

Decoration of Spindle Microtubules with Dynein: Evidence for Uniform Polarity

BRUCE R. TELZER and LEAH T. HAIMO

Department of Biology, Pomona College, Claremont, California 91711; Department of Biology, University of California, Riverside, California 92521; and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT Studies were conducted to determine whether the microtubules present within native spindles isolated from eggs of the surf clam, *Spisula solidissima*, could bind dynein obtained from axonemes of *Tetrahymena thermophila*. SDS gel electrophoresis revealed that the high molecular weight polypeptides that make up dynein cosedimented with the isolated spindles. Moreover, the ATPase activity of dynein bound to the spindle microtubules was stimulated approximately sevenfold. The birefringence retardation of spindles incubated without dynein decreased from 1.4 nm to an undetectable level within 45 min, whereas that of spindles incubated for the same period of time with dynein was 1.0 nm, ~70% of its initial value, thereby indicating that dynein stabilized spindle birefringence. Ultrastructural analysis revealed that each spindle microtubule was decorated with four to seven dynein arms attached by their "B" end, that which cross-bridges the B-subfiber within native axonemes. In addition, the polarity of the spindle microtubules could be determined by the orientation of the bound dynein arms. The results of these studies suggest that the half-spindle is composed of microtubules possessing the same polarity.

Microtubules possess structural polarity, a property which may be involved in their ability to elicit directional, intracellular movements. Within the axoneme, microtubule doublets are of the same polarity, with their assembly or "plus" end located distal to the cell body (1, 5, 7). The dynein arms on the A-subfiber of one microtubule doublet cyclically cross-bridge the B-subfiber of the adjacent doublet (15), causing it to slide toward the distal end of the axoneme (44). Accordingly, the direction of microtubule sliding may be a manifestation of intrinsic microtubule polarity.

It has been proposed that, during mitosis, sliding between microtubules of opposite polarity occurs and results in the separation of the mitotic poles (31, 34, 38) and in the procession of the chromosomes to the poles (34, 38). Recent studies involving serial sections and subsequent microtubule reconstruction of the central spindle of *Diatoma vulgare* during metaphase and anaphase demonstrate a change in microtubule profile, indicating that two groups of interdigitating, antiparallel microtubules may slide with respect to each other and result in spindle elongation (32, 33, 35).

Microtubule sliding within the mitotic apparatus would require the presence of a force-generating protein, and evidence exists for the participation of a dyneinlike ATPase in generating mitotic movements. For example, spindles isolated from sea urchin eggs contain both Mg^{2+} -stimulated ATPase activity and

proteins with electrophoretic mobilities similar to those of dynein (41). In addition, the chromosomes within lysed mammalian cells continue anaphase motions if ATP is present (10). These motions are inhibited by the addition of vanadate (11), an inhibitor of dynein ATPase (11, 19). Similarly, antibody directed against dynein inhibits chromosome motion in isolated sea urchin mitotic apparatuses (43).

We have previously demonstrated that flagellar dynein can bind to in vitro assembled microtubules and induce their ATP-dependent cross-bridging. Significantly, the binding of dynein to these microtubules revealed their structural polarity. Recently, the polarity of microtubules has also been investigated by measuring the rate and direction of microtubule assembly onto chromosomes and centrosomes (6, 25, 47) and by decorating spindle microtubules with in vitro assembled microtubule hooks (12, 24).

We have now conducted experiments seeking to determine whether dynein obtained from *Tetrahymena* ciliary axonemes can bind to the microtubules present within the meiotic spindles isolated from eggs of the surf clam *Spisula*. These studies demonstrate that the microtubules do, in fact, bind dynein and also stimulate its ATPase activity. Furthermore, observations of the orientation of the dynein arms on the meiotic microtubules suggest that the half-spindle is composed of microtubules of the same polarity.

MATERIALS AND METHODS

Isolation of Meiotic Spindles

Eggs were obtained from fertile females of the surf clam, *Spisula solidissima*, by mincing the gonads in seawater and filtering the resulting suspension through cheesecloth. The eggs were washed three times in seawater and activated by the addition of 36 mM KCl (2). Meiotic spindles were normally observed 13–17 min after activation. The eggs were then collected by centrifugation at 100 g for 2 min and resuspended in 1 M glycerol and 1 mM sodium phosphate, pH 8.0 (37, 42). They were immediately sedimented as before and resuspended in 5 mM EGTA, 0.5% Triton X-100, 1 M hexylene glycol, and 5 mM MES (2-[N-morpholino]ethanesulfonic acid) at pH 6.2 (37). The resulting suspension of lysed eggs was centrifuged at 1000 g for 5 min, and the pellet containing meiotic spindles was resuspended either in the above solution without Triton X-100 or in a solution containing or lacking dynein.

Dynein Preparation

Dynein was obtained from cilia of *Tetrahymena thermophila* by low ionic strength dialysis of axonemes (17). Cells were collected at 1,500 g for 5 min, washed in 0.5 mM CaCl₂ and 40 mM HEPES, pH 7.4, and deciliated by addition of 1 mM dibucaine. The cell bodies were sedimented at 1,000 g for 5 min, and cilia remaining in the supernate were collected at 10,000 g for 10 min at 4°C. Axonemes were prepared as previously described for *Chlamydomonas* (23) and then dialyzed at 4°C for 20 h in 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM HEPES, pH 7.4, to extract dynein. The suspension was then centrifuged at 10,000 g for 15 min, and the supernate containing dynein was clarified by centrifugation as before. The resulting supernate containing dynein was adjusted to 1.0 mM EDTA, 0.5 mM MgSO₄, 20 mM PIPES at pH 6.9 and again clarified by centrifugation. This final supernate constituted the dynein preparation used in these studies.

Dynein Binding to Meiotic Spindles

The pellets of meiotic spindles were resuspended at 18°C in the presence or absence of dynein in 1.0 mM EGTA, 0.5 mM MgSO₄, 20 mM PIPES, pH 6.9. The MgSO₄ concentration was immediately increased to 2.5 mM and the suspension incubated at 18°C for periods up to 60 min. The spindles were again collected at 1,000 g for 5 min and prepared for ATPase analysis, gel electrophoresis, or electron microscopy.

Microscopy

POLARIZATION MICROSCOPY: The presence of spindles in activated eggs and their isolation, purification, and stability were monitored in a Zeiss polarization microscope. Photographs were taken on Kodak Plus X film exposed for 0.5–3.0 s and developed in Rodinal. Birefringence retardation was measured midway between a pole and the equatorial plate of the isolated spindles, using a Zeiss photomicroscope kindly provided by Dr. E. D. Salmon and equipped with a X 20 Nikon rectified polarization objective (45).

ELECTRON MICROSCOPY: Pellets of spindles, obtained by sedimentation at 1,000 g for 5 min, were overlaid with 2% glutaraldehyde in 1 mM EGTA, 2.5 mM MgSO₄, 20 mM PIPES, pH 6.9, and then centrifuged at 11,500 g for 10 min in order to form a tight pellet. The material was fixed in the glutaraldehyde for a total of 2 h, rinsed, and then overlaid with 1% tannic acid in 20 mM sodium phosphate, pH 7.0, for 1 h (4). After several washes with 20 mM sodium phosphate, the spindles were postfixed for 1 h at 4°C in 1% OsO₄ in 20 mM sodium phosphate, rinsed, and then stained for 1 h in 1% uranyl acetate. The material was subsequently dehydrated and embedded in Epon-Araldite.

Other Procedures

ATPase assays were initiated by addition of 1 mM ATP to the spindle and/or dynein preparations in 5 mM MgSO₄, 0.5 mM EDTA, 1.0 mM dithiothreitol, 25 mM KCl, and 10 mM HEPES, pH 7.4. The release of inorganic phosphate was measured by a one-step colorimetric method (29). Electrophoresis was performed in 8% SDS polyacrylamide gels (28).

RESULTS

ATPase Analysis

ATPase activity that cosedimented with meiotic spindles was monitored as an indication of dynein binding to the spindle

microtubules. The specific ATPase activity of the *Tetrahymena* dynein preparation was 0.22 μmol of P_i/mg·min, whereas that of *Spisula* spindles was \sim 0.018 μmol of P_i/mg·min, a value similar to that reported for spindles isolated from sea urchin eggs (41). After incubation of the spindles for 45 min in a solution lacking dynein, the ATPase activity sedimentable at 1,000 g decreased to an average value of 0.005 μmol of P_i/mg·min. No detectable ATPase activity pelleted at 1,000 g after dynein was incubated in the absence of spindles. When the spindles were incubated for 45 min with dynein, however, the sedimentable ATPase activity increased. For example, when the spindles and dynein were incubated at a protein ratio of 2.5:1, respectively, the ATPase activity that cosedimented with the spindles was 0.15 μmol of P_i/mg·min, a value expected if the dynein had, in fact, bound to the spindle microtubules. When incubated at a protein ratio of 0.5:1, the resulting spindle pellet had a specific ATPase activity of 0.25 μmol of P_i/mg·min, greater, in fact, than that of the dynein preparation alone and, therefore, indicative of an activation of the dynein's ATPase activity. Estimates of the amount of dynein sedimenting with the spindles suggested that its ATPase activity was stimulated sixfold to eightfold upon binding to the spindles.

Electrophoretic Analysis

Electrophoresis also indicated that dynein bound to the meiotic spindles (Fig. 1). Shown in lane A are axonemes prepared from the sperm of *Spisula*. For reference, note the positions of tubulin and the high molecular weight dynein polypeptides. In the adjacent lane are the isolated *Spisula* meiotic spindles in which the major component is not tubulin but, instead, a protein migrating between α - and β -tubulin (lane B). In addition to containing numerous other proteins, the spindle contains several high molecular weight polypeptides that have an electrophoretic mobility similar to that of axonemal dynein (see lane A). That these bands are, in fact, dynein-like proteins has been suggested in a recent study of mitotic apparatuses isolated from sea urchin eggs (41). The *Tetrahymena* dynein preparation used in these studies is shown in lane C. Spindles incubated for 45 min in the absence of dynein and then sedimented (lane D) appear to be qualitatively similar to the original spindle preparation (lane B) although tubulin may make up a smaller percentage of the total spindle proteins. Spindles that were incubated for 45 min in dynein and collected by centrifugation contain the high molecular weight bands that make up the *Tetrahymena* dynein in addition to those proteins normally present in the spindles (lane E). Furthermore, the relative amount of dynein to tubulin in these spindles was greater than that within native axonemes (see lane A). These observations are consistent with the hypothesis that large amounts of dynein had sedimented with the isolated spindles.

Stability of Spindle Birefringence

That the sedimentation of axonemal dynein with the meiotic spindles did not represent nonspecific trapping but, in fact, reflected microtubule binding was suggested by the observation that the dynein preparation stabilized spindle birefringence. Spindles maintained in the isolation medium containing hexylene glycol appeared birefringent for long periods of time (Fig. 2a). When these spindles were then incubated in solutions lacking dynein, the birefringence remaining after 45 min was barely discernible (Fig. 2b). In contrast, spindles incubated for

45 min in the same solution but in the presence of dynein retained much of their original birefringence (Fig. 2c).

Quantitation of the retardation of light by individual spindles verified that dynein did, in fact, preserve spindle birefringence. At various times of incubation in solutions containing or

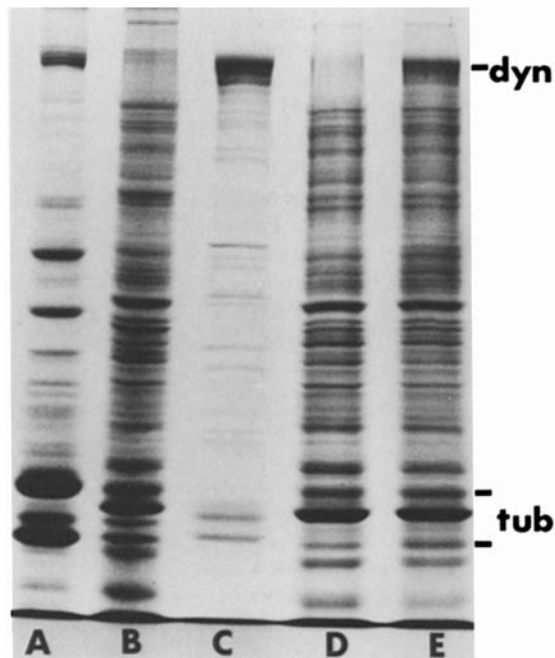


FIGURE 1 SDS polyacrylamide gel of *Spisula* spindle and *Tetrahymena* dynein preparations. Lane A, 45 μ g of axonemes obtained from *Spisula* sperm. Lane B, 45 μ g of isolated meiotic spindles. The bands corresponding to α - and β -tubulins in the spindle can be identified by their positions within the axoneme (see lane A). Note also the presence of several high molecular weight polypeptides that have electrophoretic mobilities similar to those of the axonemal dynein. Lane C, 17 μ g of *Tetrahymena* dynein preparation. Lane D, 40 μ g of a pellet of meiotic spindles obtained after a 45-min incubation in a solution lacking dynein. Lane E, 40 μ g of a pellet of meiotic spindles obtained after a 45-min incubation of 5 mg of spindles with 0.85 mg of dynein. Note that the ratio of dynein to tubulin in these spindles is greater than that in native axonemes (see lane A). *dyn*, Dynein; *tub*, tubulin.

lacking dynein, samples of the spindle preparation were fixed with 2% glutaraldehyde. In the absence of dynein the average retardation of light within a spindle decreased from an initial value of 1.44 nm to a level of no measurable birefringence within 45 min. In the presence of dynein, however, spindle birefringence was retained for longer periods, and at 45 min the retardation of light was 1.00 nm, \sim 70% of its initial value. That the retardation observed 10 min after addition of dynein to the spindles increased from 1.44 nm to 1.52 nm may reflect a contribution to the birefringence by the dynein as it decorated the meiotic microtubules.

Electron Microscopy

Ultrastructural analysis confirmed that dynein not only bound to the spindle microtubules but also revealed their structural polarity. Microtubules present in isolated spindles appeared relatively free of adhering material, although occasional projections could be observed (Fig. 3a). After incubation with dynein, however, each spindle microtubule became decorated with four to seven dynein arms (Fig. 3b), and at least one such arm could be observed to project at an angle relative to a line drawn normal to the wall of the microtubule (see arrows, Fig. 3b). Not all of the dynein arms that bound to a single microtubule lay within the same plane of sectioning. Nevertheless, those that could be clearly discerned had the same orientation (Figs. 3b and 4a). The morphology and orientation of the arms could be reinforced by Markham rotation of the micrograph, and the decorated microtubule then appeared like a pinwheel or rotary saw blade (Fig. 4b).

Dynein arms present in the outer row on the A-subfiber of axonemes have an asymmetric morphology. The arm attaches to the A-subfiber by its narrower end and broadens into a hooked structure at its distal end, that which cross-bridges the B-subfiber (see Fig. 2 of reference 3). We designate these ends of the dynein arm as its "A" and "B" ends, respectively. Analysis of the morphology of the arms attached to spindle microtubules revealed that dynein, similar in morphology to the outer arm in axonemes, bound by its broader, or B, end. These observations suggest that a rigorlike state (18) existed between the dynein and spindle microtubules (Figs. 3b and 4a and b).

Every microtubule within the spindle became decorated with dynein, but only \sim 90% of the microtubules contained one or

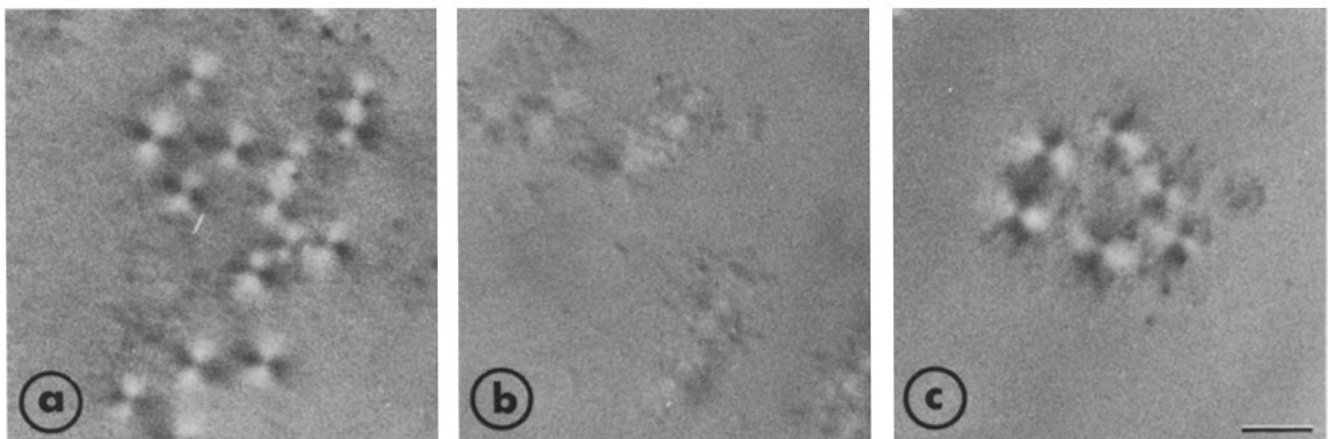


FIGURE 2 Polarization micrographs of spindle preparations. (a) Spindles isolated from meiotic *Spisula* eggs and maintained in a solution containing hexylene glycol. (b) Spindles incubated for 45 min in a solution lacking dynein. These spindles have lost most of their original birefringence. (c) Spindles incubated for 45 min in a solution containing dynein. The birefringence of these spindles appears similar to that of the original preparation (Fig. 3a). Bar, 10 μ m.

more dynein arms whose orientation could be clearly discerned (Fig. 5). It has been previously shown that when an axoneme is viewed from its base, or "minus," end toward the distal, or "plus," end, the dynein arms are oriented clockwise around the axoneme (21). Observed in this orientation, the hooklike projection on the dynein arm points clockwise around each microtubule doublet (22; see Fig. 4 reference 23). The convention of using the orientation of dynein to indicate microtubule polarity should be reversed when the dynein arm is bound to its B end. Based on the clockwise orientation of the hooks of the dynein arms bound not by their A but by their B end, it can be inferred that the microtubules in Fig. 5 are oriented with their plus end toward the observer. The pitch of dynein arms bound to 419 microtubules in >10 spindles has been scored. In any given section of the spindle, 96% of the microtubules contained dynein arms of the same orientation, either clockwise or counterclockwise, thereby suggesting that a half-spindle is composed of parallel microtubules.

DISCUSSION

Results of this study demonstrate that dynein isolated from *Tetrahymena* cilia binds to the microtubules present within the meiotic spindles of *Spisula*. We have defined the two morphologically and functionally distinguishable ends of the dynein arm according to its orientation within native axonemes as its A and B ends, which bind to the A and B subfibers, respectively. The spindle microtubules become decorated with four to seven arms, all of which are bound by their B end. Moreover,

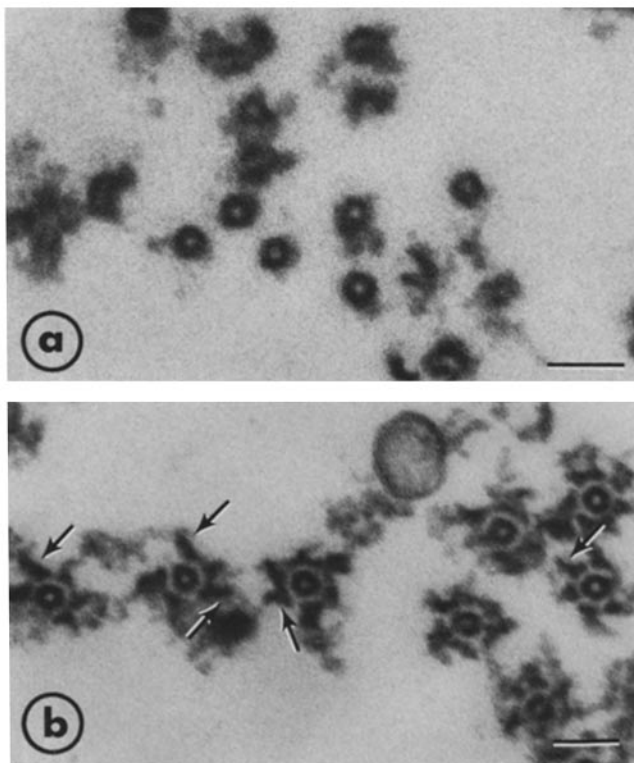


FIGURE 3 Electron micrographs of microtubules present in isolated meiotic spindles incubated in the absence (a) or presence (b) of dynein. The microtubules in b are decorated with 4-7 dynein arms. The arrows indicate some of the dynein arms that project at an angle relative to a line drawn normal to the microtubule. The dynein arms point counterclockwise around all the microtubules except the one in the center of the micrograph. Bar, 50 nm.

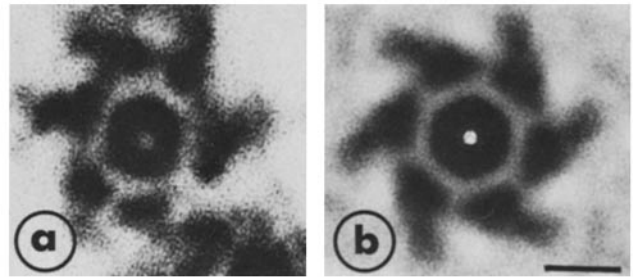


FIGURE 4 (a) Spindle microtubule decorated with six dynein arms. (b) Markham rotation of microtubule in a photographically reinforced preparation. The arms are bound to the microtubule by their "B" end, which cross-bridges the B-subfiber within native axonemes, and are pitched counterclockwise around the microtubule. Bar, 25 nm.

the ATPase activity of the dynein is activated upon binding to the spindle microtubules, and the dynein either stabilizes or contributes to the birefringence. Furthermore, the orientation of the bound dynein arms reveals intrinsic microtubule polarity within the spindle.

Initial studies on the time-course of dynein binding revealed that an interaction occurred immediately upon addition of dynein to the meiotic spindles. This observation and the fact that the dynein arms attached to the spindle microtubules by their B end suggest an association analogous to that of rigor within the axoneme (16, 18). Previous recombination studies have shown that dynein arms attached to the B-subfiber of axonemes were released upon addition of ATP (36, 48). Accordingly, we are currently studying the effects of ATP upon dynein attached to spindle microtubules.

Interestingly, the ATPase activity of dynein bound to the spindle microtubules was stimulated sixfold to eightfold. Other experiments have shown that dynein ATPase could also be activated by various microtubule preparations although no binding was demonstrated (26, 27, 39). More recent work has revealed that the ATPase activity of latent activity dynein reattached to the A-subfiber of axonemes (20, 40) or bound to in vitro assembled brain microtubules (40) was also stimulated severalfold. The present study demonstrates that the B end of the dynein arm bound to microtubules and resulted in an activation of ATPase activity, a situation analogous to the stimulation of myosin ATPase by actin (30). These observations support data suggesting that energy production within the axoneme may be coupled to cross-bridging, and, therefore, to motility (8, 9, 14).

Dynein cross-bridged adjacent in vitro assembled microtubules (23), but no such cross-bridging was observed between the spindle microtubules. Because dynein bound to and surrounded the spindle microtubules immediately upon incubation, cross-bridging may then have been prevented because all available dynein binding sites on the microtubules were occupied. Alternatively, spindle microtubules may not be free to move close enough together to permit cross-bridging, or, once bound by its B end, a dynein arm may be constrained from attaching to another microtubule by its A end.

The birefringence of meiotic spindles was maintained in the presence of dynein. It might, therefore, be expected that the dynein had stabilized the microtubules present within the spindles. However, both gel electrophoresis and electron microscopy indicated that the amount of tubulin and the number of microtubules were similar in spindle preparations incubated

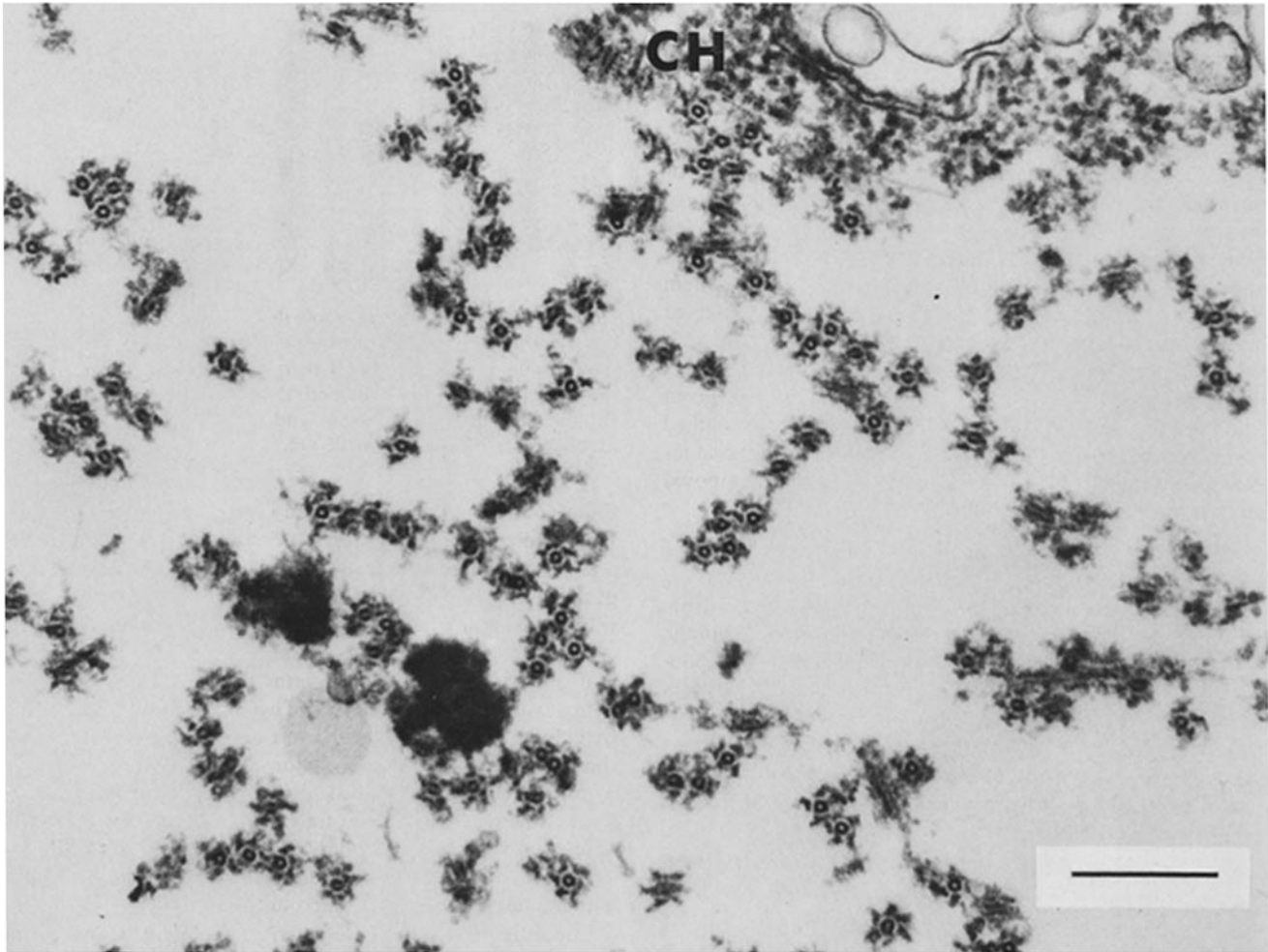


FIGURE 5 Section through an isolated *Spisula* meiotic spindle incubated in dynein. The majority of the microtubules are surrounded with clockwise-oriented dynein arms. The chromatin (CH) indicates that this section passes through the equatorial region of the spindle. Bar, 0.25 μ m.

in the presence or absence of dynein. Possibly, the binding itself of dynein arms to the microtubules contributed to the form birefringence of the spindle. The maintenance of birefringence of spindles incubated in dynein provides a convenient assay to determine that dynein has bound to spindle microtubules.

It is interesting that the spindle microtubules bound such large amounts of dynein. Previous studies have demonstrated that, several hours before ciliogenesis, cleaving sea urchin embryos contained a pool of axonemal proteins including dynein (46). Nevertheless, within these cells the dynein apparently does not bind to the mitotic microtubules. A cell, therefore, must possess some mechanism to ensure that dynein binds only to the appropriate axonemal microtubules. That this may be the case is supported by preliminary studies in which dynein isolated from *Spisula* sperm did not bind to *Spisula* meiotic spindles.

We have previously shown that the longitudinal tilt and cross-sectional orientation of *Chlamydomonas* dynein revealed polarity when bound to in vitro assembled microtubules. The outer dynein arm of *Chlamydomonas* axonemes has a distinctive morphology with an easily discernible, hooklike projection located at its B end. The orientation of this hook provided a definitive marker for the determination of microtubule polarity (23). *Tetrahymena* dynein, however, lacks such an obvious

projection, but the overall asymmetry of the arm could still be used for polarity studies. That cross sections of spindles incubated in dynein often contained chromatin (e.g., Fig. 5) confirmed that the microtubules in that section were in the region of the equatorial plate within the half-spindle and not within one of the asters. In the absence of any discernible chromatin, however, the specific location of a given microtubule within the spindle could not be determined. Nevertheless, 96% of the microtubules examined in any section, either containing or lacking chromatin, were of uniform polarity.

Longitudinal sections of the isolated spindles revealed microtubules emanating from both chromosomes and poles, and accordingly, we have inferred that the microtubule profiles observed in any given cross section containing chromatin represent both classes of spindle microtubules. That six to seven rows of dynein arms surrounded the spindle microtubules precluded a clear visualization of the tilt of these arms in longitudinal sections because of the subsequent overlap. To overcome this problem, we are currently undertaking experiments using lower dynein concentrations, resulting in a single row of dynein arms bound per microtubule. By analyzing the tilt of these individual dynein arms, it will then be verifiable that the polarity of a microtubule emanating from a kinetochore is the same as that of one emanating from the pole within the same half-spindle. Initial observations have revealed that

dynein arms or microtubules projecting from a pole tilt toward that pole. Because *Tetrahymena* dynein arms on doublet microtubules tilt toward the base or "minus" end of the axoneme (36, 44, 48), the "plus" end of these spindle microtubules should be located distal to the pole, as has been previously shown by other studies (6, 25).

Euteneuer and McIntosh (personal communication) have serially sectioned mitotic apparatuses decorated with in vitro assembled microtubule hooklike structures and have demonstrated that the microtubules within the half-spindle of PtK₁ cells possess the same polarity (13). Taken together, their work and the current investigation suggest that the chromosomes may not approach the poles by sliding of microtubules of opposite polarity.

We are most grateful to Dr. Joel L. Rosenbaum for supplies and equipment used in this study and for helpful discussions. We would like to thank Drs. Ted Salmon and David Begg for valuable advice. We acknowledge the expert technical assistance of Michael Mead.

B. R. Telzer was supported by a National Science Foundation Steps Towards Independence Fellowship from the Marine Biological Laboratory, The William and Flora Hewlett Foundation Grants of Research Corporation, and a grant from the Pomona College Research Committee. L. T. Haimo was supported by an American Cancer Society Postdoctoral Fellowship and a grant from the Committee on Research, University of California.

Received for publication 5 January 1981, and in revised form 3 February 1981.

REFERENCES

- Allen, C., and G. G. Borisy. 1974. Structural polarity and directional growth of microtubules of *Chlamydomonas* flagella. *J. Mol. Biol.* 90:381-402.
- Allen, R. D. 1954. Fertilization and artificial activation in the egg of the surf clam, *Spisula solidissima*. *Biol. Bull. (Woods Hole)*, 105:213-239.
- Allen, R. D. 1967. A reinvestigation of cross-sections of cilia. *J. Cell Biol.* 37:825-830.
- Begg, D. A., R. Rodewald, and L. I. Rebhun. 1978. The visualization of actin filament polarity in thin sections. Evidence for uniform polarity of membrane-associated filaments. *J. Cell Biol.* 79:846-852.
- Bergen, L. G., and G. G. Borisy. 1980. Head-to-tail polymerization of microtubules in vitro. Electron microscope analysis of seeded assembly. *J. Cell Biol.* 84:141-150.
- Bergen, L. G., R. Kuriyama, and G. G. Borisy. 1980. Polarity of microtubules nucleated by centrosomes and chromosomes of Chinese hamster ovary cells in vitro. *J. Cell Biol.* 84:151-159.
- Binder, L. I., W. L. Dentler, and J. L. Rosenbaum. 1975. Assembly of chick brain tubulin onto flagellar axonemes of *Chlamydomonas* and sea urchin sperm. *Proc. Natl. Acad. Sci. U. S. A.* 72:1122-1126.
- Brokaw, C. J., and T. F. Simonick. 1976. CO₂ regulation of the amplitude of flagellar bending. *Cold Spring Harbor Conf. Cell Proliferation*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 3(Book C):933-940.
- Brokaw, C. J., and T. F. Simonick. 1977. Mechanochemical coupling in flagella. V. Effects of viscosity on movement and ATP-dephosphorylation on Triton-demetempered sea urchin spermatozoa. *J. Cell Sci.* 23:227-241.
- Cande, W. Z., J. Snyder, D. Smith, K. Summers, and J. R. McIntosh. 1974. A functional mitotic spindle prepared from mammalian cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* 71:1559-1563.
- Cande, W. Z., and S. M. Wolniak. 1978. Chromosome movement in lysed mitotic cells is inhibited by vanadate. *J. Cell Biol.* 79:573-580.
- Euteneuer, U., and J. R. McIntosh. 1980. Polarity of midbody and phragmoplast microtubules. *J. Cell Biol.* 87:509-515.
- Euteneuer, U., and J. R. McIntosh. 1981. Structural polarity of kinetochore microtubules in PtK₁ cells. *J. Cell Biol.* 89:338-345.
- Gibbons, B. H., and I. R. Gibbons. 1972. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. *J. Cell Biol.* 54:75-97.
- Gibbons, B. H., and I. R. Gibbons. 1973. The effect of partial extraction of dynein arms on the movement of reactivated sea urchin sperm. *J. Cell Sci.* 13:337-357.
- Gibbons, B. H., and I. R. Gibbons. 1974. Properties of flagellar "rigor waves" formed by abrupt removal of adenosine triphosphate from actively swimming sea urchin sperm. *J. Cell Biol.* 63:970-985.
- Gibbons, I. R. 1965. Chemical dissection of cilia. *Arch. Biol.* 76:317-352.
- Gibbons, I. R. 1975. The molecular basis of flagellar motility in sea urchin spermatozoa. In *Molecules and Cell Movement*. S. Inoué and R. E. Stephens, editors. Raven Press, New York. 207-232.
- Gibbons, I. R., M. P. Cosson, J. A. Evans, B. H. Gibbons, B. Houck, K. H. Martinson, W. S. Sale, and W. Y. Tang. 1978. Potent inhibition of dynein adenosinetriphosphatase and of the motility of cilia and sperm flagella by vanadate. *Proc. Natl. Acad. Sci. U. S. A.* 75:2220-2224.
- Gibbons, I. R., and E. Fronk. 1979. A latent adenosine triphosphatase form of dynein I from sea urchin sperm flagella. *J. Biol. Chem.* 254:187-196.
- Gibbons, I. R., and A. V. Grimstone. 1960. On flagellar structure in certain flagellates. *J. Biophys. Biochem. Cytol.* 7:697-716.
- Haimo, L. T. 1981. Dynein decoration of microtubules. Determination of polarity. *Methods Cell Biol.* In press.
- Haimo, L. T., B. R. Telzer, and J. L. Rosenbaum. 1979. Dynein binds to and crossbridges cytoplasmic microtubules. *Proc. Natl. Acad. Sci. U. S. A.* 76:5759-5763.
- Heidemann, S. R., and J. R. McIntosh. 1980. Visualization of the structural polarity of microtubules. *Nature (Lond.)*, 286:517-519.
- Heidemann, S. R., G. G. Zieve, and J. R. McIntosh. 1980. Evidence for microtubule subunit addition to the distal end of mitotic structures in vitro. *J. Cell Biol.* 87:152-159.
- Hoshino, M. 1977. Interactions of *Tetrahymena* dynein with microtubule protein. Tubulin-induced stimulation of dynein ATPase activity. *Biochim. Biophys. Acta.* 462:49-62.
- Ihara, Y., T. Fujii, T. Arai, R. Tanaka, and Y. Kaziro. 1979. The presence of an adenosine-5'-triphosphatase dependent on 6S tubulin and calcium ions in rat brain microtubules. *J. Biochem. (Tokyo)*, 86:587-590.
- Laemmli, U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680-685.
- Linn, T. I., and M. F. Morales. 1977. Application of a one-step procedure for measuring inorganic phosphate in the presence of proteins: the actomyosin ATPase system. *Anal. Biochem.* 77:10-17.
- Lynn, R. W., and E. W. Taylor. 1971. Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry* 10:4617-4624.
- Margolis, R., L. Wilson, and B. Kiefer. 1978. Mitotic mechanism based on intrinsic microtubule behavior. *Nature (Lond.)*, 272:450-452.
- McDonald, K. L., M. K. Edwards, and J. R. McIntosh. 1979. Cross-sectional structure of the central mitotic spindle of *Diatoma vulgare*. Evidence for specific interactions between antiparallel microtubules. *J. Cell Biol.* 83:443-461.
- McDonald, K. L., J. D. Pickett-Heaps, J. R. McIntosh, and D. H. Tippit. 1977. On the mechanism of anaphase spindle elongation in *Diatoma vulgare*. *J. Cell Biol.* 74:377-388.
- McIntosh, J. R., P. K. Hepler, and D. G. Van Wie. 1969. Model for mitosis. *Nature (Lond.)*, 224:659-663.
- McIntosh, J. R., K. L. McDonald, M. K. Edwards, and B. M. Ross. 1979. Three-dimensional structure of the central spindle of *Diatoma vulgare*. *J. Cell Biol.* 83:428-442.
- Mitchell, D. R., and F. D. Warner. 1980. Interaction of dynein arms with B subfibers of *Tetrahymena* cilia. Quantitation of the effects of magnesium and adenosine triphosphate. *J. Cell Biol.* 87:84-97.
- Murphy, D. B. 1979. Identification of microtubule-associated proteins in the meiotic spindles of surf clam oocytes. *J. Cell Biol.* 84:235-245.
- Nicklas, R. B. 1971. Mitosis. In *Advances in Cell Biology*. D. Prescott, L. Goldstein, and H. McConkey, editors. Appleton-Century-Crofts, New York. 225-294.
- Otakawa, M. 1972. Stimulation of ATPase activity of 30S dynein with microtubular protein. *Biochim. Biophys. Acta.* 275:464-466.
- Porter, M. E. 1980. Preparation of a latent activity dynein from molluscan sperm flagella. *J. Cell Biol.* 87(2, Pt. 2):254a (Abstr.).
- Pratt, M. M., T. Otter, and E. D. Salmon. 1980. Dynein-like Mg²⁺-ATPase in mitotic spindles isolated from sea urchin embryos (*Strongylocentrotus droebachiensis*). *J. Cell Biol.* 86:738-745.
- Rebhun, L. I., and T. Sharpless. 1964. Isolation of spindles from the surf clam, *Spisula solidissima*. *J. Cell Biol.* 22:488-492.
- Sakai, H., I. Mabuchi, S. Shimoda, R. Kuriyama, K. Ogawa, and H. Mohri. 1976. Induction of chromosome motion in the glycerol isolated mitotic apparatus: nucleotide specificity and effects of anti-dynein and -myosin sera on the motion. *Dev. Growth Differ.* 18:211-219.
- Sale, W. S., and P. Satir. 1977. The direction of active sliding of microtubules in *Tetrahymena* cilia. *Proc. Natl. Acad. Sci. U. S. A.* 74:2045-2049.
- Salmon, E. D., and R. R. Segall. 1980. Calcium-labile mitotic spindles isolated from sea urchin eggs (*Lytechinus variegatus*). *J. Cell Biol.* 86:355-365.
- Stephens, R. E. 1972. Studies on the development of the sea urchin *Strongylocentrotus droebachiensis*. III. Embryonic synthesis of ciliary proteins. *Biol. Bull. (Woods Hole)*, 142:489-504.
- Summers, K. E., and M. W. Kirschner. 1979. Characterization of the polar assembly and disassembly of microtubules observed in vitro by darkfield microscopy. *J. Cell Biol.* 83:205-217.
- Takahashi, M., and Y. Tonomura. 1978. Binding of 30S dynein with the B-tubule of the outer doublet of axonemes from *Tetrahymena pyriformis* and adenosine triphosphate-induced dissociation of the complex. *J. Biochem. (Tokyo)*, 84:1339-1355.