

# SPINDLE AND KINETOCHORE MORPHOLOGY OF *Dictyostelium discoideum*

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## ABSTRACT

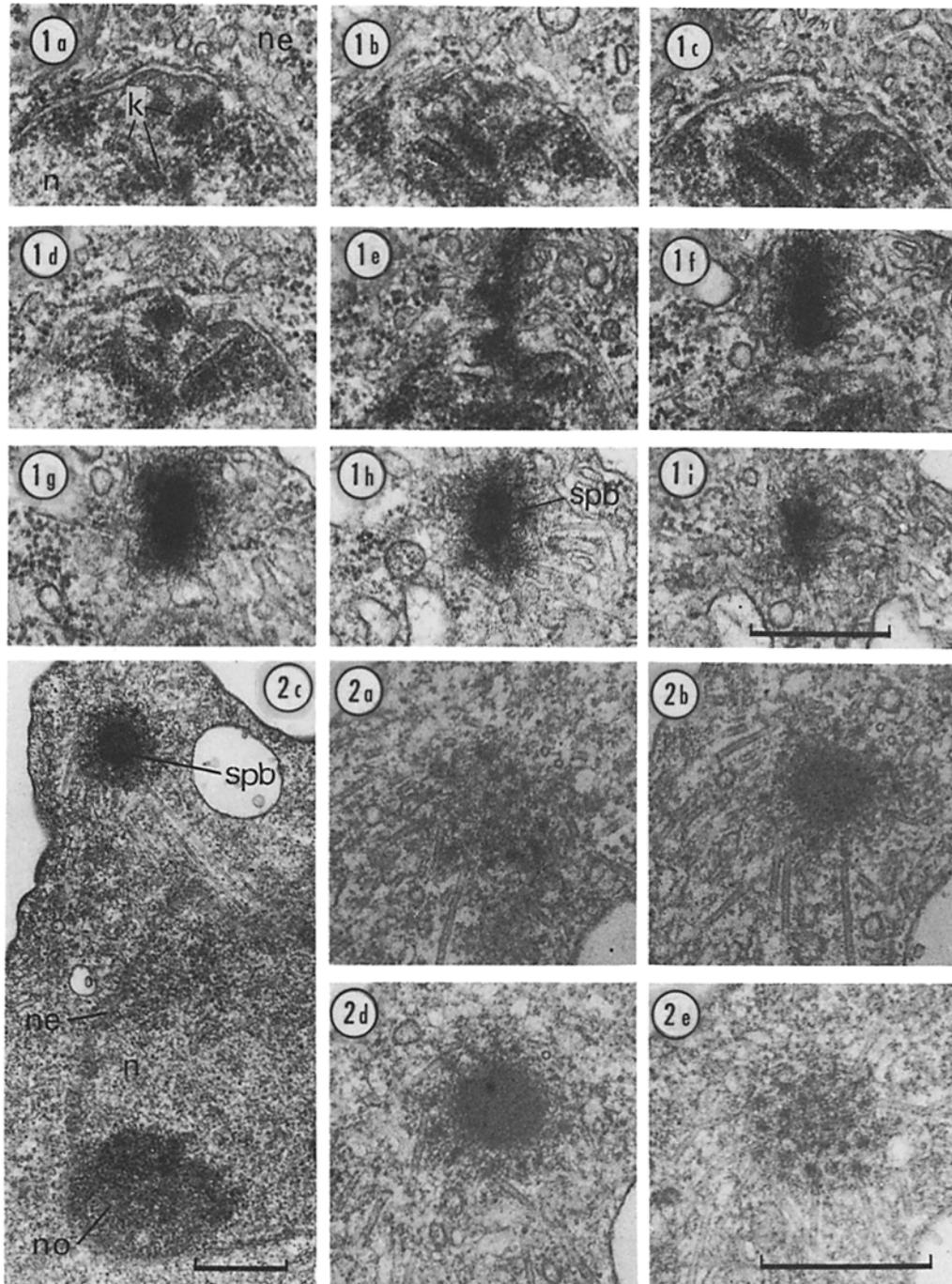
The metaphase spindle of haploid *Dictyostelium discoideum* ( $n = 7$ ) is 2  $\mu\text{m}$  long. It consists of some 20 microtubules which seem continuous between the spindle pole bodies and there are about 20 chromosomal microtubules at each end of the spindle. During anaphase the central spindle elongates and the chromosomal microtubules shorten. The spindle length and structure at this stage suggests that lengthening is caused by elongation as well as parallel sliding of the nonchromosomal microtubules. The nuclear envelope remains mostly intact during mitosis, and nuclear separation through medial constriction takes place when the spindle is 6  $\mu\text{m}$  long. Cytokinesis occurs when the spindle is 10  $\mu\text{m}$  long. At that time the kinetochores double in size. During interphase, the spindle pole body separates from the nucleus to a distance of 0.7  $\mu\text{m}$ , and it returns at the onset of the next prophase when it becomes functionally double, thereby starting the formation of a central spindle. When comparing mitosis in the cellular slime molds *Polysphondylium violaceum* and *D. discoideum*, several similarities and some differences are apparent.

The clear definition of the spindle apparatus and kinetochores in electron micrographs of the cellular slime mold *Dictyostelium discoideum* provides new information about these structures and allows comparisons with mitosis in a similar slime mold, *Polysphondylium violaceum*, reported by U.-P. Roos (10, 11). As in many protists, algae, and fungi (4, 7), the nuclear envelope remains mostly intact during division, and, like *P. violaceum*, the spindle pole body is closely associated with the nucleus only during mitosis; but, unlike *P. violaceum*, which has one chromosomal microtubule per kinetochore, *D. discoideum* has two to three microtubules attached to each kinetochore during anaphase. Novel is the observation that the spindle pole bodies and kinetochores appear to duplicate

at the completion of mitosis and become functionally double at the onset of the next mitosis.

## MATERIAL AND METHODS

Spores of *D. discoideum* NC-4 (H) were germinated on nutrient agar with *Escherichia coli* as food source for the myxamoeba. Cells were harvested at hourly intervals from 10 to 14 h by flooding the plates with a solution of 2% glutaraldehyde in phosphate buffer (pH 6.8). After 12 h of fixation, the cells were spun down at low speed, washed, and postfixed in a solution of 2% osmium tetroxide in phosphate buffer for 1 h. They were collected on filter paper and clumps suspended in a 4% agar solution at 60° C. After cooling, small agar blocks containing the cells were dehydrated in a graded alcohol series and propylene oxide and embedded in Epon. Series



**FIGURE 1** Part of a nucleus (*n*) belonging to a cell just after division. The kinetochores (*k*) are clustered near the nuclear envelope (*ne*), across from the spindle pole body (*spb*). The *spb*-associated microtubules are confined to the cytoplasm (unlike the division stages where they are also nuclear: Figs. 5, 6, 7, and 8). The *spb* measures  $240 \times 220$  nm and has an estimated thickness of 160 nm. It is not surrounded by dense 25-nm particles seen in later stages (Fig. 2). Fig. 1 *a-i* are electron micrographs of nine consecutive sections. Bar is 500 nm.  $\times 50,000$ .

**FIGURE 2** An interphase nucleus (*n*) with differentiated nucleoli (*no*) and an *spb* at some distance from the nuclear envelope (*ne*). The diameter of the disk-shaped *spb* is 260 nm. It is surrounded by some 50–60 dense particles and by numerous microtubules. At this stage, neither chromosomes nor kinetochores are visible as such, but a non-nucleolar dense-staining area is usually found in the part of the nucleus closest to the *spb*. Fig. 2 *a-e* is taken from five consecutive sections. Bars are 500 nm. (*e*)  $\times 26,000$ ; others,  $\times 48,600$ .

of 80 or more fairly thick sections (80–90 nm) collected on single hole grids, covered with Formvar film.

## OBSERVATIONS

### *Interphase*

At the beginning of interphase (Fig. 1 *a–i*), the kinetochore regions of the chromosomes are clustered in the tip of a nuclear protrusion formed at the preceding telophase (Fig. 8). Some kinetochores measure 240 nm in cross section (Fig. 1 *c, d*) and up to 270 nm (now shown) which is nearly double their size at metaphase (Fig. 5). The spindle pole body measures  $240 \times 220 \times 160$  nm and lies in the cytoplasm near the nuclear envelope, within a distance of 0–50 nm. It consists of two closely apposed disks (Fig. 1 *h*; see also Fig. 3 *b*) and it is surrounded by microtubules, vesicles, and 0–20 dense dots.

As interphase progresses, the contents of the nucleus become differentiated into dense peripheral nucleoli and a less dense central area. The kinetochores are no longer distinct, but there remains a cluster of dense, nonnucleolar material at the nuclear envelope close to the spindle pole body. The spindle pole body moves away from the envelope to a distance of 700 nm while it becomes surrounded by some 50–60 dense dots (Fig. 2). These particles correspond to the ones observed in *P. violaceum* (11), but they are more numerous in *D. discoideum*. As prophase approaches, the spindle pole body returns to the nucleus and, instead of the dots, a dense halo surrounds it (Fig. 3). General observations, and a detailed analysis of 28 complete cells, indicate that during interphase there is only one spindle pole body in cells containing a single nucleus. Of 24 measured distances, five spindle pole bodies were within 50 nm from the nuclear envelope, nine were at about 100 nm, three at 200 nm, one at 400 nm, two at 500 nm, and four at 700 nm.

### *Prophase*

At the onset of prophase the chromosomes commence to condense and the kinetochores become more distinct (Fig. 3 *a*). Following the stage shown in Fig. 3 *a*, the spindle pole body comes to lie in an indentation of the nucleus nearest to the cluster of kinetochores and it divides into two disks which separate while remaining interconnected by microtubules (Fig. 4 *a–h*). In Fig. 4 the two spindle pole bodies, marked A and B, are 170 nm apart

with several continuous microtubules between them (Fig. 4 *d*). All seven pairs of kinetochores were identified and five of them are marked and numbered in Fig. 4. For each of the chromosomes 1, 2, and 5, the pairs of kinetochores lie in a favorable plane of section, and their structure can be compared with the similar, but better defined, structures of the metaphase-anaphase kinetochores shown in Fig. 5. At prophase, microtubules pass closely by the kinetochores (e.g., dotted lines in Fig. 4 *c* pass over the kinetochores of chromosome 1 in Fig. 4 *b*) but do not make the kind of attachment seen at later stages (Fig. 5). Cytoplasmic tubules are present throughout all stages. As prophase progresses, the nucleus becomes electron-opaque, a useful diagnostic feature for the identification of cells in mitosis.

### *Metaphase, Onset of Anaphase*

At metaphase, the spindle is about 2  $\mu$ m long and the seven pairs of kinetochores are located irregularly at the equator of the spindle. Separation of partner kinetochores had just started in spindles of 2.0, 2.1, and 2.2  $\mu$ m but was more noticeable in spindles of 2.4, 2.5, 2.6, and 2.8  $\mu$ m. The spindle of 2.1  $\mu$ m length shown in Fig. 5 demonstrates the location of the seven kinetochore pairs on the spindle. All the kinetochores except those of chromosome 7 have started to separate as determined by the distance between partner kinetochores. About two to three chromosomal microtubules are connected to the dense region of the kinetochore which faces the spindle pole body. Behind the dense band is a transparent region and then a sharply defined dense curved plate of about 140 nm diameter (Fig. 5, arrows). The chromosomes cannot be distinguished. Serial cross sections of the spindle show about 40 microtubules at the poles, and 17–20 interzonal microtubules, which also suggests that two to three microtubules end at each kinetochore. In longitudinal sections of spindles at the onset of anaphase, several microtubules could be traced over nearly the entire distance between the spindle poles, but unambiguous pole-to-pole tubules as in Fig. 4 *d* could not be identified. These observations are identical to the ones reported in *P. violaceum* by Roos (10) in his Figs. 7 and 8. The spindle microtubules pass through a polar fenestra and connect with an amorphous spindle pole body which is a convex disk with a diameter of about 350 nm (Fig. 5 *d*).

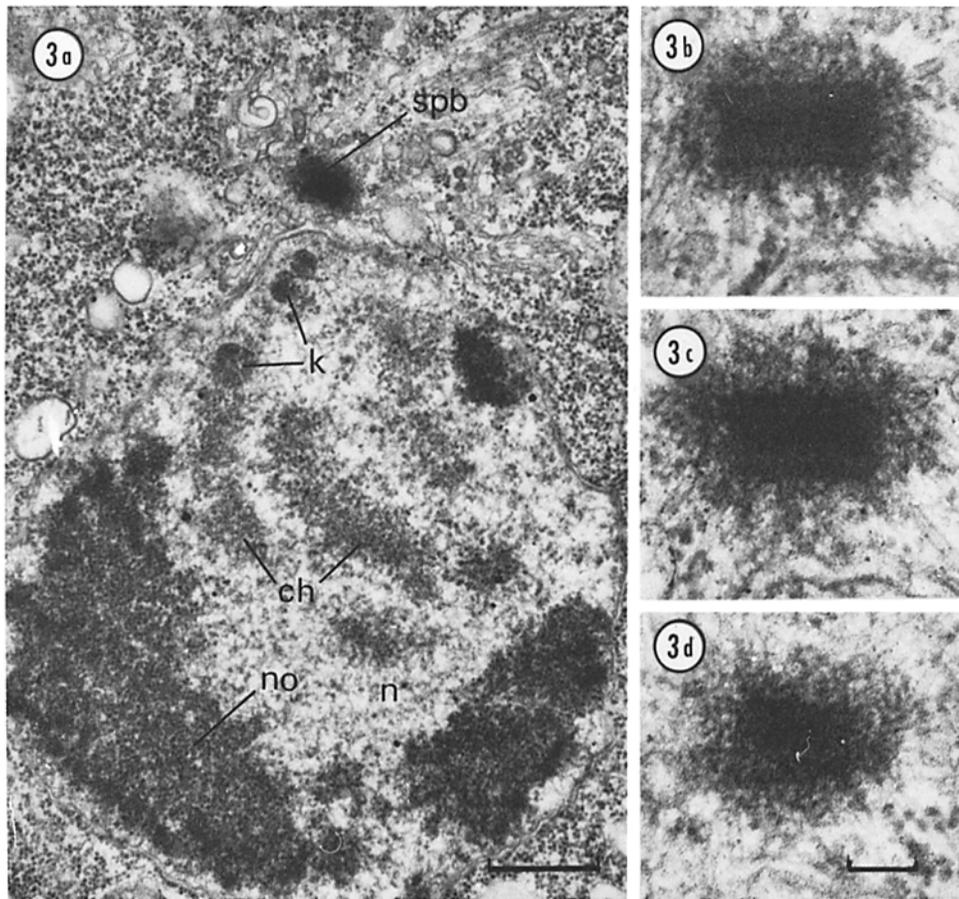


FIGURE 3 A nucleus in mitotic prophase, with chromosomes (*ch*) and kinetochores (*k*). The *spb* has a diameter of about 250 nm in the plane of section and it appears in three consecutive sections so that the diameter perpendicular to the plane of section is also about 250 nm. Its thickness is 110 nm and it appears to be composed of two disks stacked on top of each other (*b*). The dense particles have been replaced by a dense halo which surrounds the *spb* and from which microtubules extend. Fig. 3 *a-d* is taken from consecutive serial sections. (*a*) Bar is 500 nm.  $\times 30,000$ . (*b*) to (*d*) Bar is 100 nm.  $\times 90,000$ .

### Anaphase

In spindles of 4.2, 4.3, 4.8, 5.3, 5.4, and 5.6  $\mu\text{m}$  in length, the kinetochores of daughter chromosomes were widely separated and most of the distances between the spindle pole bodies and their associated kinetochores were becoming shorter, varying from 1.0 to 0.1  $\mu\text{m}$  (Fig. 6 *b*). Simultaneously, the nucleus becomes medially constricted, but the cell as a whole is not yet dividing. In the distal regions, the spindle consists of about five tightly packed microtubules, while the median region contains a larger number of more loosely bundled microtubules (Fig. 6 *a, b*).

### Telophase

Nuclear division is completed when the spindle reaches a length of 7–8  $\mu\text{m}$ . The cells now become medially constricted, and constriction is nearly completed when the spindle reaches a length of 10  $\mu\text{m}$  (Fig. 7). All kinetochores are clustered near the nuclear envelope (Fig. 8), and they appear to be increasing in size when sectioned as in Fig. 5 and compared to those kinetochores (Fig. 8 *d*,  $\pm 200$  nm). The nuclear envelope is closing, leaving only a few microtubules to pass through. The tubules are closely packed together, forming a solid bundle (Fig. 8 *b*). At this stage, the spindle pole body

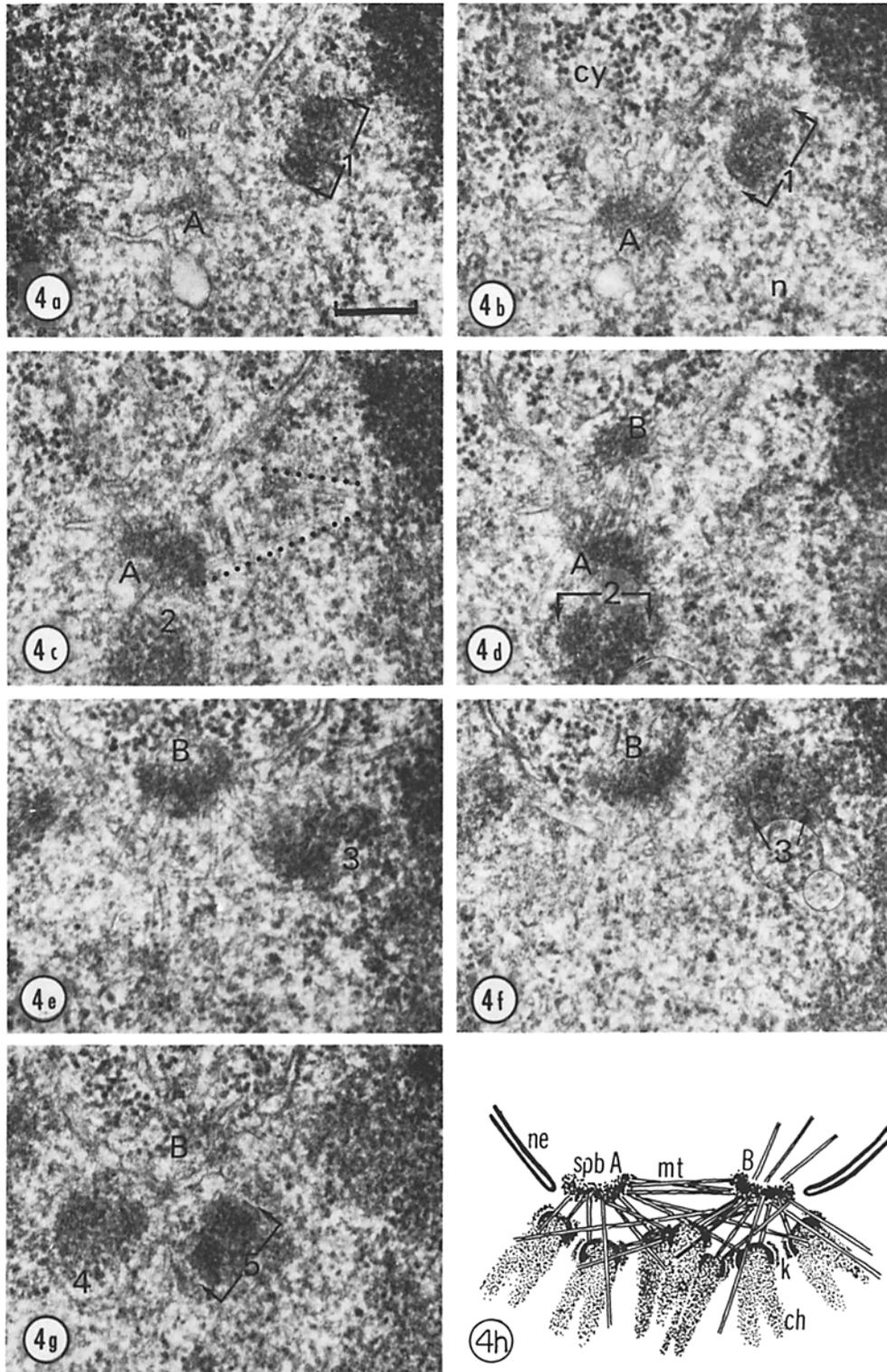


FIGURE 4 Indented area of an early prophase nucleus (*n*) containing two spindle pole bodies A and B, 170 nm apart, interconnected by a short spindle (*d*) and containing the kinetochole regions of all seven chromosomes, five of which are present in (*a*) to (*g*). In favorable sections the pairs of kinetochores can be recognized and are marked by arrows for chromosomes 1, 2, and 5. The cytoplasm is marked *cy* in (*b*). The dotted lines in (*c*) mark two microtubules which pass over the kinetochores of chromosome 1. Fig. 4 *h* is a diagrammatic representation of a reconstruction. Bar is 0.2  $\mu\text{m}$ .  $\times 60,000$ .

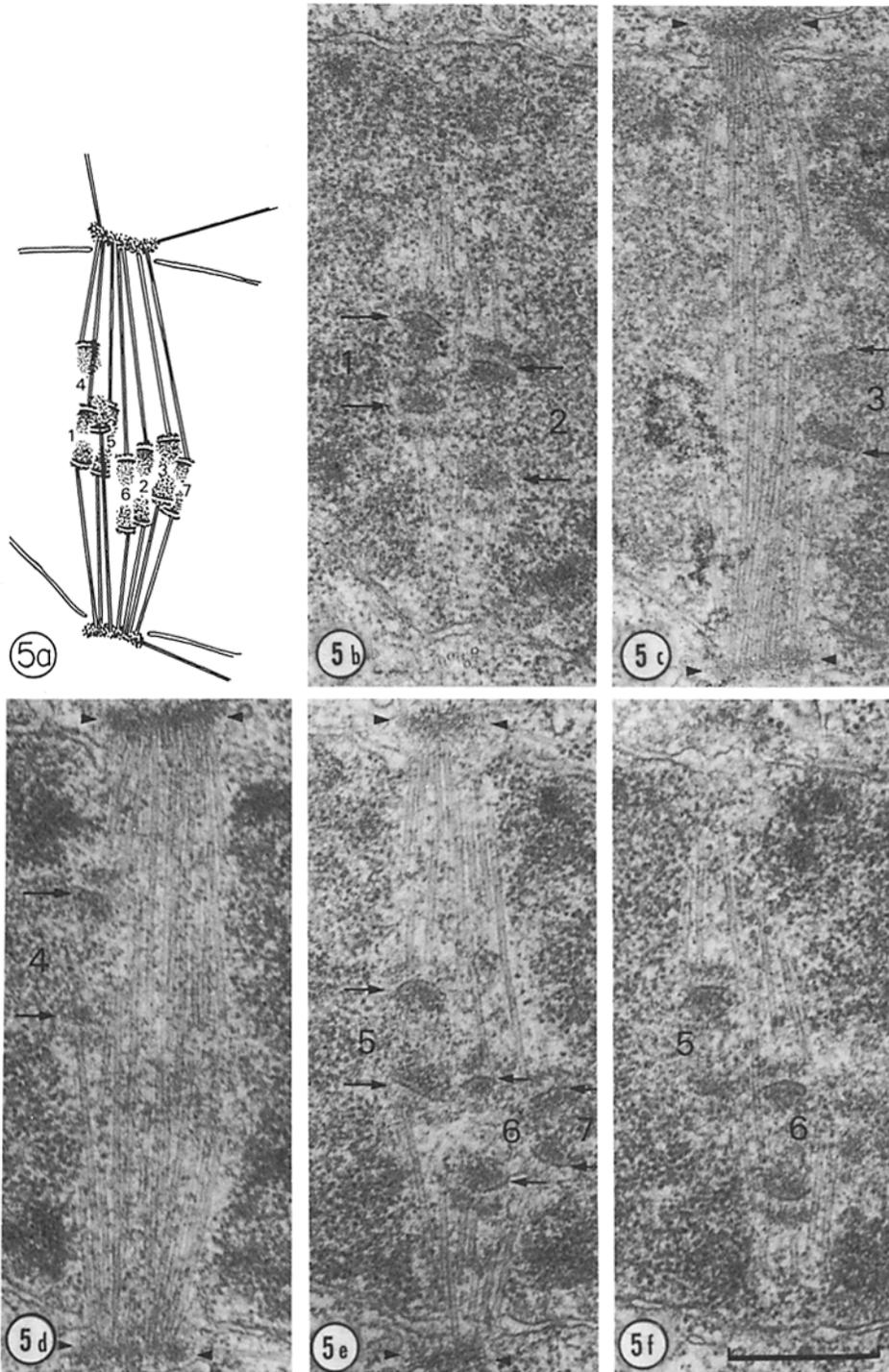


FIGURE 5 A 2- $\mu$ m spindle at late metaphase or early anaphase. The spindle pole bodies are bracketed between arrow heads and the kinetochores are marked by arrows. Partner kinetochores of chromosome 7 (*c*) have hardly separated (230 nm), while partner kinetochores of chromosome 4 are 420 nm apart. The average distance from the *spb* to the kinetochore is 0.9  $\mu$ m. Of the sections that contained the spindle, only sections 2 (*b*), 4 (*c*), 6 (*d*), 8 (*e*), and 9 (*f*) are shown. Fig. 5 *c* has stain precipitate on it. The diagram in (*a*) summarizes the positions of the kinetochores and shows a few of the microtubules. Bar is 0.5  $\mu$ m.  $\times$  42,000.

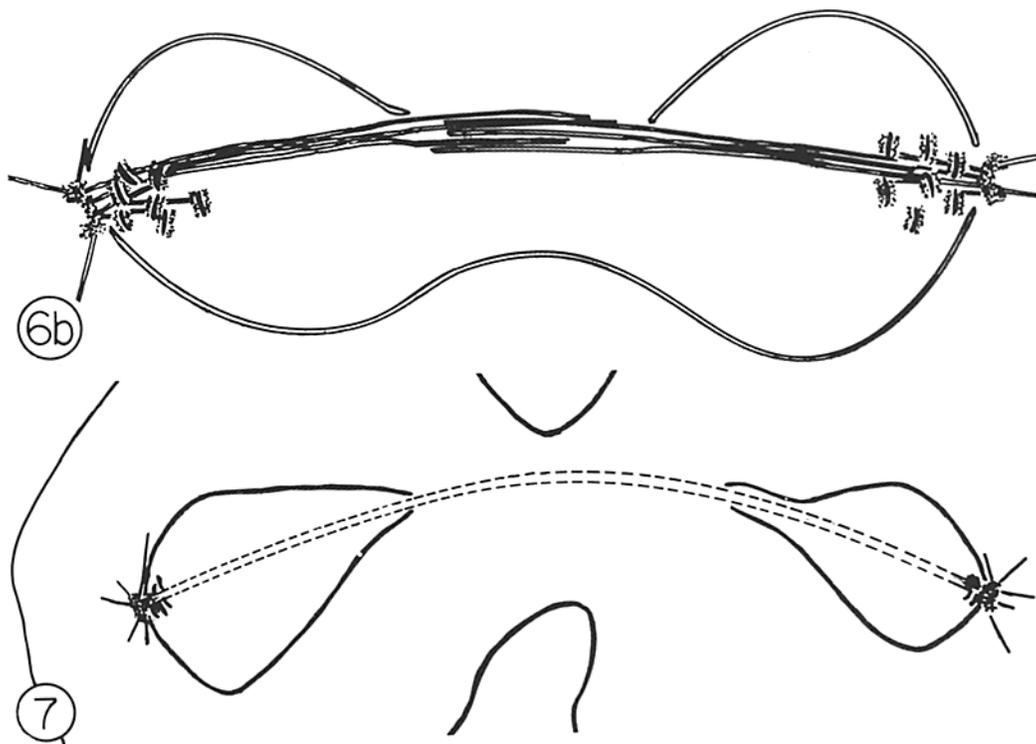
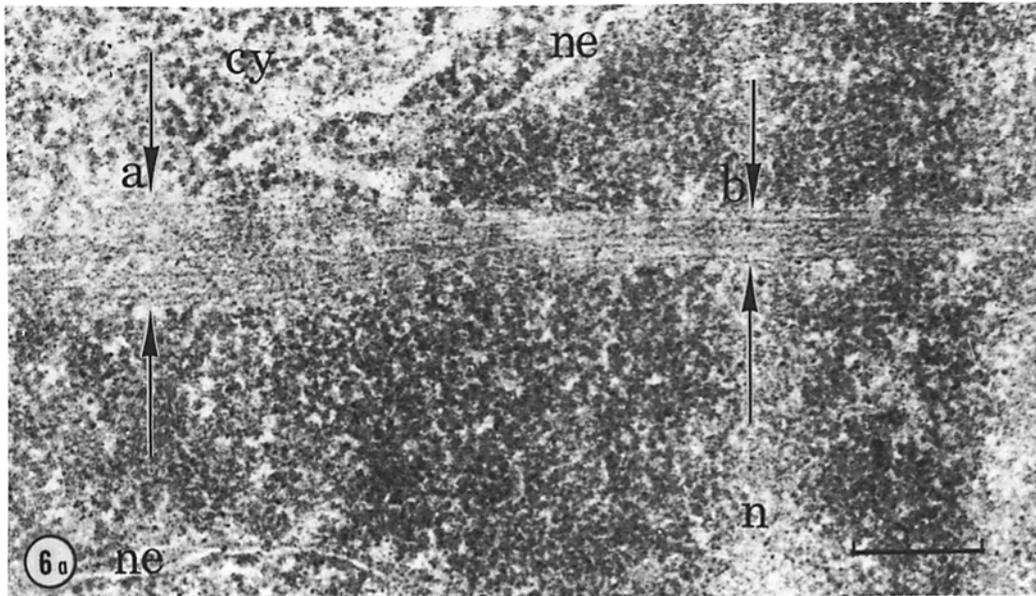


FIGURE 6 (a) Detail and (b) diagram of an anaphase spindle of  $5.4 \mu\text{m}$  in length. Fig. 6 a illustrates the narrow, compact bundle of microtubules at position *b* and the zone of overlap at position *a*. Nuclear envelope at the medial constriction, *ne*. Cytoplasm (*cy*) and nucleus (*n*). Fig 6 b is a diagram reconstructed from serial sections of the nucleus shown in 6 a. The nucleus is medially constricted and the kinetochores are at different positions close to the spindle pole bodies. The nuclear envelope is intact except in regions where the spindle passes through it. The tubules are not necessarily continuous, the bundle consists of about five tubules in the distal regions and about double that in the central region. Bar is  $0.25 \mu\text{m} \times 70,000$ .

FIGURE 7 Diagram of a  $10.3\text{-}\mu\text{m}$  spindle drawn from a cell with karyokinesis completed and cytokinesis in progress. The two daughter nuclei are well separated and the cell is constricted medially. A small bundle of microtubules extends between the two *spb* and the kinetochores are assembled close to the *spb*.

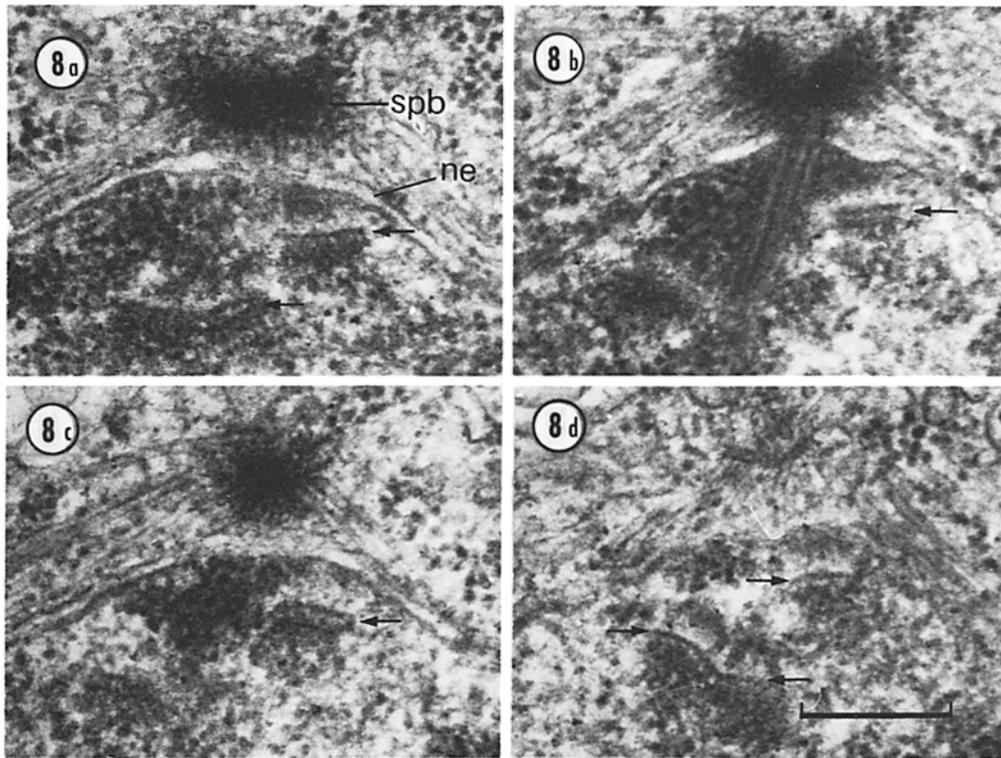


FIGURE 8 As the nuclei and the daughter cells separate the nuclear envelope (*ne*) gradually closes the spindle aperture (*b*) until the *spb* become disconnected from the nucleus (Fig. 1). In this, and other such cells, it appears that the *spb* folds double (*b*), possibly producing the two-layered structure shown elsewhere (Figs. 1 *h*, 3 *b*). It also appears that the kinetochores are much larger than during metaphase (Fig. 5), suggesting that they have doubled in preparation for the next division. Fig. 8 *a-d* is taken from four consecutive sections. Bar is 0.25  $\mu\text{m}$ .  $\times 80,000$ .

appears to be folding (Fig. 8 *d*), which suggests that the two-layered structure shown in Fig. 1 may have originated from the single spindle pole body in this manner. The spindle pole bodies in cross section are much larger (350–400 nm, see Fig. 5 *d*) during mitosis than they are at the onset of interphase (240 nm in largest dimension, Fig. 3 *g*).

## DISCUSSION

The kinetic apparatus of *D. discoideum* is not uncommon, in that it has differentiated polar regions, a central spindle, chromosomal microtubules, and kinetochores. At metaphase, the kinetochores are arranged approximately on a metaphase plate, and during anaphase the central spindle elongates while the chromosomal tubules become shorter. As is the case in many protists, algae, and fungi (4), the nuclear envelope stays

mostly intact during mitosis. Like the cellular slime mold *P. violaceum* the spindle pole bodies are associated closely with the nucleus during mitosis only (10, 11). Although this is not a common phenomenon in fungi, the dissociation of the spindle pole from the nuclear envelope has also been observed after the postmeiotic mitosis in the ascomycetous fungi *Podospora anserina* (12) and *Xylospora polymorpha* (2). Unlike the kinetic apparatus of most fungi (ref. 7, Tables I and D), the mitotic spindle pole body of *D. discoideum* or *P. violaceum* is never a part of the nuclear envelope, and when it divides the resulting two bodies are immediately interconnected by microtubules.

If the ultrastructure of mitosis is of taxonomic significance, the similarities and differences between the cellular slime molds *D. discoideum* and *P. violaceum* are of special interest. The spindles of

the two species are similar in most respects but striking is the difference in structure of the kinetochores and number of microtubular attachments. Whereas *P. violaceum* has a single microtubule attached to a relatively simple kinetochore, an arrangement common to several fungi (1, 5, 10), *D. discoideum* has complex metaphase kinetochores consisting of three distinct layers with several microtubular attachments of the type found, among others, in rat cells (6). This observation suggests that either the two slime molds are not closely related, or, more plausibly, that kinetochore structure is a species-specific adaptation, not a structure conserved in detail. Such variation is also apparent in the mitotic apparatus of closely related green algae (9).

Other differences may be, in part, due to interpretation. During interphase in *D. discoideum*, the spindle pole body becomes surrounded by an increasing number of dense particles, up to 60. In *P. violaceum*, Roos (11) reports an average of 16 such "nodules" surrounding the "nuclear-associated body." Roos (10) furthermore notes the absence of a metaphase plate in *P. violaceum*, and it is a matter of judgment whether the arrangement in *D. discoideum* shown in Fig. 5 *a* qualifies as a metaphase plate or not.

The increased size of kinetochores at late telophase and the beginning of interphase (Figs. 8 and 1) was not anticipated. The possibility of apparent fusion of adjacent kinetochores is unlikely in cells where all kinetochores could be accounted for. In such cells not all kinetochores appear equally large, probably as a result of different angles to the plane of section. It appears that the kinetochores become large plates at the end of mitosis and then curve to reappear as two curved surfaces at the next prophase. Since all kinetochores have doubled into two opposite kinetochores before interacting with microtubules, malorientation is unlikely (8), and they need not remain paired until metaphase (7). In this context, it is of interest to determine the mechanism of rapid parasexual haploidization of diploid *D. discoideum* (3). The spindle pole body folds at the end of mitosis to form a two-layered structure and becomes functionally double at the next prophase. At first, the interconnecting microtubules are very short (170 nm, Fig. 4 *d*) and continuous. At metaphase, the spindle is 2- $\mu$ m long and has many tubules of nearly that length. A ratchet, or sliding mechanism, could maximally extend the spindle to 4  $\mu$ m and would

reveal a medial zone of overlap. Spindles of 6–10  $\mu$ m with an overlap zone observed in *D. discoideum* (Fig. 6 *a*) suggest a combination of a sliding mechanism and an elongation of the tubules.

Some differences in interpretation have been suggested by Dr. U.-P. Roos of the University of Geneva (personal communications) who has also examined mitosis in *D. discoideum*. Firstly, he observed that the spindle pole bodies remain close to the nucleus even during interphase, but that the plane of sectioning may be such that the tapered point of the nucleus and the spindle pole body appear in separate sections. Secondly, he notes that the evidence for doubling of the spindle pole body through folding is not definite. Instead, duplication may take place through lateral separation, early, before other signs of prophase are noticeable.

*Dictyostelium discoideum* cells and culture methods were provided by Dr. T. Konijn of the Hubrecht Laboratory, University of Utrecht, Netherlands. The initial work was done with the electron microscope facilities of Dr. J. Bluemink of the same laboratory, while on sabbatical leave there. Extensive serial sectioning and electron microscopy was done by Mrs. L. Ebden, and the haploidy of the NC-4 (H) strain was confirmed by Dr. B. Coukell of York University. I thank these collaborators as well as Doctors A. Forer and I. B. Heath of York University for their assistance and interest in the project.

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