

ON THE MECHANISM OF ANAPHASE SPINDLE ELONGATION IN *DIATOMA VULGARE*

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ABSTRACT

Central spindles from five dividing cells (one metaphase, three anaphase, and one telophase) of *Diatoma vulgare* were reconstructed from serial sections. Each spindle is made up of two half-spindles that are composed almost entirely of polar microtubules. A small percentage of continuous microtubules and free microtubules were present in every stage except telophase. The half-spindles interdigitate at the midregion of the central spindle, forming a zone of overlap where the microtubules from one pole intermingle with those of the other. At metaphase the overlap zone is fairly extensive, but as elongation proceeds, the spindle poles move apart and the length of the overlap decreases because fewer microtubules are sufficiently long to reach from the pole to the zone of interdigitation. At telophase, only a few tubules are long enough to overlap at the midregion. Concurrent with the decrease in the length of the overlap zone is an increase in the staining density of the intermicrotubule matrix at the same region. These changes in morphology can most easily be explained by assuming some mechanochemical interaction between microtubules in the overlap zone which results in a sliding apart of the two half-spindles.

The main problems hindering investigations of the structural basis of anaphase chromosome segregation can be grouped into two broad categories: problems arising from the structural complexity of mitotic systems, and problems inherent in the tools and techniques used to study anaphase motion.

Within the first category we can list the following specific examples:

(a) *The structural elements of most mitotic systems are not well ordered.* To understand how any machine works (biological machines included), it is important to know how the parts move relative to one another. Knowledge of the precise spatial relationships of the structural elements in striated muscle has allowed some insight into how that

particular biological machine works. In most mitotic systems, however, the components (at least those that have been identified) are not so well ordered and, consequently, the nature of their interactions is much more difficult to determine.

(b) *There may be more than one mechanochemical system operating simultaneously within the mitotic apparatus (MA).* In many mitotic systems, anaphase segregation of chromosomes involves two distinct motions: the movement of chromosomes from the metaphase plate to the poles, and the further separation of the poles, or so-called spindle elongation. Each of these motions may have a different mechanochemical basis. Failure to discriminate between the fiber systems associated with each type of motion may account, in

part, for the diverse interpretations that have arisen from previous structural studies.

(c) *The number of energy transducing molecules required to effect chromosome segregation may be very small.* The calculations that lead to this conclusion are discussed elsewhere (16) and need not concern us here, but what is significant is the realization that so few important molecules in such a relatively large space are likely to be very difficult to identify.

Within the second class of problems we can cite the following specific examples:

(a) *Individual fibers of the MA cannot be visualized with the light microscope.* The great advantage of the light microscope is that it allows us to follow the events of mitosis in living cells; however, its main disadvantage is that it does not resolve the interaction of individual structural units, e.g., microtubules (MTs), within the spindle. Electron microscopy (EM) provides the needed resolution, but it introduces other problems.

(b) *Standard EM preparations may not preserve important structural elements of the MA.* Before the general use of glutaraldehyde as a fixative, MTs of the spindle were partially or totally destroyed by processing techniques for electron microscopy. Likewise, although there is some evidence (2, 17, 19) that actin (or actin-like protein) may be an important component of the spindle, conventional EM preparations fail to show filaments of the appropriate size as a conspicuous part of spindle structure. Pollard et al. (18) have suggested that the failure to see such fibers, if present, may be due to their destruction by OsO_4 . Thus, artifacts of preparation and loss of structural detail remain serious problems in EM preparations.

(c) *EM is applicable only to dead cells.* Therefore, to understand changes in MA structure during anaphase, it is necessary to compare different cells at various stages of anaphase movement. Complete characterization of any one stage requires serial section reconstruction of the entire MA. This kind of analysis for many different stages represents a considerable investment in work, especially with large, disordered spindles.

As a result of such problems, there is little precise information to use in formulating models or hypotheses that attempt to explain the structural basis of anaphase chromosome motions. However, several recently published papers (1, 3-13, 15) have given quantitative data on the redistribution of spindle MTs during mitosis, and have

shown important qualitative distinctions between the kinds of spindle MTs. Four types are recognized: *kinetochore MTs*—those with one end at a kinetochore and the other at a pole or free in the spindle; *polar MTs*—those with one end at a spindle pole and the other free; *free MTs*—those with both ends free in the spindle; and *continuous MTs*—those with each end at a pole. The work of Heath (5) and of Heath and Heath (6) provides an example of the kind of spindle structure data obtainable by serial section analysis. These authors have identified the point at which each MT begins and ends within several spindles and how the different kinds of MTs redistribute during anaphase. Thus, it was possible to correlate particular anaphase motions with changes in the distribution of particular kinds of microtubules. Those papers also illustrate another important point: that MAs of some organisms are much more amenable to serial section analysis than others.

The spindle of *Diatoma vulgare* has characteristics that make it well suited for the structural analysis of anaphase spindle elongation by serial section reconstructions. It consists of two spatially separate and distinct fiber systems, one of which radiates out from the poles into the chromatin and cytoplasm, and another which constitutes the so-called central spindle, a paracrystalline array of about 250-300 MTs which run from pole to pole. In this paper, we focus specifically on the changes in central spindle structure and how they relate to the phenomenon of spindle elongation. The mechanism of chromosome-to-pole motion is not considered at all. A detailed description of the general features of the ultrastructure of *Diatoma* mitosis has been published previously (17).

MATERIALS AND METHODS

Electron Microscopy

Fixation and embedding procedures were the same as those reported previously (17). Flat-embedded cells were scanned at $\times 400$ with phase-contrast optics, and dividing cells were excised and remounted in an orientation that allowed their spindles to be cut accurately in cross-section. Sections approximately 70-nm thick were cut on either a Reichert OM 2 or Porter-Blum MT-2 ultramicrotome and picked up in known order on slot grids coated with 0.6% Formvar (Monsanto Co., St. Louis, Mo.) and stabilized with carbon. Specimens were viewed at 60 kV on a Philips 300 electron microscope equipped with a goniometer stage allowing 45° tilt in any direction. Micrographs of each section were taken at approximately $\times 30,000$ which permits the entire area of

each spindle cross section to be recorded on a single $3\frac{1}{4} \times 4$ in. piece of film.

Serial Section Analysis

Central spindles from five cells were analyzed: one in metaphase, three in anaphase, and one in telophase. In cross-section, the stages of mitosis were determined by the following criteria, derived from a previous paper (17): (a) at *metaphase*, the chromatin encircles the midregion of the spindle; (b) at *anaphase*, the chromatin is at or near the poles; the staining intensity of the intermicrotubule matrix of the central spindle midzone is intermediate between that of metaphase and that of telophase, and the persistent polar complexes (PPCs; see reference 17) are still located at the ends of the central spindle; (c) at *telophase*, the chromatin is at the poles, the intermicrotubule matrix at the central spindle midzone is densely osmiophilic, and the PPCs are no longer at the spindle ends.

For tracking MTs, each negative of a cross section was printed to a final magnification of approximately $\times 150,000$. The print of each cross section was numbered, and the set was arranged to correspond with the sequence of the sections. In a preliminary analysis of the central spindle, we counted the number of tubules in each section. Because of the ordered nature of the spindle, these counts are precise; for example, counts for a given section by different individuals agree to within 1%. Near the spindle poles, the oblique tubules which fan out into the chromatin (17) can almost always be distinguished from the central spindle by their orientation.

For a more thorough analysis of spindle structure, we tracked each central spindle MT from the section where it began to the section where it ended. This was accomplished by placing a sheet of clear acetate over the first print of a given series (corresponding to one spindle pole) and marking the position of every MT with a fine-tipped pen. Each "mark" was, in fact, a code which allowed each MT to be identified separately. By using a two-element code of a letter plus a single digit, we had enough combinations to identify all the MTs in each spindle. When all the MTs on the first print were marked, the fact that they were present in that section was recorded on graph paper on which the *x*-axis represented section number (equivalent to position along the spindle axis) and the *y*-axis was a column of all the microtubule codes. The acetate sheet from the first print was then superimposed upon the print of the adjacent serial section so that all MTs that were continuous from this section to the next were in register. These were then marked on a new acetate sheet, using their same codes from the previous print. All new MTs appearing in that section were given a new code. Any MT that was present in the previous section but not in the next was recorded as having ended in that section. The overlay from the second print was then put in register with the print of the next section and so on until all the prints were processed. The product of such an analysis is a record of the total

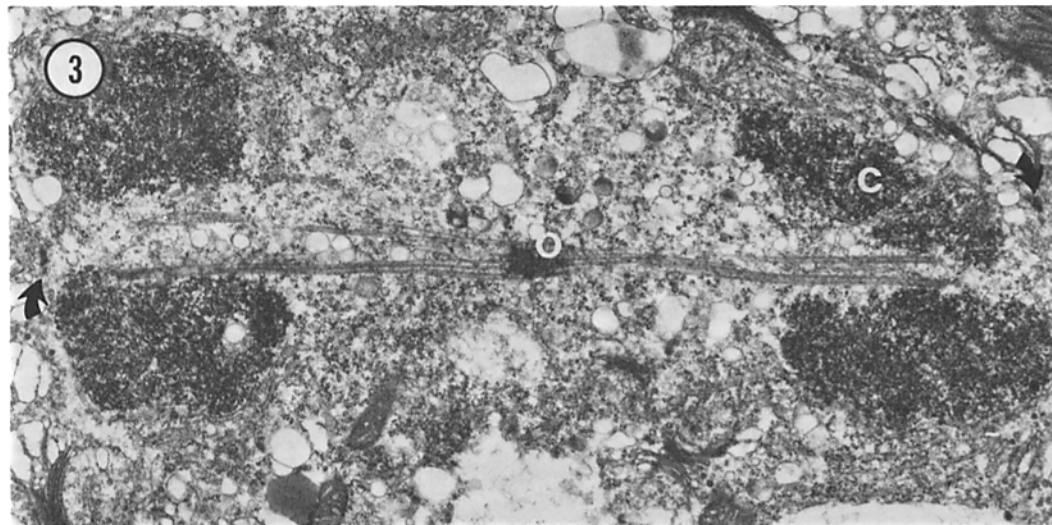
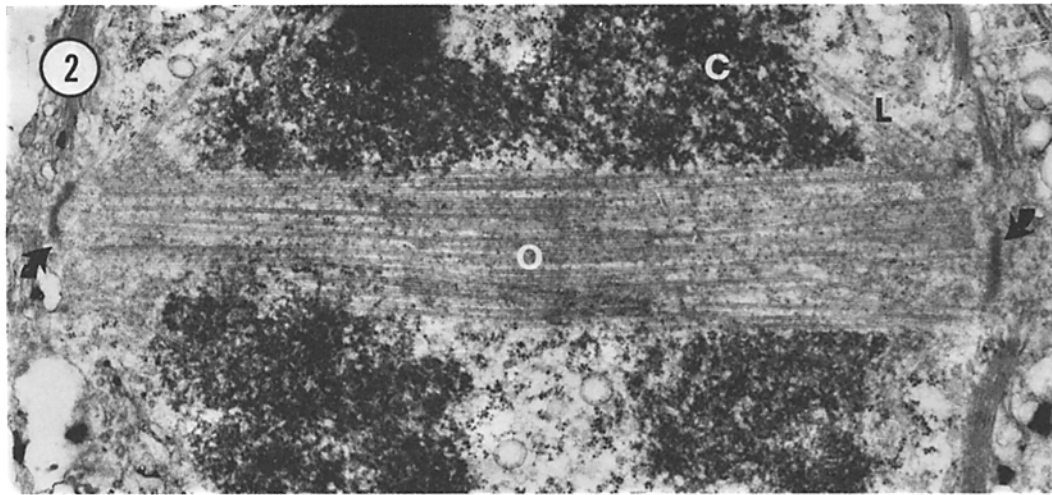
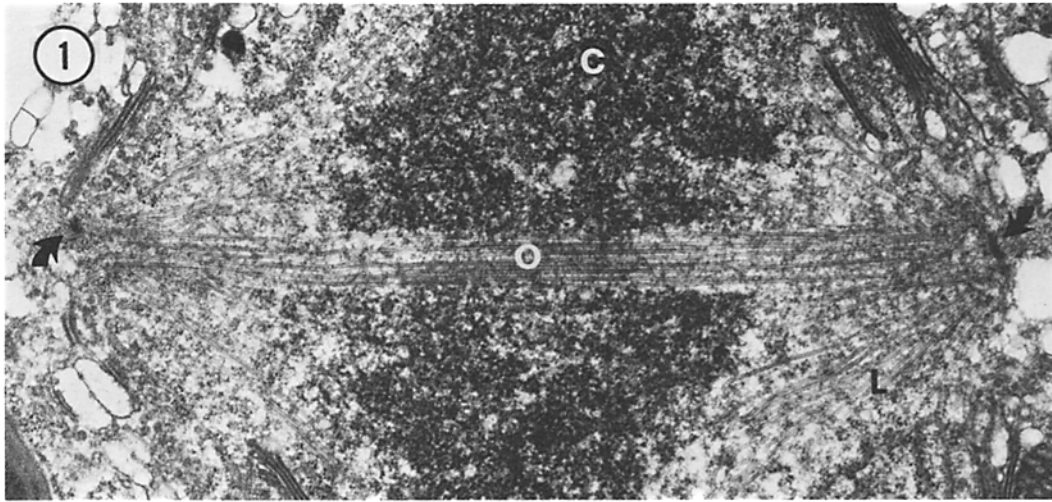
length of each MT measured in number of sections and a one-dimensional record of the position of the tubule ends. These data can then be reorganized into a form suitable for easy viewing.

OBSERVATIONS

Figs. 1–3 are longitudinal sections through the MA at metaphase, early anaphase, and telophase. The central spindle is the bundle of essentially parallel MTs between the poles, and it always contains a prominent dense region in the middle. Other MTs end near each pole but diverge from them at various angles (Figs. 1 and 2). At metaphase (Fig. 1) and anaphase (Fig. 2), the poles are marked by the PPCs but at telophase (Fig. 3) these structures have separated from the spindle. The chromatin encircles the midregion of the central spindle at metaphase (Fig. 1); it moves toward the poles *before* spindle elongation begins (e.g., the spindles in Figs. 1 and 2 are approximately the same length— $5 \mu\text{m}$). After the chromatin reaches the poles, the spindle elongates, attaining a length of about $7 \mu\text{m}$ at telophase (Fig. 3). Note that Fig. 3 is printed at a lower magnification than are Figs. 1 and 2. During elongation, the dense region of the central spindle changes in appearance. At metaphase (Fig. 1), it is stained more intensely than the rest of the central spindle. At telophase (Fig. 3), its staining intensity has increased, whereas its extent along the central spindle has decreased. These changes are further illustrated in Figs. 4–7.

Fig. 4 is a cross section taken outside the dense region of a metaphase central spindle; the appearance of this part of the spindle does not change significantly during spindle elongation. Figs. 4–7 also show that the central spindle is a flattened bundle of MTs, which accounts for its different appearance when seen in longitudinal sections of different paraxial orientations (Figs. 1 and 2). Cross sections through the midregion at metaphase (Fig. 5), anaphase (Fig. 6), and telophase (Fig. 7) show how the density of the intermicrotubule matrix increases as spindle elongation proceeds. Finally, Figs. 4–7 illustrate some of the sections used in the serial section analyses. It is clear how the ordered parallel arrangement of these MTs facilitates their accurate counting in each section and then their tracking from section to section.

Graphs of the number of microtubules in the central spindle versus position along the spindle axis help to clarify the structure of the tubule bundle and show how its structure changes during



anaphase. The distribution profile at metaphase (Fig. 8) shows that the dense region of the central spindle contains considerably more tubules than the region on either side. An equivalent graph for two early to mid-anaphase spindles (Figs. 9 and 10) reveals a similar profile, but by late anaphase (Fig. 11) the MT distribution is altered, showing a much lower peak in the midzone. By telophase (Fig. 12) the profile is further changed, showing a trough instead of a peak in the middle region. These graphs are also labeled to show the distribution of chromatin (*C*) and osmiophilic intermicrotubule matrix (*M*) along the axis for each stage. The actual position of the sections in Figs. 4–7 is marked on graphs 8, 10, and 12, respectively. Figs. 13–17 show the length of each MT and the position of its ends in central spindles at metaphase, anaphase, and telophase. In these figures, MTs that appear near the left spindle pole are at the top of the graph, arranged according to the positions of their distal ends. The bottom part of the graph displays the distribution of the tubules ending near the right pole. Represented at the middle of the graph are those MTs that run from one end of the spindle to the other.

In every cell analyzed, the central spindle is seen to be made up of two half-spindles, which in turn are made up of MTs of different lengths (Figs. 13–17). The peaks in the MT distribution profiles at metaphase (Fig. 8) and early to mid-anaphase (Figs. 9 and 10) are the result of the interdigitation of the two populations comprising each half-spindle (Figs. 13–15). By late anaphase (Figs. 11 and 16), the peak has flattened out because the number of MTs beginning and ending in any section in the overlap zone is almost the same, and by telophase (Figs. 12 and 17) there is a

dip in the profile because only the longest MTs of each half-spindle are still overlapping.

Table I lists the frequencies of the different kinds of MTs present in each central spindle. Most of the MTs are polar MTs, and in one case (telophase, Fig. 17) *all* are polar MTs. Free MTs appeared with low frequency in the anaphase spindles (Figs. 14–16), and a few continuous MTs are present at each stage except telophase.

DISCUSSION

This study has confirmed what we suspected from longitudinal sections of these spindles (17), namely, that the dense region in the middle of the central spindle is a zone of overlap between the two half-spindles. Furthermore, we now know the kinds of MTs that make up the central spindle and the fractions of the total MT population each represents. About 3% are continuous MTs, free MTs are very rare, and the remainder are polar MTs. These proportions remained relatively constant for all spindles studied except the telophase spindle which contained neither continuous nor free MTs. The total number of MTs in each spindle was quite similar. This is not surprising if the

TABLE I
Frequency of Different MT Types in Central Spindles of Diatoma vulgare

Spindle	Total no. of MTs	Polar MTs		Continuous MTs		Free MTs	
		<i>no.</i>	<i>%</i>	<i>no.</i>	<i>%</i>	<i>no.</i>	<i>%</i>
Metaphase	271	262	96.6	8	3.0	1	0.4
Anaphase-1	254	249	98.0	4	1.6	1	0.4
Anaphase-2	222	214	96.4	6	2.7	2	0.9
Anaphase-3	232	218	93.9	8	3.5	6	2.6
Telophase	241	241	100	0	0	0	0

FIGURE 1 Longitudinal section through a metaphase spindle. The chromatin (*C*) occupies an equatorial position. The microtubules between the spindle poles (arrows) comprise the central spindle, whose midregion (*O*) stains more densely than the regions on either side. Other microtubules (*L*) diverge from the poles at various angles. Total spindle length = 5.3 μm . \times 22,800.

FIGURE 2 Longitudinal section through a spindle in early anaphase. The chromatin (*C*) is midway between the central region (*O*) and the poles (arrows). This spindle is sectioned at a different angle from those in Figs. 1 and 3 to show that the central spindle is a flattened band of MTs (cf., Figs. 4–7). Oblique, noncentral spindle MTs (*L*) end near each pole. Total spindle length = 5.4 μm . \times 22,800.

FIGURE 3 Longitudinal section through a telophase central spindle. The chromatin (*C*) is at the poles but the PPCs (arrows) have separated from the spindle ends. The midregion (*O*) of the central spindle is densely stained. Total spindle length = 7.3 μm . \times 15,200.

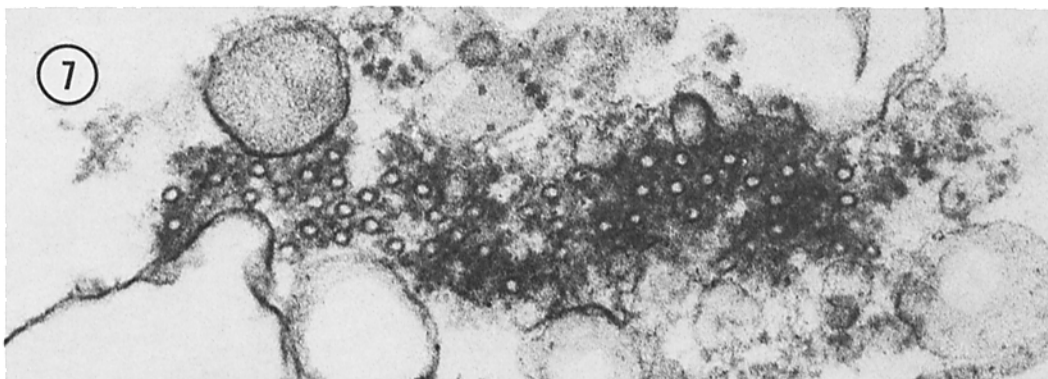
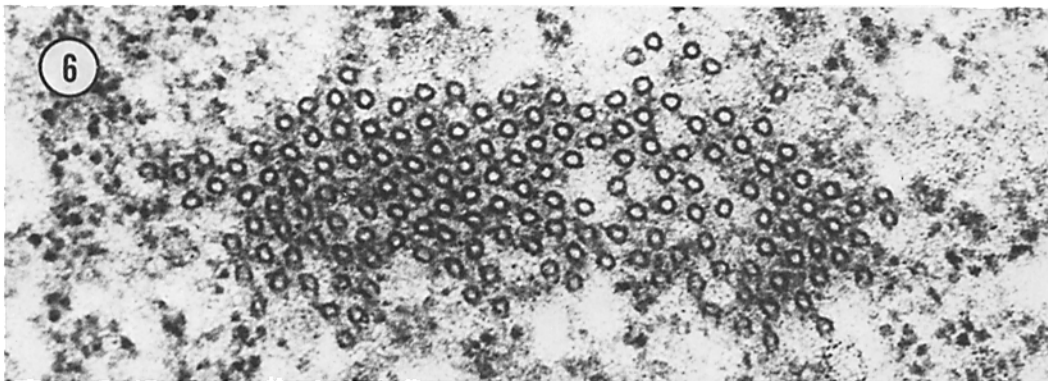
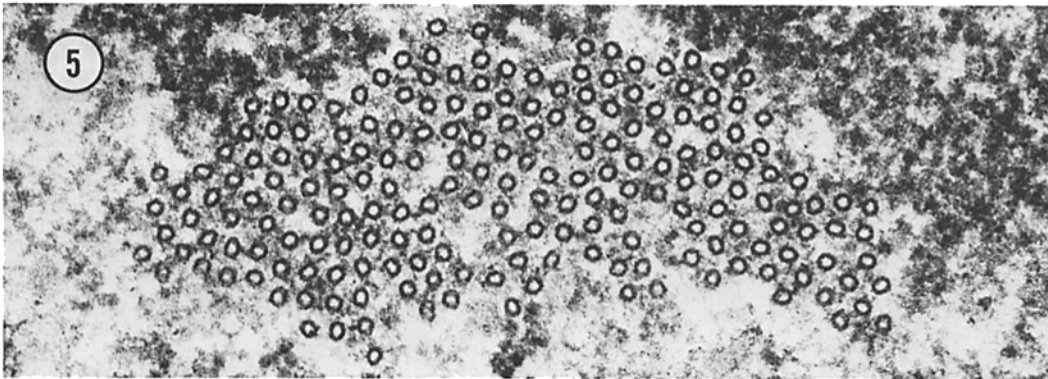
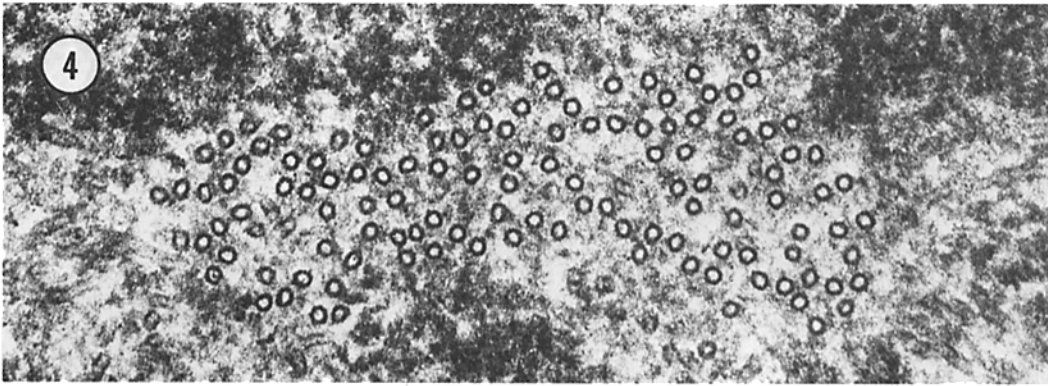


FIGURE 4 Cross section through a metaphase central spindle outside the region of overlap. $\times 100,000$.

FIGURE 5 Cross section from the same spindle but in the region of overlap. $\times 100,000$.

FIGURE 6 Cross section from an anaphase spindle in the region of overlap. Compare the staining intensity of the intermicrotubule matrix with that of the same region in Figs. 5 and 7. $\times 100,000$.

FIGURE 7 Cross section from a telophase spindle in the region of overlap. The intermicrotubule matrix is densely stained. $\times 100,000$.

PPCs, which are of approximately constant size and shape from cell to cell (17), are somehow involved in the organization of the central spindle.

These data also reveal the structural changes in the central spindle as it proceeds from metaphase to telophase: (a) as the total spindle length increases, the number of MTs in the overlap region decreases; (b) there is elongation of the few continuous MTs from metaphase through late anaphase; (c) there is an increase in the density and a decrease in the volume of the osmiophilic material in the overlap region. What do these structural changes imply about the possible mechanism of anaphase spindle elongation?

It is our opinion that the most likely mechanism for spindle elongation in *D. vulgare* is the sliding apart of half-spindles as a result of some MT-MT interaction in the overlap region. We favor this interpretation because it is the simplest one that explains our observations. Other interpretations are possible but much more complicated. For example, one could imagine that the redistribution of MTs from the metaphase configuration (Fig. 13) to the telophase configuration (Fig. 17) is accomplished by the removal of subunits from the free end of the MTs in the overlap, concurrent with the addition of subunits to the polar ends. This model requires a microenvironment at the poles of the spindle that favors microtubule assembly and a microenvironment at the overlap that promotes disassembly. The vacuoles at each pole (17) and the dense intermicrotubule matrix at the midregion could be the morphological expressions of these physiological differences. Still, there remains the problem of the origin of the subunits for polar growth. Is it newly synthesized tubulin or the disassembled tubulin from the midregion? And, if the latter, how is it moved specifically from the overlap to the poles? Because of these complications, we are inclined to regard this explanation as unlikely until such time as our simpler hypothesis is disproved.

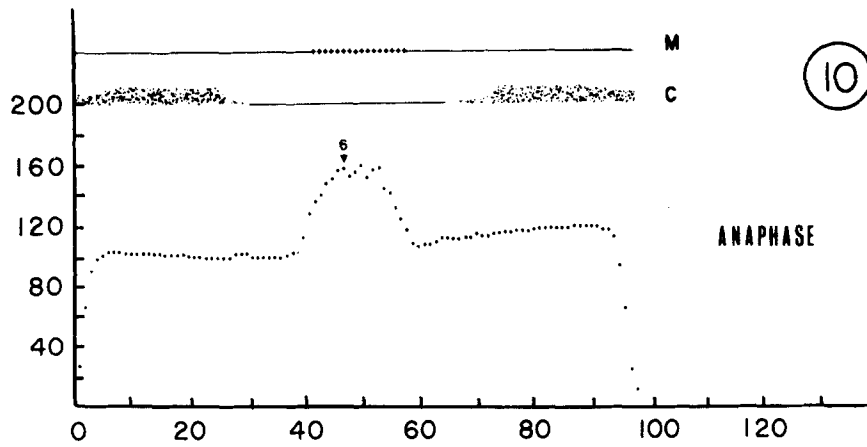
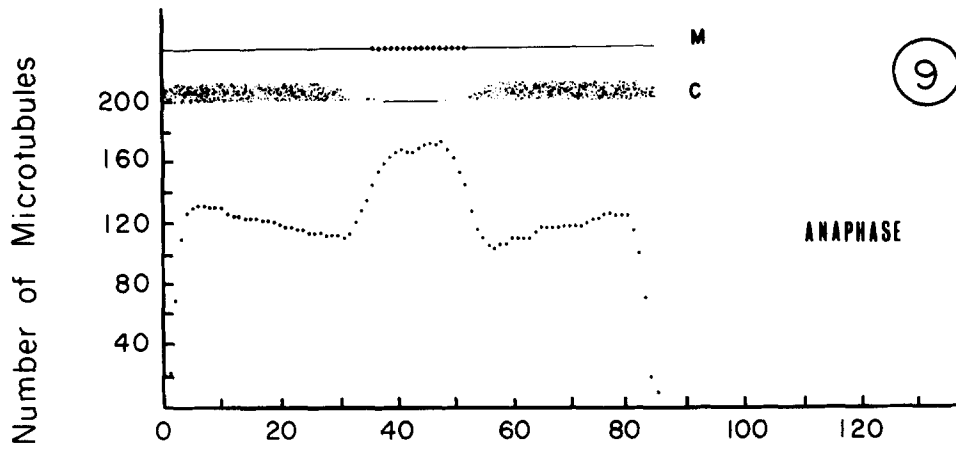
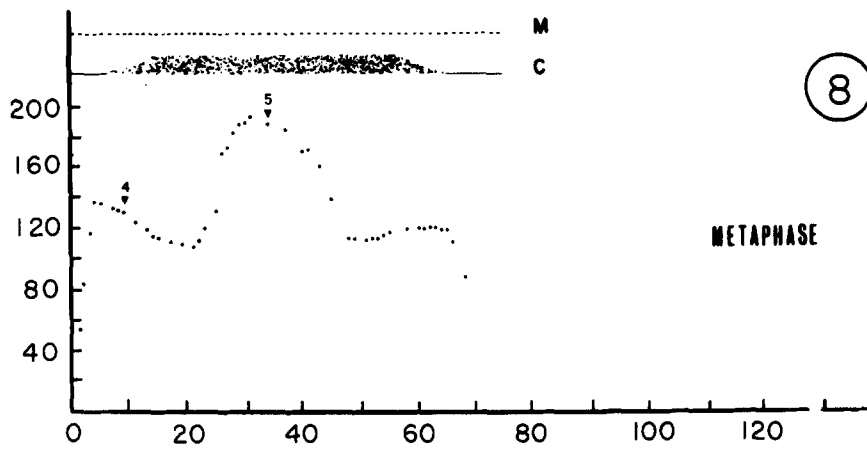
A MT-MT sliding mechanism requires the MTs in the overlap region to be packed tightly enough to allow forces to be generated between them. As one can see from Figs. 4-7, this requirement is satisfied. Also, MTs from one pole must be preferentially associated with MTs from the opposite pole. Our preliminary investigation of this relationship in the overlap zone shows that this is indeed the case. The spatial analysis of the MT-MT interdigitation in the overlap will be the subject of a separate paper. Finally, the morphological changes in the overlap zone during anaphase

(increased density of the intermicrotubule matrix) suggest that it may be a functionally active region of the spindle. However, whether or not that activity is mechanochemical remains to be seen. It is noteworthy that these changes in density closely resemble what one observes during midbody formation in mammalian cells (11).

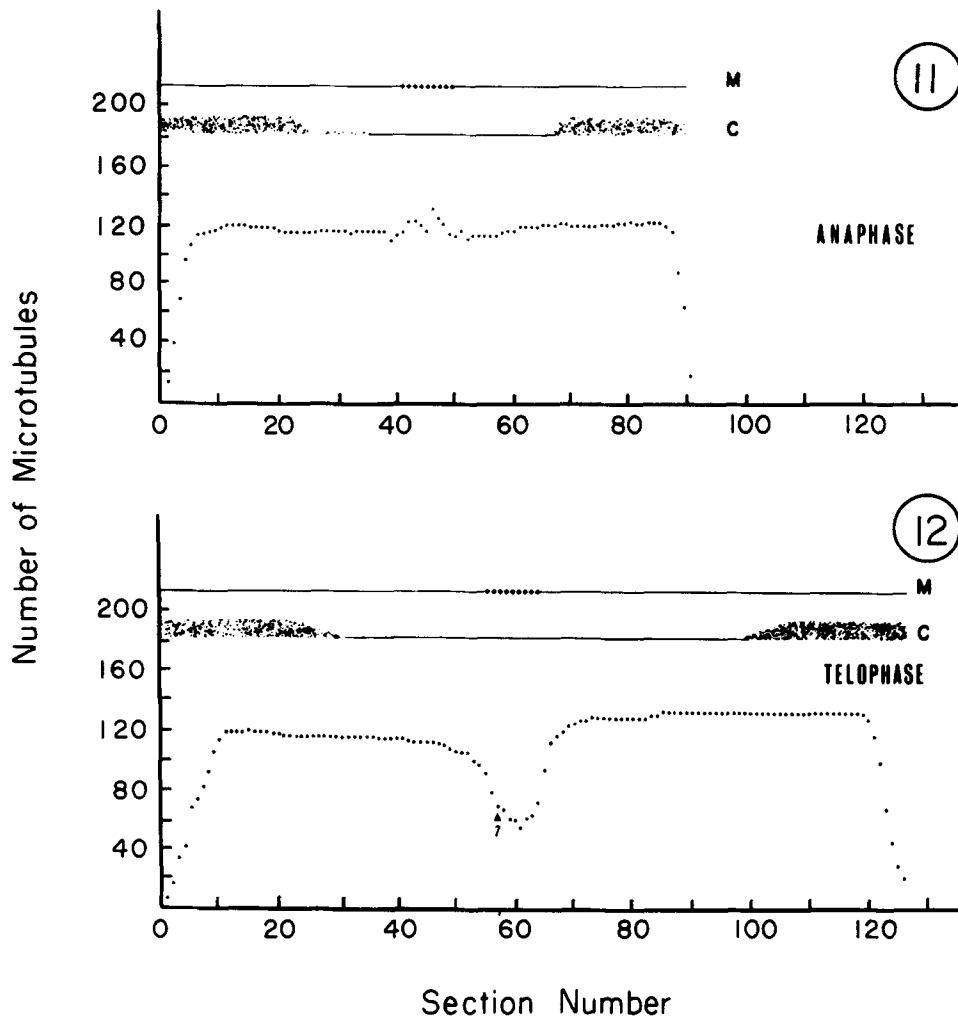
In some organisms (6) there is reason to believe that growth of continuous MTs is responsible for spindle elongation. Because there are no continuous MTs at telophase in *D. vulgare*, we believe this explanation to be unlikely. There are continuous MTs in the earlier stages of spindle elongation and perhaps they are functionally important, but because of their low frequency we believe that they do not constitute a significant component involved in spindle elongation. Although there are probably enough of these continuous MTs to generate the force required for spindle elongation (16), it seems unlikely that such a critical cellular event as spindle pole separation would be entrusted to such a small fraction of the spindle. The possibility also exists that there are *no* continuous MTs in the central spindle of *D. vulgare*, i.e., that the few we describe are a result of tracking mismatches. This would occur if the end of a polar MT from one half-spindle were sufficiently close (within one section thickness and in approximate register along the spindle axis) to the beginning of a MT from the other half-spindle. Under these circumstances, they would appear as one MT profile in cross-section. When one considers that up to 200 MTs are intermeshed in the overlap region, one could expect this coincidence to happen in a small percentage of cases.

Comparisons of length between spindles (where length is measured in number of sections) must be made with caution. In general, our length data from serial sections agree with actual measurements from longitudinal sections, but there are some problems. For example, our late anaphase spindle (defined by morphological criteria such as density of the intermicrotubule matrix) was shorter (in number of sections) than the spindle of the earlier anaphase cells. This could be an accurate reflection of spindle length variation in *D. vulgare* (whose cells, it should be remembered, vary in size as in other diatoms), or it could be due to differences in section thickness. We are currently trying to obtain more accurate estimates of spindle and MT lengths from longitudinal sections and by measuring the actual thickness of serial cross sections.

Motility systems such as muscle, flagella, and



Section Number



FIGURES 8-12 MT distribution profiles of central spindles in metaphase (Fig. 8), early to mid-anaphase (Figs. 9 and 10), late anaphase (Fig. 11), and telophase (Fig. 12). Above each graph the distribution along the spindle axis of chromatin (stippled areas on line labeled C) and densely staining intermicrotubule matrix material (diamonds on line labeled M) is indicated. The exception to this is Fig. 8, where no distribution is indicated for the matrix material because it was not dense enough to distinguish from other areas of the spindle. The arrows in Figs. 8, 10, and 12 indicate the positions of the sections used for Figs. 4-7.

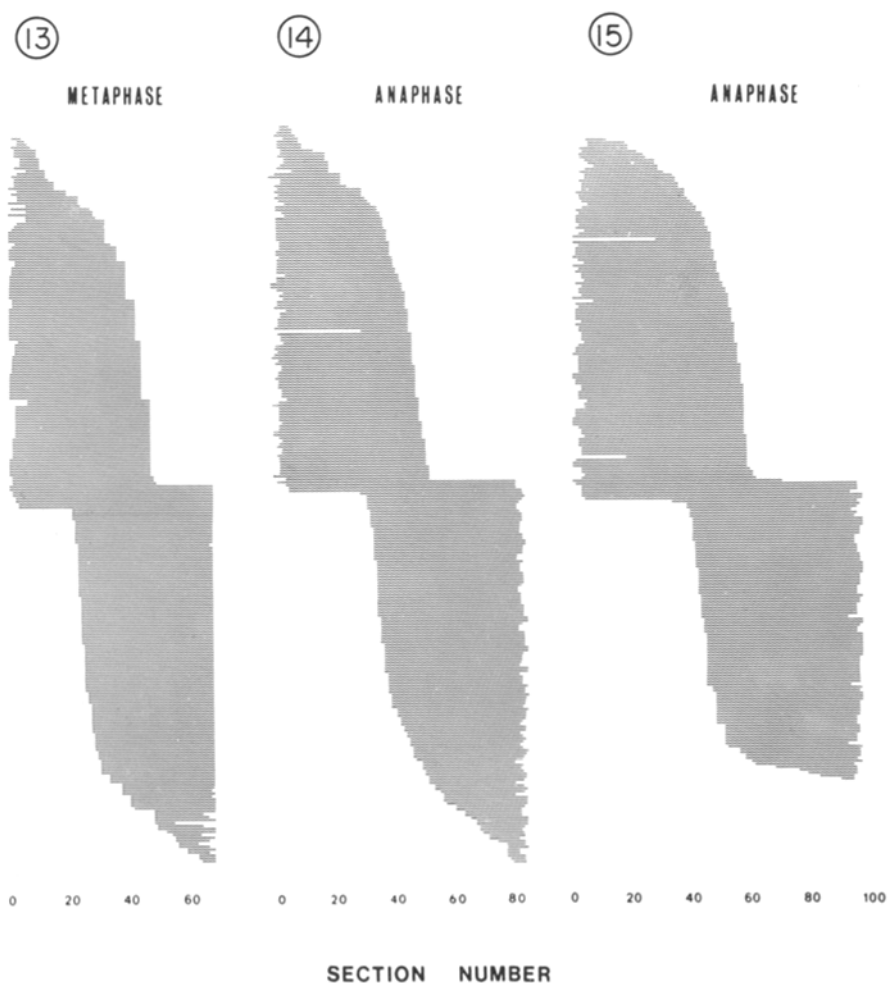


FIGURE 13 The distribution of individual MTs from a central spindle at metaphase. Each MT was tracked through serial sections, then arranged along the spindle axis (= x -axis) according to its point of origin and point of termination. The y -axis has no significance. In this spindle there are eight MTs at the center of the figure which extend from pole to pole. The simultaneous ending of many MTs at the right hand pole is due to the loss of two sections in that area.

FIGURE 14 Distribution of individual MTs in an early to mid-anaphase spindle. Four MTs extend from pole to pole.

FIGURE 15 As for Fig. 14, except that 6 MTs are continuous from pole to pole.

axostyles have a common structural characteristic which has been of great advantage to those working on their mechanochemistry. They are all highly ordered systems. Thus, it is possible to detect changes in structure at the macromolecular level and to correlate these with structural changes at higher levels of organization. By comparison, mitotic systems are usually much less ordered.

They have the further complications that less energy is required to produce chromosome separation (16) and that more than one motility system may be operating concurrently in the spindle (14). The structural features of the *Diatoma* central spindle allow us to overcome some of these difficulties, and for this reason we are encouraged that further work with this system will provide

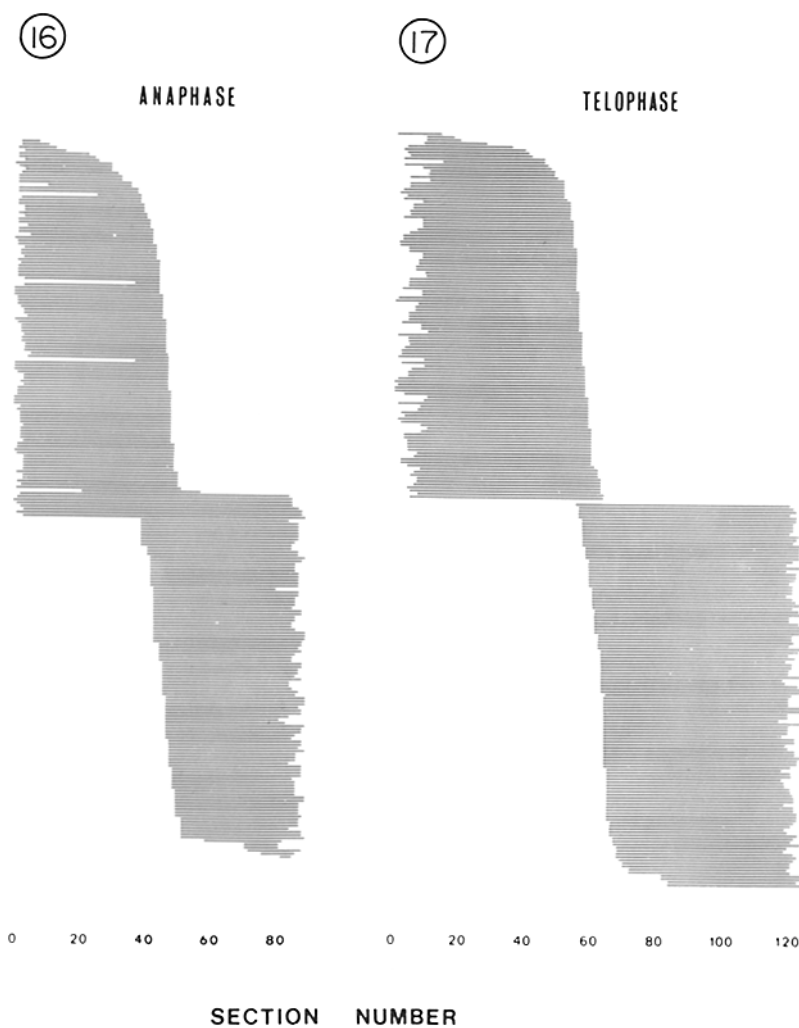


FIGURE 16 Distribution of MTs in a late anaphase central spindle. Eight continuous MTs.

FIGURE 17 Distribution of individual MTs in a telophase central spindle. All MTs are polar MTs.

new insights into the mechanochemical basis of spindle elongation.

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