MITOSIS IN THE PENNATE DIATOM *SURIRELLA OVALIS*

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ABSTRACT

Mitosis in *Surirella* is described; this organism displays a number of unusual features including an unorthodox method of chromosome attachment to the spindle, and the differentiation of an extranuclear central spindle from a large spherical organelle named the microtubule center (MC). The MC, present during interphase, breaks down at late prophase as the central spindle is formed. Later, the spindle enters the nucleus; the chromatin, in association with microtubules (MTs) from the poles, increasingly aggregates around the middle "overlap" region of the central spindle, and by metaphase completely encircles it. Throughout, MTs usually associate laterally with the chromatin. We were not able to identify kinetochore MTs with confidence at either metaphase or anaphase. Instead, at anaphase the leading point of the chromosomes is embedded in a ring of electron-dense material, named the "collar," which encircles each half spindle and extends from the chromatin to the pole. Anaphase separation of the chromosomes is achieved by at least three separate mechanisms: (*a*) between metaphase and late anaphase the central spindle increases in length by addition of MT subunits; (*b*) at late anaphase the central spindle elongates concurrent with a reduction in the overlap; this apparently results from an MT/MT sliding mechanism; (*c*) each set of chromosomes moves to the poles by a thus far unknown mechanism; however, we anticipate some interaction of the collar and central spindle. At telophase, the polar complexes, (i.e., structures at the spindle pole) separate from the spindle, and later a new MC is formed near each polar complex, after which the polar complexes break down. Aspects of the complex differentiation of the MC, spindle formation, and some unusual characteristics of the diatom spindle as they relate to anaphase motion and spindle function are discussed.

In 1896, Lauterborn (12) published a detailed account of nuclear and cell division in various diatoms, with emphasis on the pennate diatom *Surirella*. He noted several unusual features characteristic of mitosis in these organisms, which were not later confirmed by workers using the light microscope (see Discussion). More recently, Manton et al. (14–17) observed with the electron microscope that the spindle of the centric *Lithodesmium* does indeed have unusual characteristics. They illustrated in considerable detail the formation of the extranuclear "central spindle" which runs from one pole to the other, and indicated that by metaphase it consists essentially of two half-spindles, each a highly ordered set of microtubules (MTs) which interdigitate in the central "overlap" region. They also demonstrated that the centriole involved in flagellum formation during sexual re-

production forms *de novo* at each pole. We have documented all stages of mitosis and cytokinesis in two diatoms; several further interesting observations were made, including that the central spindle of *Diatoma* (20) elongates during anaphase, concurrent with a reduction in the overlap region. Additionally, we have shown in *Melosira* (28) that the overlap region during prophase consists of a very precise arrangement of MTs; one MT from one pole is usually surrounded by four from the other pole. These observations together clearly hinted that sliding of parallel/antiparallel MTs in the overlap could be responsible for generating that part of anaphase motion due specifically to that spindle elongation. Further work on the structural analysis of the *Diatoma* spindle[1] confirms that sliding generated by such MT/MT interaction provides the most logical and satisfying explanation for the reduction of the overlap during spindle elongation.

One aspect of the diatom spindle which is especially puzzling concerns the existence of typical kinetochore MTs characteristic of conventional spindles. Manton and her colleagues did not address this point; our work on both *Diatoma* and *Melosira* suggested that such MTs could be present, although the matter was not resolved. We have therefore embarked on studies of other diatoms, in conjunction with diverse experimental manipulations of the diatom spindle.

We selected the pennate diatom *Surirella* for the present study for two reasons. First, Lauterborn's descriptions of mitosis in this genus is exceedingly detailed, and we were particularly interested in his observation that no spindle fibers are seen attaching to the chromosomes during anaphase in living cells. Secondly, we had fortuitously observed in an interphase cell the large spherical organelle situated near the nucleus which is associated with numerous MTs. This organelle, previously described (8), may be similar to other functionally similar but morphologically different "microtubule organizing centers" (MTOCs) in other diatoms, and in the case of *Melosira* (28) it clearly takes part in forming the spindle. *Surirella* therefore offered interesting possibilities for the study of spindle formation from such an interphase MTOC.

[1] McDonald, K. L., J. D. Pickett-Heaps, J. R. McIntosh, and D. H. Tippit. 1977. On anaphase spindle elongation in *Diatoma vulgare*. *J. Cell Biol.* **74.**

MATERIALS AND METHODS

Surirella ovalis (Breb) was isolated from a local stream, and unialgal cultures were grown under constant illumination at 18°C in diatom medium (22) containing the following supplement of vitamins per 1,000 ml of medium: 10 μg of vitamin B12, 5 μg of biotin, and 0.5 μg of thiamine. For transmission microscopy, the cells were fixed in 1% glutaraldehyde made up in the medium; about 5 s later, a small vol of 2% osmium tetroxide was added, and the cells were fixed concurrently in glutaraldehyde and osmium tetroxide for 20 min. The amount of osmium tetroxide was not important, and we estimated its final concentration at around 0.2%. The cells were washed in water, then dehydrated slowly in acetone and flat embedded in Spurr's (26) resin. Sections were cut on diamond knives and collected on Formvar-coated grids, then stained with uranyl acetate and lead citrate before examination in a Philips EM 200 electron microscope. For high voltage electron microscopy, 0.3–0.5-μm thick sections were collected on Formvar-coated slot grids. The sections were stained for 20 min in methanolic uranyl acetate and for 5 min in lead citrate, then examined at 1,000 kV. For scanning electron microscopy, cells were concentrated by centrifugation, treated with concentrated nitric acid for 5 min, washed several times, and dried on glass cover slips. The specimens were coated with carbon and gold, and viewed in a Cambridge Stereoscan S4 scanning electron microscope. Light micrographs were taken with Zeiss Nomarski differential interference contrast optics.

RESULTS

Interphase

The interphase structure of *S. ovalis* will not be described in detail here. As in other diatoms, the cell is enclosed by a beautifully sculptured siliceous wall, consisting of two halves or valves interconnected by girdle bands (Fig. 1). These diatoms are conveniently described from two aspects: girdle view, in which the girdle bands are seen, and valve view, in which the face of the valve is observed (Figs. 1 and 2). Internally, the cell is largely filled by a vacuole; a chloroplast, apparently single, lies predominantly against each valve surface (Fig. 2) in a thin layer of peripheral cytoplasm containing the usual assortment of organelles. Within this peripheral cytoplasm, underlying the raphe, a small bundle of microfilaments can sometimes be found; these have also been observed in several other diatoms where they seem to be involved in generating the gliding motion characteristic of those algae (7). The centrally located nucleus (Fig. 2) is surrounded by numerous Golgi bodies (as in

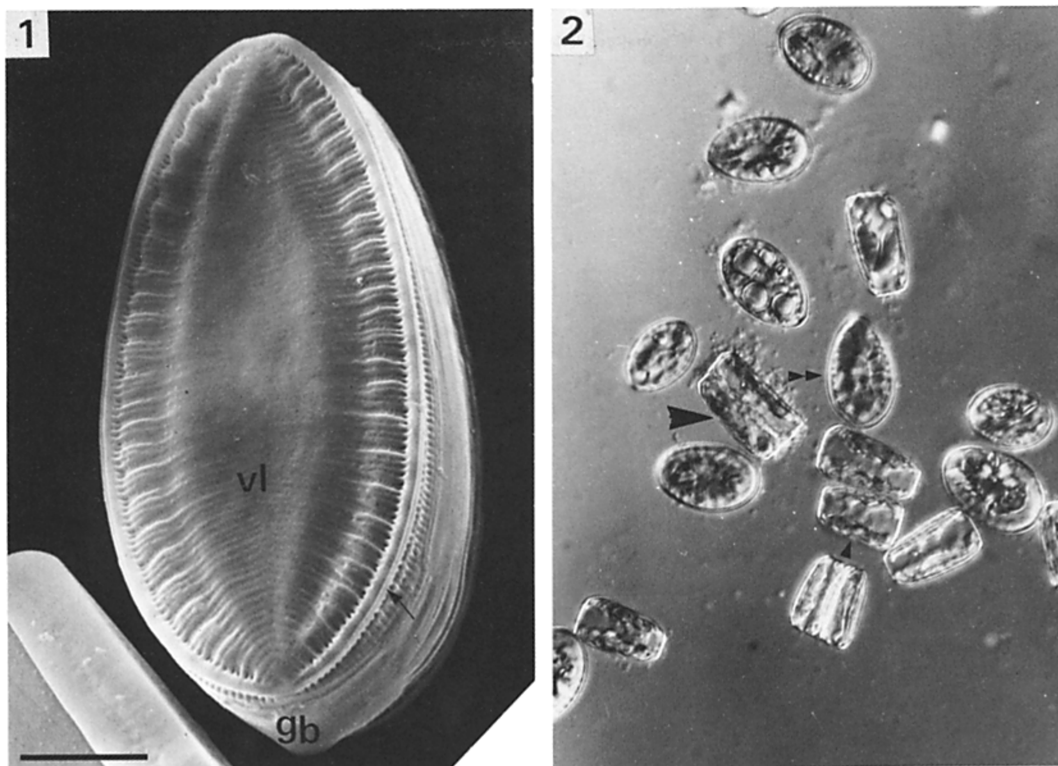


FIGURE 1 Scanning electron micrograph of *S. ovalis* in valve view, showing the ornamented face of the valve (*vl*), the girdle bands (*gb*) and the raphe (arrow). × 1,700. Bar, 10 μm.

FIGURE 2 Live cells in both girdle (single arrow) and valve view (double arrow). The large arrow indicates a recently divided cell with a clear cleavage furrow. × 430.

Fig. 22). Before division, it migrates to the more rounded end of the cell whereupon it undergoes mitosis; after cleavage, each daughter nucleus moves back to the middle of its cell. The way the rigid wall or frustule accommodates cell expansion is complex and not considered here.

Microtubule Center (MC)

In a depression of the interphase nucleus lies a small spherical organelle which we call the MC (Fig. 3). The interior of the MC is evenly granular, and numerous MTs emanate from its slightly more dense surface layer; many of these MTs lie tangential to the nucleus (see also references 8 and 9). The MC is present only during interphase, and the earliest and latest stages of mitosis; it is significantly associated with spindle formation. The MC breaks down during mitosis and a new MC later forms in each daughter cell after cytoplasmic cleavage (these events have been summarized in Fig. 23).

Certain other diatoms contain an organelle associated with the interphase nucleus which is undoubtedly equivalent to the MC. In *Diatoma* and *Melosira* (20, 28), we previously called this structure the "persistent polar complex" since (in *Melosira*) it persists through the cell cycle, replicating at prophase to form the structures at the spindle poles, and then, after mitosis, separating from the spindle and reverting to its interphase form. Unfortunately, this terminology now turns out to be confusing if applied to *S. ovalis* since the MC breaks down at prometaphase and then reforms at telophase, and so we have been obliged to introduce the term MC to describe this organelle.

Central Spindle, Collar, and Polar Complex

The central spindle in diatoms is the conspicuous set of MTs which extends between the spindle poles (20). It consists essentially of two half spin-

dles whose MTs interdigitate at the middle "overlap" region (Figs. 9, 11, and 15). We use the term collar to describe the electron-dense material which encircles certain portions of the central spindle (see Metaphase). At anaphase, such material extends between the chromatin and the pole on each half spindle; at metaphase, similar material surrounds the end half spindle but is not in contact with the chromatin. The structures at the poles of the spindle are now referred to as "polar complexes" (Figs. 6, 15, and 17). Each polar complex forms at prophase near the MC; they separate from the spindle at anaphase, and then break down after cleavage (Fig. 23).

Prophase

Before division, the MC followed by the nucleus migrates to the end of the cell. The development of the central spindle is initiated before migration when a small striated structure assembles next to the MC (Fig. 4, arrow). This structure enlarges during early prophase, and then separates from the MC, which is occasionally flattened on the side facing the forming spindle and is still the focus of cytoplasmic MTs (Fig. 5). The material for the forming spindle does not appear to be derived entirely from the MC since the MC does not become appreciably smaller during early prophase.

The forming central spindle in Figs. 4 and 5 is a double structure consisting of several different components arranged symmetrically on either side of a dense central core. These components are more clearly seen in an early prophase cell (Fig. 7) sectioned at right angle to the cell in Fig. 5. Next to the core (Fig. 7, arrow) on either side are alternating light and dark bands, and the whole complex is surrounded by an amorphous layer. Invariably, there is at least one small vacuole partially embedded in the outermost layer of the entire complex (Figs. 5 and 6).

Careful inspection of the early forming spindle in longitudinal view reveals faint tubular structures lying across the dense central core. Later, the core of the forming spindle breaks down, and the two resultant outer structures separate; we now call each of these a polar complex (Fig. 6). Numerous parallel MTs increase in length between the separating polar complexes; one end of these MTs terminates in the dense layer of each polar complex (Fig. 6, arrow), which we previously called the "spindle insertion" (20). The polar complexes, sometimes curved, are slightly thickened at their end nearest the MC; the other end is closely associated with the nuclear envelope (Figs. 6 and 8*a*).

The central spindle continues to elongate, and its MTs appear to extend from one polar complex to the other during all of prophase (Fig. 8*a*). In cross sections, these MTs are arranged like those in the overlap of the anaphase spindle (Fig. 16*a*). The MC remains beside the elongating central spindle, but by late prophase no cytoplasmic MTs radiate from it, and its morphology is slightly altered; it is no longer spherical and appears to be disintegrating at its surface (Fig. 8*b*, arrow). Later, the MC apparently breaks down completely; it is not seen between late prophase and telophase, when a new MC is differentiated in

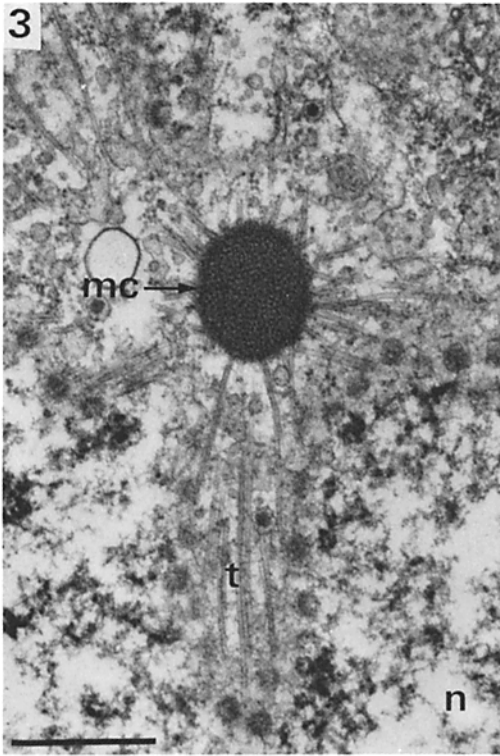
FIGURE 3 The interphase microtubule center (*mc*), the focus of numerous microtubules (*t*), is located in an indentation in the nucleus (*n*). × 38,000. Bar, 0.5 μm.

FIGURE 4 Preprophase. The central spindle is initiated as a small striated organelle (arrow) arising near the MC. × 37,000. Bar, 0.5 μm.

FIGURE 5 Preprophase. The forming central spindle is the doubled structure now separated from the MC. A vacuole (*v*) is invariably associated with the forming spindle which remains outside the nucleus (*n*). × 37,000. Bar, 0.5 μm.

FIGURE 6 The doubled structure (shown in Fig. 5) later divides as MTs of the central spindle (*s*) assemble between the two resultant halves, now called the polar complexes (*pc*). These MTs embed in a dense layer of the polar complex, named the spindle insertion (arrow). One end of each polar complex is associated with the nuclear envelope (*ne*). × 34,000. Bar, 0.5 μm.

FIGURE 7 Early prophase, forming central spindle sectioned in end view (at right angle to the spindle in Fig. 5). Numerous components of differing densities are symmetrically arranged on either side of the dark central core (arrow). × 45,000. Bar, 0.5 μm.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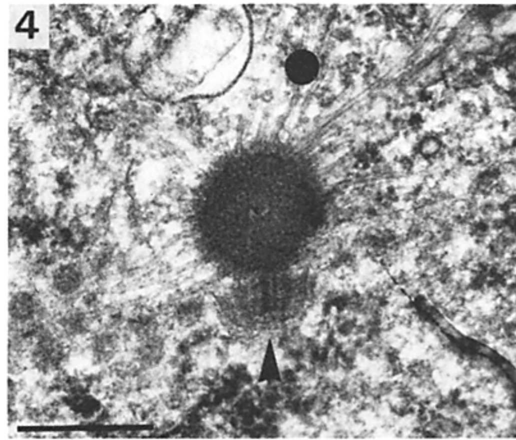
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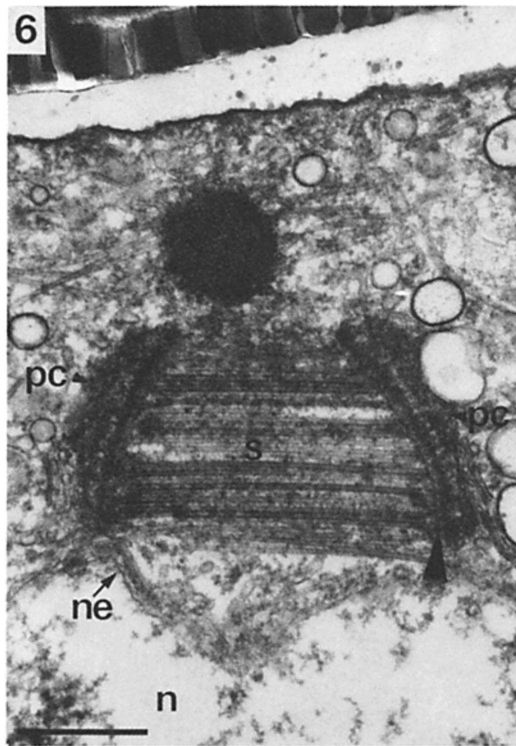
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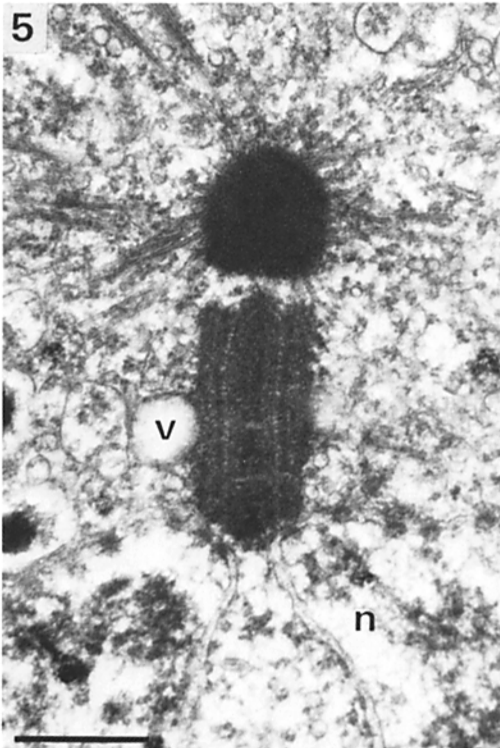
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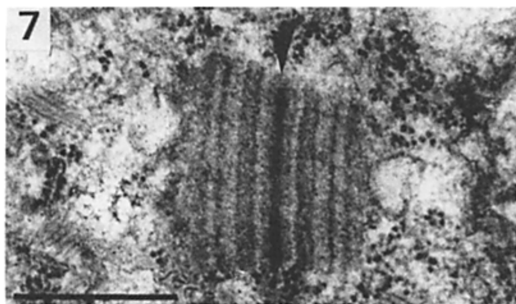
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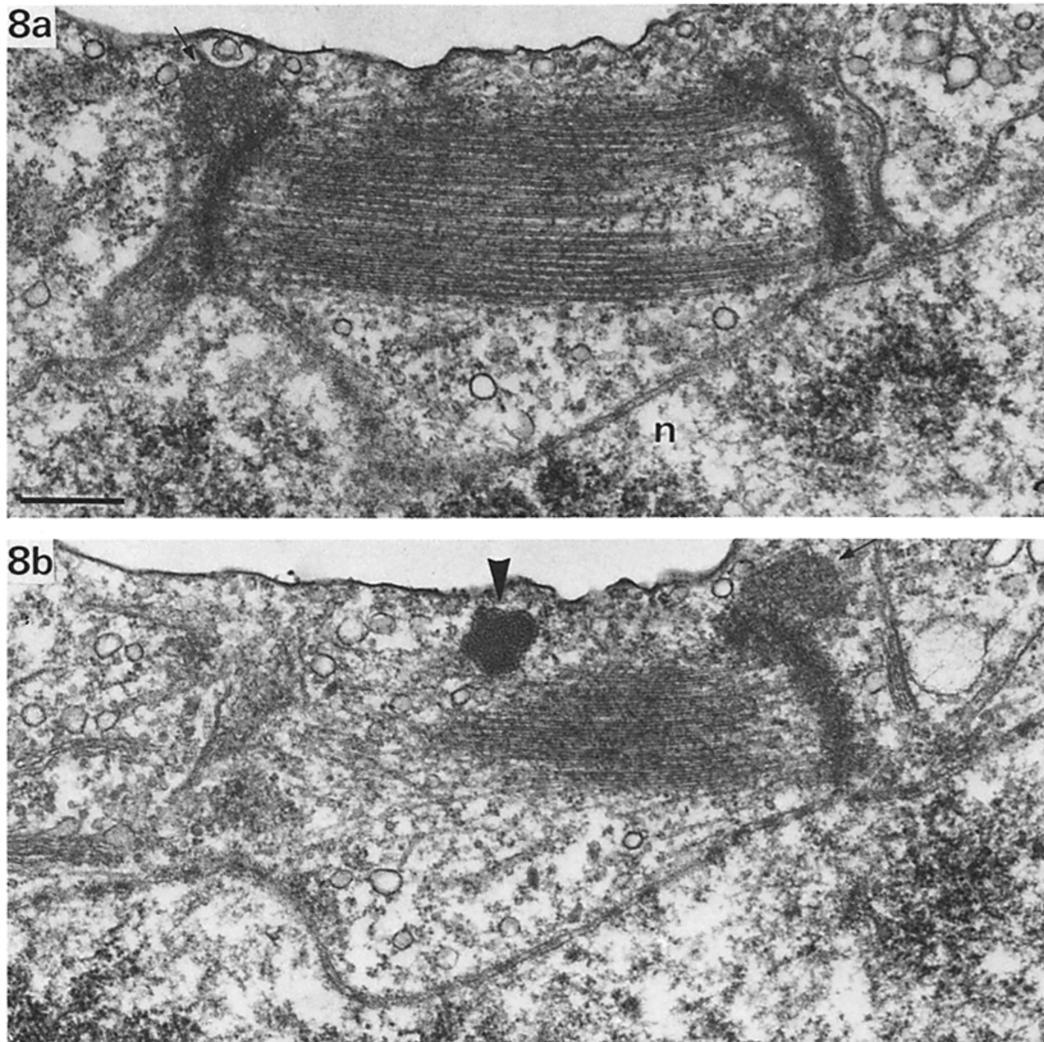
each cell (see section on Telophase). The end of each polar complex which faces the wall now contains a considerable thickening of amorphous material (Fig. 8*a* and *b*, small arrows). As the spindle elongates, other MTs which are distinct from those of the forming central spindle radiate from each polar complex and invaginate the nuclear envelope (Fig. 8*a* and *b*), whose surface here consequently becomes highly indented and irregular. Later, the nuclear envelope breaks down in this region, thereby allowing MTs and, later, the spindle to enter the nucleus. Within the nucleus, the chromatin condenses, and the nucleolus disperses.

Prometaphase

As in other diatoms, the forming central spindle sinks into the nucleus during prometaphase (Figs. 9 and 10). As it does so, the polar complexes remain accurately aligned with the unbroken remainder of the nuclear envelope, which is partially intact throughout mitosis with its complement of associated Golgi bodies.

The central spindle has become considerably



FIGURES 8*a* and *b* Two sections through the same late prophase spindle. Each polar complex has a thickening at one end (small arrows). The MTs of the central spindle appear to run from pole to pole (Fig. 8*a*); other MTs invaginate the nuclear envelope. The MC (Fig. 8*b*, large arrow) appears to disintegrate, and is not observed at metaphase and anaphase. × 28,500. Bar, 0.5 μm.

longer by prometaphase and reveals a concomitant change in morphology. At prophase, the MTs appear to overlap over the entire spindle length, but from prometaphase until anaphase the central spindle consists of two half spindles whose MTs interdigitate only in the central overlap (Figs. 10 and 15). It appears furthermore that the length of the overlap at late prophase is greater (by up to 30%) than the length of the overlap at prometaphase and metaphase (cf. Fig. 8*a* with Figs. 10 and 11); thus, the overlap decreases in size even during prometaphase while each half spindle continues to grow in length.

At prometaphase, the condensed chromatin is dispersed throughout the nucleus. The incoming central spindle soon makes contact with some of the chromatin (Fig. 9) which increasingly aggregates around the overlap (Fig. 10). Initially, this chromatin appears to attach to and possibly be stretched along the central spindle (Fig. 9, large arrow); numerous MTs (Fig. 9, small arrows) radiating from or near the spindle poles penetrate this chromatin, often passing tangentially to it for variable distances. Other MTs extend elsewhere into the nucleoplasm and laterally associate with the chromatin farther from the spindle.

Metaphase

By metaphase, most of the chromatin encircles the central spindle, although smaller masses may still be moving inwards (as in Fig. 11) in association with MTs from the poles. Eventually, the central spindle is evenly surrounded by chromatin (cf. Figs. 10 and 11) which becomes more compact and diamond-shaped as metaphase progresses (cf. Figs. 11 and 12). The MTs of the central spindle do not now terminate in the spindle insertion but end in nearby amorphous material (Fig. 14); the PCs are slightly curved and have no thickening at one end as they did during late prophase.

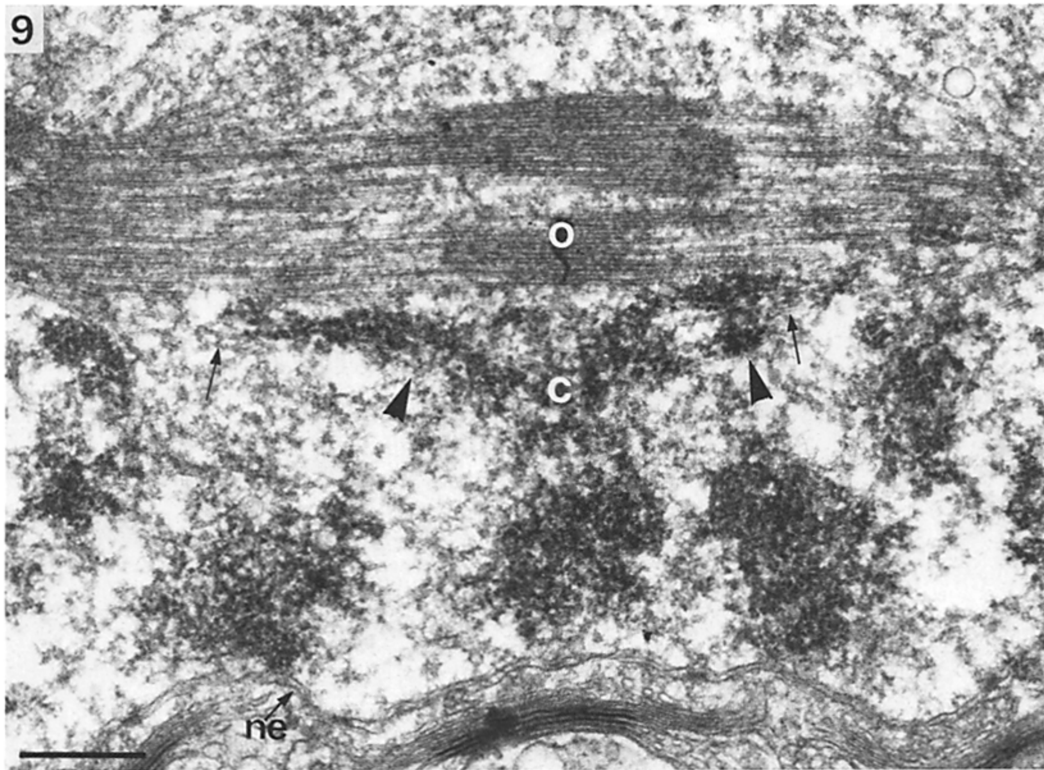
The way in which the chromosomes are attached to the spindle in diatoms has become increasingly puzzling to us. For this reason, we examined metaphase spindles of *S. ovalis* in considerable detail, searching in particular for the classical kinetochore-MT system common to so many other spindles. Our observations remain enigmatic.

Chromatin is always associated with spindle MTs in two possibly equivalent ways. First, strands of chromatin extend polewards over the surface of the central spindle (Fig. 11, small arrows). Secondly, other strands farther out on the metaphase plate also extend polewards, in association with MTs which are not part of the central spindle (Fig. 11, large arrow). Such MTs radiate from the poles or possibly from along the central spindle near the pole (Figs. 11, 13, and 14) into the nucleoplasm, many of them penetrating the chromatin deeply; some extend past the chromatin and overlap with equivalent MTs from the other pole. We particularly examined the relationship of these MTs to the outer strands of chromatin (i.e., Fig. 11, large arrow) and find it difficult to make a definitive statement about this most important association. Some MTs apparently end at the strands, but these may have simply been passing from the plane of sectioning. Many other MTs clearly lie tangentially very close to the strands, passing beside them for variable distances. The impression most strongly gained is that the chromatin associates laterally with many such MTs and that this may be equivalent to the lateral association between the chromatin and the MTs of the central spindle. By late metaphase, the chromatin is more compact, and the outer strands previously extending polewards are not so prominent.

The MTs of the central spindle are often more densely stained near the poles and in the overlap than the MTs just outside the overlap (Fig. 11). Those MTs near the pole embed into dense material which encircles the central spindle; this is more clearly visualized in thick sections. Occasionally, a ring of electron-dense material surrounds each half spindle between the chromatin and the poles (Figs. 12 and 13, arrows); we call these structures "collars." At other times, the collar is not a well-defined ring; instead, such material, less concentrated, surrounds the central spindle extending from the pole to, but not touching, the chromatin. The collars are most easily seen in thick sections grazing the central spindle (Fig. 13) and are sometimes detectable in sections through the middle of the spindle and in thin sections. The collars have not been detected before metaphase, but we suspect that they may be present on the prophase spindle. Since the collars are not structurally well-defined, the term is necessarily, and deliberately, vague.

Anaphase

Anaphase spindles were rarely encountered, and so our structural analysis of this stage is less complete compared with the other stages described. During early anaphase, each polar com-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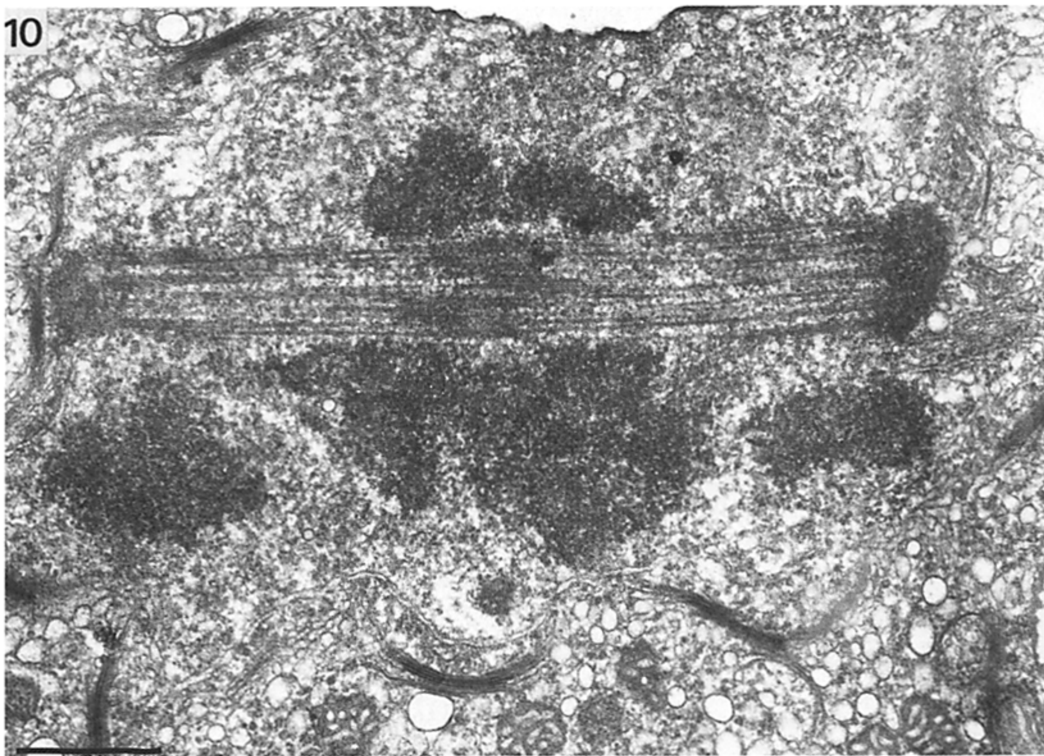
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plex separates from the central spindle, although this becomes more evident by early telophase (Fig. 17, arrows). At mid-anaphase, the chromatin has clearly separated into two doughnut-shaped masses penetrated by the central spindle (Fig. 15). As before, numerous MTs which are not part of the central spindle radiate from the poles or from along the central spindle between the chromatin and the poles. Some of these MTs clearly penetrate through the chromatin, others appear to end at the chromatin, but again these may have passed from the plane of sectioning. Most of the MTs radiating from the poles appear to associate laterally with the leading edge of the chromatin. As with metaphase, we have not been able to identify kinetochore MTs with confidence. Instead, the leading edge of each chromatin mass is in contact with an ill-defined ring of amorphous material which encircles the central spindle (Fig. 15, small arrows indicate one such ring). We believe that these two rings (one on each half spindle) are the collars visible earlier at metaphase. The collars again are difficult to see in longitudinal thin sections; transverse sections reveal them more clearly. Figs. 16*a–f* are selected from transverse sections taken along a spindle equivalent to that in Fig. 15. It is important to visualize in longitudinal view where along the spindle each of these cross sections is located. Accordingly, the arrows labeled with a letter along the spindle in Fig. 15 correspond positionally to the micrographs identified by the same subscript in the series of Fig. 16*a–f*.

Fig. 16*a* is a transverse section through the overlap region of the central spindle. Here, this central spindle contains approx. 370 MTs which display a tendency towards square-packing around its periphery but are more randomly arranged in the center. Fig. 16*b* is taken outside the overlap; it passes through the trailing ends of one chromatin mass, and the central spindle here contains approx. 219 MTs. Between many of the MTs are noticeable gaps which correspond positionally to those MTs (e.g., some of those in Fig. 16*a*) derived from the opposite pole which have dropped out of the plane of sectioning. Thus, MTs from one pole tend to be surrounded by MTs from the opposite pole in the overlap, although this regularity in arrangement is not so precise as in certain other diatoms (28). The regular packing of MTs in the overlap obviously influences their arrangement in those sections adjacent to the overlap. When the plane of the section is taken progressively closer to the pole (Fig. 16*c*), the arrangement of MTs and spaces is not so precise, as more MTs appear to line the outside edge of the central spindle. In this region, the chromatin now completely encircles the central spindle.

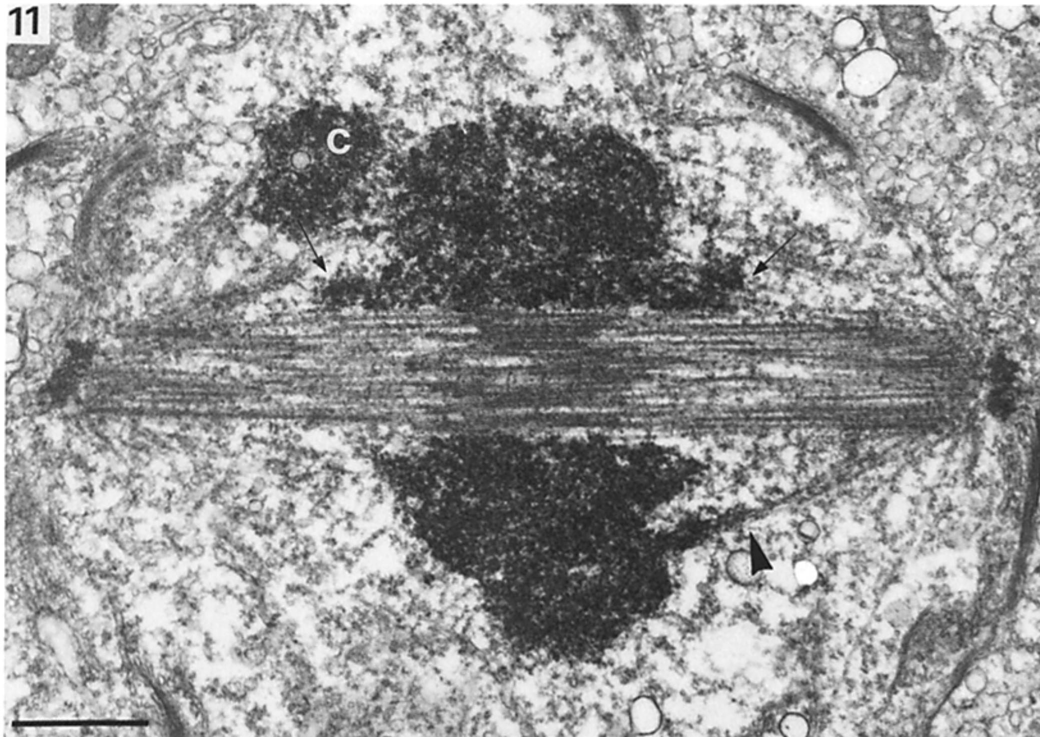
When sections are taken closer to the poles, they pass through the collars; each is seen as a ring of amorphous material which encircles the spindle (Fig. 16*e* and *f*). This material penetrates into the MTs around the central spindle's periphery, and to a lesser extent into the inner MTs (Fig. 16*e*). A striking picture of the diatom spindle emerges from sections in which parts of individual chromosomes can be identified; the apexes of these elongated or V-shaped masses of chromatin embed in the collar. Back from this region, the chromosomes merge to form the typical, rather structureless mass (Fig. 16*c*). After examination of numerous sections through the collars, we conclude that the chromatin (chromosomes) attaches here to the central spindle.

In those sections which pass through the collars, the MTs of the central spindle are concentrated around its outer edge, which results in comparatively large gaps devoid of MTs in the middle of the central spindle (Fig. 16*f*). This is in contrast to the more even distribution of the MTs just outside the overlap (Fig. 16*b*). Thus, it appears that the arrangement of MTs within the central spindle could be influenced by the attachment of the chromatin to it.

Figure 16*d*, taken between the leading edge of the chromatin and the pole, displays no obvious

FIGURE 9 Prometaphase. The spindle sinks into the nucleus which is still partially bounded by portions of the nuclear envelope (*ne*). Some chromatin (*c*) (large arrows) appears to attach to, and possibly stretch along the central spindle near the overlap (*o*). MTs from the poles (small arrows) associate with this chromatin. × 33,500. Bar, 0.5 μm.

FIGURE 10 Late prometaphase. Most chromatin has aggregated around the central spindle. × 19,000. Bar, 1 μm.



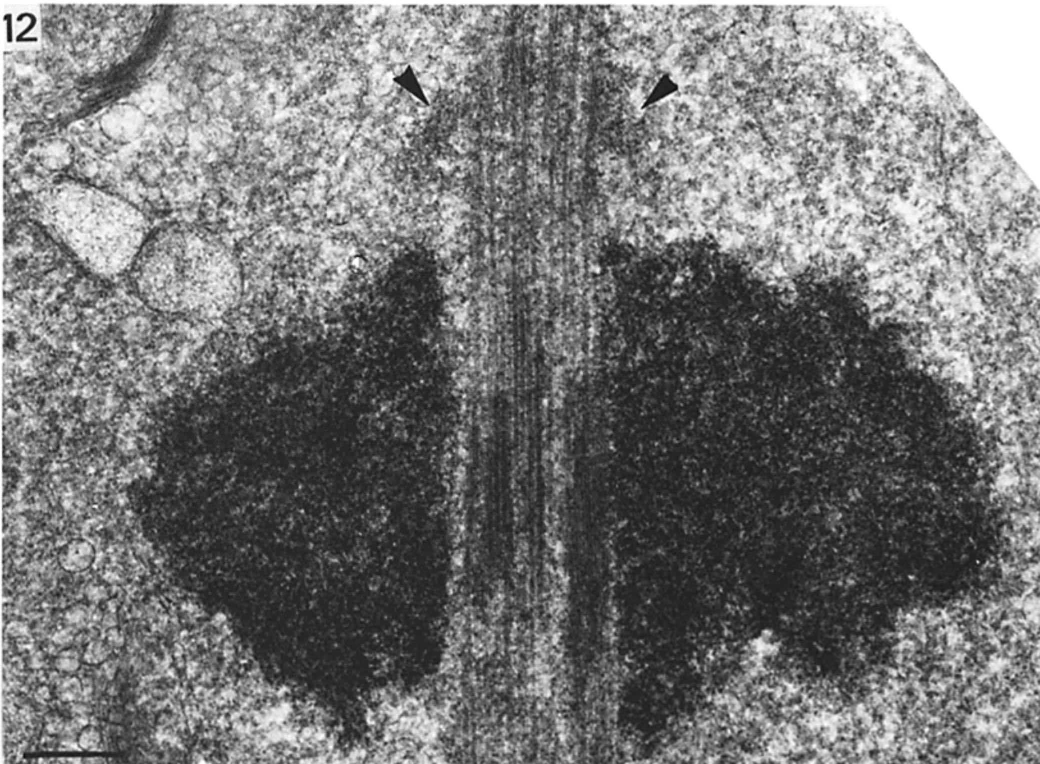


FIGURE 11 Metaphase. Strands of chromatin (small arrows) extend polewards along the surface of the central spindle. Similar strands (large arrow), farther away from the central spindle, are drawn polewards in association with MTs from the poles; elsewhere, other chromatin (*c*) may be moving inwards to the overlap, also in association with such MTs. × 17,500. Bar, 1 μm.

FIGURE 12 High voltage micrograph of a thick section of late metaphase cell showing "the collar" (arrows), which is usually not visible in longitudinal thin sections of the central spindle, i.e., Fig. 11. × 26,000. Bar, 0.5 μm.

external collar, but apparently the same amorphous material penetrates the MTs of the central spindle. Such material extends exclusively along the inside of the central spindle, between the leading edge of the chromatin and the poles (cf. Fig. 16*d*, *e*, *f* and with 16*b*).

The central spindle, at anaphase, does not appear to contain a population of typical kinetochore MTs, since no significant portion of MTs terminate where the chromatin is attached to the spindle. This is shown by counting the MTs in sections along the spindle; the number in the sections along each half spindle is fairly constant (±7 MT, until sections are taken close to the pole) and there is no appreciable change in the number as one passes through the chromatin and collar slightly towards the pole. For example, there are 219 MTs in section 16*b*, 226 MTs in sections 16*c* and *e*, and 213 MTs in section 16*d* (the number has actually decreased slightly closer to the pole). One would expect a distinct increase in this latter region if indeed the chromatin were attached to a population of kinetochore MTs which constituted part of the central spindle.

MT counts of the other half spindle of the cell shown in Fig. 16*a–f* reveal the same organization. As may be usual in diatoms, the total number of MTs in each half spindle add up to more than the total in the overlap; this matter is discussed at length in another paper.[1]

Between metaphase and anaphase the spindle elongates, while the length of the overlap region at first remains the same size or possibly even increases slightly (cf. Figs. 11 and 15). However, as in other diatoms (20), the central spindle later increases in length during late anaphase or early telophase concurrent with a reduction in the size of the overlap (cf. Figs. 15 and 17).

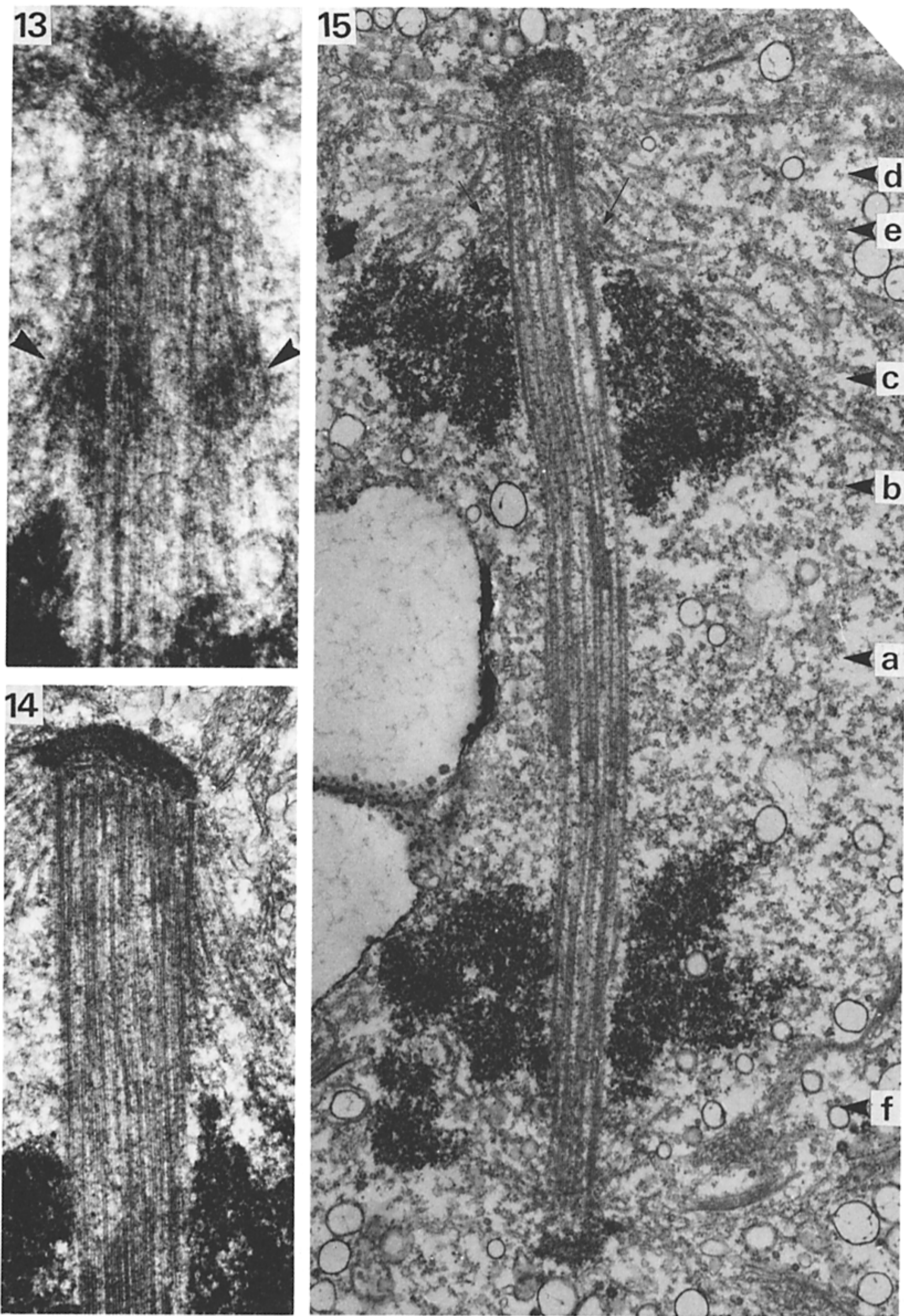
Telophase and the Formation of the New MC

By telophase, each polar complex separates from the central spindle, after which MTs rarely radiate from the polar complex (Fig. 17). Occasionally, those MTs which are not part of the central spindle continue to radiate from around the poles and penetrate the chromatin; many others pass into the cytoplasm, often tangential to and behind the doughnut-shaped chromatin, some of which at this stage has actually moved past the poles. The collar is not detectable on the telophase spindle between the two chromatin masses. Soon the ingrowing cleavage furrow breaks the central spindle near the remaining overlap (Fig. 18); the remains of the central spindle now rapidly disintegrate as the daughter nuclei reform. The chromatin rounds out, and the polar complexes stay outside the forming nuclear envelope which is surrounded by numerous Golgi bodies.

After separating from the central spindle, a new MC forms near each polar complex. When cytoplasmic cleavage is complete, one (Fig. 19) or more (Fig. 20) spherical aggregates begin to form on the edge of each polar complex which still possesses its laminate substructure and is associated with the nucleus. Later, only one such aggregate, now separate from the polar complex, develops into the new MC. The forming MC and the polar complex, followed by the nucleus, begin to migrate closer to the blunt end of the cell where cleavage was initiated. Each new MC soon acquires its typical interphase substructure and, as it enlarges, it clearly is separate from the polar complex (Fig. 21). The MC then moves close to the plasmalemma and becomes the focus of numerous MTs. During this movement, it is followed by the slowly disintegrating polar complex and the nucleus. Later, no trace of the polar complex can be found. The daughter nuclei enlarge, reform their nucleoli, and move even closer to the MC (Fig. 22); the nuclei become cone-shaped with their vertices ensheathed by MTs directed toward the MCs. Next, the nucleus migrates back into the center of the cell and becomes flattened against the chloroplast. During this movement, the MC does not precede the nucleus; instead, it faces the cleavage furrow, and later it assumes its interphase location against the side of the nucleus, roughly in the center of the cell.

Cytoplasmic Cleavage and Valve Formation

Cytoplasmic cleavage is initiated at late anaphase. A broad invagination appears first in the plasmalemma around the cell periphery. Soon afterwards, a narrow furrow grows inwards from the invagination (Fig. 18). The ingrowing edge of this furrow is lined with a dense layer of material which can be resolved into a layer of microfilaments, as shown in *Diatoma* (20), and which is now known to be a consistent feature of the cleavage furrow in several other diatoms. Cleavage proceeds as in *Diatoma* and is thus not described here in detail.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As in other diatoms, the silicalemma appears as a flattened vesicle which grows over the surface of the completed cleavage furrow; how it arises and then grows is not known. After the MC has migrated to the corner of the cell, the silicalemma is initiated near the region where the plasmalemma has become thickened and electron dense (Fig. 22, small arrows). The corner of the cell then rounds up to form a circular rim, lined internally by the growing silicalemma, and soon the siliceous wall begins to be secreted here. Thus arises the circular rim around the edge of the valve, characteristic of the species (Fig. 1). While the silicalemma extends over the plasmalemma, the siliceous wall also grows outwards inside this membrane, initially being thickest where formed at the rim. Thus, morphogenesis of the new cell wall involves several organelles, including briefly the MC. These most interesting events are being investigated in other diatoms, and are the subject of another paper.[2]

DISCUSSION

In his monograph of 1896, Lauterborn (12) described nuclear and cell division in several diatoms, and gave a particularly detailed description of *Surirella calcarata*. Several features of this division were reported to be unusual, for example, the behavior of the centrosome (MC) in generating a distinct, extranuclear spindle which later sank into the nucleus, the degeneration of the original centrosome, and the subsequent creation of a new centrosome at each daughter pole of the spindle. Fritsch (11; p. 609), in reviewing subsequent papers on this general subject, notes that many of Lauterborn's results were not supported by other investigators; Fritsch states: "A reinvestigation of *Surirella* would probably, with better methods now available, afford results in line with this conclusion. The peculiar mode of spindle formation in diatoms would scarcely have been so long accepted had the work on *Surirella* not been undertaken by so excellent an investigator as Lauterborn." Our results here, obtained 80 yr later, fill us with admiration of the completeness and accuracy of Lauterborn's work. Indeed, at times we have felt that we have simply been filling in details and confirming observations that Lauterborn technically should not have been able to make! His monograph is an instructive, and rather sobering, example of what can be achieved by patience and dedication in the pursuit of accurate scientific observation. Comparison of his elegant water colors with our electron micrographs has proved invaluable to us in the interpretation of our current work on this and other diatoms, e.g., chromosomal attachment to the spindle, Fig. 24.

The MC: Spindle Initiation and Valve Morphogenesis

The MC in *Surirella* is one of the largest and most structured microtubule organizing centers (MTOCs) known to us; it is somewhat similar to the "centrosphere" in certain heliozoan's (5). The MC has a distinctive substructure, similar to that of the taxonomically related *Cymatopleura* (authors' unpublished data) but quite different from the functionally similar, rather dense amorphous organelles in the unrelated *Melosira* (28) and *Pinnularia*.[3] Since the two species of *Pinnularia*

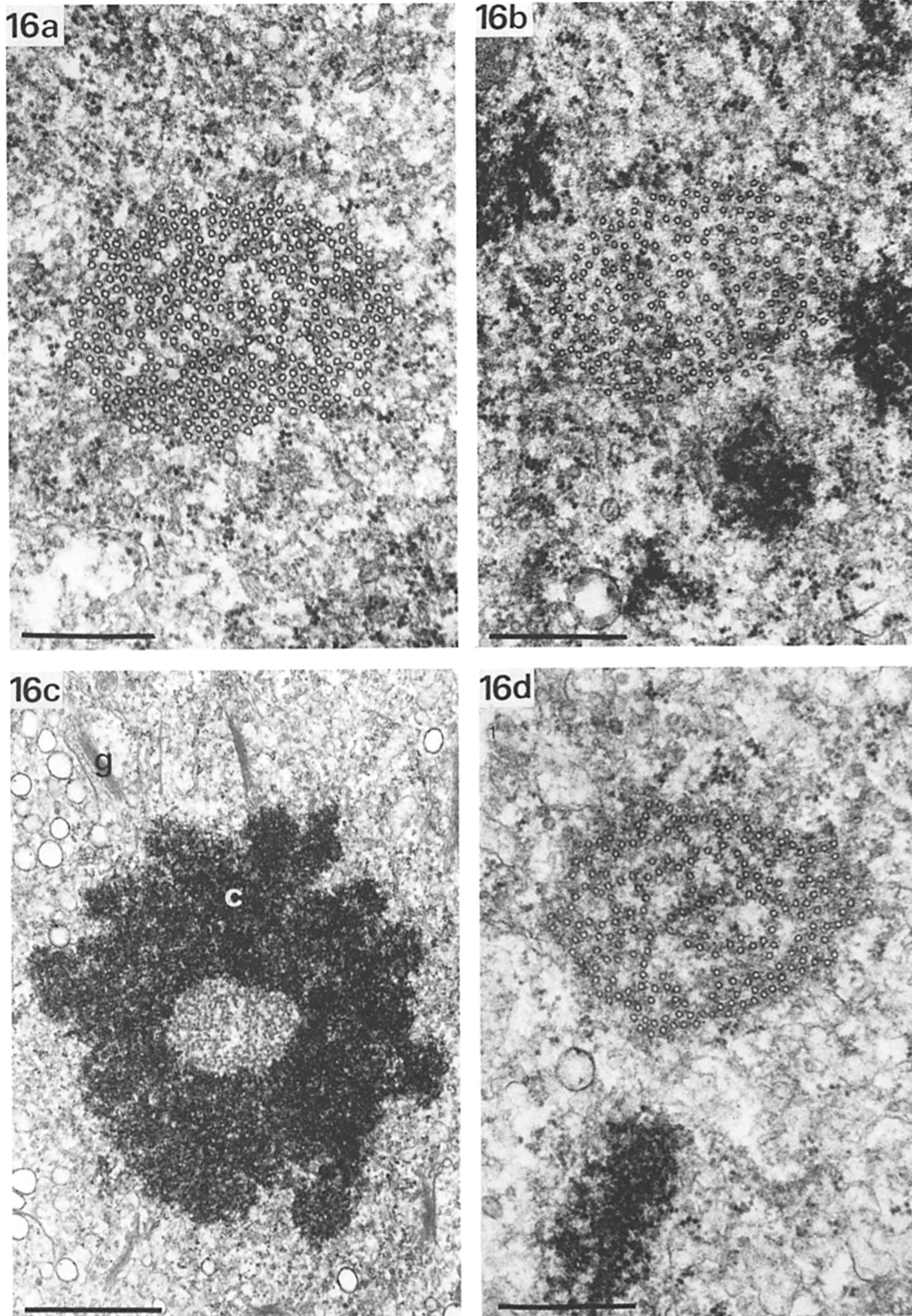
[2] Pickett-Heaps, J. D., and D. H. Tippit. Cell division in the pennate diatom *Pinnularia*. II. Valve Morphogenesis. Manuscript in preparation.

[3] Pickett-Heaps, J. D., and D. H. Tippit. Cell division in the pennate diatom *Pinnularia*. I. Mitosis. Manuscript in preparation.

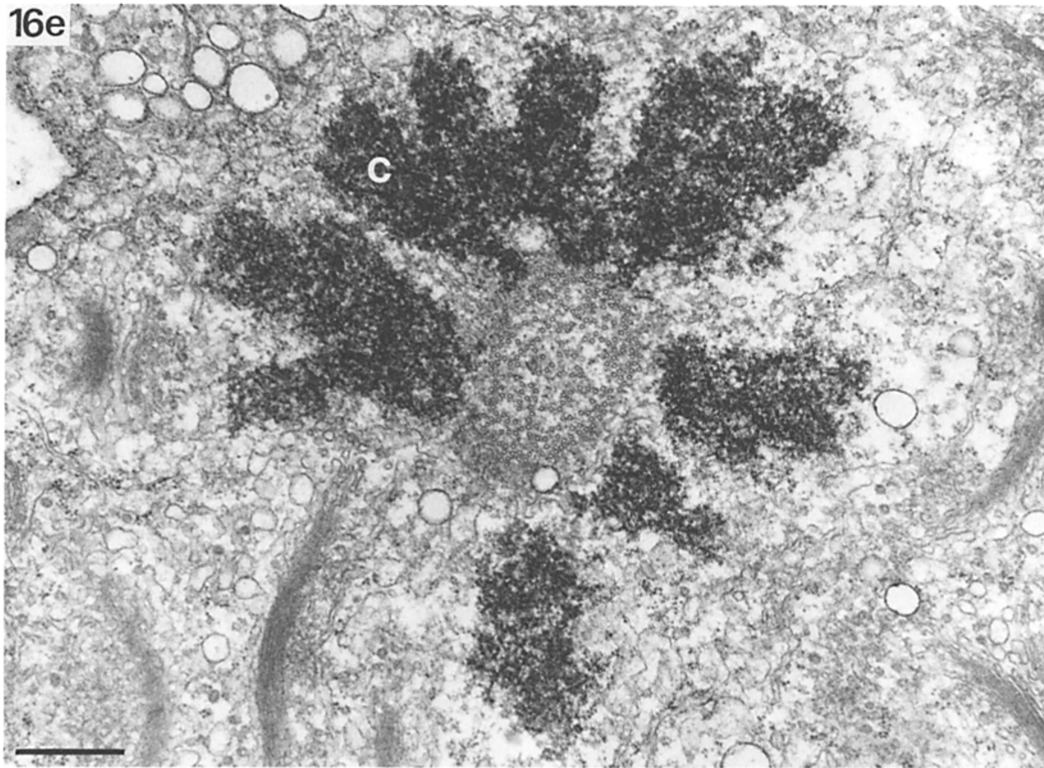
FIGURE 13 As for Fig. 12, except that this section grazing the central spindle reveals the collar as a dense ring. × 26,500. Bar, 0.5 μm.

FIGURE 14 One pole of a metaphase spindle. The MTs of the central spindle are highly ordered and do not terminate in the spindle insertion (see Fig. 4). Other MTs radiate from the poles, or possibly from along the central spindle to the chromatin. × 26,000. Bar, 0.5 μm.

FIGURE 15 Anaphase. The chromatin has separated into two masses penetrated by the central spindle; the leading edge of chromatin appears in contact with the collar (arrows) (see Figs. 16*e–f*). Some MTs radiating from or near the poles penetrate the chromatin; others laterally associate with it. The arrows labeled by the letters *a–f* show the position of those sections in Fig. 16 labeled *a–f*, all taken from a similar anaphase cell sectioned transversely. × 20,000. Bar, 0.5 μm.



FIGURES 16a–f Transverse sections through an anaphase spindle. The approximate positions of these sections along the spindle are indicated by the arrows a–f in Fig. 15. (a) The overlap region. × 51,000. Bar, 0.5 μm. (b) This section is taken beyond the overlap and therefore contains fewer MTs than Fig. 16a: these are arranged so that they generally associate in the overlap with MTs from the opposite pole. × 51,000. Bar, 0.5 μm. (c) This section passes through the chromatin (c) encircling the central spindle. Numerous Golgi (g) bodies line the nucleoplasm. × 21,000. Bar, 1 μm. (d) This section shows that the amorphous material of the collar extends along the central spindle between the chromatin and the poles. × 51,000. Bar, 0.5 μm.



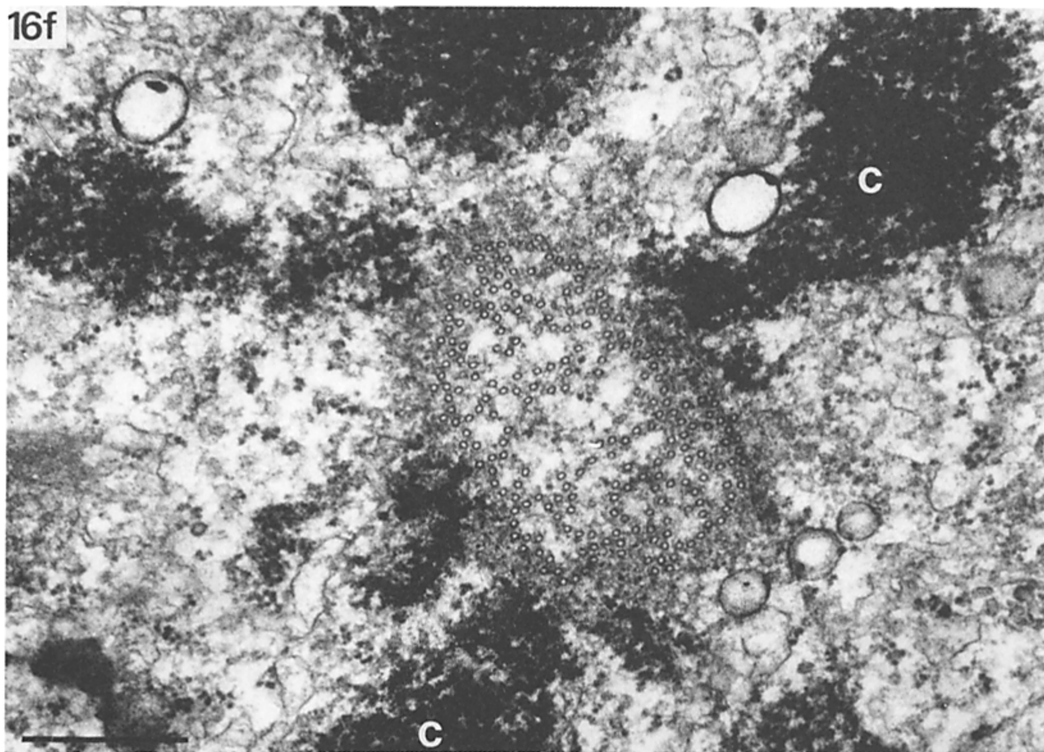


FIGURE 16 (*e*) This section passes through the collar and the leading edge of the chromatin. The V-shaped chromosomes (*c*) are attached at their vertices to the amorphous material (collar) which encircles the central spindle. × 29,000. Bar, 0.5 μm. (*f*) As for Fig. 16*e*, but through the other half spindle (see Fig. 15). × 55,000. Bar, 0.5 μm.

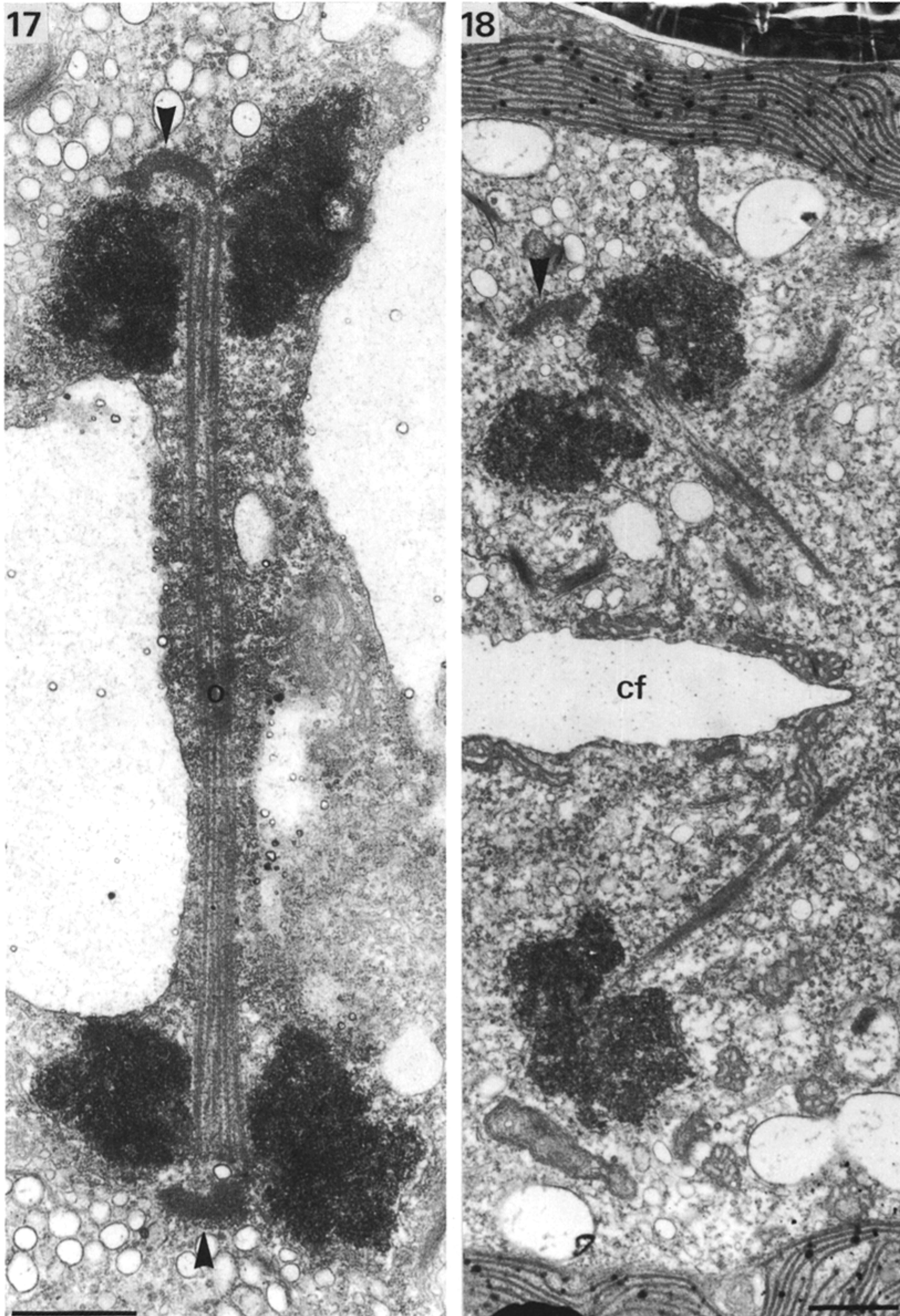


FIGURE 17 Telophase. The length of the overlap (*o*) has decreased compared with anaphase (Fig. 15), concurrent with spindle elongation. Some of the chromatin lies behind the poles. Each polar complex (arrows) is separated from the central spindle. × 19,000. Bar, 1 μm.

FIGURE 18 The ingrowing cleavage furrow (*cf*) cuts the intact central spindle. One polar complex (arrow) in the plane of this section has moved away from its reforming daughter nucleus. × 15,000. Bar, 1 μm.

that we have examined contain virtually identical interphase MCs, these organelles may turn out to be useful taxonomic indicators. In *Lithodesmium*, the interphase "spindle precursor" (equivalent to the MC) near the nuclear envelope is described (14, 15) as containing two dense terminal plates, i.e., it is already apparently a doubled structure, the possibility exists that the presence of such spindle precursors indicates a very early mitotic stage as appears in the case of *Pinnularia*, for example. In *Diatoma*, we have not been able to recognize an interphase MC with confidence, although a similar double structure becomes conspicuous before prophase.

The function of such MCs at interphase is not clear. However, in the few diatoms studied in detail (*Pinnularia, Melosira, Cymatopleura,* and *Surirella*), the MC is clearly associated with the creation of the polar complexes. In *Surirella,* the polar complexes arise next to the MC, after which the MC disintegrates; later, each polar complex in turn figures prominently in the appearance of the new interphase MC, and then the polar complexes disappear (these events have been summarized in Fig. 23). Such interdependence and cyclical creation and disintegration of the two types of MTOCs constitute more complex behavior than we had expected (it was incidentally, clearly visualized by Lauterborn). Our observations suggest that whatever their nature these MTOCs cannot necessarily transform directly from one structure into another, even if they are perhaps performing the same basic function of controlling and/or orienting MTs. In contrast, the rhizoplast of *Ochromonas* serves as an MTOC during interphase, involved in control of the cell shape (6) and also in mitosis as the spindle pole (6, 25). Certain fungi contain slightly more complex MTOCs; the interphase MTOC directly transforms into the structure later found at the spindle poles, which after mitosis reverts to its interphase form (23).

Each new MC, after its formation, appears significantly involved in valve initiation. Its movement to the corner of the newly formed daughter cells in *Surirella* coincides with the initiation of the silicalemma. In *Pinnularia*[2] this involvement is more easily visualized, as the MC, formerly at each pole of the spindle, drops back through a hole in the nucleus and comes to rest on the flattened surface of the completed cleavage furrow in the center of the cell, where the silicalemma appears. Thus, in both *Surirella* and *Pinnularia*, the MC is present near the initiation site of the silicalemma although this event occurs in spatially different regions of these cells.

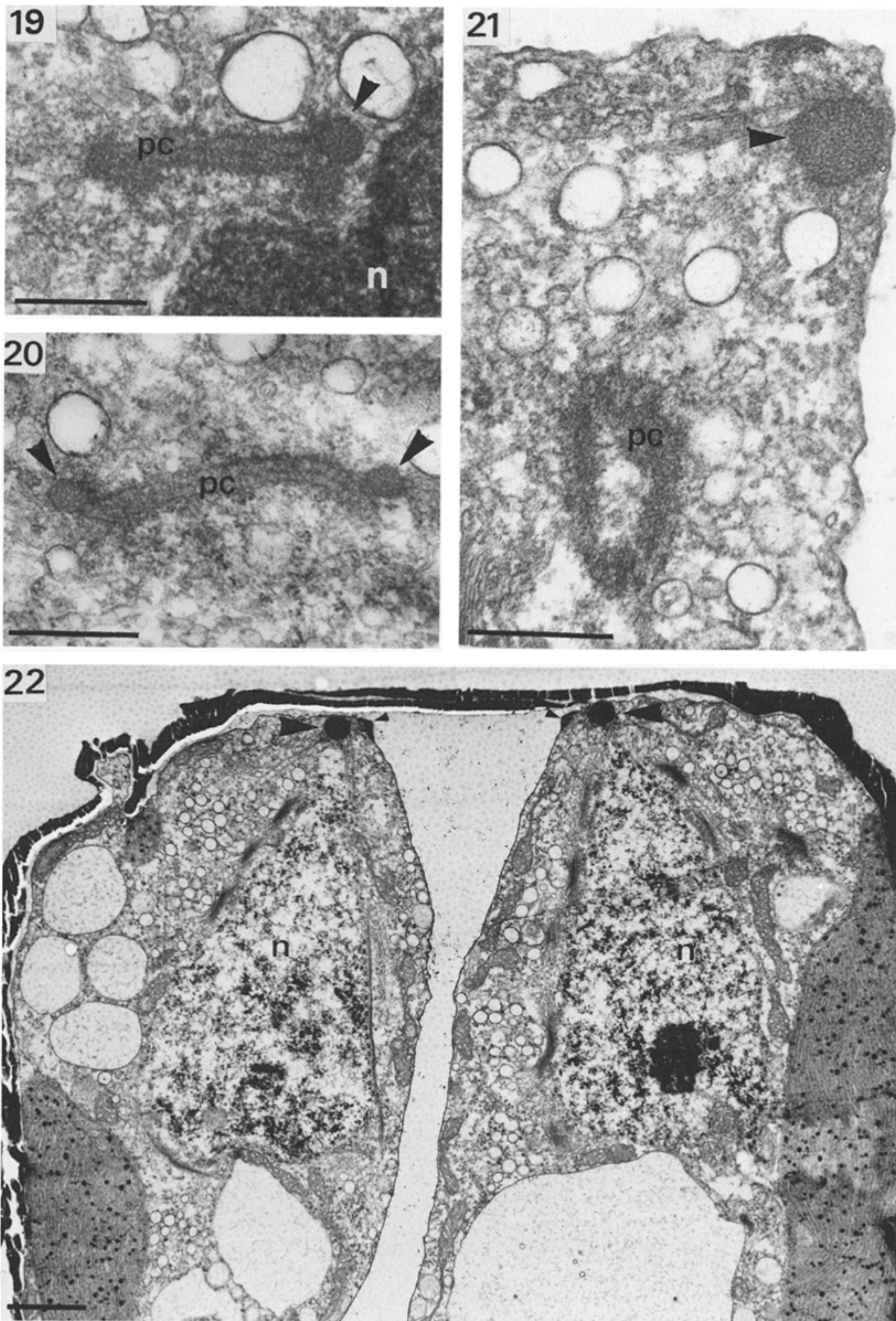
Chromosomal Attachment to the Spindle

This and another paper[3] offer some new and intriguing information concerning the attachment of chromosomes to the spindle in diatoms. The quality of fixation achieved in the work presented in these two papers is greatly improved in comparison with our previous work, and this may be responsible for the recent visualization of a major component of the spindle, a ring of amorphous material encircling portions of each half spindle which we have named the "collar."

For some time, we have been trying to clarify whether chromosomes in diatoms generally are attached to the spindle by the kinetochore/MT system common to many other organisms. Our earlier observations could be interpreted to comply with current models of such chromosomal attachment although we considered the matter unresolved (20, 28). We now are sure that diatoms are unusual in their chromosomal attachment to the spindle, and this has some unusual implications concerning the mechanism of anaphase motion in these organisms. The important relevant observations in this regard can be summarized as follows:

(*a*) In our early work (20, 28), we never encountered clear evidence for the attachment of the leading points of chromatin to MTs. Instead, we always gained the strong impression that, as in *Surirella* and *Lithodesmium* (16, 17), many of the MTs radiating out from the poles penetrated the chromatin and/or were laterally associated with strands of chromatin drawn polewards.

(*b*) We have included a set of illustrations (Fig. 24) of division in *Surirella calcarata* redrawn from Lauterborn's work (12). These drawings show the positioning of the chromosomes during mitosis, which is exceedingly difficult to observe by electron microscopy. In live cells, Lauterborn observed that during prophase each of the V-shaped chromosomes attach their apex (kinetochore region?) nonsimultaneously, to the central spindle (Fig. 24 *a–d*). At anaphase, the leading point of each chromosome is tightly appressed to the surface of the central spindle (Fig. 24 *d* and *e*); such an intimate association is clear under the electron microscope, but occasionally we have also observed strands of chromatin farther out from the central spindle (particularly during metaphase) which are drawn polewards. In live cells, Lauterborn could see no spindle fibers attaching to the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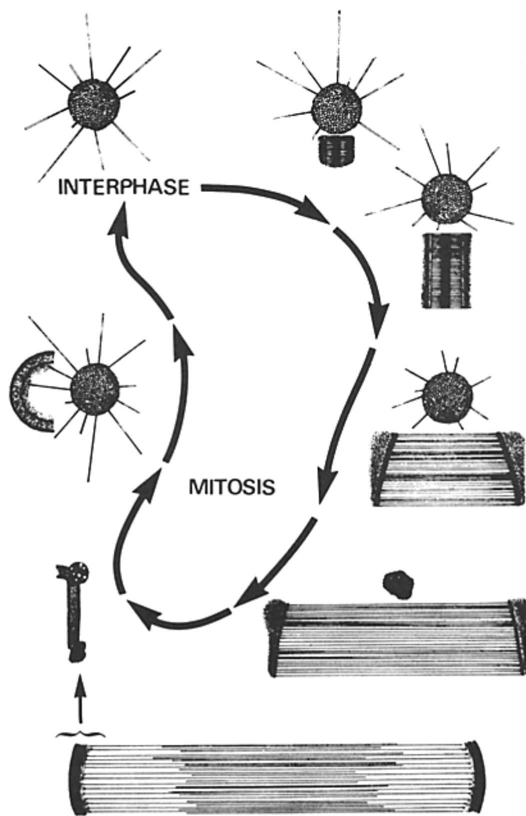

FIGURE 23 This diagram illustrates the behavior of the MC during the cell cycle. During interphase, the MC is the focus of numerous MTs. At preprophase, the MC is intimately associated with the formation of the central spindle. Soon afterwards, the MC disappears; it is not observed at metaphase. At late anaphase, each polar complex (the structure at the spindle poles) separates from the central spindle. A new MC is differentiated near each polar complex in the daughter cells; later, the polar complex disappears. For simplicity, the diagram does not illustrate any changes in the size of the MC during spindle formation.

chromosomes; he therefore concluded that the motion of the chromosomes must come from the "chromosomes themselves."

(*c*) Because of the highly ordered nature of the central spindle, we can with great accuracy count the MTs comprising it at different points along its length, and in the case of *Diatoma* each MT has been tracked through consecutive serial sections.[1] We have never observed any significant increase in MT number at the leading point of the chromosome masses at anaphase, in either *Surirella* or *Diatoma*, indicating that the chromosomes do not attach to kinetochore MTs which are part of the central spindle. A few MTs of the central spindle end short of the overlap; many of these are situated inside, e.g., in the middle, of the central spindle which would make conventional attachment of chromosomes to them impossible. However, it is more difficult to locate the end points of other MTs which are not part of the central spindle but which also radiate from the spindle poles and are therefore not so easily visualized in our transverse sections of the spindle. We cannot rule out the possibility that a few or even a single kinetochore MT per chromosome could exist. However, such MTs, if attached to the leading point of the chromatin, should lie close to the central spindle, and we would have expected them to be visible.

(*d*) Our present work shows that the leading points of the chromosomes at anaphase embed in the ill-defined collar that permeates the outer MTs of each half spindle between the leading edge of the chromatin to the pole. This result is, at first, puzzling and rather difficult to accept. However, our work on *Pinnularia*[3] and *Synedra* (authors' unpublished data) reveals an entirely equivalent and even more striking collar, which is clearly present at prophase even when chromosomes are dispersed throughout the nucleus.

FIGURE 19 The polar complex (*pc*), still situated near one reforming nucleus (*n*), differentiates a small spherical aggregate (arrow) that later becomes the new MC. × 40,500. Bar, 0.5 μm.

FIGURE 20 As for Fig. 19, except that two of these spherical aggregates (arrows) are associated with the polar complex; the significance of two such structures is not understood. × 41,000. Bar, 0.5 μm.

FIGURE 21 The newly formed MC (arrow) migrates to a corner of the cell where cleavage was initiated. The polar complex (*pc*) then disappears. × 46,500. Bar, 0.5 μm.

FIGURE 22 The reformed daughter nuclei (*n*) are ensheathed with MTs radiating from each new MC (large arrow). The silicalemma is initiated near each MC at the differentiated area of the plasma membrane (small arrows). × 6,700. Bar, 2 μm.

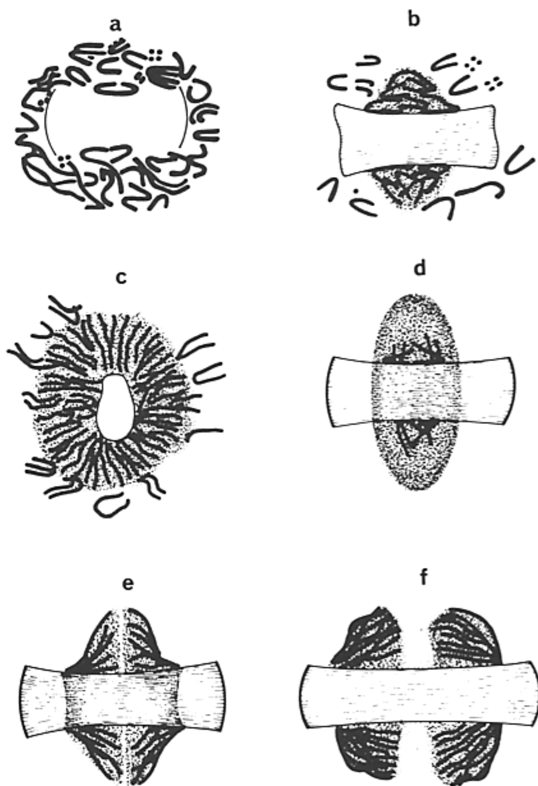

FIGURE 24 This diagram shows some stages of mitosis in *Surirella calcarata* redrawn from Lauterborn's (12) monograph (certain details have been omitted from Lauterborn's original watercolors; only the chromosomes and the central spindle are shown). (*a*) Prophase. The central spindle sinks into the nucleus which contains numerous paired chromosomes. (*b*) Prometaphase. The chromosomes begin to aggregate around the central spindle. (*c*) Prometaphase in transverse view. The V-shaped chromosomes clearly are attached at their vertex to the central spindle. (*d*) Metaphase, (*e* and *f*) At anaphase, the chromosomes are attached to the central spindle as they move polewards. The leading point of motion is the chromatin next to the central spindle.

These observations, and the consistent impressions gained from looking at hundreds of diatom spindles in several species, now make us confident that the chromosomes attach to the spindle in what appears to be an unorthodox manner. The nature and significance of the collar naturally excites speculation, particularly since it appears that the collar and the chromatin move concurrently to the poles. Even more puzzling is that although typical kinetochore MTs have not been identified, kinetochore structures have now been found on the chromosomes of *Pinnularia*[3] and *Cymatopleura* (authors' unpublished data) which are unattached to the central spindle, e.g., during prometaphase or after colchicine treatment. In *Surirella* we suspect that ill-defined, lightly stained areas on the chromatin may represent such kinetochores (one is visible in the left piece of chromatin in Fig. 10) although they are not so distinct as in *Pinnularia*. Hopefully, further work will clarify some of the interesting possibilities that these results suggest.

Nonkinetochore or Passive Transport

The transport of various kinds of particles along systems of MTs has been quite widely reported. Some examples include axons (29), certain protozoa which have axopods (10, 24, 27), melanophores and erythrophores (18, 21), and the phragmoplast of higher plants (1, 3). In some cases it appears that MTs may influence the direction of this motility but may not provide the motive force. In the mitotic apparatus, forces directed poleward act on more than just the kinetochores (4, 13, 19); various particles in living cells, (e.g., persistent nucleoli and chromosomal fragments, if present) move polewards during both metaphase and anaphase at approximately the same rates at which chromosomes move; this movement, although not understood, appears to be in some manner MT-dependent, since such granules move polewards when adjacent to a chromosomal fiber (2). There is no reason why such forces could not also contribute to achieving chromosome separation. Indeed, several workers have speculated that material associated with MTs may be responsible for movements within the mitotic spindle. Bajer and his colleagues have always emphasized that the kinetochore fiber itself is transported polewards during anaphase and, thus, it too could be subjected to some kind of MT-mediated movement.

If Lauterborn's drawings (e.g., Fig. 24) are closely examined, a consistent feature of anaphase is that part of the chromosomes is appressed to the surface of the central spindle. In our studies on numerous diatoms, this phenomenon is also clear. Furthermore, at early telophase, the chromatin, sometimes resolvable into chromosomes, which arrives first at the pole is subsequently pushed around past the pole as telophase proceeds (present paper, footnote 3, and reference 28). Such morphological evidence suggests that the chromosomes themselves may be transported polewards

due to their lateral association with the surface of the central spindle. This type of phenomenon would be consistent with the transport properties associated with the MTs in many other spindles.

Formation and Elongation of the Central Spindle

The diatom's central spindle at metaphase is clearly composed of two half spindles interdigitated to form the central overlap. Micrographs suggest that the overlap is generated during prophase, when the MTs of the spindle run from one pole to the other; even now the spindle could be composed of two half spindles. If one reflects upon the way in which the MTs grow by addition of subunits to the ends (intussusception of subunits will not be considered here), several ways of forming this spindle can be imagined with interesting alternative implications for spindle function.

Let us assume first that the MTs comprising each half spindle grow only either at the "free" end *or* at the "attached" end, i.e., that end embedded in the polar complex. If they grow only at their free end, to generate the overlap while the spindle is increasing in length (i.e., Figs. 6 and 8*a*) the MTs from either pole must slide (or shift) past one another in the same direction in which they appear to move when the overlap is diminished during anaphase. If they grow only from their attached end, then they must slide in the opposite sense of what they do later. Similar results are obtained assuming MT-biased polar growth. Another alternative is that the MTs can grow during prophase at an equal rate from both ends; in this event, the overlap can be generated without any movement of MTs past one another. However, something must happen to stop further growth at one end of the MTs comprising each half spindle, once sufficient overlap has been created.

We cannot yet resolve these (or a host of variant) possibilities. But this reasoning suggests that variations in the size of the overlap during early stages of mitosis may become significant. It is risky to compare directly the size of the overlap from one spindle to another. But the impression we gain from the study of many diatom spindles is that, after a length of overlap is generated at prophase, it decreases somewhat by prometaphase, and then may increase slightly during metaphase-early anaphase, while the central spindle is increasing in length, before it (rather abruptly) decreases to almost nothing at late anaphase. MT/MT sliding apparently is responsible for the diminution of the overlap during late anaphase,[1] and thus a similar mechanism could be responsible for its diminution at early prometaphase. The changes in the size of the overlap during mid-mitosis may be related to the differing rates of MT polymerization at both ends of each half spindle. Obviously, the central spindle of diatoms has many possibilities for the study of MT polymerization as well as for MT/MT interaction in the generation of movement.

Multiple Mechanisms of Chromosomal Separation

A most important feature of the diatom spindle is that it allows the separate identification of at least three mechanisms which collectively move the chromosomes apart.

(*a*) In *S. ovalis*, the central spindle increases in length during metaphase and anaphase, while the size of the overlap remains the same or increases slightly. Since the overlap does not decrease, and since most MTs comprising each half spindle are known to have one end at the overlap and the other at the pole, increase in the length of these MTs (by addition of MT subunits) must accompany this phase of spindle elongation.

(*b*) Later, the central spindle elongates, concurrent with a marked reduction in the size of the overlap; as in other diatoms, this phase of spindle elongation apparently results from a MT/MT sliding mechanism, although it remains to be shown if this is active sliding generated by a mechanochemical mechanism operative in the overlap, or passive sliding apart of the two half spindles.

(*c*) Each set of chromosomes obviously moves towards its pole. The means by which this particular phase of movement is achieved is not clear; an interaction of the collar with the central spindle could be anticipated.

In summary, it appears that both MT/MT sliding and MT assembly figure prominently in spindle elongation, while chromosomal movement from the middle of the spindle to the poles is achieved separately. It is a debatable question whether diatom mitosis is equivalent to other types of mitosis. We believe it likely that more than one mechanism is acting to create the complex movements that accompany mitosis in many organisms. Distinguishing between these separate mechanisms that collectively achieve chromosomal separation may be more difficult than has been the case with the highly structured diatom spindle.

We are grateful to Dr. David McIntire, who kindly identified this species of *Surirella* for us.

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