av</sub> | 302 ± 8        | 203 ± 9        | 305 ± 9        |
| **ΣL** | 8,970          | 1,132          | 854            |
| **ΣN** | 444            | 119            | 96             |

Values were calculated for anterior lateral microtubule cells from animals of the indicated genotype. Animals were examined at 48 h after hatching (20°C).

* N<sub>av</sub>, the average number of MTs per section in a continuous series of electron micrographs [180 sections (total length, 9 μm) for wild type and 120 sections for the mutants]. Mean ± SEM, n = 3, 3, and 7, respectively.

† l<sub>av</sub>, the average length (μm) of an individual MT. Values calculated as described in Chalfie and Thomson (11). The mean ± SEM (n = 3) is shown for the wild type, but, because relatively few MTs and hence few terminations are seen in the mutants, the data from all cells have been pooled.

§ L, the length (μm) of the microtubule cell process. The length was estimated as the distance from the cell body to the middle of the first pharyngeal bulb. Mean ± SEM, n = 6, 5, and 5, respectively.

¶ ΣL, summed length (μm) of MTs within the process (equal to N<sub>av</sub> x L). Values calculated as δ N<sub>av</sub> x ΣL<sub>av</sub>.

Values were taken from three MT length and number experiments with e1343/+ and e1522/+ heterozygotes. Times are given in 25°C equivalents.

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**FIGURE 6** Effect of temperature on mec-7/+ heterozygotes. Each strain is designated by the mec-7 mutation it contains (+, wild type). All are of the genotype dpy-7 mec-7/szT1. At least 250 heterozygotes of each strain were tested for touch sensitivity. Clear, stippled, and solid bars represent the percentage of animals that are touch sensitive, partially touch insensitive, and completely touch insensitive, respectively.

**FIGURE 7** Temperature shift experiments with e1343/+ and e1522/+ heterozygotes. Values are given in 25°C equivalents.

**FIGURE 8** Total MT number (solid line) and length (dashed line) in anterior lateral microtubule cells during C. elegans development (20°C). Values taken from Table II.

**FIGURE 9** Dose response of the effect of colchicine on touch sensitivity.
From 36 to 48 h, there is an additional increase in MT number and length to the adult level. Using these data and estimates for the lengths of the cells, we have calculated the total number and length of MTs within the cells. As seen in Fig. 8, although there is an exponential increase in the summed length of MTs throughout larval growth, the number of MTs does not increase beyond 36 h (20°C). The temperature-sensitive period of the mec-7/+ heterozygotes thus corresponds to the period during which the MTs increase in number rather than length.

Effect of Colchicine and Other Antimitotic Drugs

Because of the distinctive MTs in the microtubule cells and the correlation between MT loss and touch insensitivity in mec-7 mutants, we tested drugs that disrupt MT organization for their effects on touch sensitivity and MT structure. Some antimitotic drugs differentially affect the two types of MTs. C. elegans grown in the presence of colchicine (0.5-2.0 mM) are completely touch insensitive (Fig. 9). At these concentrations there are no other detectable abnormalities in the animals; growth rate and number of progeny are identical to those of untreated animals. At concentrations above 2 mM, the animals grow slowly and are uncoordinated.

Colchicine acts specifically on the microtubule cells. Electron micrographs indicate that the microtubule cell processes of animals grown in colchicine are devoid of MTs (Fig. 10). (Often the processes cannot be found.) MTs are present in other neurons, e.g., those of the ventral cord, under these same conditions (0.5 and 1.0 mM colchicine). Thus, colchicine at these concentrations appears to eliminate selectively all the MTs from the microtubule cells. The effect is not specific to the 15-p MTs, since the 11-p MTs are eliminated from the touch cells (but not other cells) of the mec-7 mutants (three alleles, e1343, e1506, and e1522, have been examined). The action of colchicine appears specific since 1 mM lumicolchicine, the UV-inactivated analogue of colchicine, has no detectable effect on either touch sensitivity or the microtubule cell MTs (Fig. 10).

The time of action and the reversibility of the colchicine effect were tested by growing the animals in the presence or absence of the drug and shifting the animals at various times to the opposite conditions (Fig. 11 a). Animals are touch sensitive in the presence of colchicine for the first 6 h after hatching but become irreversibly touch insensitive by 12 h. If C. elegans is reared in the absence of the drug and then transferred to plates containing colchicine, they will become at least partially touch insensitive as long as the shift occurs before 36 h after hatching. Colchicine, however, has little effect on adults that have been placed on the drug for >2 d. The lack of response in adults may be due to a greater impermeability afforded by the adult cuticle or to a greater stability of the MTs.

Podophyllotoxin acts like colchicine; at concentrations >100 μM, podophyllotoxin-treated animals are touch insensitive and their microtubule cells lack MTs; MTs in other neurons are normal (data not shown). The action of podophyllotoxin is more reversible than that of colchicine (Fig. 11 b). If removed from the drug at 24 h, animals will develop into touch-sensitive adults; if removed as late as 48 h, they recover partially.

A variety of other compounds known to disrupt MTs have been tested on C. elegans but do not have any selective effect on touch sensitivity. They either produce uncoordinated or paralyzed animals (nocodazole >2 μM and benomyl >6 μM) or stop larval growth (vinblastine >0.5 mM). Electron micrographs of the paralyzed animals produced by benomyl or nocodazole treatment show that the microtubule cells and their 15-p MTs are intact, but the number of cell processes of ventral cord neurons is reduced. Untreated animals have 49 ± 1 ventral processes (mean ± SEM, n = 4); animals grown in 14 μM benomyl have 34 ± 4 processes (n = 5). Unlike colchicine or podophyllotoxin, benomyl appears to have little effect upon young larvae; newly hatched animals placed on benomyl become uncoordinated at ~18 h. The effects of the drug are reversible until ~36 h post-hatching.

Effect of Benomyl on mec-7 Animals

The general effects of benomyl suggest an action on the 11-p MTs. We, therefore, expected that the drug would affect microtubule cell outgrowth in mec-7. As indicated in Table III, benomyl does not affect process growth in the wild type but does in the mutants. There are very few lateral microtubule cell processes and no detectable ventral microtubule cell processes in electron micrographs of benomyl-treated mec-7 animals. These data have been confirmed using Nomarski optics: lateral processes, which can be seen in the living animal (10), are either missing or misdirected in drug-treated mutants. The processes are normal in benomyl-treated wild types. Thus, in the absence of the 15-p MTs, benomyl prevents proper microtubule cell outgrowth.

DISCUSSION

Although most known MTs have 13 protofilaments, MTs with different numbers of protofilaments have been identified in crayfish and lobster neurons (12-p; 8, 9), crayfish sperm (15-p; 9), and cockroach epidermis (15-p; 22). The finding that C. elegans tissues contain 11-p and 15-p MTs not only adds another species to this list but brings additional support for the
FIGURE 10 Effect of colchicine on *C. elegans* MTs. (a) A lateral microtubule cell (ALM) from an animal grown in 1 mM colchicine (G + A fixation). (b) The same type of cell from an animal grown in 1 mM lumicolchicine (OsO₄ fixation). (c) A section of the ventral cord from an animal grown in 1 mM colchicine (G + A fixation). Note that the small (11-p) MTs are present in all neurons except the microtubule cell (AVM). Compare with Fig. 4a. Arrows indicate the cell-specific mantle. x 60,000.

concept of cell-specific MTs. The 11-p MTs are found in all examined cells of the animal, but the 15-p MTs are found only in the microtubule cells.

A surprising finding of this study is that *C. elegans* lacks 13-p MTs (excepting the ends of the A subfibers of sensory cilia; mitotic MTs have not been examined). 11-p MTs apparently replace 13-p MTs as the principal MT structure, except in the microtubule cells where 15-p MTs are used. Thus, it is unlikely that any common cellular role involving MTs, e.g., as cytoskeleton, in cell division or in intracellular transport, absolutely requires a 13-p structure. (The finding that ciliary outer doublets have the typical 13-p and 11-p structure of many species suggests that this may be important for their production and, perhaps, function. But in these cilia the inner singlet MTs have 11 protofilaments (not 13), suggesting a separation of their organization from that of the outer doublets.)

One cellular function that appears to require MTs, irrespective of the number of protofilaments, is the correct outgrowth of neuronal processes. A number of in vitro studies involving the use of anti-MT drugs have indicated that MTs (presumably 13-p) are important for the maintenance of neuronal shape (e.g., 5, 12, 33), but the exact role of the MTs is not fully understood (27). Our data suggest that in *C. elegans* the 11-p and 15-p MTs are also involved in the production of neuronal processes. The importance of these MTs is seen not only in the absence of microtubule cell processes in colchicine-treated wild types, in some dominant mec-7 mutants, and in benomyl-treated mec-7 mutants, but also in the reduction of the number of ventral processes in benomyl-treated wild types. However, continuity of either set of MTs may not be essential during process outgrowth since the array of 15-p MTs in newly hatched larvae and of the 11-p MTs in the microtubule cells of the mec-7 mutants can be discontinuous.

Various data suggest that the 15-p MTs have an additional, more specific function: they appear necessary for mechanosensation. The strongest evidence for a role in sensory transduction is that the mec-7 mutations and colchicine or podophyllotoxin lead to the concomitant loss of the 15-p MTs and touch sensitivity. Moreover, newly hatched wild-type larvae, before shifting onto colchicine, and temperature-sensitive mec-7/+...
larvae before shifting to the restrictive temperature, are touch sensitive. Thus, functional synapses can form, and animals still become touch insensitive. This suggests that colchicine and mec-7 mutations are less likely to be causing their effects by indirectly disrupting synaptic organization than by directly resulting in the loss of the sensory apparatus. The replacement of the 15-p MTs by 11-p MTs in mec-7 indicates that the structure of the MT is important. The hypothesis that the 15-p MTs serve a specialized role in touch reception is strengthened by the findings that (a) the 15-p MTs are found only in the six touch receptor cells (10) and principally in the receptor portion of these cells (M. Chalfie, unpublished data), (b) the number of MTs in cross sections of these cells far exceeds that of 11-p MTs in other neurons (11), and (c) most if not all of the 15-p MTs appear after functional synapses have formed.

The role of the 15-p MTs in the sensory response is unknown. The association between the distal ends of the MTs and the plasma membrane (11) may indicate a direct role of the MTs in sensory transduction. However, it seems equally likely that the MTs serve the more passive role of providing a strong substructure against which the cells may be deformed by the touch stimulus. Cross-bridges (see Fig. 6 in reference 11) between the MTs may increase the structural integrity of the MT bundle. It is intriguing that the two other known 15-p MTs (9, 22) are also arranged in bundles (see also reference 1). In addition, the 11-p MTs in the mec-7 touch receptors do not form bundles. Perhaps, the 15-p structure facilitates the close association of these MTs.

The 11-p and 15-p MTs differ not only in protofilament number, cellular distribution, and, perhaps, function but also in a number of physical and chemical properties. Unlike the 11-p MTs, the 15-p MTs are easily fixed in osmium tetroxide and are more cold stable. In addition, they can be selectively eliminated by the mec-7 mutations. The 11-p MTs appear more sensitive to benomyl and nocodazole, because the number of ventral processes and mec-7 microtubule cell processes are reduced, whereas wild-type microtubule cell processes are unaffected by these drugs.

These in vivo studies cannot reveal whether these differences are consequences of the 11-p and 15-p structures or are special characteristics of the C. elegans MTs. Two observations suggest that the protofilament structure may affect MT properties. First, Nagano and Suzuki (22) found that the 15-p MTs of cockroach epidermis also fixed easily with osmium tetroxide and remarked that the differential effect of osmium tetroxide might serve to identify such MTs. Second, in their demonstration of cold sensitivity of axonemal MTs, Tilney and Porter (30) found some large-diameter MTs (outer diameter = 34 nm) after cold treatment. Although those authors hypothesized that these MTs had the same number of protofilaments as the cold-sensitive one, only pitched to give a wider diameter, it is possible that these MTs had more protofilaments. Such MTs would then be similar in size and cold-resistance to the 15-p MTs in C. elegans. It is thus possible that at least some of the differential characteristics of the 11-p and 15-p MTs of C. elegans are consequences of their protofilament substructure.

We have found 11-p and 15-p MTs (but no 13-p MTs) in another nematode species, Panagrellus redivivus (M. Chalfie, unpublished data). Cells identical to the microtubule cells (with larger, and presumably 15-p, MTs) have been identified in a variety of nematode species [hookworm (26), Strongyloides papillosa (M. Chalfie, unpublished data), Ascaris lumbricoides (A. Stretton, University of Wisconsin, Madison, WI, personal communication)]. Thus, it appears that the 15-p MT, and perhaps also the 11-p MT, may be common to many members of the phylum. If some properties of these MTs are affected by the number of protofilaments they contain, these difference might be exploited as a means of producing selective anthelmintics. Benzimidazoles are powerful antinematode reagents (e.g., 4). Perhaps their selectivity is enhanced by the fact that they work on MTs with a structure (11-p) different from that of MTs of the host (13-p). This may not be the only factor since Friedman and Platzer (13) have shown that the binding of benzimidazoles to Ascaris tubulin differs from their binding to beef tubulin.

The sensitivity of the MTs of the microtubule cells to colchicine does not appear to be related to the number of protofilaments. Both the 15-p MTs in the wild-type cells and the 11-p MTs in the mec-7 cells appear to be selectively sensitive to the drug. These findings could result from differential uptake of colchicine into the cells or from a different colchicine-sensitive component of the microtubule cell MTs.

A genetic distinction between the 11-p and 15-p MTs is indicated by the action of the mec-7 gene. Mutations in this gene result in the elimination of the 15-p MTs from the microtubule cells and their partial replacement by 11-p MTs. This appearance of a new form of MT in the mutant cells, not seen in colchicine-treated animals, suggests that the mec-7 product is responsible for a component that determines the number of protofilaments. The finding of colchicine-sensitive MTs in the mec-7 cells argues that MTs can form, but with an altered structure. Thus, it seems unlikely that mec-7 codes for a cell-specific tubulin. Nematode tubulins copurify with pig brain tubulin through rounds of assembly and disassembly (M. Chalfie, unpublished data) and no differences can be detected on two-dimensional gels between the tubulins of wild type, mec-7 mutants, or mutants in the gene unc-86 (which completely lack the microtubule cells; see reference 10).

The defect in the mec-7 mutants need not be in a tubulin. A priori, the number of protofilaments might be determined by other MT proteins, factors required for elongation, or elements of the nucleation sites. Recent observations by Scheele et al. (25) suggest that nucleation does determine the number of protofilaments. It is an intriguing possibility that mec-7 may be a gene required for MT initiation. The correspondence of the temperature-sensitive periods of the mec-7 alleles with the time of increase of number of the 15-p MTs is consistent with this idea. Moreover, this may explain why the microtubule cell process is sometimes absent in the dominant mec-7 mutants: an altered gene product poisons the nucleating sites so that no MTs can be produced and a process cannot form. In the other mutants no product is made and the resulting unmodified nucleating sites initiate 11-p MTs. If this hypothesis is correct, then identification of the mec-7 product will not only allow the characterization of a component of MT nucleation sites but also will aid in an understanding of how these sites are modified to produce particular MT structures.

We are grateful to Kay Buck for assisting with the electron microscopy. This work was done during the tenure of a British American Research Fellowship of the American Heart Association and the British Heart Foundation and of a Research Fellowship from the Muscular Dystrophy Associations of American to M. Chalfie.

Received for publication 14 September 1981, and in revised form 2 November 1981.
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