Organization of Spindle Microtubules in *Ochromonas danica*

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ABSTRACT   The entire framework of microtubules (MTs) in the mitotic apparatus of *Ochromonas danica* is reconstructed (except at the spindle poles) from transverse serial sections. Eleven spindles were sectioned and used for numerical data, but only four were reconstructed: a metaphase, an early anaphase, a late anaphase, and telophase. Four major classes of MTs are observed: (a) free MTs (MTs not attached to either pole); (b) interdigitated MTs (MTs attached to one pole which laterally associate with MTs from the opposite pole); (c) polar MTs (MTs attached to one pole); (d) kinetochore MTs (kMTs). Pole-to-pole MTs are rare and may be caused by tracking errors. During anaphase, the kMTs, free MTs, and polar MTs shorten until most disappear, while interdigitated MTs lengthen. In the four reconstructed spindles, the number of MTs decreases between early anaphase and telophase from 881 to 285, while their average length increases from 1.66 to 4.98 μm. The total length of all the MTs in the spindle (placed end to end) remains at 1.42 ± 0.04 mm between these stages. At late anaphase and telophase the spindle is comprised mainly of groups of interdigitated MTs. Such MTs from opposite poles form a region of overlap in the middle of the spindle. During spindle elongation (separation of the poles), the length of the overlap region does not decrease. These results are compatible with theories that suggest that MTs directly provide the force that elongates the spindle, either by MT polymerization alone or by MT sliding with concomitant MT polymerization.

Microtubules (MTs) undergo positional rearrangements and changes in length during mitosis. Consequently, many theories of chromosome movement postulate that MTs produce the force that moves chromosomes at anaphase (i.e., by sliding etc.). One important aspect of research into mitosis involves unraveling the complex structure of the spindle. The vast amount of literature on the fine structure of the spindle conceals the fact that there are almost no precise data about the number of MTs, the position of their end points and their spatial organization at different mitotic stages (8, and references therein). The most reliable technique for acquiring such data involves serially sectioning the entire spindle and then reconstructing it by tracking each circular MT profile through the series of transverse serial sections. Besides the enormous technical problems presented by this approach, a major obstacle is the limited selection of cell types available whose spindle MTs are straight enough to be tracked. The small cells of algae and fungi have thus far proved to be the best experimental material.

The mitotic apparatus of diatoms contains a central bundle of MTs, named the central spindle, which was first described by Lauterborn in 1896 (14). The central spindle has been reconstructed by the method described above at each mitotic stage (28); other MTs radiating from the poles could not be reconstructed. In these diatoms, as in nearly all other cell types, the chromosome movements at anaphase occur in two distinct stages: (*a*) the chromosomes move to the poles; (*b*) the spindle elongates (the poles move apart) which further separates the chromosomes. The mechanism of chromosome-to-pole movement is not understood. During spindle elongation in diatoms, the MTs of the central spindle change their distribution in a way that suggests that MTs generate the force that separates the spindle poles. The most likely mechanism is MT-MT sliding, although alternate mechanisms involving polymerization and depolymerization of MTs also fit the data. The unusual structure of the diatom spindle (which allows the central spindle MTs to be tracked) prompted the suggestion that it may not be representative of more conventional spindles. In this paper, we reconstruct the entire framework of spindle MTs (except near the poles) in the Chrysophyte alga *Ochromonas danica* at metaphase, anaphase, and telophase, to further characterize the structure of the mitotic apparatus and the mechanism of spindle elongation. This organism contains a spindle characteristic of higher animal and plant mitosis (25, 3). *Ochromonas* is one of the few cell types known in which

every MT in the spindle can be reconstructed from serial sections; it has been found to have a central spindle analogous in organization but not in behavior to that of the phyletically related diatoms.

MATERIALS AND METHODS

Terminology

Fig. 1 shows our classification for different types of spindle MTs (after reference 19): continuous MTs run from pole to pole; free MTs are not attached at either pole, but instead have both ends free in the spindle; polar MTs have one end at the pole and the other end free; kinetochore MTs (kMTs) have one end at a kinetochore. MTs with one end at a kinetochore and the other at a pole are by definition kMTs; interdigitated MTs (from reference 22) have one end attached to the pole and the other end (or any point along its length) laterally associated (within a distance of 40 nm center to center) with an MT attached to the opposite pole. The distance of 40 nm was chosen because it is an upper limit for the length of MT bridges (22, and references therein). This distance was trial tested on the late anaphase spindle; the results showed that nearly all of the MTs from opposite poles which are near neighbors are within 40 nm of each other. Interdigitated MTs from opposite poles create a region of overlap (Fig. 1). One end of each half spindle is defined by the edge of the overlap, while the other end is at the pole. This is most easily visualized in the telophase reconstruction (Fig. 15), where all the MTs on the top half of the diagram belong to one half spindle and all the remaining MTs (excluding free MTs) belong to the other. Late metaphase is distinguished from early metaphase by the arrangement of spindle MTs. The MTs in late metaphase spindles are more highly organized (straight and thus trackable) than during prometaphase and early metaphase.

Electron Microscopy and Culturing

Cultures of Ochromonas danica were obtained from the University of Texas algae culture collection and were grown in the following aqueous solution containing: 0.1% tryptone, glucose, and yeast extract (Difco Laboratories, Detroit, Mich.), and 0.02% liver infusion extract (Sigma Chemical Co., St. Louis Mo.) For electron microscopy, cells were fixed in 1% glutaraldehyde and phosphate buffer for 1 h, then rinsed three times in buffer and fixed 4 h in osmium tetroxide. After a water rinse, the cells were placed in a 1% solution of uranyl acetate for 8 h followed by dehydration in acetone and embedding in Spurr's (26) resin. Preselected dividing cells were serially sectioned as in previous studies (17).

MT Tracking and the Organization of the Spindle Reconstruction

Techniques for tracking MTs through serial sections are described elsewhere (17, 28, 27). Briefly, this technique is accomplished as follows: an entire spindle is serially sectioned, photographed, and then printed at approx. × 110,000. An outline of each MT in section No. 1 is traced onto clear acetate (28 × 33 cm). This acetate overlay is placed onto section No. 2 and the best fit of MTs is determined. Similarly, the MTs in section No. 2 are identified in section No. 3 and so on. A graph is then generated showing in one-dimensional form the end points of each MT in the spindle (Figs. 5, 6, 9, 15); we call these graphs reconstructions. Along the horizontal axis of the reconstructions is the number of sections beginning at one spindle pole and extending to the other. Along the vertical axis, each line represents a single MT. The kMTs, polar MTs, and interdigitated MTs are not separated into groups in these diagrams; instead the MTs are arranged by length. Free MTs are grouped separately at the bottom of each reconstruction and are arranged according to their starting point. The
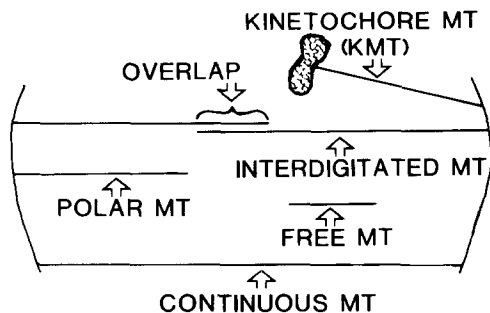
position of the chromatin along the spindle axis is also displayed at the bottom of each reconstruction.

The spindle pole is operationally defined as the region where the polar MTs, interdigitated MTs, etc. terminate, and is represented as a box in the three reconstructions in Figs. 5, 6 and 9; in Fig. 5 the spindle pole extends between section Nos. 1-12 and 62-72. In the late metaphase (Fig. 5), early anaphase (Fig. 6), and late anaphase (Fig. 9) reconstructions, the MTs near the poles are terminated where tracking was stopped. Thus, in these reconstructions, one end of each MT (except free MTs) in both half spindles terminates in a section close to the spindle pole. For example, all the MTs from the left pole of the metaphase reconstruction in Fig. 5, terminate in section No. 15. These MTs are not tracked into the spindle pole to their end points, because the MTs converge slightly inward at the pole, making tracking difficult and inaccurate.

Figs. 16 and 17 are silhouettes of the reconstruction in Figs. 5, 6, 9, and 15. These two figures were made as follows: the outline of the MTs in Fig. 5, for example, is traced (free MTs are omitted). The midpoint of each spindle pole is used as the polewards side of the tracing (sections Nos. 6 and 67 in Fig. 5). The outlines of the different spindles are placed on top of one another using the overlap as the center point.

Method of Obtaining Indirect Length Measurements of MTs

The total length of all the MTs (placed end to end) in the spindle (Table II) is calculated by first determining the length of each MT by counting the number of sections an MT traverses and multiplying by section thickness (80 nm). All of the individual MT lengths are then summed. Because the MTs are not tracked into the spindle pole in Figs. 5, 6, and 9, we have assigned the end point of these MTs at the midpoint of each spindle pole. For example, to calculate total length of the MTs in Fig. 5, the MTs from the left pole are terminated in section No. 6, those from the right pole in section No. 67.

The overlap length in Table II was calculated by two different methods. Both of these methods use the data in the reconstructions (Figs. 5, 6, 9, and 15). We use the term overlap to describe the region that contains all the overlapping MTs. Method No. 1: the limits of the overlap are defined by the longest MTs from each half spindle. As one abnormally long MT (in relation to other MTs in that half spindle) may disproportionally affect the size of the overlap, we disregard the two longest MTs from each half spindle. Thus, in the telophase spindle (Fig. 15) the overlap in method No. 1 is defined as the region between section Nos. 58 and 93; there are 256 MTs that terminated between these two sections. The length (in sections) of each one of the 256 MTs is determined and then these individual MT lengths are summed (5,485 U at telophase. Table II). The overlap in the other spindles are similarly analyzed; in each only the 256 longest MTs (128 from each pole) are used in this calculation (the number of MTs at telophase is used as a reference). Thus we are comparing at different stages the length of the overlap that is generated by the 128 longest MTs from each pole. At metaphase and anaphase, there are >256 MTs that overlap (Figs. 5, 6, and 9). As the half spindle at metaphase is shorter than at telophase, many of the short polar MTs at metaphase overlap with the longest MTs from the opposite pole. This will bias any simple measure of the length of overlap. For example, if we calculate the length of all the overlapping MTs at metaphase (instead of 256), the overlap length is 13,718 U, which is not a useful number for comparison to the telophase overlap. Method No. 2: a straight line is fitted to the nonpolar end of the MTs in the reconstructions (Figs. 5, 6, 9, and 15) and is extrapolated to meet the horizontal line that defines the boundary between the two half spindles (as in reference 21). The distance along the abscissa between the two intersection points is the overlap length in sections (section thickness used is 80 nm).

Determination of Different Types of MTs

Interdigitated MTs by definition are those MTs from one pole within 40 nm (in any section along their length) of an MT from the opposite pole. At metaphase (Table I), to separate interdigitated MTs from the group of MTs that terminate in the chromatin, we first analyze the end points of all MTs and determine which are interdigitated MTs. The remaining MTs are checked in each section through the chromatin to determine their classification.

RESULTS

General Features of Mitosis

We have examined three species of Ochromonas (O. danica, O. minuta, O. malhamensis) and have determined O. danica to be the most suitable for MT tracking. Between metaphase (Fig. 2a) and telophase (Fig. 2b), the spindle of O. danica elongates

FIGURE 1   This diagram illustrates our classification of spindle MTs.

Stage of mitosis	Total MTs	Free MTs		Polar MTs		Continuous MTs		Interdigitated MTs		MTs that terminate in chromatin	
			% Total		% Total		% Total		% Total		% Total
Late metaphase	953	62	6.5	274	29.0	2	0.2	236	24.8	379	39.8
Early anaphase	881	21	2.4	201	22.8	1	0.1	351	39.8	307	34.8
Late anaphase	540	2	0.4	107	19.8	0	0	262	48.5	169	31.3
Telophase	285	4	1.4	25	8.7	0	0	253	88.8	3	1
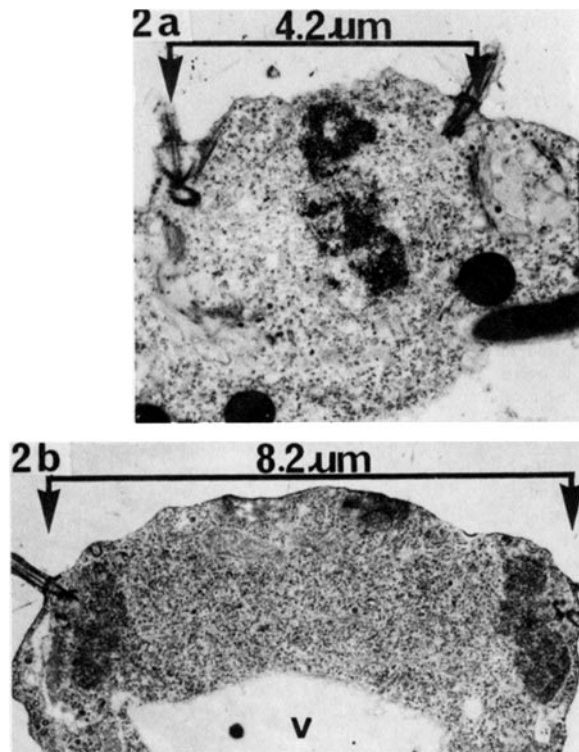
FIGURE 2   These micrographs show the elongation of the spindle during later mitosis. Fig. 2a is a metaphase spindle and Fig. 2b is a late telophase. A large vacuole (v) occupies most of the cell.

at least 4 μm. All of the spindle MTs contain a characteristic central core of unknown composition (Figs. 4 and 10). At metaphase, the chromatin is tightly compacted into the metaphase plate and individual chromosomes are not discernible. Kinetochores are difficult to identify, but we suspect that a lightly staining indentation in the chromatin is the kinetochore (Fig. 3). A single MT usually terminates at each kinetochore after metaphase (Fig. 3, large arrow and Fig. 4a-c). We have not observed two or more MTs ending at a kinetochore, although many MTs terminate nearby at the leading edge of chromatin (Fig. 3, double arrow). It is not known if these latter MTs function as kMTs or if they are polar MTs which by chance terminate at the leading edge of the chromatin.

Spindles at prophase, prometaphase, and early metaphase are not reconstructed because the disorganized arrangement of MTs during early mitosis does not permit MT tracking. At prophase, the spindle forms between two rhizoplasts (this structure is present during interphase and is associated with the basal bodies; it replicates at prophase and acts as the spindle
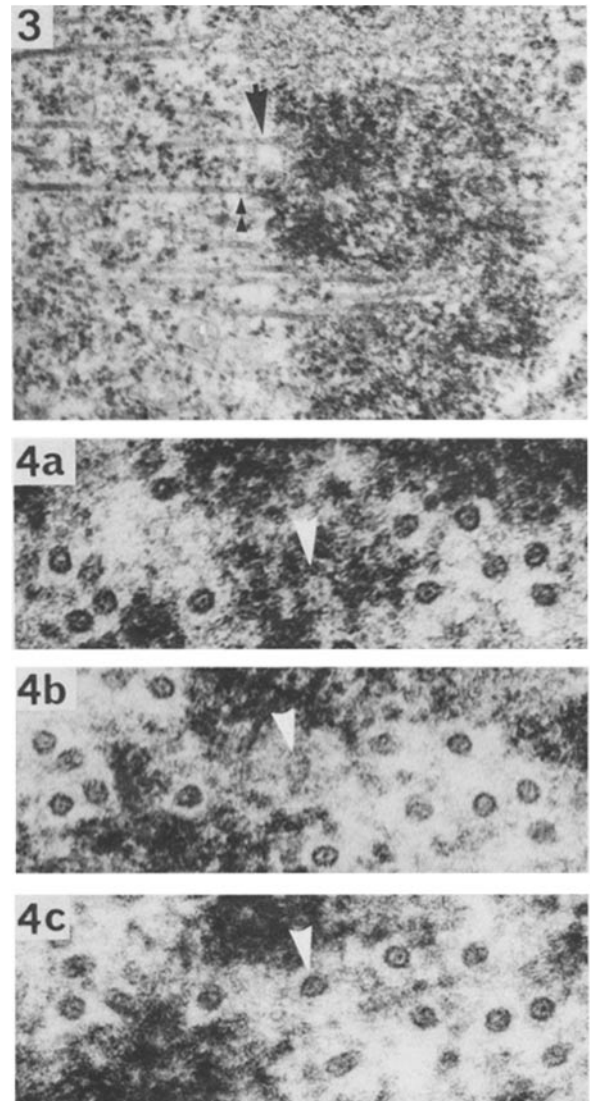
FIGURE 3   A longitudinal section from a metaphase spindle. A single MT (large arrow) terminates in a lightly staining indentation in the chromatin that we believe is the kinetochore (see Fig. 4 a-c). A nearby MT (double arrow) terminates at the leading edge of the chromatin; it is not known if such MTs function as kMTs or if they are polar MTs which by chance terminate at the edge of the chromatin.

FIGURE 4   Three consecutive serial transverse sections of a metaphase spindle showing the starting point of a kMT (arrow) similar to the one in Fig. 3 (large arrow). The cuplike kinetochore region is in Fig. 4b. × 103,000.
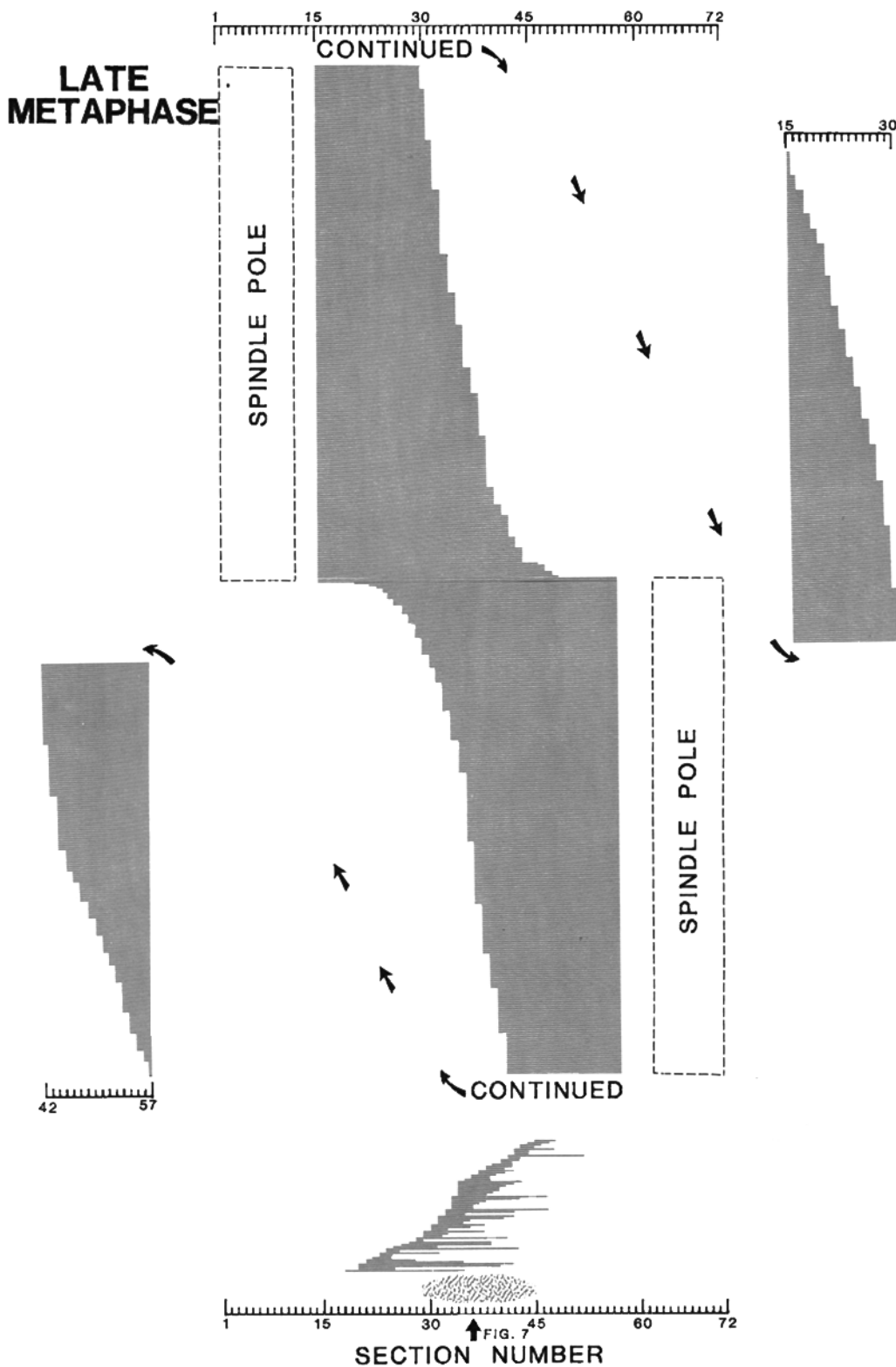
FIGURE 5 This diagram and Figs. 6, 9, and 15 are reconstructions of spindle MTs. Each reconstruction shows the end point of all the MTs within a mitotic apparatus; different MT types (kMTs, polar MTs, etc.) are not designated in these diagrams. Along the horizontal axis is the section number starting at one pole and extending to the other. Along the vertical axis each line represents an individual MT; collectively these lines show the end point of each MT in the spindle. These diagrams clearly do not show the spatial arrangement of MTs within the spindle. The two spindle poles are defined as the region where polar MTs, kMTs, etc. terminate and are labeled and boxed. For example, the spindle poles at late metaphase (Fig. 5) are at section Nos. 1–12 and 62–72. The MTs from each pole are arranged according to length. Free MTs are displayed as a separate group of lines at the bottom of each diagram. The MTs are not tracked into the spindle pole (all the MTs from the left pole in Fig. 5 were tracked only to section No. 15; see Materials and Methods). All the MTs from one pole or half spindle are set on top of the MTs from the other pole, however, because of space limitations, the extreme top and bottom of some of these reconstructions is displaced (follow arrows) to the side. The position of the chromatin along the spindle axis is displayed at the bottom of each diagram by cross-hatching. Many of the micrographs in this report are taken from these reconstructions; this is indicated at the bottom of each reconstruction. For example, Fig. 7 is a micrograph of section No. 36 in the late metaphase reconstruction (Fig. 5).

534

EARLY
ANAPHASE

SPINDLE POLE

CONTINUED

SPINDLE POLE
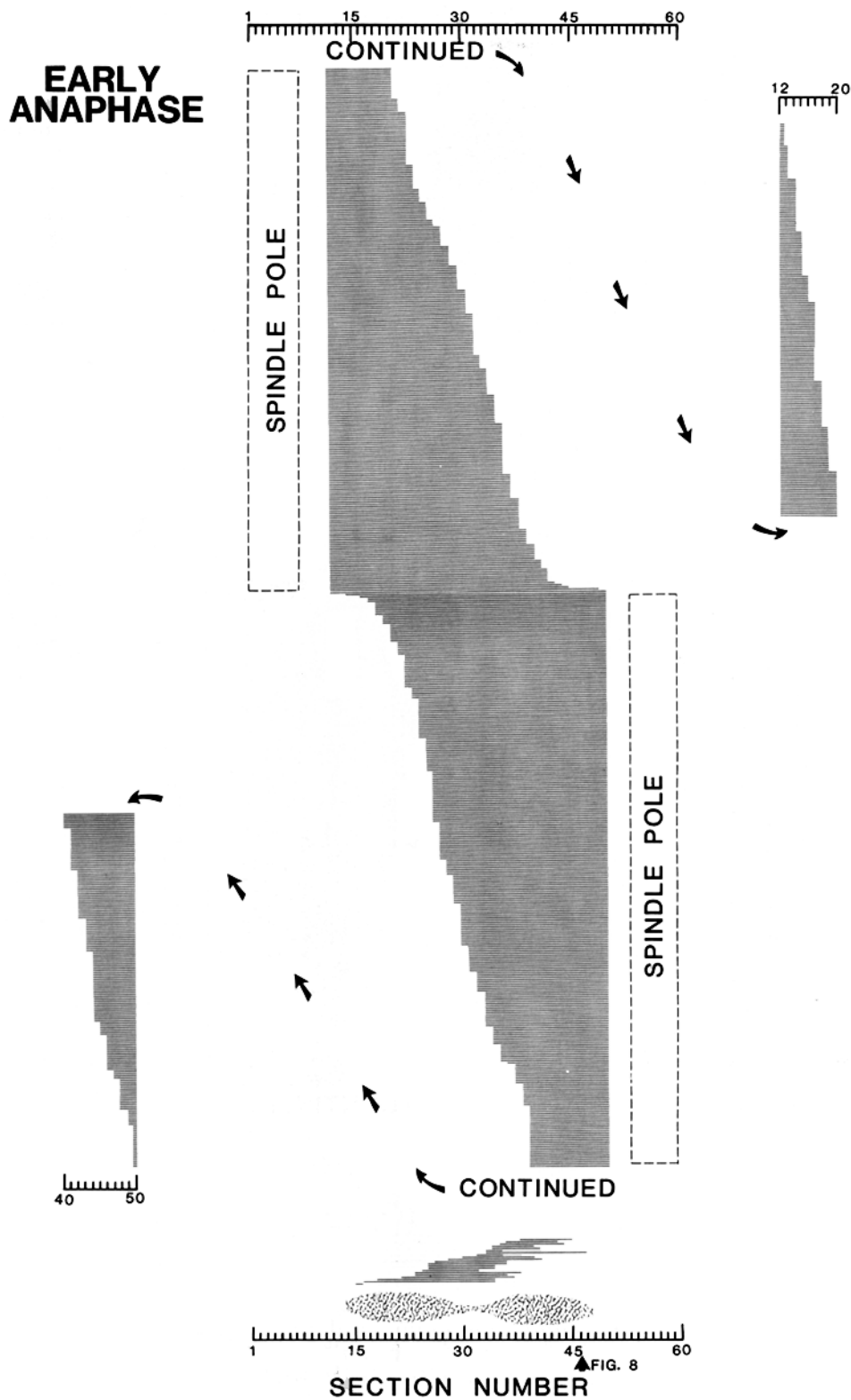
CONTINUED

SECTION NUMBER

FIG. 8

FIGURE 6   Early anaphase reconstruction of spindle MTs. See figure legend 5 for an explanation of this type of diagram.

pole body during mitosis [3]). At the spindle pole at metaphase and later is a diffuse platelike structure where most of the MTs terminate (3, 25). As the spindle forms, MTs attached to opposite poles laterally associate and aggregate into small bundles. Thus, the unordered array of MTs radiating from the poles of the prophase spindle is transformed into an ordered array of MTs by metaphase (Fig. 7).

Fixation of MTs

The validity of these results is dependent upon the assumption that MTs are not altered by fixation. However, we cannot eliminate the possibility that length changes of MTs or loss of free MTs could take place during fixation (see comments in references 5 and 9).
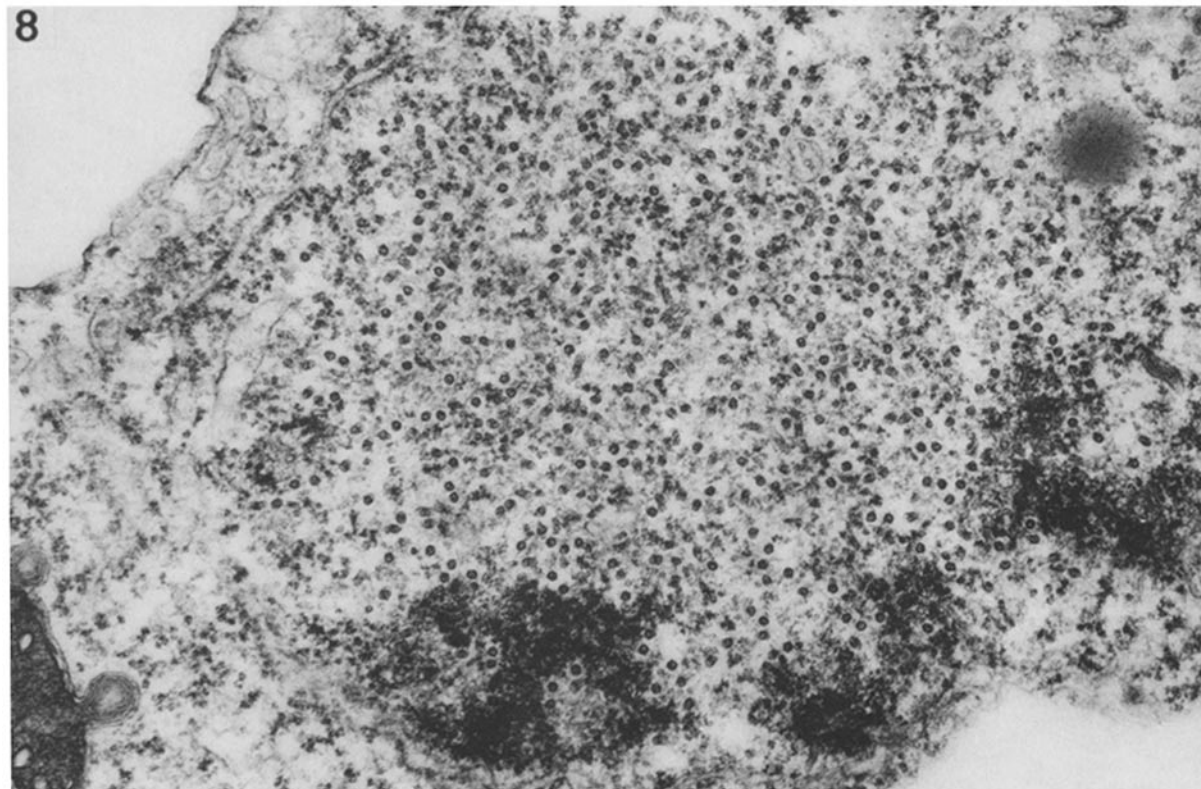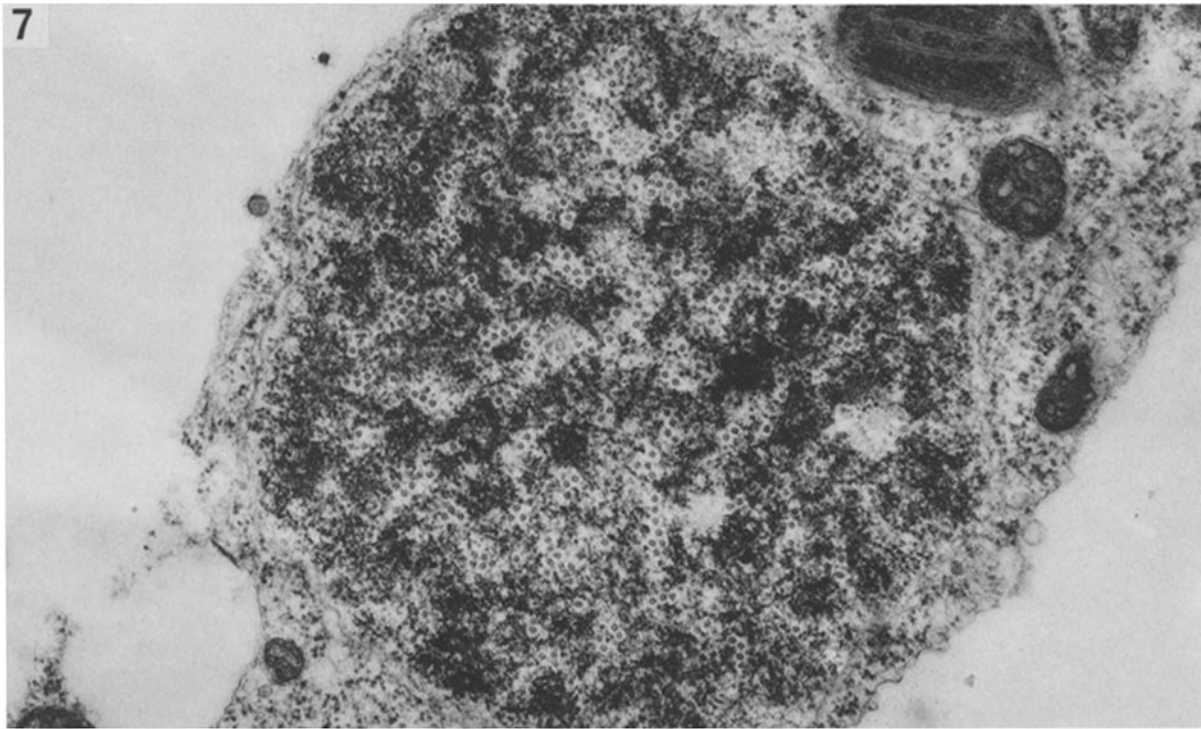
FIGURE 7 A micrograph of section No. 36 in the late metaphase reconstruction (Fig. 5). The chromatin is tightly compacted into the metaphase plate. The MTs from opposite poles aggregate into small bundles. Many of the 265 MTs in this section are not well observed at this magnification; the MTs are normally tracked at × 110,000. The presence of a MT in any particular section is determined by analyzing the position occupied by each MT in the preceding and following sections. × 24,000.

FIGURE 8 A micrograph of section No. 46 in the early anaphase reconstruction (Fig. 6). The leading edge of the chromatin is visible in the lower part of the picture. The 360 MTs in this micrograph (see comments in legend of Fig. 7) are more evenly spaced and unclustered in comparison with the overlap (Figs. 7 and 10). × 68,200.
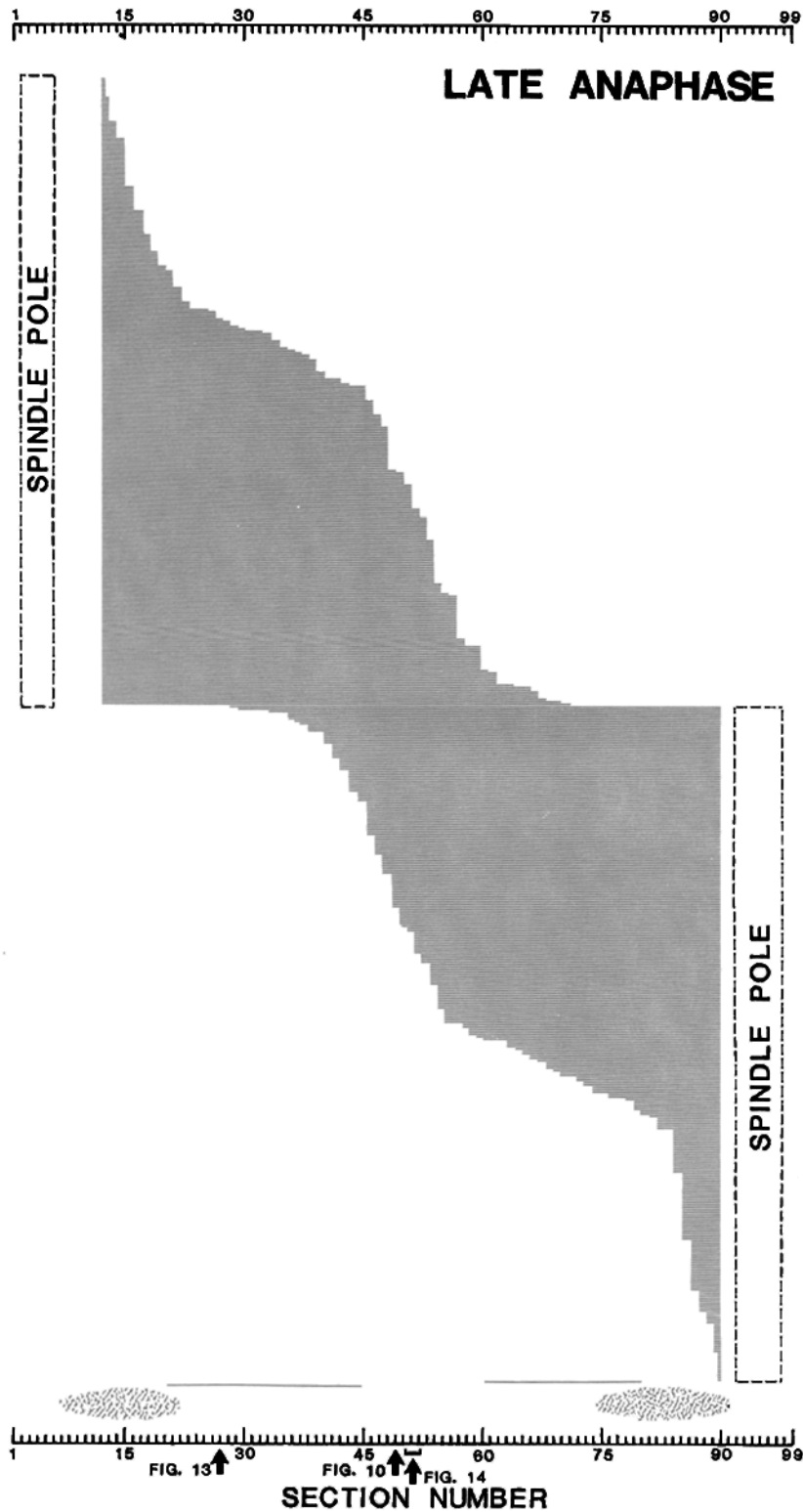
FIGURE 9   Late anaphase reconstruction of spindle MTs. See figure legend 5 for an explanation of this type of diagram.

Changes in the Distribution of MTs During Mitosis

Each MT from four spindles (late metaphase, early anaphase, late anaphase, and telophase) has been reconstructed from transverse serial sections (Figs. 5, 6, 9, and 15). Seven other spindles at similar stages of mitosis were sectioned and photographed but not fully reconstructed; numerical data from these spindles appears in Table III. All of the micrographs in this paper are taken from the reconstructed spindles and the position of each micrograph is noted at the bottom of the reconstructions. For example, Fig. 7 is a micrograph of section No. 36 from the metaphase spindle reconstructed in Fig. 5. Occasionally the number of MTs in the micrograph does not
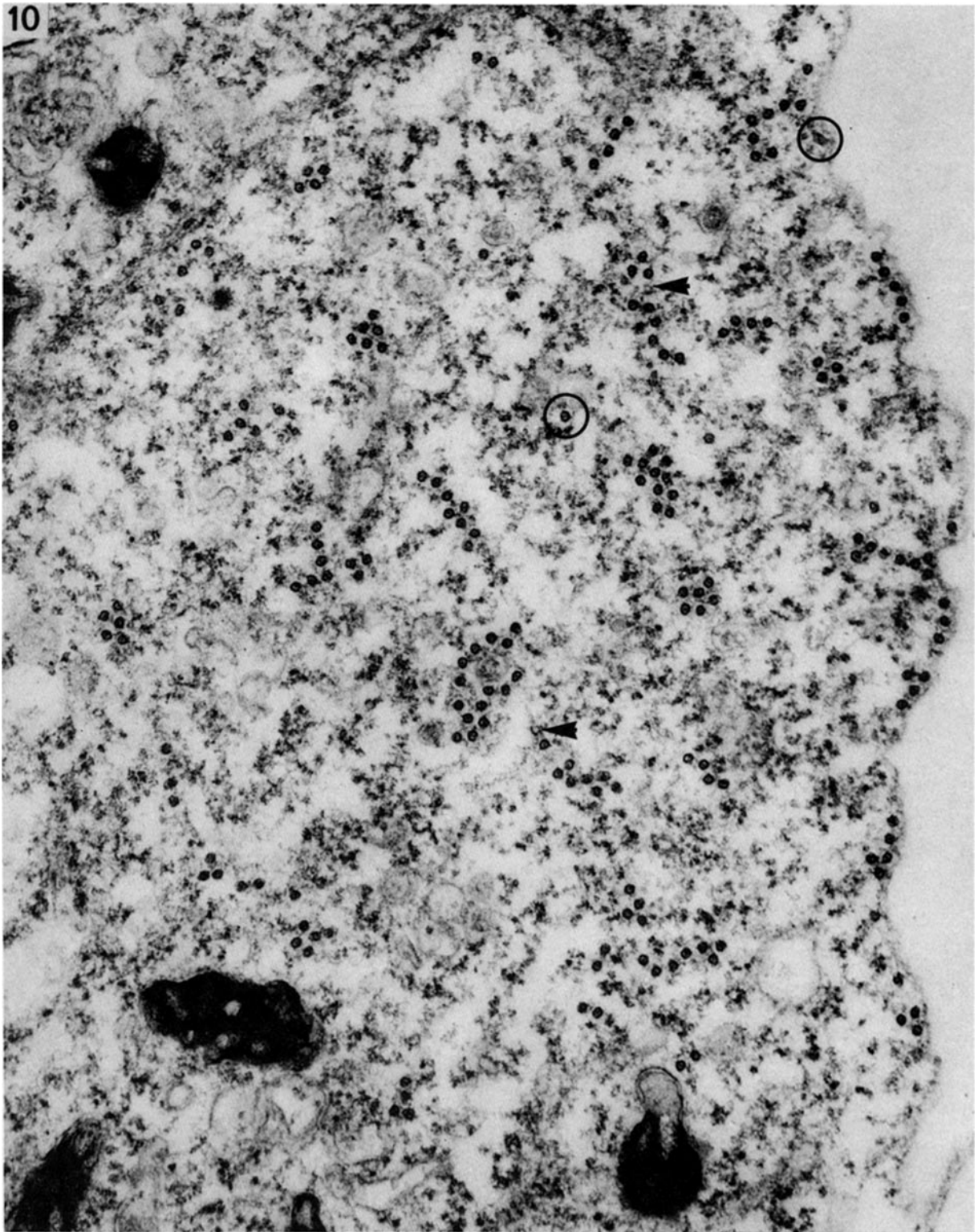
FIGURE 10   A micrograph of the overlap region (section No. 49) from the late anaphase reconstruction (Fig. 9). The MTs are clustered into bundles. This section contains 190 MTs; 188 of these are interdigitated MTs and the remaining two circled MTs are polar MTs, which do not laterally associate with MTs from the opposite pole. The two arrows show examples of MTs about to terminate. × 54,900.

correspond exactly to the number of MTs in the reconstruction (Figs. 7 and 8) because of a minor ambiguity in positively assigning the end points of MTs to one of two adjacent sections. In Fig. 10, for example, the two arrows denote MTs which are about to terminate and could be assigned an end point in any

of three sections. There may occasionally be up to 20 such MTs in a selected section.

The MTs in the late metaphase, early anaphase, and late anaphase reconstructions are not tracked into the spindle pole region; instead all the MTs are drawn as terminating in one

section where tracking was stopped near the spindle pole (see Materials and Methods). This does not influence the descriptions of the distribution of MTs which follows.

In this section we describe the length changes of all MTs without reference to different types of MTs. The kMTs in the reconstructions (Figs. 5, 6, 9, 15) can be identified, if necessary, as some of those MTs that terminate at the leading four sections of the chromatin (e.g., sections 28–31 in Fig. 5). The kinetochores in Figs. 3 and 4 cannot always be confidently identified and thus their distribution is not displayed on the reconstructions. Throughout mitosis, 97% of the spindle MTs are attached to the poles; these MTs extend a variable distance toward the middle of the spindle and then terminate at the kinetochore or have an unattached end. Between late metaphase and telophase, some MTs lengthen thereby increasing the length of the spindle, and other MTs shorten as the chromosomes move polewards (compare Figs. 5, 6, 9, and 15). This affects the distribution of MTs in the middle of the spindle. At metaphase (Fig. 5), 82 MTs from one pole and 67 from the other (149 total) are not long enough to overlap with the single longest MT from the opposite pole. By telophase (Fig. 15), only a total of 19 such MTs are present.

The changes in MT lengths can be visualized in Figs. 16 and 17, which are silhouettes of the reconstructions in Figs. 5, 6, 9, and 15. Fig. 16 is a superposition of the late metaphase and early anaphase reconstructions. The late metaphase spindle is 12 sections longer than the early anaphase. In comparison with other metaphase spindles we have examined, this appears to be abnormally long, (72 sections vs. 57 and 53 sections, Table III). Disregarding the overall length variation, there is a difference in the length of MTs in the middle of the reconstructed spindles. The single arrow in Fig. 16 in the bottom half of the spindle marks MTs in one half spindle that are longer at anaphase than at metaphase; the double arrow marks MTs that are shorter. The later stages of mitosis are shown in Fig. 17 which is drawn on the same scale as Fig. 16. Certain MTs continue to lengthen while others shorten and finally disappear. The length of the overlap region remains fairly constant during this transition.

Frequency of Different Types of MTs

Table I displays the frequency of different types of MTs in the reconstructed spindles in Figs. 5, 6, 9, and 15. The kMTs are not included as a class in this table, because we cannot positively identify every kinetochore, nor can we exclude the possibility that MTs that terminate at the leading edge of the chromatin (see Fig. 3, double arrow) also function as kMTs. Therefore we designate a new group of MTs for this analysis: MTs that terminate in the chromatin which are not interdigitated MTs or free MTs (see Materials and Methods section entitled Determination of Different Types of MTs). This group contains all the kMTs, and undoubtedly numerous polar MTs which terminate in the chromatin but do not attach to a kinetochore. The number of MTs in this group decreases from 379 at metaphase to 3 at telophase. Both the number and the percentage (of the total spindle MTs) of free MTs and polar MTs decrease after metaphase until telophase. The percentage of interdigitated MTs increases during this transition, although their number remains at 294 ± 58. Continuous MTs are rare and are not observed after early anaphase. The continuous MTs may result from tracking errors of the type illustrated in Figs. 14 a–c (see figure legend).

It is not known if the higher number of interdigitated MTs

in the early anaphase spindle is a result of cellular variability or reflects a real increase at this stage; this increase is also evident in Fig. 18 (see Results section entitled Number of MTs in the Spindle).

Spatial Arrangement of MTs

The spatial arrangement of the MTs in the half spindle near the pole at early anaphase is shown in Fig. 8; the metaphase half spindle is similar. In each, the MTs are somewhat evenly spaced and are not clustered together; during late anaphase and telophase, the MTs are also arranged thus (Fig. 13), although there are fewer MTs during later mitosis. In contrast to the half spindle, the MTs in the overlap are clustered together into small groups at all stages of mitosis (Figs. 7 and 10). At metaphase, these groups of MTs contain both polar and interdigitated MTs. During later mitosis (Fig. 10), the grouping of MTs is more pronounced. The interdigitated MTs now comprise >99% of the MTs in the overlap.

Fig. 10 is a micrograph of section No. 49 from the anaphase reconstruction (Fig. 9); Fig. 11 is a tracing of all the interdigitated MTs in Fig. 10 (see figure legends 10 and 11). Fig. 11 shows that the MTs are clustered into 27 groups of variable size. We determine which MTs constitute a group as follows: the overlap of the spindle is defined as the region between the longest MTs of each half spindle (between section Nos. 28–72 in Fig. 9). Any MT which terminates in the overlap region and is within 50 nm of another MT is placed into a group; MTs which are further than 50 nm apart cannot be placed into the same group. Although some MTs in a group are clearly more than 50 nm apart, (Fig. 11, arrow), these spaces are filled in by MTs that are out of the plane of this section. For example, Fig. 11 is a micrograph of section No. 49; the space in group 14 (arrow) is filled in by MTs appearing in section No. 60, thereby connecting together the MTs of this group. The 27 groups of MTs in Fig. 11 are displayed in Fig. 12, which is a longitudinal representation of these groupings and shows the end points of each MT within the 27 groups. For example, group no. 6 in
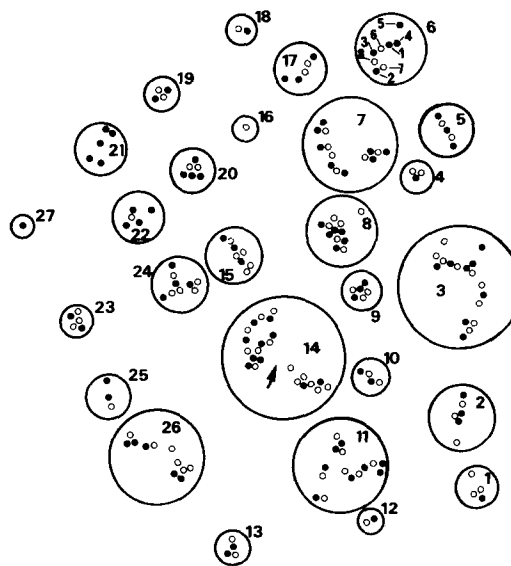

Figure 11   A tracing of the 188 interdigitated MTs in Fig. 10. The 97 solid circles denote MTs from the left pole in Fig. 9, while the 91 circles show MTs from the right pole. MTs from one pole seek out as near neighbors MTs from the opposite pole. The MTs cluster into 27 groups which are circled and are labeled 1-27. The MTs of these 27 groups are displayed in longitudinal form in Fig. 12.
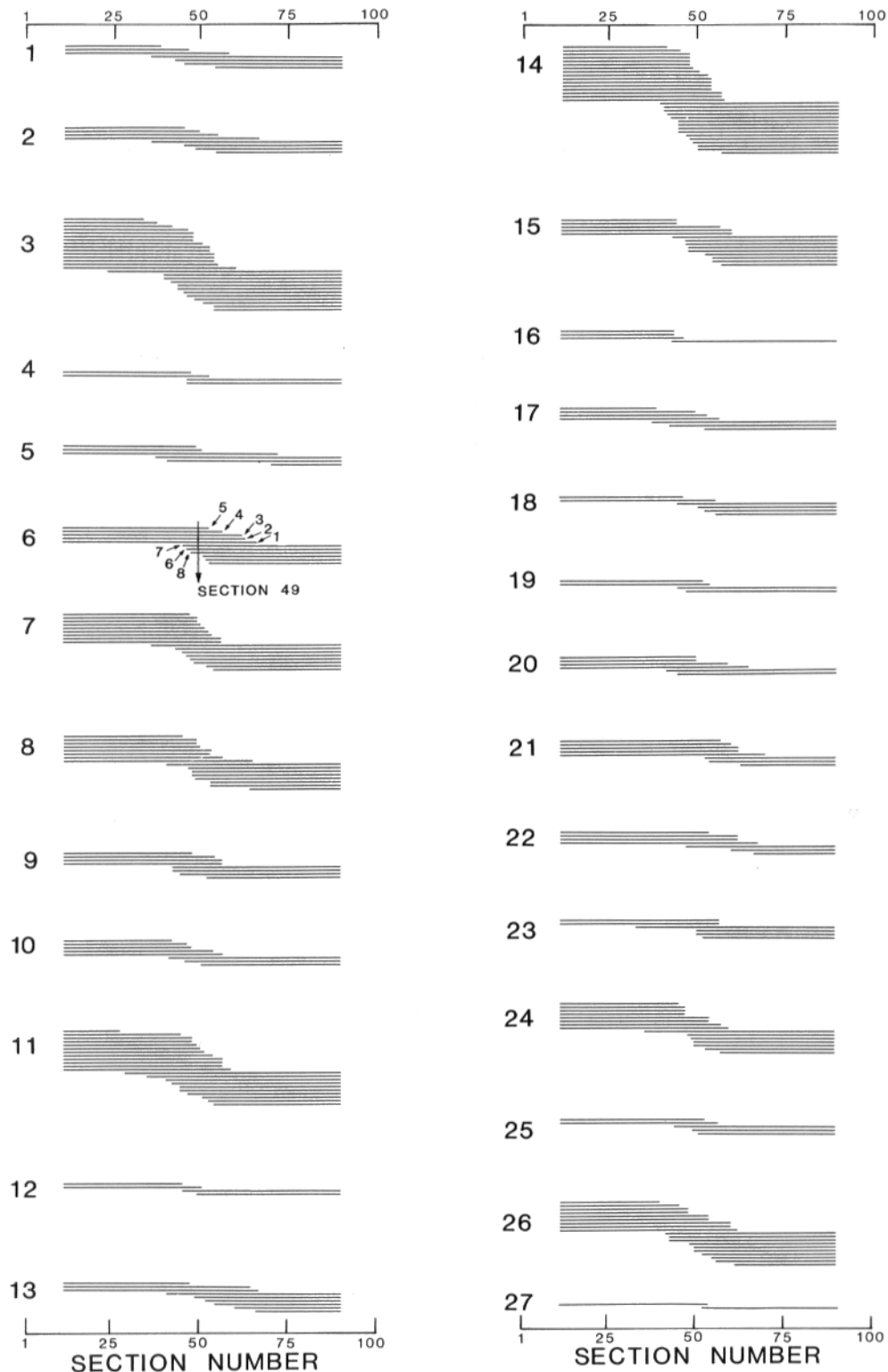
FIGURE 12   This diagram shows the end points of the 27 bundles of MTs from the late anaphase spindle which are displayed in Fig. 11. For example, group No. 6 in Fig. 11 contains eight MTs numbered 1–8, the same eight MTs are labeled in Fig. 12 in group 6. There are three MTs in group 6 in Fig. 12 which do not appear in Fig. 11 because they were out of the plane of the section (Fig. 11 is section No. 49). Similarly, group 27 in Fig. 11 contains one MT, in Fig. 12 this MT overlaps with one other MT in sections Nos. 53–55. This diagram indicates that the interdigitated MTs in the midregion of this spindle are confined to numerous small bundles which vary in their position and extent of overlap.

Fig. 11 contains eight MTs numbered 1–8; these same MTs are labeled 1–8 in group 6 in Fig. 12. There are three unnumbered MTs in group 6 in Fig. 12 that do not reach to section 49 and are thus not in Fig. 11. Every MT in Fig. 12 is also present in the late anaphase reconstruction (Fig. 9). However, there are 54 MTs in Fig. 9 (27 at each pole) that terminate between the two chromatin masses (i.e., between section Nos. 22 and 74), but do not overlap with MTs from the opposite pole and are not included in Fig. 12.

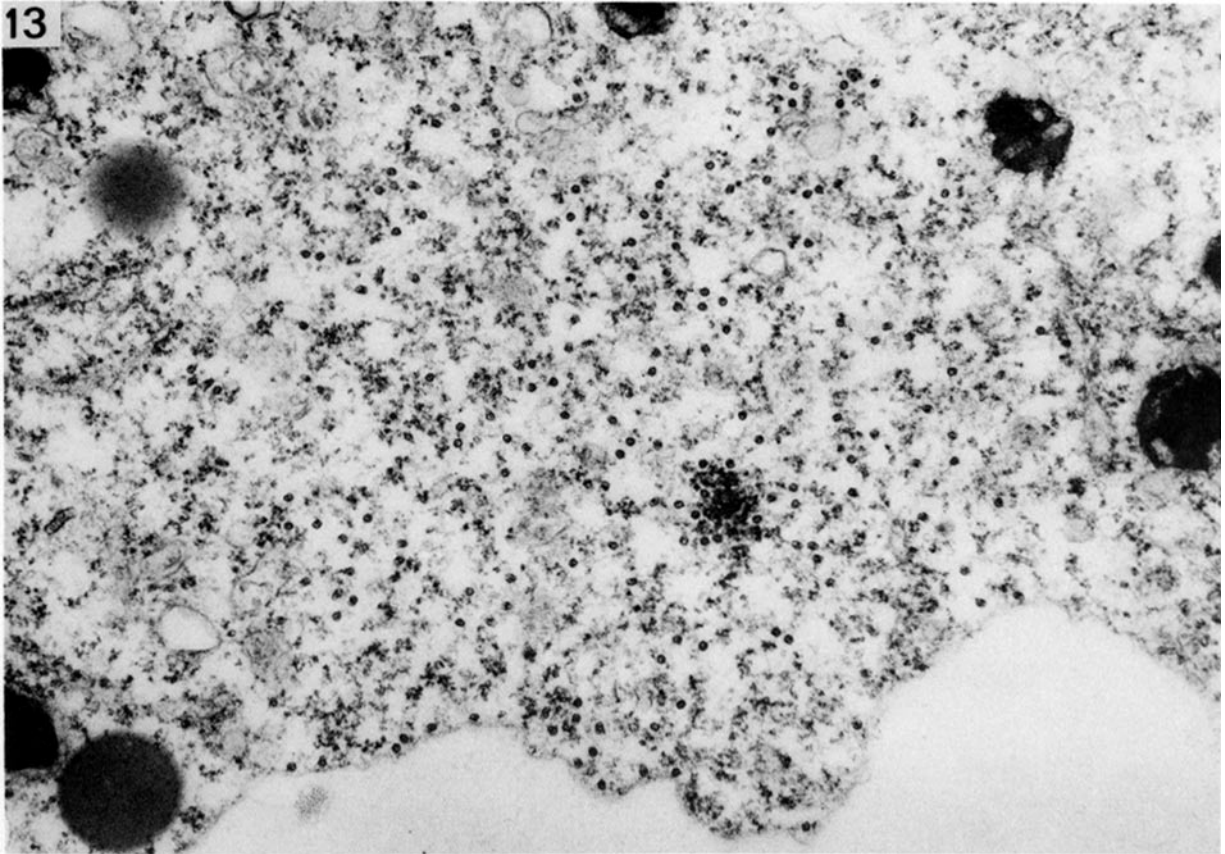Examination of Figs. 10–12 reveals that these spindles con-

FIGURE 13   A micrograph of section No. 27 of the late anaphase reconstruction (Fig. 9). The 164 MTs in the half spindle are more evenly spaced in comparison with the grouping of MTs in the overlap (Fig. 10). × 36,700.
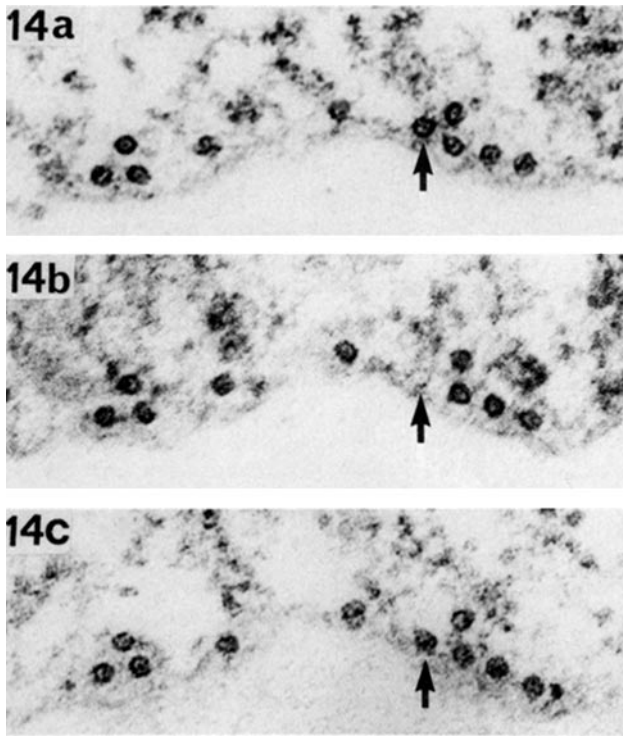

FIGURE 14   Three micrographs are taken from the overlap (section Nos. 50–52) of the late anaphase reconstruction (Fig. 9). An MT from the left pole in Fig. 14a (arrow) terminates in Fig. 14b, and a new MT from the right pole (arrow) starts in the same position in Fig. 14c. This illustrates how a continuous MT may result from a tracking error in which MTs from opposite poles are linked together; if Fig.

tain a distinct framework of interdigitated MTs. The amount of overlap varies between each group of MTs; in Fig. 12, group 27, the MTs overlap three sections, while in group 3 there are 32 sections of overlap. Similarly, the position of the overlap can also vary; group 21 contains MTs that overlap between section Nos. 54 and 70 while the MTs in group 16 overlap between section Nos. 43 and 47. The number of MTs comprising a group varies between 2 and 31. Other anaphase and telophase cells that were not reconstructed (Table III) contain a similar grouping of MTs. Examination of the telophase spindle (Fig. 15) reveals that there are only 10 MTs that extend past the chromatin but do not belong to a group, 44 less than at late anaphase. This indicates that noninterdigitated MTs are shortening and eventually disappear even at this late stage.

Indirect Length Measurements of MTs

The length of the MTs in these spindles may be calculated by counting the number of sections they traverse and then multiplying by section thickness (see Materials and Methods). This calculation is subject to numerous sources of error, including variation in section thickness, and must be treated with caution (see comments in reference 28). Table II shows the total length of all the MTs (placed end to end) in a single spindle, the average length of each MT and the overlap length from the four reconstructions in Figs. 5, 6, 9, and 15. As previously noted, the metaphase cell in Table II is abnormally long in comparison to the two other metaphase cells that were

14b were a thicker section the resulting tracking would erroneously show a single MT in this section. × 85,800.
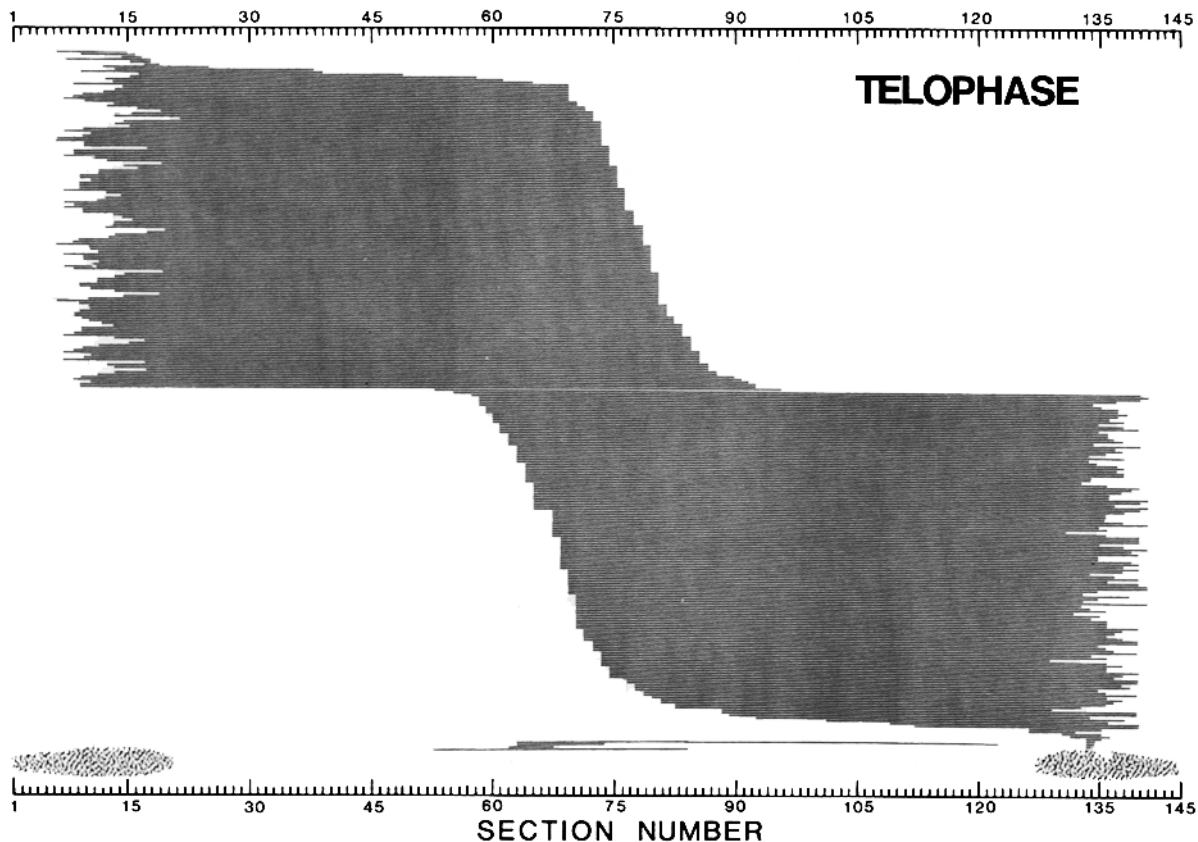
FIGURE 15 Telophase reconstruction of spindle MTs. See figure legend 5 for an explanation of this type of diagram.

TABLE II

Length Measurements of MTs

Stage of mitosis	Total length of all MTs (placed end to end)	Average MT length	Average overlap length	
			Method 1	Method 2
	mm	μm		μm
Late metaphase	1.79	1.88	4,237*	13.6
Early anaphase	1.46	1.66	5,035	17.6
Late anaphase	1.39	2.57	4,918	20.8
Telophase	1.42	4.98	5,485	22.4

* See Materials and Methods for units.

TABLE III

Number of MTs in Spindles not Reconstructed

Stage of mitosis	Section No. (position)	No. of MTs	Total length of spindle in sections
Metaphase	22 (o)*	325	57
Metaphase	17 (h.s.)‡	369	53
Anaphase	45 (o)	189	86
	62 (h.s.)	145	
Anaphase	40 (o)	131	80
	53 (h.s.)	94	
Anaphase	67 (o)	197	136
	24 (h.s.)	161	
Telophase	51 (o)	107	104
	29 (h.s.)	70	

* o Denotes overlap or middle of the spindle.
‡ h.s. Denotes half spindle—between the middle of the spindle and the pole.

sectioned (Table III). Consequently, we suspect that the total MT length and average MT length at metaphase in Table II are larger than normally expected. From early anaphase to telophase, the average length of the individual MTs increases, from 1.66 to 4.98 μm, while the total MT length remains quite constant (1.42 ± 0.04 mm).

The overlap length is qualitatively measured using two separate methods which give differing results (Table II). Consequently, Figs. 16 and 17 can be used to approximate the changes in overlap length. The overlap length may increase between metaphase and telophase (Figs. 16, 17, and Table II).

Number of MTs in the Spindle

Fig. 18 shows the number of MTs in each section of the four reconstructed spindles in Figs. 5, 6, 9, and 15; the total number of MTs in these reconstructions is 953, 881, 540, and 285, respectively. Table III shows MT counts taken from selected sections of other spindles that were not reconstructed; these correspond to the counts in Fig. 18. The V-shaped curve in Fig. 18 reflects the large number of short polar MTs in the metaphase spindle that are not long enough to overlap with MTs from the opposite pole. In contrast, the early anaphase, late anaphase, and telophase spindles have a peak in the number of MTs in the middle of the spindle corresponding to the overlap that is composed primarily of interdigitated MTs.

DISCUSSION

Arrangement of MTs in the Spindle

In most diatoms studied thus far, the central spindle (see Introduction) is a single bundle of interdigitated MTs (17, 28, 23). In certain diatoms (15), the interdigitated MTs are orga-

nized into several bundles. In contrast, the *Ochromonas* spindle contains ~30 smaller but similar bundles, averaging ~10 MTs each and the smallest bundle (No. 27 in Fig. 12) contains only one MT from each pole. Therefore, the *Ochromonas* spindle is structurally similar to that of diatoms. Longitudinal sections of other diverse spindles are difficult to interpret, but indicate

FIGURES 16 AND 17 These two figures contain silhouettes of the data from the spindle reconstructions in Figs. 5, 6, 9, and 15. Fig. 16 is a superposition of the late metaphase and early anaphase reconstructions; the arrows denote length changes of MTs between these stages (see text). Similarly, Fig. 17 compares the three late stages of mitosis. All the spindles are drawn to the same scale and Figs. 16 and 17 are on the same scale.
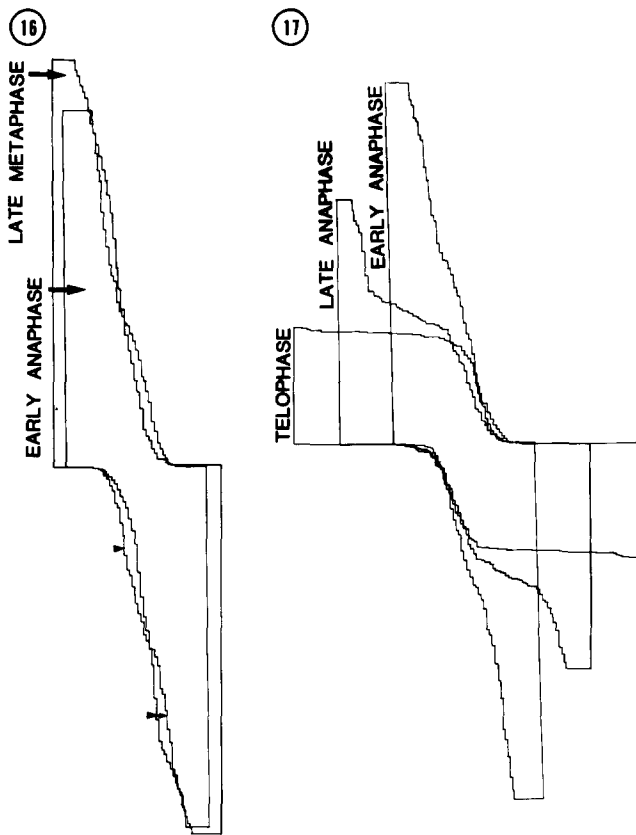
that they also could contain such a framework of interdigitated MTs (4, 6, 8, 10, 18, 22); this may be the predominant form of spindle construction. Large spindles (12, 7) or those that elongate significantly during anaphase (13), appear to contain many free MTs. There is not sufficient structural data to determine if these spindles at metaphase also contain a framework of MTs from opposite poles that overlap, or if bundles of overlapping free MTs extend between the poles. Such an overlap region may be necessary to establish a bipolar spindle and keep the metaphase spindle from collapsing upon itself as tension is induced between kinetochores stretching toward opposite poles.

During prophase and prometaphase, the spindle MTs in *Ochromonas* are not discernibly ordered, but by metaphase there are distinct populations of polar and interdigitated MTs. The fate of an MT at prophase, to become either a polar MT or an interdigitated MT by metaphase, appears to be determined by random lateral associations. The longest MTs from each pole are most likely to associate with an MT from the opposite pole, thus becoming interdigitated MTs, while the shorter MTs are likely to remain polar MTs. At anaphase and telophase, the interdigitated MTs progressively clump together into discrete bundles.

Mechanism of Spindle Elongation

The relevant observations concerning the changes in MT length and distribution during spindle elongation in *Ochromonas* are: (*a*) interdigitated MTs increase in length; this increases the length of each half spindle and separates the spindle poles; (*b*) the region of overlap does not decrease during spindle elongation. These observations are used to construct two possible methods of spindle elongation based upon polarity of growth of interdigitated MTs (Fig. 19).

Assume that all interdigitated MTs from one pole are of one polarity (the intrinsic polarity of MTs need not be considered) and that the MTs add subunits predominately at one end. If the MTs lengthen by adding subunits at the pole (Fig. 19 *b*), then polymerization of MTs could provide the force that elongates the spindle. Alternatively, if the MTs lengthen by adding subunits at their other end (the equatorial region of the spindle, Fig. 19 *c*), then sliding between MTs from opposite poles could generate the force, because the MTs must slide
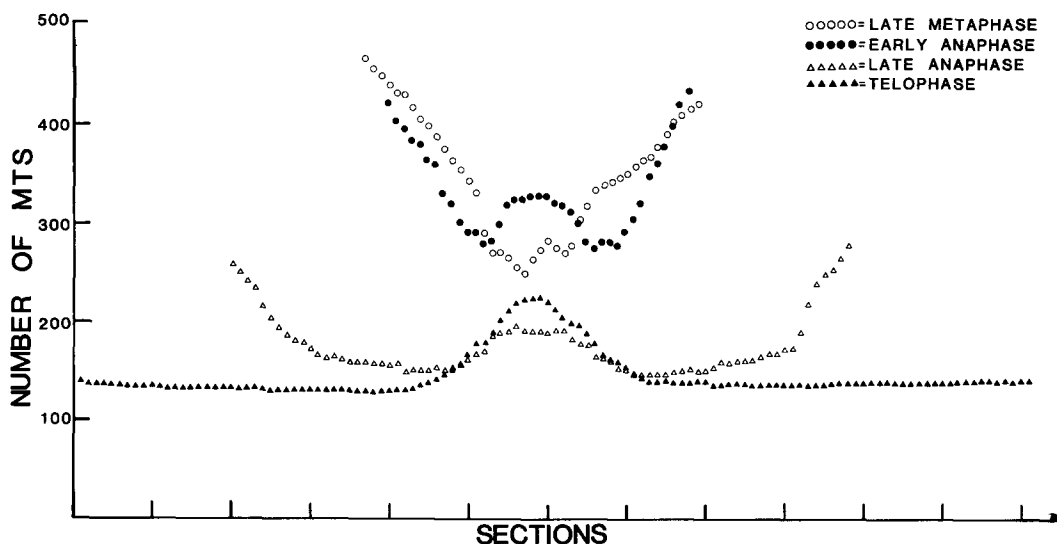

FIGURE 18 This graph shows the total number of MTs in each section from the four reconstructed spindles in Figs. 5, 6, 9, and 15.
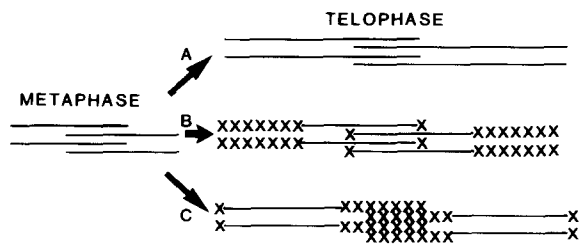
Figure 19 This figure illustrates two methods for elongating the spindle based upon growth polarity of interdigitated MTs. Each line represents a single MT. Path A shows the morphology of the interdigitated MTs at metaphase and telophase. This change may be accomplished by adding subunits (X) either at the pole (path B) or at the overlap (path C). Addition of subunits at the overlap requires that MTs from opposite poles must slide past each other to keep the overlap length constant (see Discussion).

apart to keep the overlap length relatively constant as the MTs increase in length. We cannot distinguish between these two possibilities. However, in the second proposal (Fig. 19 c), sliding would have to be precisely synchronized with polymerization to keep the overlap from substantially increasing or decreasing. Furthermore, if subunits add to all non-kMTs at the equatorial region at anaphase, as suggested by Margolis et al. (16), then we would expect free MTs and many polar MTs to lengthen; because these MTs in fact shorten, the second model (Fig. 19 c) can function only if the interdigitated MTs alone add subunits at the overlap.

The mechanism of spindle elongation has been most extensively examined in diatoms. These cells at metaphase contain a centrally located bundle of MTs, the central spindle, which consists of two half spindles that overlap. During spindle elongation in *Fragilaria* (28), the overlap region decreases in length concurrently with spindle elongation, while the half spindles remain the same length. In *Diatoma* (17, 21), the overlap also decreases with spindle elongation; this does not account for all the elongation because the length of the half spindles also increases between early anaphase and telophase. Cine observations in *Hantzschia* are difficult to interpret, but indicate that some lengthening of the half spindle may occur (24). We have suggested that the decrease in overlap length is best explained by assuming that the MTs from each half spindle either passively or actively slide apart; alternate methods involving polymerization and depolymerization of MTs cannot be excluded. Thus, the primary structural difference between spindle elongation in diatoms and that of *Ochromonas*, is that the overlap region decreases during spindle elongation in diatoms, but does not in *Ochromonas*. Perhaps, spindle elongation in most cell types will resemble that of *Ochromonas*, where lengthening of the half spindles proceeds without a decrease in the overlap.

The structural data from both diatoms and *Ochromonas* is compatible with theories that suggest spindle elongation is driven by a mechanism involving MTs. We still cannot rule out the possibility that spindle elongation is accomplished by a non-MT mediated mechanism and that MT polymerization (sliding) is a passive, accompanying process.

Polymerization and Depolymerization of MTs at Anaphase

The number and length of MTs increases during prophase in most cell types. In contrast, during anaphase certain MTs are thought to lengthen, thereby contributing to spindle elon-

gation, while kMTs must shorten to accommodate poleward movement of chromosomes. Any theory of mitosis must account for physiological conditions within the spindle which promote polymerization (lengthening) of certain MTs concurrent with depolymerization (shortening) of others. The complexity of this problem even in the small spindle of *Ochromonas* is enormous. Our data indicate that none of the spindle MTs are a constant length between metaphase and telophase; instead they either polymerize or depolymerize. Specifically, interdigitated MTs lengthen, while all other MTs shorten until most disappear. For example, the number of polar MTs decreases from 274 at metaphase to 25 at telophase. Similarly, MTs that terminate in the chromatin (including kMTs) decrease from 379 to 3, while interdigitated MTs remain at 294 ± 58 during this transition (see Table I) and lengthen considerably (Table II). Free MTs decrease in number from 62 at metaphase to 4 at telophase; the free MTs during late mitosis may be in the process of depolymerizing as indicated by their unusual spatial placement within the spindle. It is not clear why the six free MTs at anaphase and telophase are longer than the free MTs at metaphase. Perhaps they lengthen between early anaphase and late anaphase. Alternately, all the free MTs present at metaphase completely depolymerize and then at late anaphase new ones are created by the breaking of existing MTs.

These results show that there is a profound and significant redistribution of MT subunits during anaphase (11). It is generally assumed that only kMTs shorten as chromosomes move to the poles during anaphase, but we unequivocally show in this cell type that anaphase is accompanied by depolymerization of three classes of MTs (kMTs, free MTs, and polar MTs), while only one MT class lengthens (interdigitated MTs). We suggest that the spatial arrangement of MTs within the spindle influences their polymerization and depolymerization properties. The interdigitated MTs are the only class of MTs stabilized against depolymerization during anaphase, possibly because of lateral association between MTs from opposite poles and presumably of opposite polarity. In contrast, MTs that have a free end in the spindle or at a kinetochore (polar MTs, free and kMTs) are not stabilized in this manner and depolymerize at anaphase. We emphasize that MTs from the same pole that laterally associate (within 40 nm of each other) are not resistant to depolymerization. McIntosh et al. (21) have also proposed that the interdigitated MTs in *Diatoma* are resistant to depolymerization at prometaphase, while the short polar MTs depolymerize. The mechanism of selective MT depolymerization could be the same in *Ochromonas* and *Diatoma*, although such depolymerization begins at different mitotic stages (prometaphase in *Diatoma*, anaphase in *Ochromonas*).

Polarity of spindle MTs is also relevant to understanding the functions of, and changes in length of, MTs during anaphase. The problem of polarity of spindle MTs has two separate aspects (20, 2, 28, 1): (a) the polarity of growth and shortening of MTs (i.e., the site of addition of MT subunits to interdigitated MTs as in Fig. 19; and the site of loss of subunits from kMTs, polar MTs); (b) the intrinsic polarity of different classes of spindle MTs and its consequent involvement in spindle function. Bergen et al. (1) have deduced the intrinsic and growth polarity of MTs nucleated from centrosomes and kinetochores in vitro, by analyzing growth kinetics of MTs. Structural data of the type in this report is relevant to the problem of growth polarity, because it identifies which classes of MTs grow and shorten at different mitotic stages. The

spindle of *Ochromonas* is a simple system for a theoretical consideration of polarity of spindle MTs, because 97% of the MTs are attached to the poles (there are only 3% free MTs). This problem is analyzed further in a paper in preparation.

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