MITOSIS IN THE CELLULAR SLIME MOLD *POLYSPHONDYLIUM VIOLACEUM*

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ABSTRACT

Myxamebas of *Polysphondylium violaceum* were grown in liquid medium and processed for electron microscopy. Mitosis is characterized by a persistent nuclear envelope, ring-shaped extranuclear spindle pole bodies (SPBs), a central spindle spatially separated from the chromosomal microtubules, well-differentiated kinetochores, and dispersion of the nucleoli. SPBs originate from the division, during prophase, of an electron-opaque body associated with the interphase nucleus. The nuclear nevelope becomes fenestrated in their vicinity, allowing the build-up of the intranuclear, central spindle and chromosomal microtubules as the SPBs migrate to opposite poles. At metaphase the chromosomes are in amphitelic orientation, each sister chromatid being directly connected to the corresponding SPB by a single microtubule. During ana- and telophase the central spindle elongates, the daughter chromosomes approach the SPBs, and the nucleus constricts in the equatorial region. The cytoplasm cleaves by furrowing in late telophase, which is in other respects characterized by a re-establishment of the interphase condition. Spindle elongation and poleward movement of chromosomes are discussed in relation to hypotheses of the mechanism of mitosis.

The cellular slime molds (Acrasiales or Acrasiomycetes; Bonner, 1967; Raper, 1973) are eukaryotes with a simple life cycle. A period of cell multiplication, during which myxamebas exist as independent cells, is followed, upon depletion of food organisms, by aggregation into cell masses. Differentiation and morphogenesis culminate in the formation of stalked fruiting bodies containing spores. These release myxamebas upon germination to start a new cycle (for reviews see Bonner, 1967, 1971).

The taxonomic position of the cellular slime molds is still controversial. They are included in the protozoa by some authors, but also appear in treatises on fungi (Ainsworth, 1973; Raper, 1973). Knowledge of mitosis in an ever-increasing number of organisms has established the possibility that ultrastructural features of the mitotic apparatus can serve as taxonomic criteria (Pickett-Heaps, 1972; Pickett-Heaps and Marchant, 1972). Applied to *Polysphondylium violaceum* this principle might indicate whether the cellular slime molds are more closely related to protozoa or to fungi.

Investigations of the mechanism of mitosis have greatly profited from new techniques combining light microscopy with electron microscopy of selected cells sectioned at a determined angle (Brinkley and Cartwright, 1971; McIntosh and Landis, 1971). This approach is impracticable with cellular slime molds because of the small size of the nuclei. Furthermore, the duration of mitosis is short (Ross, 1960). The only possibility, therefore, was to cut random sections of random cells, but the disadvantages of this approach were largely

compensated for by the use of a mass culture method which yielded a satisfactory number of cells in different stages of mitosis.

The ultrastructure of mitosis in *P. violaceum* proved interesting from two points of view. First, the persistent nuclear envelope, the presence of spindle pole bodies (SPBs), (Aist and Williams, 1972), the tightly organized central spindle, and the simple but well-differentiated kinetochores are features of mitosis in fungi rather than protozoa. Second, because of the simple architecture of the mitotic apparatus it was possible to distinguish clearly between two spatially independent components, viz. the central spindle and the chromosomal microtubules. Functional independence of the two components was deduced by comparing early and late stages of division. In light of these observations some of the current hypotheses of spindle function were ruled as not applicable to mitosis in *P. violaceum*.

MATERIALS AND METHODS

Myxamebas of a clone of *Polysphondylium violaceum* Brefeld were grown in association with *Escherichia coli* B in Sussman's (1961) medium. Cultures were incubated to log phase on a reciprocal shaker at 25°C in the dark. For fixation, a working solution of 6.5% glutaraldehyde was prepared by diluting the 50% stock solution in 0.07 M Sörensen's (1912) phosphate buffer, pH 6.8. Of this working solution, 1 vol equal to that of culture medium was added to each flask to give a final concentration of 3.2% glutaraldehyde (final concentration of buffer approximately 0.03 M). Amebas were fixed for 1.5 h at room temperature with intermittent agitation, centrifuged for 2–3 min at 300–500 *g*, rinsed three times in buffer, and postfixed in buffered 1% OsO_4 for 1 h at room temperature. After another rinse in buffer the amebas were pelleted in 2% agar, and the pellets were diced, rinsed three times in cold distilled water, and prestained in cold aqueous 2% uranyl acetate for 1 h. The agar dices were dehydrated in a graded series of cold ethanol, transferred to propyleneoxide, and embedded in Epon. Serial sections in the silver range were cut with a diamond knife on a Reichert OmU-2 ultramicrotome, transferred to single-hole grids coated with Formvar and carbon, and stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Sections were examined in an A.E.I. EM 6B electron microscope at 60 kV, with objective apertures of 25 or 50 μm. The microscope was calibrated with a carbon replica grating.

OBSERVATIONS

Interphase

Most myxamebas are uninucleate. Each interphase nucleus is associated with a compact, very electron-opaque body (NAB, nucleus-associated body; Figs. 1, 2). In sections passing through both this body and the nucleus the latter has typically the shape of a pear or teardrop (Fig. 1). The NAB always lies near the tapered end of the nucleus. Microtubules radiate from it into the cytoplasm. Some of the tubules run very close and almost parallel to the nuclear envelope (Fig. 2). In its morphology, osmiophilia, and relationship to microtubules the NAB resembles a microtubule-organizing center as defined by Pickett-Heaps (1969).

The nuclear matrix consists of moderately electron-opaque, granulofibrillar material. The peripheral nucleoli (Mercer and Shaffer, 1960; Raper, 1973) are in intimate contact with the nuclear envelope (Fig. 1). They are very electron-opaque and coarsely granular.

Prophase and Prometaphase

An extensive search for early stages of mitosis yielded only one incomplete series of sections of an apparent prophase nucleus (Figs. 3–6). The nuclear envelope is intact, except for the bottom of an invagination, where two electron-opaque bodies are located (Fig. 3). These are foci for cytoplasmic and intranuclear microtubules (Figs. 4–6). Tubules connecting the two bodies constitute a rudimentary central spindle (Figs. 5, 6). Other microtubules extend to the peripheral mass of electron-opaque material (Fig. 4), which is less condensed than the nucleoli of interphase cells and less homogeneous. By analogy with later stages (see below) the more finely fibrillar and more electron-opaque patches in this mass can be identified as profiles of chromosomes. Accordingly, putative microtubule-to-chromosome connections can be recognized (Fig. 4).

Metaphase

This stage is characterized by as yet unseparated sister chromatids attached to kinetochore microtubules (Fig. 7). In two favorable sections I observed pairs of sister chromatids approximately at the equator of the spindle, but eccentric positions also occur, with one chromatid connected to the near pole, while its sister is connected to the far pole (Fig. 7). The dispersed nucleoli fill the nucleus except for a central channel, in which the mitotic spindle lies. The mitotic apparatus consists of four components: (*a*) spindle pole bodies; (*b*) a central spindle; (*c*) chromosomal microtubules; (*d*) astral microtubules.

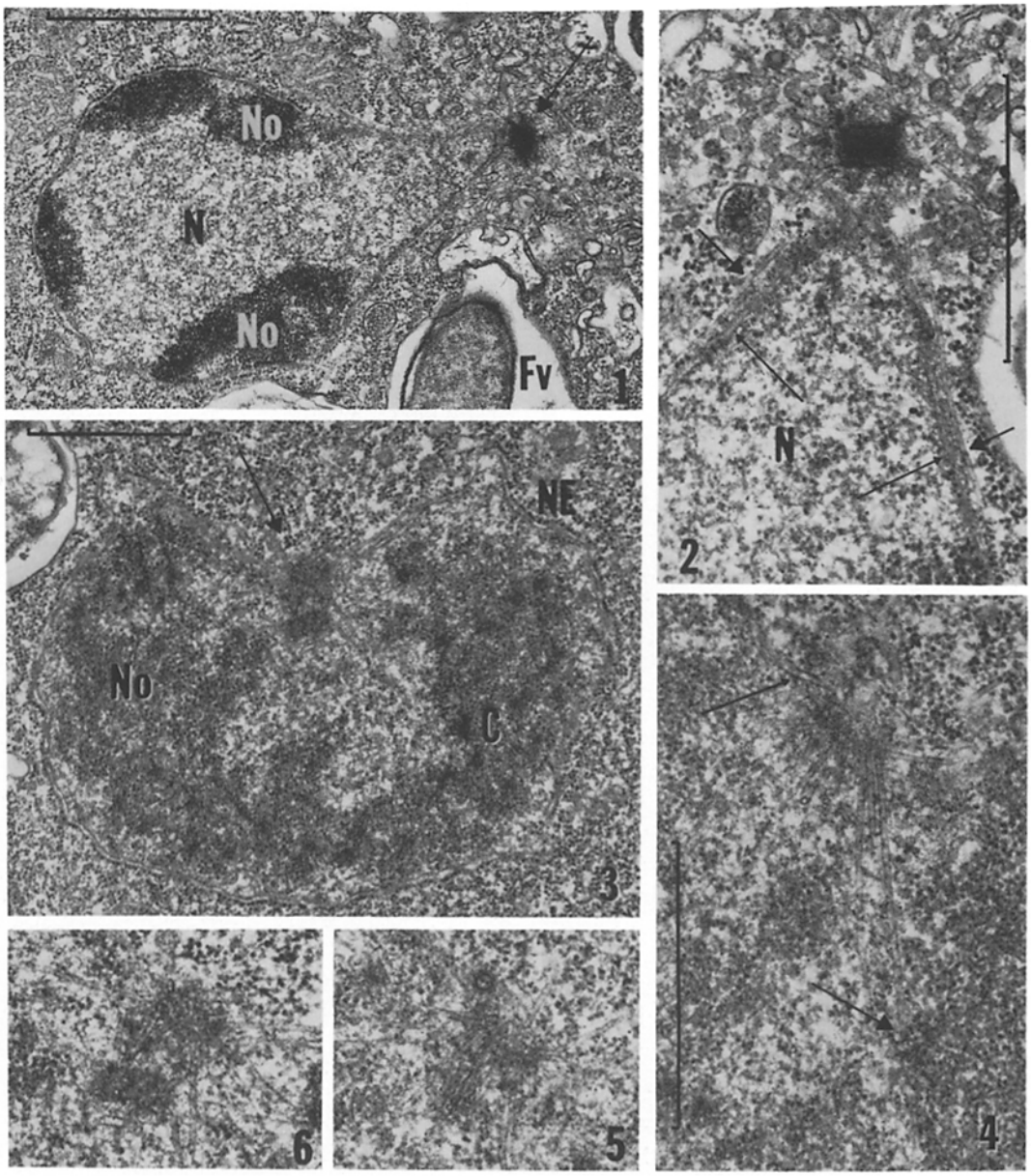


FIGURE 1 Interphase nucleus (*N*). Note the electron-opaque body associated with the nucleus (*NAB*, arrow), and the nucleoli (*No*). *Fv* is a food vacuole containing a bacterium. × 22,500. Scale = 1 μm.

FIGURE 2 Interphase nucleus (*N*) and NAB. From the latter, microtubules run in various directions. Two microtubules (large arrows) are close and parallel to the nuclear envelope (small arrows). × 39,600. Scale = 1 μm.

FIGURE 3 Prophase nucleus. Most of the granular, electron-opaque mass (*No*) represents the dispersed nucleoli. Denser patches (*C*) are chromosomes. The nuclear envelope (*NE*) is discontinuous at the bottom of an invagination (arrow), where two electron-opaque bodies lie. × 22,500. Scale = 1 μm.

FIGURES 4–6 Three serial sections through the electron-opaque bodies of the nucleus of Fig. 3. One of the bodies is closely associated with the nuclear envelope (small arrow in Fig. 4). Microtubules radiate from the bodies into the nucleus (large arrow in Fig. 4: a putative microtubule-chromosome contact) and out into the cytoplasm. Short microtubules seem to connect the two bodies (Figs. 5, 6). Scale = 1 μm. × 39,600.

(*a*) The extranuclear SPBs are electron opaque and of granulofibrillar structure (Figs. 7, 8, 10), From serial sections I concluded that they are rings approximately 300 nm in diameter and 100 nm thick (cf. Fig. 18).

(*b*) The central spindle consists of approximately parallel, straight or slightly curved microtubules. These tubules originate in an SPB, penetrate into the nucleus through polar openings (fenestrae) in the nuclear envelope, and most likely terminate in the SPB at the opposite spindle pole (Figs. 7, 8, 10). I have not obtained a section illustrating the pole-to-pole continuity of microtubules unequivocally, but images such as Fig. 8 make this interpretation a realistic one.

(*c*) Each chromatid is directly linked to an SPB by a single microtubule (Fig. 7; also verified from serial sections). When both members of a pair of chromatids are visible in a section it is clear that they are oriented to opposite poles (amphitelic orientation; Figs. 7, 9). Chromosomal microtubules terminate at a distance of approximately 500 Å from the surface of the chromatids (Fig. 9). The tip of these tubules is embedded in finely fibrillar osmiophilic material often in the shape of a band approximately 200 Å wide (see also Figs. 12, 16). This band is commonly separated from the chromatid proper by an electron-lucent space approximately 300 Å wide.

(*d*) Astral microtubules extend from SPBs into the cytoplasm (Figs. 7, 8). The asters appear rather modest in general, for only a few microtubules occur in any one section. The longest astral microtubules run along the nuclear envelope in a manner similar to the microtubules associated with the NAB of interphase cells.

Anaphase and Telophase

Anaphase nuclei have a characteristic shape (Fig. 11). The polar faces are formed into oblique cones and a constriction is apparent in the equatorial region. The length of the spindle is greater than at metaphase and the microtubules of the central spindle are more tightly bundled. Chromosomes remain condensed and their section profiles are scattered throughout the dispersed nucleolar material. Kinetochores are predominantly located in the central channel, near the spindle poles (Fig. 12).

Spindle elongation and equatorial constriction of the nucleus seem to be concurrent events during anaphase. In late anaphase the incipient daughter nuclei are connected by a narrow nuclear bridge (Fig. 13). The central spindle is a shaft of microtubules connecting the SPBs. It lies either within the nuclear bridge or it is parallel to the latter, passing out of one daughter nucleus and into the other (Fig. 14). Therefore, the nuclear envelope is either intact in the region of the isthmus or discontinuous at the points of penetration of the tubular shaft (Fig. 14).

Telophase nuclei are dumbbell shaped (Fig. 15). It is evident that the central microtubules still insert into the SPBs (cf. Fig. 14), but the length of the spindle makes it very difficult to determine whether they form a single, continuous shaft, or two interdigitating bundles. Kinetochores are grouped near the SPB, into which the very short chromosomal microtubules insert (Fig. 16). For comparison the mean length of chromosomal microtubules is given in Table I for meta-, ana-, and telophase nuclei.

The farther advanced a cell is in telophase the more pronounced is the conical shape of the polar faces of the daughter nuclei (Figs. 13–15, 19). The envelope of each daughter nucleus is drawn out to a beak on the interzonal side, where it encloses the microtubular shaft (Fig. 17). There are fewer shaft tubules in late than in early telophase, and in very late telophase (cytokinesis) the extranuclear portion of the shaft is absent, while an intranuclear remnant persists (Fig. 20). There is no evidence of cytokinesis in cells that have not completed karyokinesis (Fig. 19). I did not observe microfibrils in the region of the cleavage furrow, although they are common at the base of phagocytic vacuoles and in pseudopods.

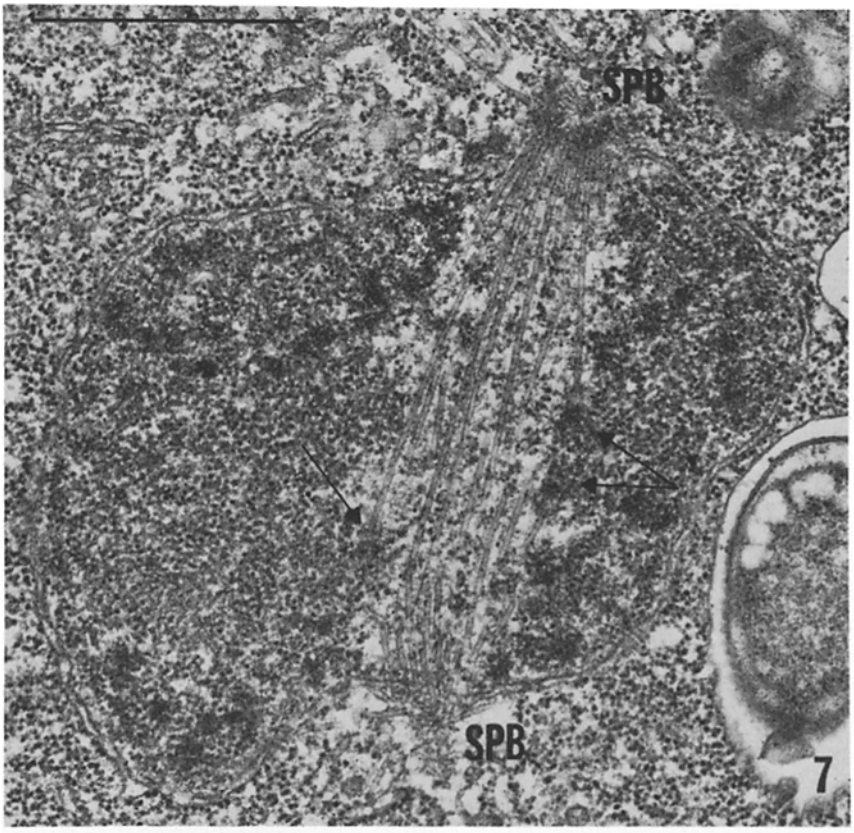
Nuclei in very late telophase resemble interphase nuclei (compare Figs. 19, 21, with Figs. 1, 2). The beak and polar fenestrae are closed, the chromosomes are dispersed, the nucleoli are recognizable, and the SPBs, lying at the tapered end of the nucleus, are no longer ring shaped. Rather, they are compact and electron opaque, thus resembling NABs of interphase cells (Figs. 19–21).

DISCUSSION

Spindle Components and Sequence of Mitotic Events

The principal stages of mitosis as they can be reconstructed from ultrathin sections are summarized in Fig. 22 *a–f*.

(*a*, *b*) The transition from interphase to prophase is indicated by the dispersal of the nucleoli and the condensation of the chromosomes. Spin-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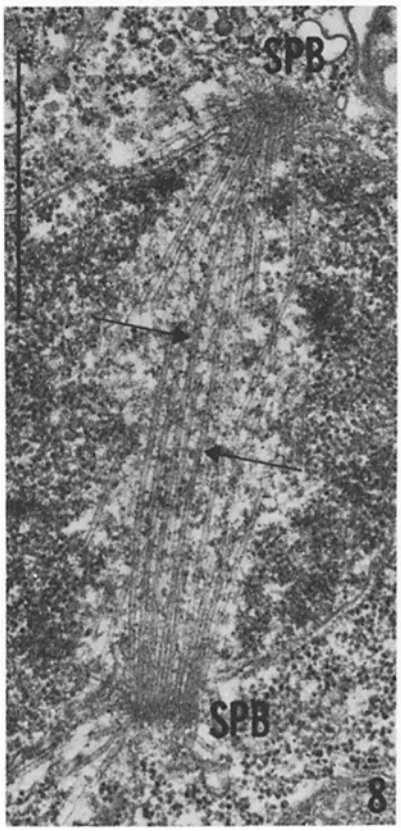
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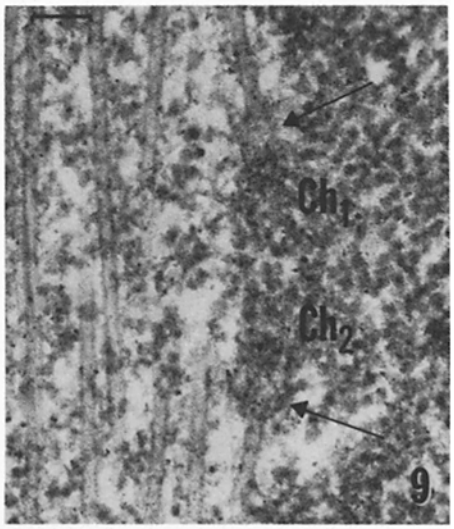
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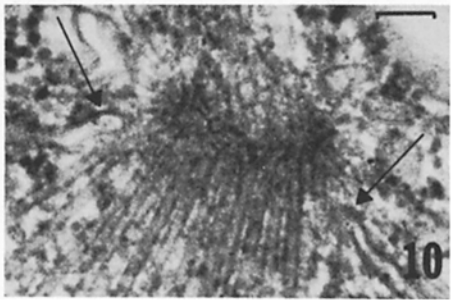
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dle pole bodies are formed by division of the electron-opaque body associated with the interphase nucleus. This event is incompletely documented due to the lack of sections from nuclei in the appropriate stages. However, the genesis from a single unit, in the early prophase, of two organelles that later occupy a position at the spindle poles is well documented, for example, in ascomycetes (McCully and Robinow, 1971; Moens and Rapport, 1971), basidiomycetes (Girbardt, 1971; Lerbs and Thielke, 1969), and fungi imperfecti (Aist and Williams, 1972) and there is no reason to assume that it is different in *P. violaceum*. More evidence, though circumstantial, lies in the observation that SPBs as well as NABs are foci for microtubules. Perhaps the most convincing argument in support of the above contention is the transition from SPBs to NABs at the end of division (see below).

The central spindle is most probably formed between the SPBs which move apart as the spindle elongates (Figs. 5, 6). Examples of such behavior involving other types of polar organelles are the alga *Lithodesmium undulatum* (Manton et al., 1969) and the fungi *Thraustotheca clavata* (Heath, 1974), *Saprolegnia ferax* (Heath and Greenwood, 1970), and *Polystictus versicolor* (Girbardt, 1968).

The SPBs possibly play the role of organizing centers (Nicklas, 1971) directing the construction of the central spindle. The nuclear envelope opens up opposite the spindle pole bodies during prophase (Fig. 3). The chromosomes nearest these sites can thus establish microtubular connections to one or both SPBs before the central spindle reaches full metaphase length in a way similar to mammalian cells (Roos, 1973).

(*c*) Elongation of the central spindle and concomitant migration of the SPBs to opposite poles results in the metaphase configuration (Figs. 7, 8). The SPBs are fully differentiated and no longer resemble the NAB of interphase cells. They are unique and unlike SPBs in fungi (Aist and Williams, 1972). The chromosomes are in amphitelic orientation (Bauer et al., 1961), i.e., sister chromatids are attached to microtubules linking them to opposite spindle poles. There is no typical metaphase plate, an observation in agreement with many reports on mitosis in fungi (e.g., Aist and Williams, 1972; Heath, 1974; McCully and Robinow, 1971; Robinow and Caten, 1969). However, as noted by Heath (1974) this could simply be a consequence of rapid oscillations of the chromosomes at the time of fixation.

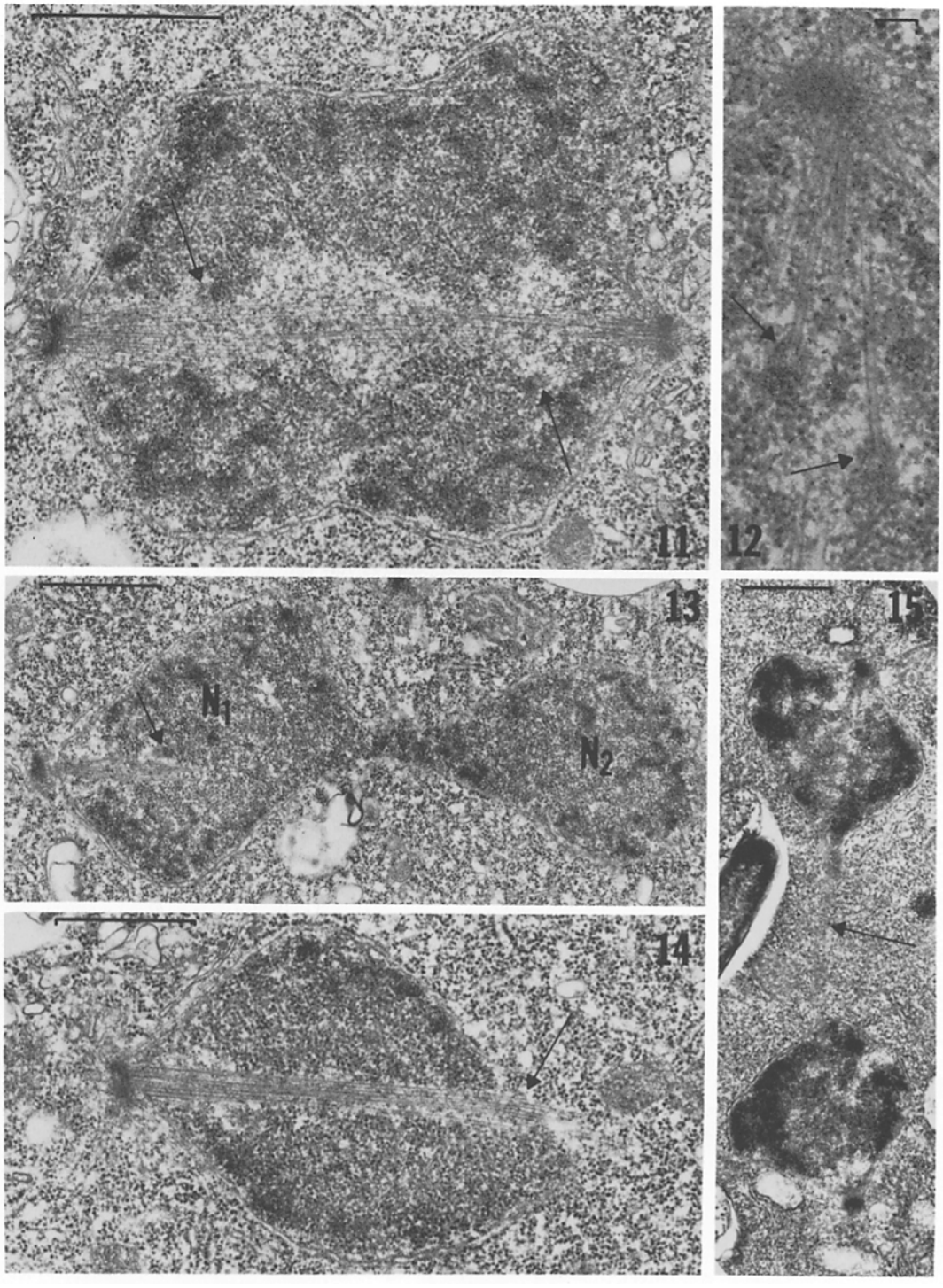
I have carefully checked serial sections of several nuclei to make certain that a single microtubule links each chromatid directly to the corresponding SPB. Such a relationship may also apply to certain protozoa (Aikawa et al., 1972), but it is quite common in fungi and related organisms. Examples are the myxomycete *Physarum polycephalum* (Ryser, 1970), the oomycetes *S. ferax* (Heath and Greenwood, 1968) and *T. clavata* (Heath, 1974), and the imperfect fungus *Fusarium oxysporum* (Aist and Williams, 1972).

The kinetochores of *P. violaceum* are very distinct (Fig. 9) and very similar to those of the organisms listed above. As in mammalian cells (Roos, 1973) and many algae (Mughal and Godward, 1973; Pickett-Heaps, 1973 *a*; Pickett-Heaps

FIGURES 7, 8 Two serial (not adjacent) sections of a metaphase nucleus. The central spindle lies in an electron-lucent channel. In Fig. 7 a pair of sister chromatids (forked arrow) lies approximately midway between the spindle pole bodies (*SPB*). A single microtubule connects the upper chromatid directly to the upper SPB. Another chromatid, lying off the spindle equator (single arrow), is also connected to the upper pole. In Fig. 8 two microtubules (arrows) extend from the lower SPB almost all the way to the upper one. Note the astral microtubules emanating from both SPBs. The length of the spindle is 2.2 μm. Scale = 1 μm. × 35,500.

FIGURE 9 The sister chromatids (Ch_1, Ch_2) with their kinetochores (arrows) of Fig. 7 at higher magnification. The upper kinetochore is fully sectioned. The microtubule terminates in finely fibrillar material at a distance of approximately 400 Å from the surface of the more electron-opaque chromatid. The lower kinetochore is obliquely cut and its appearance therefore less typical. Scale = 0.1 μm. × 80,000.

FIGURE 10 Detail of an SPB from another metaphase nucleus. Microtubules originating from the SPB penetrate into the nucleus through a polar opening (fenestra) of the nuclear envelope (between arrows). Scale = 0.1 μm. × 80,000.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and Fowke, 1969) with kinetochores consisting of several layers, a chromosomal microtubule terminates in amorphous or finely fibrillar material at a certain distance from the surface of the chromatid proper. One can consider these organelles linking a chromatid to a *single* microtubule as "unit kinetochores" representing a primitive condition and peculiar to small chromosomes.

(*d*, *e*) During anaphase and telophase the distribution of daughter chromosomes to the future daughter nuclei is accomplished by shortening of the chromosomal microtubules and elongation of the central spindle. These two mechanisms of segregation act concurrently as is evident from the observation that chromosomal tubules attain minimal length only in late telophase (Figs. 11, 12, 16; Table I).

The shape of the nucleus at anaphase and telophase is characteristic of most organisms in which the nuclear envelope remains essentially intact throughout mitosis (fungi: Hemmes and Hohl, 1973; McCully and Robinow, 1971, 1973; Van Winkle et al., 1971; algae: Marchant and Pickett-Heaps, 1970; Pickett-Heaps, 1973 *b*). There is no evidence for any extra- or intranuclear organelles or structures that could actively constrict the nucleus in the equatorial region.

(*f*) The most remarkable changes in very late telophase concern the SPBs. These lose their ring shape and become more compact. Their position at the tapered end of the nuclei and their persistent association with cytoplasmic microtubules (Figs. 19, 21) clearly indicate that they become again the NABs of interphase cells. It thus appears that they undergo cyclic changes related to mitosis: NAB → ring-shaped SPBs → NAB.

TABLE I

Mean Length of Chromosomal Microtubules

Number of nuclei and mitotic stage	Number of microtubules measured	Mean length (± SD) from kinetochore to pole
		μm
2 metaphase	10	1.09 ± 0.26
1 very early anaphase	6	1.07 ± 0.42
1 midanaphase	8	0.88 ± 0.50
4 mid and late telophase	14	0.50 ± 0.28

Spindle Function

The small size of the nuclei and the relatively small number of microtubules in the mitotic apparatus of *P. violaceum* make it easy to distinguish two spatially separated components involved in the segregation of chromosomes: the central spindle and the chromosomal microtubules. The central spindle has the function of a "pushing body". This can be inferred by comparing the shape of nuclei in meta-, ana-, and telophase. At metaphase the SPBs may lie in slight depressions of the polar faces of the nucleus (Figs. 7, 8, lower pole), but during ana- and telophase they are the leading points (Figs. 11–15). The chromosomal microtubules, on the other hand, have the task of moving the daughter chromosomes towards the SPBs. This is evident from direct observation (Figs. 7, 11, 12, 16) and measurements (Table I). In *F. oxysporum*, chromosome

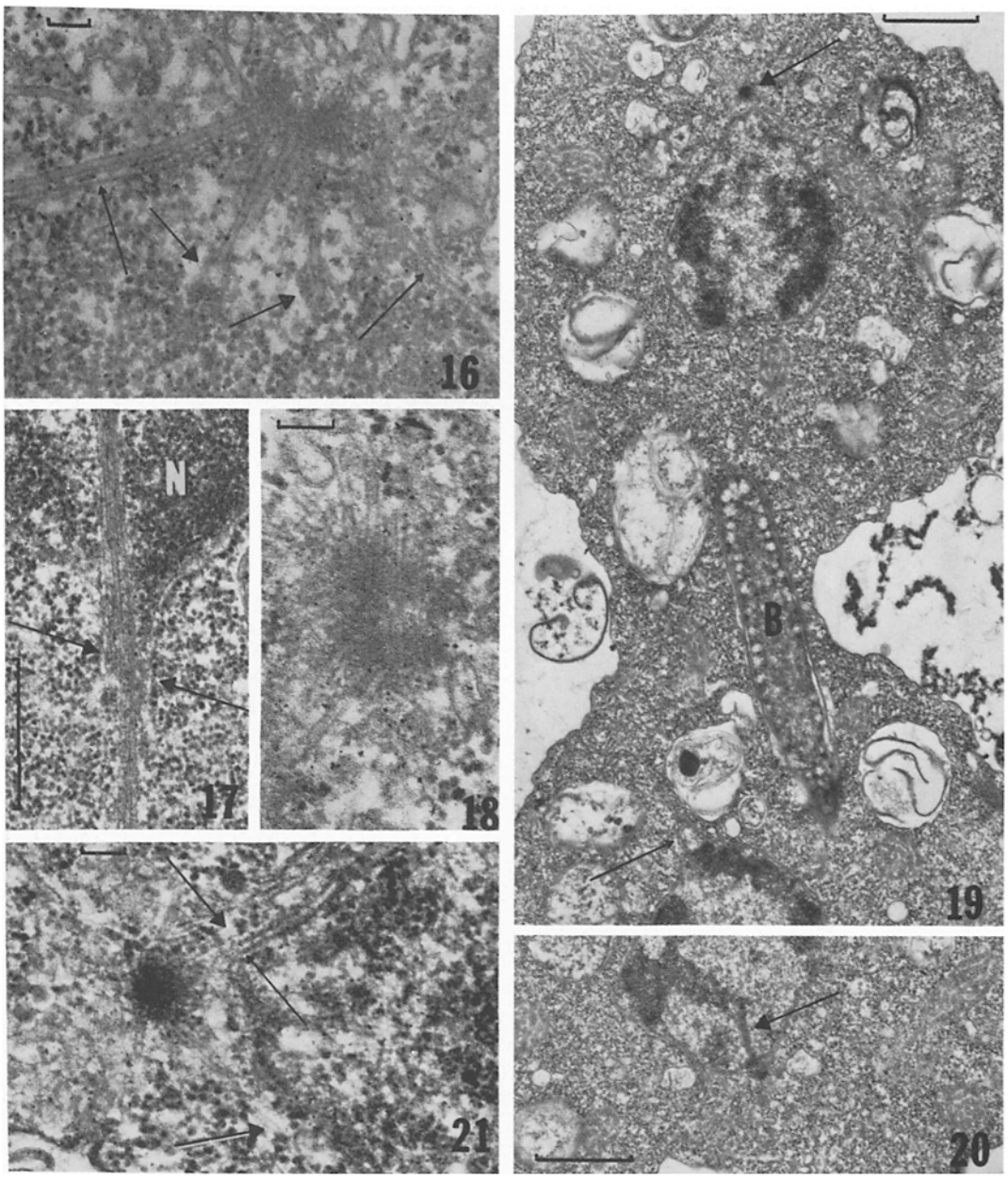
FIGURE 11 Anaphase nucleus. The polar faces are shaped into flat cones and a constriction appears in the equatorial region. A kinetochore near each pole is marked by an arrow. The length of the spindle is 3.2 μm. Scale = 1 μm. × 28,400.

FIGURE 12 Serial section of the nucleus of Fig. 11. Two kinetochores (arrows) lie in the central channel near the spindle pole. Scale = 0.1 μm. × 62,000.

FIGURE 13 Late anaphase. The section was oblique relative to the spindle axis. The two incipient daughter nuclei (N_1, N_2) are connected by a bridge resulting from the advancing equatorial constriction. A kinetochore is visible near the left spindle pole (arrow). Scale = 1 μm. × 18,000.

FIGURE 14 Serial section of the left daughter nucleus of Fig. 13. The parallel microtubules of the central spindle exit through the nuclear envelope on the interzonal face (arrow). × 21,300. Scale = 1 μm.

FIGURE 15 Full telophase (accidental thicker than normal section). The shaft formed by the tightly bundled microtubules of the central spindle (arrow) runs from one SPB to the other. The length of the spindle is 5.9 μm. Scale = 1 μm. × 13,500.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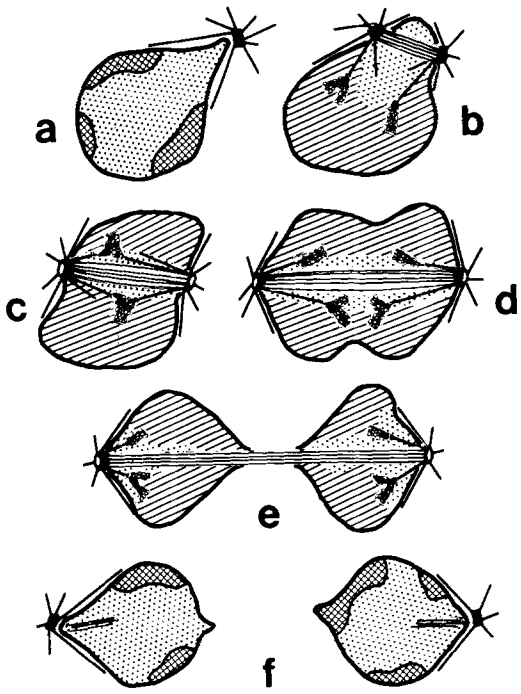

FIGURE 22 Diagrammatic summary of mitosis in *P. violaceum*. Numbers in parentheses denote the number of cells examined for each stage. (*a*) Interphase. (*b*) Prophase (1). (*c*) Metaphase (2). (*d*) Anaphase (5). (*e*) Telophase (9). (*f*) Very late telophase (cytokinesis) (4). See text for explanation.

movement precedes spindle elongation (Aist and Williams, 1972), but in *P. violaceum* they occur concurrently.

Since each chromatid is directly linked to an SPB, elongation of the central spindle without concomitant shortening of the chromosomal microtubules would be a sufficiently effective mechanism of chromosome segregation, much as it is in the protozoan *Syndinium* (Ris and Kubai, 1974). Such mechanisms are, however, relatively primitive (Ris and Kubai, 1974) and, in comparison to them, mitosis in *P. violaceum* is orthodox.

Poleward motion of daughter chromosomes during anaphase is most easily explained in terms of a depolymerization of their microtubules according to the "dynamic equilibrium" hypothesis advanced by Inoué and co-workers (Inoué, 1964; Inoué and Sato, 1967), or the "assembly" hypothesis of Dietz (1972). Spindle pole bodies and/or kinetochores could assume a controlling function governing the equilibrium. The clear spatial separation between the central spindle and the chromosomal microtubules makes a sliding mechanism (McIntosh et al., 1969; Nicklas, 1971) highly unlikely. Heath (1974) and Ryser (1970) reached similar conclusions regarding mitosis in *T. clavata* and *P. polycephalum*, respectively.

The situation is not so clear for the central spindle. Even though it appears from metaphase nuclei (Figs. 7, 8) that interpolar microtubules are

FIGURE 16 Polar region of a nucleus in late telophase. Two kinetochores with their short microtubules are marked by large arrows. A cytoplasmic microtubule (upper left) lies close to the nuclear envelope (small arrows). Scale = 0.1 μm. × 60,000.

FIGURE 17 Late telophase. The nuclear envelope of a daughter nucleus (*N*) encloses the microtubular shaft on the interzonal face (arrows). Scale = 0.5 μm. × 42,600.

FIGURE 18 Spindle pole body of a telophase cell (the third of five serial sections). The ring shape is recognizable but incomplete because of the slightly oblique angle of sectioning. Scale = 0.1 μm. × 80,000.

FIGURE 19 Very late telophase (cytokinesis). The upper daughter nucleus with its SPB (large arrow) resembles an interphase nucleus (compare with Fig. 1), except for the incompletely reconstructed nucleoli. The beak remaining after the closure of the nuclear envelope is indicated on the interzonal face of the lower nucleus (small arrow). A bacterium (*B*) lies within a food vacuole in the cleavage region. Scale = 1 μm. × 13,200.

FIGURE 20 Serial (not adjacent) section of the lower nucleus of Fig. 19. Note the SPB and the intranuclear remnant of the microtubular shaft (arrow). Scale = 1 μm. × 13,800.

FIGURE 21 SPB of the upper nucleus of Fig. 19 at higher magnification. The nuclear envelope seems continuous (small arrow) even though two intranuclear microtubules can be seen. Some extranuclear microtubules are very close to the nuclear envelope (large arrows). The SPB is more compact and electron opaque than at earlier stages of mitosis. Scale = 0.1 μm. × 62,000.

truly continuous from pole to pole the possibility cannot be ruled out that they inderdigitate during ana- and telophase. In the former case elongation of the central spindle could be explained by the assumption of microtubule growth by polymerization from subunits (Inoué, 1964; Dietz, 1972), but in the latter case a sliding mechanism with concomitant elongation of microtubules would be a more probable explanation.

Taxonomic Considerations

How representative mitosis in *P. violaceum* is of the cellular slime molds must await observations on other species. In the myxomycetes there are two types of mitosis (Aldrich, 1969). The open spindle with polar centrioles of the myxamebas bears no resemblance to the spindle of *P. violaceum*. Kinetochores in plasmodial mitosis (Ryser, 1970) do resemble those of *P. violaceum* both in structure and in the number of associated microtubules, but the absence of a central spindle and SPBs, and the partial breakdown of the nuclear envelope during ana- and telophase (Aldrich, 1969) are significant differences supporting the hypothesis that the two groups are not closely related (Bonner, 1967). Mitosis in *P. violaceum* is also quite unlike that in protists, notably the ameboid protozoa (cf. Roth and Daniels, 1962).

There is, on the other hand, a striking similarity with certain fungi. Spindle pole bodies and a tightly organized central spindle in the late stages of division have been documented for zygomycetes (Franke and Reau, 1973; McCully and Robinow, 1973), ascomycetes (McCully and Robinow, 1971; Van Winkle et al., 1971), basidiomycetous yeasts (McCully and Robinow, 1972), and an imperfect fungus (Aist and Williams, 1972). The variation in the shape and location of the SPBs is clearly of secondary importance, for uniformity does not even exist within a class (e.g., the ascomycetes: Van Winkle et al., 1972; Zickler, 1970).

Kinetochores of the type observed in *P. violaceum* occur in fungi (Aist and Williams, 1972; Heath, 1974; Heath and Greenwood, 1968), in the myxomycete *P. polycephalum* (Ryser, 1970), and apparently in certain coccidian protozoa (Aikawa et al., 1972). Only a systematic study within a group of closely related organisms can reveal the degree of variation or constancy, and therefore their value in taxonomy, of ultrastructural features of mitosis (a case in point are the algae, see Pickett-Heaps and Marchant, 1972), but the ensemble of similarities pointed out above indicates that cellular slime molds may be more closely related to extant fungi than to protozoa.

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