

# ORGANIZATION OF NEURONAL MICROTUBULES IN THE NEMATODE *CAENORHABDITIS ELEGANS*

MARTIN CHALFIE and J. NICHOL THOMSON

From the MRC Laboratory of Molecular Biology, University Postgraduate Medical School, Cambridge, CB2 2QH, England

## ABSTRACT

We have studied the organization of microtubules in neurons of the nematode *Caenorhabditis elegans*. Six neurons, which we call the microtubule cells, contain bundles of darkly staining microtubules which can be followed easily in serial-section electron micrographs. Reconstruction of individual microtubules in these cells indicates that most, if not all, microtubules are short compared with the length of the cell process. Average microtubule length varies characteristically with cell type. The arrangement of microtubules gives an overall polarity to each bundle: the distal ends of the microtubules are on the outside of the bundle, whereas the proximal ends are preferentially inside. The distal and proximal ends each have a characteristic appearance indicating that these microtubules may have a polarity of their own. Short microtubules in processes of other neurons in *C. elegans* have also been observed.

**KEY WORDS** neuronal microtubules · microtubule length · microtubule polarity · *Caenorhabditis elegans* · serial-section electron microscopy

Microtubules are common organelles in neuronal processes and are thought to participate in such diverse functions as the organization and maintenance of cell shape, the transport of materials within the cell, and sensory transduction (13, 16, 26). One unsettled question concerning neuronal microtubules is whether or not they extend the entire length of the processes.

In an attempt to investigate this problem, Weiss and Mayr (25) counted the number of microtubules along rat axons before and after branch points. They found that the total number of microtubules in the branches equalled the number of microtubules in the process before the branch and concluded that most of the microtubules extended throughout the branch and were likely to be con-

tinuous for the entire length of the process. Other studies using similar methods have both confirmed (11) and contradicted (11, 27) these conclusions. In none of these studies, however, were individual microtubules reconstructed.

During an examination of the ultrastructure of the nervous system of the free-living soil nematode *Caenorhabditis elegans*, we identified six neurons with microtubules that are particularly favorable for studying microtubule continuity. These six cells, which we call the microtubule cells, can be identified in electron micrographs because they contain bundles of darkly staining microtubules. (Microtubules in other neurons stain less darkly.) Genetic and laser ablation studies identify the microtubule cells as mechanosensory cells which mediate a response to light touch. The role of the microtubules in these cells is unknown, but it may involve sensory transduction: animals whose microtubule cells lack the microtubules, either through mutation or through treatment of early

larvae with colchicine, are touch insensitive.<sup>1</sup> Smith and Croll (18) saw similar microtubule cells in other nematodes and noted a difference in microtubule number at two different positions in the same process. The organization and staining properties of these microtubules facilitate their reconstruction from transverse serial sections. This paper reports our data on the overall structure and extent of microtubules in these cells and in some other neurons in *C. elegans*.

## MATERIALS AND METHODS

The wild type strain N2 of *C. elegans* (var. Bristol) was grown at 20°C as described by Brenner (1).

Initial observations on the microtubule cells were made using serial electron micrographs in the MRC Laboratory of Molecular Biology collection.<sup>2</sup> These pictures of transverse serial sections were prepared by the method of Ward et al. (22), except that the worms were fixed in 1% OsO<sub>4</sub> instead of 0.5%.

When individual microtubules were to be examined, samples were prepared by a modification of a procedure developed for the fixation of microtubules in *C. elegans* larvae.<sup>3</sup> Worms were prefixed for 1 min in a solution containing 2.5% glutaraldehyde (TAAB Laboratories, Reading, England), 1% acrolein (TAAB Laboratories), 1% 1-phenoxy-2-propanol, 2 mM MgCl<sub>2</sub>, and 100 mM sodium phosphate buffer, pH 7.4. The animals were washed three times in 2.5% glutaraldehyde, 2 mM MgCl<sub>2</sub>, and 100 mM sodium phosphate buffer, pH 7.4, and fixed in this solution for 1 h. To accelerate penetration of the fixative, worms were cut in half with a scalpel after 1 min. After fixation, the worms were washed in 100 mM sodium phosphate buffer, pH 7.4, and postfixed in 1% OsO<sub>4</sub> as before. Preparation and electron microscopy of serial sections from these animals followed the procedure of Ward et al. (22).

A few animals were fixed in glutaraldehyde in tubulin reassembly buffer as modified from Luftig et al. (8). Worms were washed in a few milliliters of tubulin reassembly buffer containing 1 mM GTP, 1 mM ethylene-glycol-bis-( $\beta$ -amino-ethyl ether) N,N'-tetraacetic acid, 1 mM MgSO<sub>4</sub>, and 100 mM piperazine-N,N'-bis-(2-ethanesulphonic acid), pH 6.9, and then fixed in the same buffer containing 2.5% glutaraldehyde. As before, the animals were cut in half after 1 min and the fixation was allowed to continue for 1 h. After fixation, the worms were washed three times in 100 mM sodium phosphate

buffer, pH 7.4, over a period of 1 h and postfixed in 1% OsO<sub>4</sub> for 1 h. Subsequent handling was as described above.

When microtubules were to be reconstructed, consecutive serial sections were collected on slotted grids, and every section was photographed at  $\times 30,000$ . Microtubules could be conveniently followed from one section to the next in contact prints of these pictures with the aid of a  $\times 2$  viewer.

The average microtubule length ( $L$ ) in a given series was calculated using the formula  $L = 2Na/T$ , where  $N$ ,  $a$ , and  $T$  are, respectively, the average number of microtubules per section, the length of the series, and the number of terminations in the series (5). Series lengths were determined by multiplying the number of sections by an assumed average section thickness of 50 nm. This estimate is in good agreement with measured values of 44, 44, and 50 nm obtained using the procedure of Gunning and Hardham (4).

## RESULTS

### *General Description of the Microtubule Cells and Their Microtubules*

The positions of the microtubule cells in the adult *C. elegans* hermaphrodite are shown in Fig. 1. A detailed description of the structure and connectivity of these six cells is outside the scope of this paper. Briefly, however, there are three pairs of microtubule cells: (a) a bilaterally symmetric pair of anterior lateral cells with cell bodies located anterior to the vulva, (b) a bilaterally symmetric pair of bipolar posterior lateral cells with cell bodies in the tail, and (c) a nonsymmetric pair of cells with cell bodies  $\sim 1/4$  of the body length anterior (the right side) or posterior (the left side) to the vulva; both of these cells send anteriorly-directed processes into the ventral cord.<sup>4</sup> Of these six cells, only the latter two arise from post-embryonic cell divisions; they are products of the Q-cell lineages (19). As seen in Fig. 1, all of the processes of these cells are unbranched, except for branches extending into the circumpharyngeal nerve ring. The anterior processes of these six cells

<sup>4</sup> Recently a nomenclature for naming all the neurons in *C. elegans* has been developed by J. White (unpublished). In this system each type of neuron is assigned a three-symbol designation; neurons within a set are distinguished by an additional symbol to denote symmetry. Thus the six microtubule cells are: AVM, anterior ventral microtubule cell; PVM, posterior ventral microtubule cell; ALML and ALMR, the left and right anterior lateral microtubule cells; PLML and PLMR, the left and right posterior lateral microtubule cells.

<sup>1</sup> Chalfie, M. and J. E. Sulston, manuscript in preparation.

<sup>2</sup> White, J., J. E. Sulston, D. Albertson, J. N. Thomson, E. Southgate, and S. Brenner, unpublished data.

<sup>3</sup> McIntosh, J. R., MRC Laboratory of Molecular Biology and University of Colorado, personal communication.

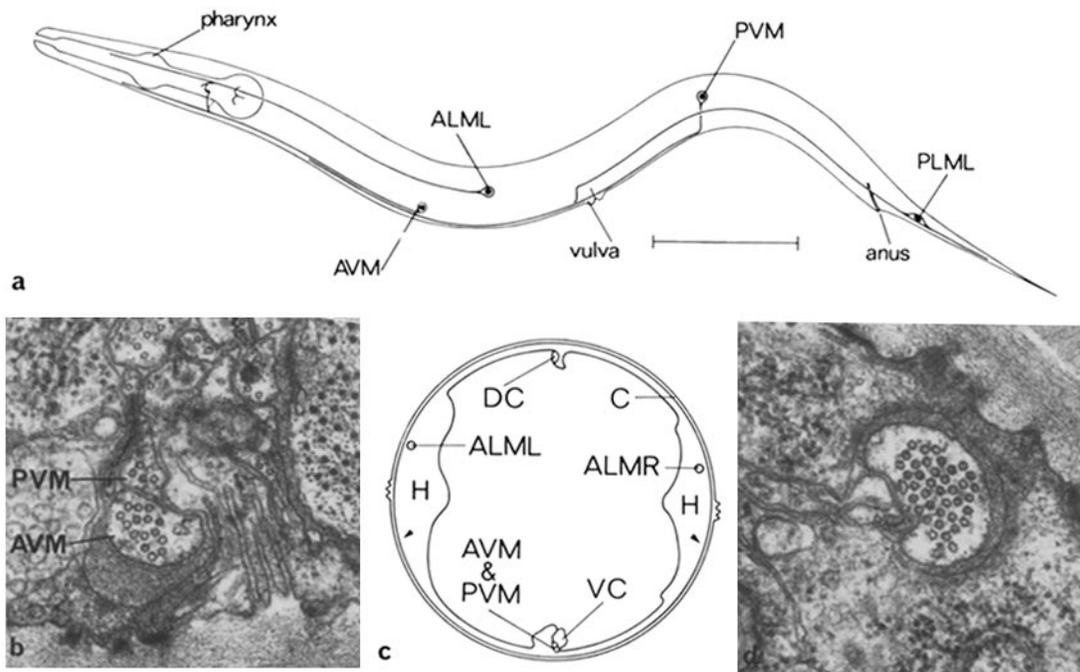


FIGURE 1 Location of the microtubule cells in *C. elegans*. (a) Diagram of the left side of a young adult hermaphrodite showing the positions of the microtubule cells. The figure is based on camera lucida drawings of the worm under Nomarski optics (18). Under these conditions the cell bodies and the positions of the lateral processes can be easily identified. The fine structure of the neurons and their endings was obtained from serial electron micrographs. Dotted structures represent parts of cells which are on the right side of the worm. The right lateral microtubule cells are not drawn. Cell abbreviations are described in footnote 4. Bar, 100  $\mu\text{m}$ . (b) Electron micrograph of the anterior and posterior ventral cord microtubule cells. Note the difference in appearance between the microtubules in these cells and those in other ventral cord neurons. Fixation here and in Fig. 1d was in glutaraldehyde and acrolein as described in Materials and Methods.  $\times 60,000$ . (c) Diagram of a transverse section of an adult hermaphrodite at a level  $\sim 1/4$  of the body length from the anterior end of the worm showing the positions of the microtubule cells. The figure is based on a tracing of montaged electron micrographs. The nerve cells are embedded within the hypodermis (H) which underlies the cuticle (C). The ventral (VC) and dorsal (DC) cord are also shown. (d) Electron micrograph of the right anterior lateral microtubule cell.  $\times 45,600$ .

extend for  $1/4$ – $1/2$  of the length of the animal; thus, in the adult, these processes can be 400–500  $\mu\text{m}$  long. All six cells are found very near the outer surface of the worm, with only a thin layer of hypodermis separating them from the cuticle (Fig. 1b, c, and d).

Because they contain darkly staining microtubules, the six microtubule cells are easily identified in electron micrographs of transverse sections of *C. elegans* (Fig. 1b and d). These microtubules are distinguishable from microtubules in other neurons by their arrangement in a bundle, their darker staining, and their greater apparent diameter (Fig. 1b). Microtubule-cell microtubules in calibrated electron micrographs of glutaraldehyde- and ac-

rolein-fixed worms have an outer diameter of  $29.6 \pm 0.4$  nm (mean  $\pm$  SEM,  $N = 14$ ) and an inner diameter of  $14.7 \pm 0.4$  nm. In contrast, microtubules in other neurons in the ventral cord have an outer diameter of  $24.0 \pm 0.3$  nm and an inner diameter of  $10.8 \pm 0.3$  nm ( $N = 7$ ).

#### Fluctuation in Microtubule Number along Microtubule Cell Processes

To understand more fully the organization and extent of microtubules within these cells, we have counted the number of microtubules at intervals of 0.7–1.5  $\mu\text{m}$  along the length of the cell processes. We find that the number of microtubules

is not constant but fluctuates along the unbranched processes of all six cells and under all three fixation conditions (Figs. 2 and 3; Table I). The number of microtubules also fluctuates in the lateral cells of L1 stage larval worms (Table I) (there are no ventral microtubule cells at this time). These data suggest that many of these microtubules do not extend the entire length of the process.

Although the number of microtubules varies considerably along a given cell process, each cell type has a characteristic mean number of microtubules (Table I). In particular, the lateral cell processes contain more microtubules than the ventral cell processes. The lateral cell processes in adults have many more microtubules than those in larvae.

#### *Reconstruction of Microtubules from Consecutive Serial Sections*

To examine microtubule structure in more detail, we have cut consecutive serial sections which were photographed at high magnification on slotted grids. The spatial array of microtubules in the bundle was well preserved in adjacent sections,

allowing us to follow individual microtubules. Schematic reconstructions of microtubules in portions of three anterior ventral cells and one left anterior lateral cell are shown in Figs. 4 and 5, respectively. Both cells contain extensive microtubule terminations within the examined series. In fact, in one of the series (Fig. 4*b*) all of the microtubules had at least one end within the series. Terminating microtubules have been seen in all series examined (at least three each) of the anterior ventral cells, the anterior lateral cells, and the posterior lateral cells; the posterior ventral cells were not examined. These data confirm that most, and probably all, of the microtubules in these cells do not extend the entire length of the neuronal processes.

The length of the microtubules that have both termini within a series can be accurately determined; the 13 microtubules of this type in the series in Fig. 4 vary in length from 1.20 to 10.70  $\mu\text{m}$ . However, since most of the microtubules have one end which extends beyond the examined sections, the lengths of the complete microtubules are not representative of all the microtubules, but are in the lower part of the distribution of microtubule

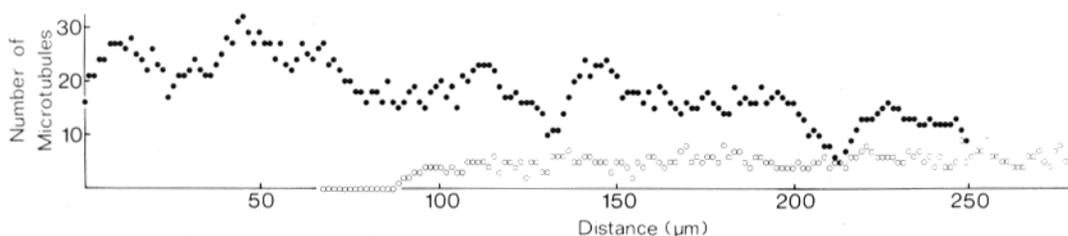


FIGURE 2 Changes in microtubule number along the length of portions of the anterior and posterior ventral microtubule cells in the ventral cord. The anterior ventral cell (●) leaves the ventral cord by a commissure at a position 250  $\mu\text{m}$  into the series. The posterior ventral cell (○) begins 70  $\mu\text{m}$  into the series. Posterior is to the right.



FIGURE 3 Electron micrographs showing fluctuations in microtubule number in the ventral microtubule cells at intervals along the ventral cord. The series *a-e* proceeds posteriorly. The anterior ventral microtubule cell (*A*) and the posterior ventral microtubule cell (*P*) are indicated in each frame because the relative positions of the cells change along the ventral cord. The distances between the sections are *a-b*, 5.7  $\mu\text{m}$ ; *b-c*, 6.0  $\mu\text{m}$ ; *c-d*, 7.5  $\mu\text{m}$ ; *d-e*, 5.7  $\mu\text{m}$ .  $\times 45,000$ .

TABLE I  
Variations in Microtubule Numbers in Microtubule Cells

Cell	Series	Fixation	Approx. series length	No. of microtubules	
				Mean $\pm$ SD	Range
			$\mu\text{m}$		
AVM	a	OsO <sub>4</sub>	250	18 $\pm$ 5	5-32
	b	OsO <sub>4</sub>	160	13 $\pm$ 5	4-24
	c	Glut. in T.A.B.	30	13 $\pm$ 3	9-17
	d	Glut. in T.A.B.	30	12 $\pm$ 2	10-15
	e	Glut. in T.A.B.	30	12 $\pm$ 2	8-16
	f	Glut. + Ac.	10	14 $\pm$ 2	11-19
	g	Glut. + Ac.	10	14 $\pm$ 1	12-17
	h	Glut. + Ac.	10	6 $\pm$ 2	5-9
PVM	a	OsO <sub>4</sub>	210	7 $\pm$ 2	1-12
	b	OsO <sub>4</sub>	190	5 $\pm$ 1	2-9
ALM (adult)	a	OsO <sub>4</sub>	10	29 $\pm$ 9	18-45
	b	OsO <sub>4</sub>	10	37 $\pm$ 3	30-44
	c	Glut. in T.A.B.	30	31 $\pm$ 2	28-33
	d	Glut. in T.A.B.	30	25 $\pm$ 2	22-29
	e	Glut. in T.A.B.	30	25 $\pm$ 4	19-32
	f	Glut. in T.A.B.	30	25 $\pm$ 3	19-31
	g	Glut. in T.A.B.	30	25 $\pm$ 2	20-28
	h	Glut. in T.A.B.	30	31 $\pm$ 2	26-33
	i	Glut. + Ac.	10	29 $\pm$ 2	26-32
PLM (adult)	a	OsO <sub>4</sub>	40	22 $\pm$ 8	3-32
	b	OsO <sub>4</sub>	30	46 $\pm$ 2	42-52
	c	OsO <sub>4</sub>	30	49 $\pm$ 3	46-56
ALM and PLM (L1 larvae)	a	OsO <sub>4</sub>	130	5 $\pm$ 3	1-14
	b	OsO <sub>4</sub>	110	4 $\pm$ 2	2-9
	c	OsO <sub>4</sub>	80	7 $\pm$ 2	3-11
	d	OsO <sub>4</sub>	70	6 $\pm$ 1	2-8
	e	OsO <sub>4</sub>	70	6 $\pm$ 1	4-8
	f	OsO <sub>4</sub>	60	5 $\pm$ 3	3-10
	g	OsO <sub>4</sub>	40	5 $\pm$ 1	2-6

Values for the above means utilize data collected on average once every 0.7-1.5  $\mu\text{m}$  (15-30 sections). Samples were prepared by fixation in OsO<sub>4</sub>, glutaraldehyde in tubulin assembly buffer (Glut. in T.A.B.), or glutaraldehyde and acrolein (Glut. + Ac.) as described in Materials and Methods.

lengths. An estimate of the mean microtubule length of the series can be obtained as described in Materials and Methods. Average lengths estimated in this way are shown in Table II. Two points can be made about these data. First, the average microtubule lengths are short compared to the length of the process (not more than 5% of a presumed length of 500  $\mu\text{m}$ ). Second, the average lengths are quite similar for a given cell type and yet dissimilar between cell types, i.e., the microtubule lengths are apparently cell specific. This can be demonstrated in different cells (AVM and ALML) examined in the same series of micrographs from a single nematode (e.g., Figs. 4a and 5).

#### Evidence for Microtubule Polarity in the Microtubule Cells

The termini of microtubule cell microtubules can be distinguished by their position and structure (Fig. 6). In the anterior processes of the examined cells the distal ends are usually found on the outer edge of the bundle. All of the 94 distal microtubule ends of the anterior cells and 47 of 50 distal ends of the posterior lateral cells fit this pattern. The pattern of proximal terminations is less striking, but here, too, the distribution appears to be non-random. There are more internal proximal ends than one would predict from the number of internal microtubules (Table III). This

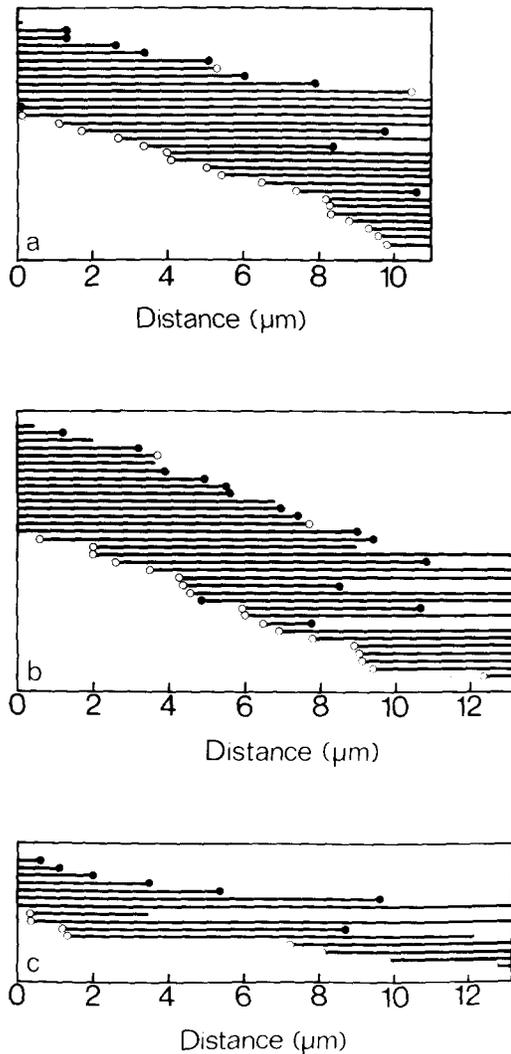


FIGURE 4 Schematic diagram of microtubules along the length of portions of the anterior ventral microtubule cell. *a*, *b*, and *c*, represent the microtubules in cells of three separate animals. The vertical axis here and in Figs. 5, 7, and 8 has no significance. Posterior is to the right in all of these figures. Diffuse (○) and filled (●) endings are indicated for all ends which could be classified.

is particularly clear in the case of the anterior lateral cells. Thus, there is an overall orientation of each microtubule: the distal end is located almost exclusively on the outside of the bundle, the proximal end preferentially within it.

The placement of the distal ends on the outside of the microtubule bundle could result from an

association of these ends with the plasma membrane, especially since most peripheral microtubules are within a microtubule's diameter of the membrane. Although few microtubules contact the plasma membrane, 94 of 132 distal ends touched the membrane with the diffusely staining material that surrounds the ends (see below). Thus, these ends may be associated with the plasma membrane.

The arrangement of the microtubules with their proximal ends primarily within the bundle and distal ends on the periphery gives an overall polarity to the array of microtubules, but does not necessarily imply a polarity of the individual microtubules. However, a second feature of the ends is also seen which may indicate such a polarity. 95% of the endings can be grouped into two broad categories on the basis of ultrastructure. In the first, the microtubule outline gives way to a diffuse patch of stained material with a diameter up to twice that of the microtubule. This diffuse patch often appears in two consecutive sections, and frequently the outline of all or part of the microtubule can be seen within it. For convenience, this type of termination will be called a diffuse ending. The ends of microtubules 2-5 in Fig. 6 are examples of this type.

In the second type of ending the microtubule terminates with a darkly stained core. (Stain is also seen in the core at intervals along the length of the microtubule, but it is fainter than the core-staining at the ends.) The overall shape and size of this ending is similar to that of the microtubule but may be slightly larger and less distinct around the edge. In some cases the subsequent section

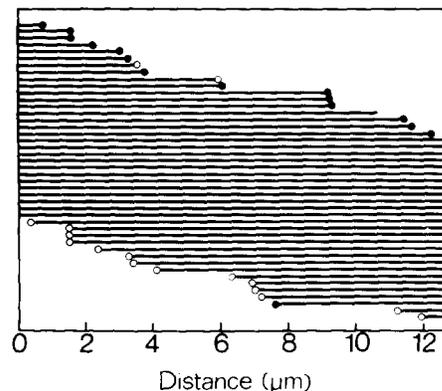


FIGURE 5 Diagram of microtubules along a portion of the left anterior lateral microtubule cell. Microtubule endings are symbolized as in Fig. 4.

TABLE II  
Average Length of Microtubules in Microtubule Cell Processes

Cell	Series	Length of series $\mu\text{m}$	Avg. No. of microtubules	No. of termi- nations	Calculated avg.
					microtubule lengths $\mu\text{m}$
AVM	1	11.05	13.5	33	9.1
	2	13.20	13.0	40	9.3
	3	13.20	6.7	18	9.8
ALM	1*	12.80	29.0	33	23.0
	1a*	3.40	43.4	11	26.9
	2	13.40	27.0	37	19.6
PLM anterior process	3	4.50	21.3	10	19.2
	1‡	3.50	18.1	14	9.4
	2§	5.65	41.4	59	7.9
PLM posterior process	2a§	2.25	47.2	17	12.5
	3	1.75	36.7	16	8.0
	1‡	2.00	16.7	11	6.1
	2	4.10	17.8	10	14.6

Average microtubule lengths were calculated using the formula  $L = 2Na/T$  as described in Materials and Methods. The series marked with either \*, ‡, or § came from the same cell.

contains a small dot in the same position as the terminated microtubule. This ending will be referred to as a filled ending. Examples of this type of termination are seen in Fig. 6, microtubules 1 and 6.

The ultrastructure of endings correlates with the proximal and distal position of the endings in the anteriorly directed processes (Figs. 4 and 5). 80% of the distal termini have diffuse endings, whereas ~20% of the proximal ends have this structure. Conversely, 75% of the proximal ends and 15% of the distal ends are filled ends. It should be noted that this classification is somewhat subjective and that ~5% of the endings do not readily fit into either category. In particular, some of the proximal ends appear as large filled structures, i.e., taking on the appearance of both types of endings. The filled appearance of the proximal ends cannot be ascribed to their position within the bundle, e.g., as a consequence of crowdings, since filled endings are seen on microtubules that end peripherally as well as those that end centrally.

Of the 14 microtubules that lie completely within the examined anterior cell series, 9 have both the filled proximal ends and diffuse distal ends. Of the remaining microtubules, most have one or the other of these termini on the appropriate end and an unclassifiable one on the other end. No microtubule, however, had a diffuse proximal end and filled distal end. Thus, the structure of these complete microtubules supports the hy-

potheses that the distal and proximal ends of individual microtubules can be distinguished ultrastructurally, and that the microtubules in the bundles have a common orientation. Whether the orientation reflects the underlying molecular polarity of the microtubules is unknown.

#### *Microtubule Organization in the Cell Body and Posterior Process of the Posterior Lateral Microtubule Cells*

We have examined sections through the cell bodies of the bipolar posterior lateral cells to see if there was any difference in the organization of the microtubules in the two processes and within the cell body. The schematic reconstruction of the microtubules in one such series is shown in Fig. 7. The number of microtubules in the bundle drops sharply as the processes join the cell body and again as the bundle approaches the nucleus. There are very few microtubules in the bundle as it passes the nucleus. There is always at least one microtubule in the bundle throughout the entire series, yet no single microtubule extends from the anterior to the posterior process. This general pattern has been seen in all four cells examined. No apparent microtubule organizing structures were seen in the cell body.

Attempts to see if the organization of the microtubules in regions close to the cell body is similar to that in the processes described above have been

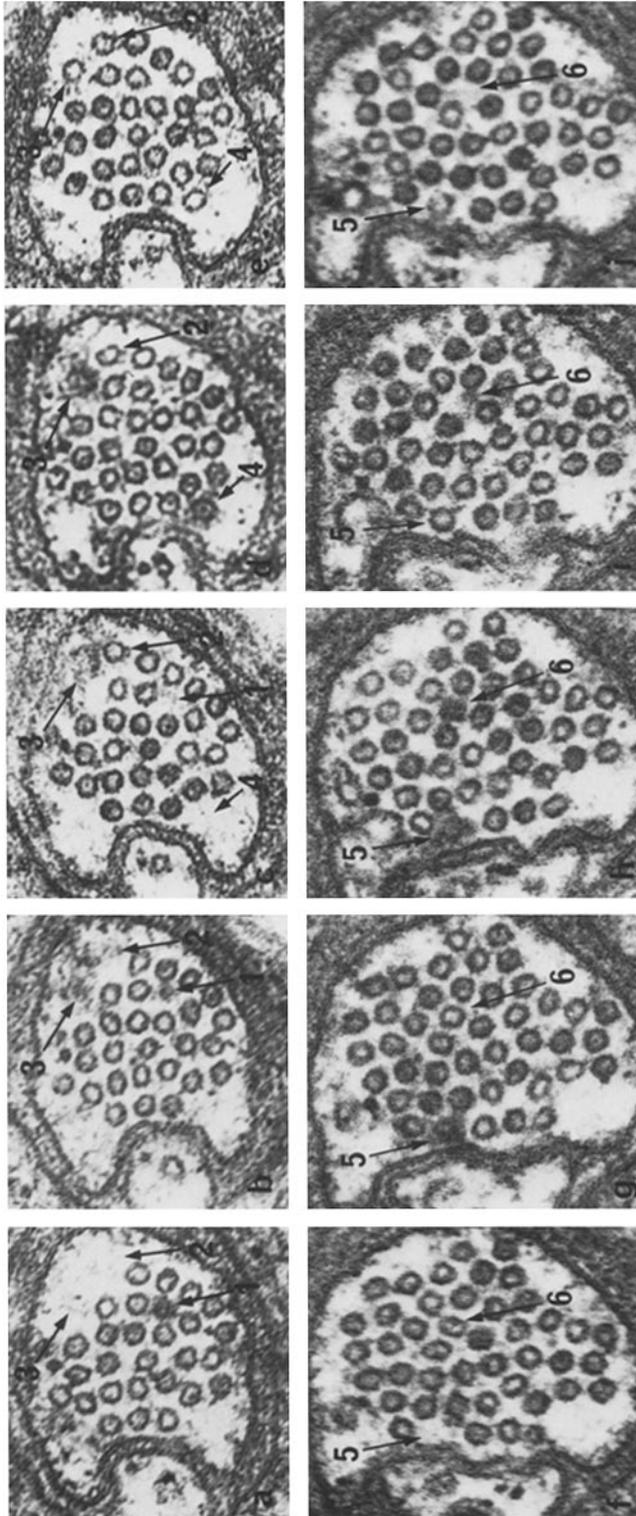


FIGURE 6 Electron micrographs showing microtubule ends. Two consecutive series (*a-e* and *f-j*) of transverse sections of anterior lateral cells are shown. Microtubules 1 and 6 have filled proximal ends; microtubules 2-5 have diffuse distal ends.  $\times 116,000$ .

TABLE III  
Observed and Expected Internal Proximal Terminations in Anterior Processes of  
Microtubule Cells

Cell	Series	Avg. No. of microtubules	No. of proximal terminations	Avg. fraction of internal microtubules	Expected No. of internal terminations	Actual No. of internal terminations
AVM	1	13.5	14	0.24	3	9
	2	13.0	21	0.13	3	4
	3	6.7	9	0.04	0	1
ALM	1	29.0	18	0.43	8	15
	1a	43.4	4	0.45	2	4
	2	27.0	18	0.32	6	11
PLM	3	21.3	4	0.29	1	3
	2	41.4	26	0.40	10	16
	2a	47.2	9	0.48	4	5
	3	36.7	7	0.36	3	4

The expected number of internal terminations is calculated by multiplying the average fraction of internal microtubules by the number of proximal terminations. This estimate assumes that a microtubule has the same probability of terminating proximally regardless of its position in the microtubule array. The examined series are numbered as in Table II.

frustrated by the relatively small number of microtubules in these bundles and a larger spacing of the tubules in some of the posterior processes. Hence, no significant data are available on the posterior of the proximal and distal ends in the bundle in the anterior and posterior processes near the cell body.

The proximal and distal ends of microtubules in the anterior process near the cell body tend to be filled and diffuse, respectively, as in the other anterior processes described above. The ends within the cell body and in the posterior process are more difficult to characterize because of the dense staining of the surrounding cell inclusions. Both types of ending are seen on both anterior and posterior ends of the microtubules. Of the complete microtubules diagrammed in Fig. 7, only eight could have both ends characterized. Two microtubules in the anterior portion of the cell and three in the posterior had diffuse anterior ends and filled posterior ends, i.e., they had the same orientation as that seen in the anterior process. Three other microtubules in the posterior portion of the cell had the reverse orientation. No microtubule was seen with the same type of ending on both ends. Thus, it appears that there is at least a partial reversal of microtubule polarity in the posterior portion of these cells.

#### Microtubule Organization in Processes of Non-Microtubule Cell Neurons

Since it is possible that short microtubules are

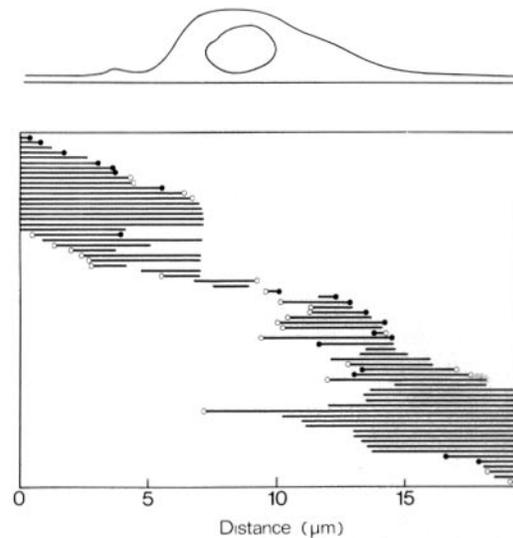


FIGURE 7 Diagram of microtubules in the cell body of a posterior lateral microtubule cell. Only microtubules in the bundle were followed. (A very small number of microtubules were seen in other portions of the cytoplasm.) Microtubule endings are symbolized as in Fig. 4. A computer generated projection of the examined cell is shown above the diagram.

peculiar to these mechanosensory cells, we have followed microtubules in other nerve processes in *C. elegans*. Following microtubules in these cells is difficult because of cell inclusions, displacements of the microtubules, and the fainter appearance of the microtubules. Nonetheless, these microtubules

can be reconstructed, and they, too, terminate within the neuronal process (Fig. 8). In the two ventral cord cells diagrammed in Fig. 8, average microtubule lengths of 6.4 and 6.9  $\mu\text{m}$  (for the series in a and b, respectively) were calculated. Terminating microtubules were found in six ventral, lateral, and tail neurons. Thus, other neurons in addition to the microtubule cells have discontinuous microtubules. Cell inclusions make characterization of the end structure of these microtubules difficult. Two of these termini are shown in Fig. 9. Although some ends appear to be similar to the diffuse and filled ends described above, many are ambiguous. Furthermore, there was no apparent relationship between position and end structure of these microtubules.

#### DISCUSSION

The microtubule cells of *C. elegans* provide a convenient system in which to study the structure of neuronal microtubules: the microtubules are stable under a variety of fixation conditions, are conspicuously stained, and are arranged in a bundle. Examination of the microtubules in these cells shows that their average length is short compared to that of the process and that probably none extends the entire length of the process. Short microtubules are not specific to these cells since they are also found in non-microtubule neurons.

It is unlikely that the discontinuity of the microtubules we have observed is a consequence of fixation, for a number of reasons. First, the fluctuation in microtubule number in a given cell is seen under a variety of fixation conditions. Second, several series include different cell types in which different cell specific microtubule lengths are observed. Finally, the polarity of the ends both in their position in the bundle and in their struc-

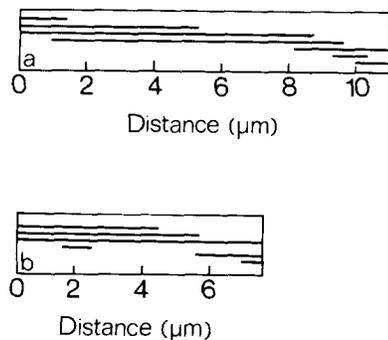


FIGURE 8 Diagram of microtubules in two ventral cord neurons (a and b).

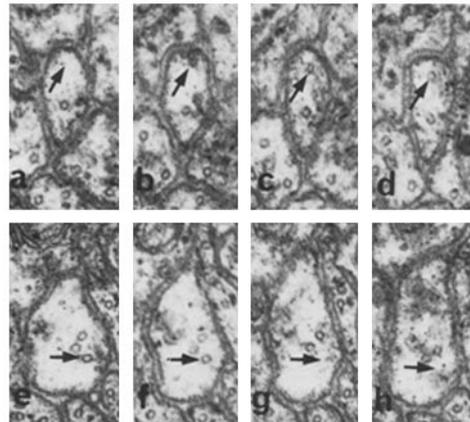


FIGURE 9 Electron micrographs of microtubule terminations in non-microtubule ventral cord neurons. Arrows indicate an anterior end (a-d) and a posterior end (e-h). The proximo-distal direction is not known.  $\times 60,000$ .

ture argues against the ends being created by breakage of the microtubules during sample preparation.

In the past, the idea of continuous neuronal microtubules was based on the constancy of microtubule numbers in a series of separated transverse sections (11, 25). However, as exemplified by the series in Figs. 4, 5, and 9, microtubule numbers can be fairly constant, in situations where few microtubules extend through the series. Thus, these previous studies do not prove microtubule continuity.

Some investigators, however, have found a lack of constancy in microtubule numbers in some nerves. Nadelhaft (11) found two cell types in the crayfish abdominal nerve cord that did not have constant microtubule numbers. Zenker and Hohberg (27) calculated the total number of microtubules in the terminal branches of rat A- $\gamma$ -motor nerve fibers to be eleven times the number in the stem fiber. In both of these studies the authors concluded that many microtubules could not be continuous in these cells. Moreover, microtubule counts given by Weiss and Mayr (25) for a single rat sensory neuron show considerable variability. It is possible that this variation was not entirely a result of counting errors, as these authors suggest, but also represented an underlying fluctuation in microtubule number as a result of microtubule termination. Thus, it is likely that non-continuous microtubules are a common structural feature of processes in both vertebrate and invertebrate neurons.

The absence of continuous microtubules in neuronal processes means that some theories on microtubule function in nerve cells must be modified. For example, as Nadelhaft (11) has noted, the existence of short microtubules implies that microtubules cannot serve as continuous guides in axonal transport.

One advantage of short overlapping microtubules over continuous ones is that they could provide local cytoskeletal support of process shape but also accommodate changes in process length. As *C. elegans* bends its body ventrally and dorsally, it is likely that portions of the ventral cord neurons undergo as much as a twofold change in length.<sup>5</sup> Continuous microtubules would presumably buckle under these length changes, whereas short microtubules could slide over each other. An additional advantage is that small, local length changes could be accommodated by short microtubules. Conversely, continuous microtubules, such as those in cilia, may provide structural rigidity where length changes are undesirable.

In teleost cone myoids (structures that contain short, overlapping microtubules) Warren and Burnside (24) found that the cross-sectional number of microtubules decreases during elongation. Although these authors state that myoid elongation could be a consequence of active microtubule sliding, they also suggest that the changes in microtubule number could have resulted from passive movements that accompanied cell elongation.

In recent years a number of investigators have examined microtubule structure in non-neuronal cells using consecutive serial electron micrographs (3, 5, 6, 7, 9, 10, 12, 17, 23, 24). These studies, with the exception of that of Schliwa (17) (who reported very few microtubule terminations in serial sections of angelfish melanophores) find that many microtubules in a variety of cell types and structures are not continuous, and that some microtubule lengths are short relative to the cell dimensions that the collection of microtubules is thought to span. Our data show that these conclusions are also true for neurons in *C. elegans* and add support to the suggestion that discontinuous microtubules may be a common structural element of cellular architecture. However, observations with immunofluorescence microscopy suggest that some cells contain long continuous microtubules (e.g., reference 14).

<sup>5</sup> Chalfie, M., unpublished measurements from videotape data.

Most of the microtubule termini in microtubule cells fall into two categories: diffuse ends and filled ends. These structures imply that the microtubules have individual polarity. We find that, especially in the anterior processes of the microtubule cells, these structural characteristics correlate with the position of the termination, whether distal or proximal. These data suggest that most if not all microtubules are aligned in the same manner in the bundle.

We do not know the significance of the microtubule orientation seen in this study, but it may reflect a polarity of growth of the microtubules with one end or the other acting as a microtubule organizing center. Alternatively, the diffuse and filled ends may reflect a functional specialization of these microtubules. Structures similar to both ends have been seen by other authors, but both have been interpreted as microtubule organizing centers. A number of investigators have suggested that diffusely staining regions often seen at one end of a microtubule are microtubule organizing centers (15, 16, 20, 21). However, Byers et al. (2) find that microtubules growing from spindle pole bodies have closed proximal ends. These ends, if seen in transverse sections, would probably look like the filled ends of the present study. Regardless of the significance of the two termination structures we observe in the microtubule cells, our data indicate that the ends of these microtubules can be distinguished.

We would like to thank our many colleagues at the Molecular Biology Laboratory for their helpful discussions and suggestions, particularly Gary Borisy, Jonathan Hodgkin, John White, Donna Albertson, John Sulston, and Phil Anderson. Sydney Brenner kindly provided laboratory space and encouragement.

This work was done during the tenure of a British American Research Fellowship of the American Heart Association and the British Heart Foundation to M. Chalfie.

Received for publication 23 October 1978, and in revised form 20 February 1979.

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