

## Polarity of Midbody and Phragmoplast Microtubules

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**ABSTRACT** A newly discovered method (Heidemann and McIntosh, 1980, *Nature [Lond.]*, 286:517) for displaying the molecular polarity of microtubules (MTs) has been slightly modified and applied to the midbodies of cultured mammalian cells and the phragmoplasts of *Haemanthus* endosperm. The method involves the decoration of preexisting MTs in lysed cells with curved ribbons of tubulin protofilaments; the direction of curvature of these C-shaped appendages as seen in cross section reflects the intrinsic polarity of the MTs. In transverse sections of midbodies from HeLa and PtK cells, we find that essentially all the MTs in a given region of the structures have the same direction of hook curvature, and hence the same polarity. The midbody MTs that lie on one side of the spindle equator show the opposite polarity from those on the other side, indicating that the midbody is constructed from two families of antiparallel MTs. Midbody MTs are arranged with their fast-growing ends overlapping at the spindle equator, consistent with the hypothesis that the midbody is formed by the interdigitation of aster MTs. The polarities of the MTs from the phragmoplast of endosperm cells are the same as those found in the mammalian midbody. Our results eliminate one model for mitosis, but are consistent with others. The systematic and reproducible polarities observed favor the concept that MT polarity is an important factor in the formation and/or the function of these two mitotic structures.

Microtubules (MTs) possess an intrinsic polarity that derives from their construction out of asymmetric subunits polymerized head-to-tail (for a review, see reference 2). Functional manifestations of this polarity are found in the different rate constants for assembly and disassembly at the two ends of an MT (1, 3, 10, 20, 31) and in the polar orientation of dynein arms sometimes seen on flagellar MTs (1, 12, 33). Both of these properties have been used as probes for the polarity of MTs in particular cellular systems (4, 5, 12, 31). Recently, however, an alternate method for MT polarity determination has been described that is based upon the capacities of tubulin for polymorphic assembly. Burton and Himes (9) have identified conditions in which tubulin will form diverse protofilament bundles and abnormal wall junctions. In cross section, some of these aggregates display enantiomorphic images which have the potential of revealing the intrinsic polarity of the polymers (19). Heidemann and McIntosh (13) have found related conditions in which spontaneous tubulin assembly forms variegated protofilament bundles, but when the assembly reaction is seeded by MTs, the polymerizing material more closely resembles normal MTs. The preexisting MTs elongate in the usual fashion, but in addition, one observes the decoration of those MTs with C-shaped sheets of laterally associated protofilaments that make an unusual wall-to-wall junction with the true MT. Such sheets seen in cross section appear as hooks that curve clockwise or counterclockwise about the MT (Fig. 1a).

When MTs of defined polarity, such as astral MTs and MTs grown from a basal body, are viewed from their fast-growing, or plus end, toward their slow-growing, or minus end (3), then ~90% of all hooks curve clockwise (13). We can therefore define a "right-hand rule" for relating hook curvature to MT polarity: curl the fingers of your right hand in the direction of hook curvature; your thumb will now point toward the minus end of the MT.

In this paper, we show that hooks can form directly on preexisting MTs in lysed cells, facilitating polarity determination for MTs not readily studied by a method requiring MT elongation. We have used these hooks to determine the polarities of the MTs in the mitotic midbody of mammalian cells and in the phragmoplast of *Haemanthus* endosperm. These systems were selected for study because the polarities of their MTs may be informative about mitotic mechanism. The midbody is an array of MTs found in telophase and postdivision animal cells. It is located in the zone between the separated chromosomes or sister nuclei and is believed to be derived from the interzone fibers of anaphase (22). Serial sections in both longitudinal (26, 29) and transverse orientation (7, 22-24) suggest that the midbody is constructed from two families of interdigitating MTs with a number of short MT fragments scattered throughout (Fig. 1b). The phragmoplast is the organelle that forms the cell plate, i.e., the wall that develops to achieve cytokinesis in plant cells. Phragmoplast origin is in

most circumstances related to the spindle in a fashion reminiscent of the midbody (15), but there are many subtleties of phragmoplast formation and function that deny a strict homology (18). In this paper, we present data which define the polarities of midbody and phragmoplast MTs and relate the polarities observed to those predicted by several models for mitosis.

## MATERIALS AND METHODS

### Tubulin Preparation

Microtubule protein (MTP) was prepared from bovine brain by two cycles of temperature-dependent polymerization and depolymerization in a buffer containing 0.1 M 2[*N*-morpholino]ethane sulfonate (MES), pH 6.5, 1 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM GTP, and 8 M glycerol (30, 34). An MTP pellet was resuspended in 0.5 M PIPES, pH 6.9, 1 mM MgCl<sub>2</sub>, 1 mM EDTA, and 1 mM GTP at 0°C. We call this buffer 0.5 PMEG. The resuspended MTP was cycled once through assembly and disassembly in 0.5 PMEG by the customary temperature changes and then spun at 250,000 *g* for 3 h at 4°C to remove oligomers and retard spontaneous polymer initiation. The resulting high speed supernate, which we will call tubulin, was frozen in small aliquots and kept in liquid nitrogen for subsequent use. Protein concentrations were determined by the method of Bradford (6).

### Cells

HeLa cells were grown on coated microscope slides in petri dishes using Eagle's minimum essential medium supplemented with 10% calf serum (Grand Island Biological Co. [GIBCO] Grand Island, New York) and 1% nonessential amino acids (GIBCO). The slides used for cell growth were coated with Teflon (Spray-Mate, dry lubricant, 3 M Co., St. Paul, Minn.) and then dipped in 1 mg/ml polylysine, rinsed in H<sub>2</sub>O, dried, and sterilized with ultraviolet light. PtK<sub>1</sub> cells were similarly grown but in Ham's F12 (GIBCO) plus 10% fetal calf serum.

### Cell Lysis and Growth of Hooks

Cells were rinsed quickly with warm 0.5 PMEG and then treated with a mixture containing 1% Triton X-165, 0.5% deoxycholate, 0.02% sodium dodecyl sulfate, 2.5% dimethyl sulfoxide (DMSO), and 0.5 mg/ml tubulin in 0.5 PMEG at 37°C for 30 min. This solution lyses the cells, stabilizes cellular MTs, and induces hook formation (13; for detailed instructions in making this detergent mixture, see 14).

### Isolation and Treatment of Cilia

*Tetrahymena pyriformis* was grown in 0.75% proteose peptone (Difco Laboratories, Detroit, Mich.), 0.75% yeast extract, 1.5% sucrose, 1 mM MgSO<sub>4</sub>, 0.05 mM CaCl<sub>2</sub>, 2 mM KH<sub>2</sub>PO<sub>4</sub>, and 30 μg/ml Sequestrene (Ciba-Geigy Corp., Pharmaceuticals Div., Summit, N. J.). Cells in midlog growth were deciliated by a 2-min treatment with 4 mM Dibucain (Pfaltz & Bauer Inc., Stamford, Conn.). The cells were pelleted at 800 *g* for 5 min; then the cilia present in the supernate were sedimented at 17,000 *g* for 15 min, washed in 0.1 M PIPES, 1 mM EGTA, 1 mM MgCl<sub>2</sub>, 1 mM GTP, pH 6.9 (0.1 PMEG), and resuspended in 0.5 PMEG. Cilia were then incubated in a buffer containing 2.5% DMSO and 2 mg/ml tubulin in 0.5 PMEG with or without 0.1% Triton X-100 for 20 min at 34°C to induce the formation of hooks on the ciliary MTs.

### Preparation and Treatment of Endosperm Phragmoplasts

Fruits of *Haemanthus katherinae* Baker were generously provided by W. T. Jackson (Dartmouth College, Hanover, N. H.). Endosperm cells were gently squeezed onto slides coated with Teflon, polylysine, and a thin layer of 0.5% agar in water with 3.5% glucose. The cells were allowed to settle and spread at room temperature for 20–30 min in a wet chamber. They were then treated with a mixture containing 2.5% DMSO, 0.04% Saponin (Sigma Chemical Co., St. Louis, Mo.) or 0.3% polyoxyethylene-20 cetyl ether (Brij 58, Sigma Chemical Co.), and 0.5 mg/ml tubulin in 0.5 PMEG for 30 min at 37°C to lyse the cells and form hooks.

### Electron Microscopy

Cells and cilia were fixed with 2% glutaraldehyde in 0.1 PMEG for 30 min,

washed twice in 0.1 M cacodylate buffer, pH 7.1, and then postfixed with 1% OsO<sub>4</sub> in the same cacodylate buffer. In some cases 1% tannic acid was added to the glutaraldehyde-containing buffer. Dehydration, including en bloc staining with uranyl acetate and phosphotungstic acid and embedding were carried out according to a standard procedure (11). The cilia were embedded as a pellet in an Eppendorf tube while the cells were flat embedded between two Teflon-coated slides separated by chips of coverslips to serve as spacers. Specimens were sectioned on an ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.; or LKB Instruments, Inc., Rockville, Md.) and observed either in a Philips EM 300 or a JEOL 100C electron microscope. From the cutting of the sections through the printing of the electron micrographs, the original orientation of the sections was always considered to assure that the final pictures were not mirror images of the true structures. Handedness of the hooks was scored on prints with a final magnification of about × 50,000. Fig. 1a shows some of the images observed and the ways that we classified them.

## RESULTS

### Polarity-revealing Hooks Can Form on Preexisting Microtubules

In our earlier work with conditions for hook formation, MT appendages were found predominantly, if not entirely, on the newly polymerized portion of the MTs studied (14). Here we have asked if conditions could be found in which hooks would form on preexisting MTs. Isolated cilia were used as a system in which the preexisting MTs could be unambiguously distinguished from the newly grown polymer, although these MTs with so many microtubule-associated proteins (MAPs) may not form hooks as well as other MTs. After a 20-min incubation of cilia with tubulin at 2 mg/ml in 0.5 PMEG plus 2.5% DMSO some hooks are seen (Fig. 2). Shorter incubation times are not adequate to produce hooks in this system. These conditions are similar to the ones used earlier, except that the time of incu-

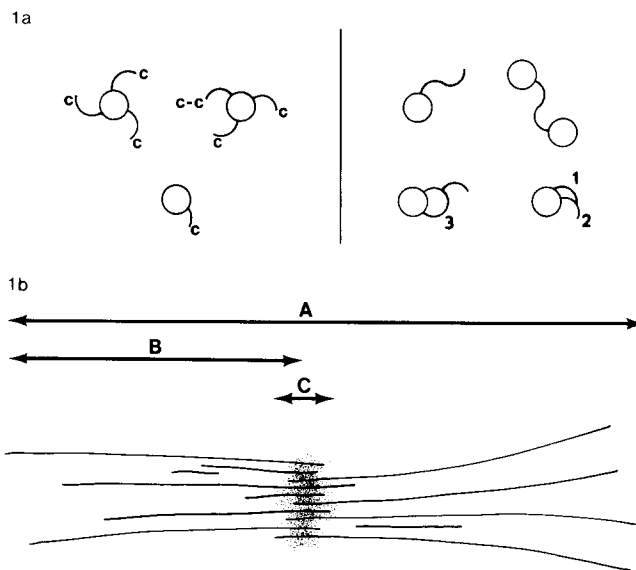


FIGURE 1 (a) Diagram illustrating the observed hook images. All hook images on the left side of the diagram were counted either as counterclockwise hooks (*c-c*) or as clockwise hooks (*c*); those on the right side (with the exception of the image with the No. 2) were not considered, because they do not permit an unambiguous interpretation of hook handedness. The doublet (3) has developed by the closure of a hook; hooks on hooks were not counted. (b) Diagram illustrating a midbody in longitudinal orientation. The word "midbody" refers to the entire structure (A), which is composed of two halves (B), one located in each sister cell. The midregion of the midbody (C) is identified by dark-staining material found between the MTs. MTs are represented by lines; the electron-dense material is indicated by stippling.

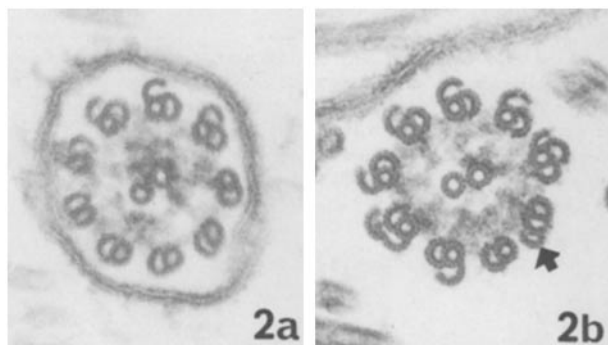


FIGURE 2 Cross sections of isolated cilia with (a) and without (b) their original membrane. Several hook images are present after a 20-min treatment with tubulin in 2.5% DMSO and 0.5 PMEG; hooks are predominantly found on the A subfiber of the cilia MTs. The closure of a hook (arrow) to form a B-subfiber-like image will occur if a hook gets long enough to touch the wall of a preexisting MT or another hook (Fig. 1a).  $\times 100,000$ .

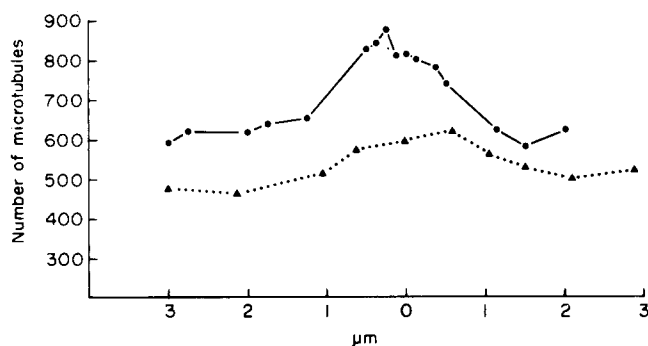


FIGURE 3 Distribution of midbody MTs in PtK<sub>1</sub> cells. MTs were counted in cross sections along the length of the midbodies. The circles represent a midbody lysed under hook-forming conditions. The triangles depict a midbody fixed in vivo (data from 23). The very middle of the midbody, which is characterized by electron-dense material in between the MTs, was taken as a marker (0 point on the abscissa); the distance from this area was determined by counting the sections in either direction, assuming that the thickness of a silver section is  $\sim 900$  Å.

bation is longer. This change seems to favor hook formation relative to MT elongation.

A detailed structural analysis of midbodies exposed to our lysis conditions has confirmed that hooks can add to preexisting MTs. The midregion of a midbody is characterized by an accumulation of electron-dense material (8, 26, 29; for a diagram of midbody structure and nomenclature, see Fig. 1b). Using this material as a marker, one can relate the electron microscope image of a serially sectioned midbody from a cell lysed as described above to that derived from a cell fixed in vivo. Fig. 3 shows the distribution of microtubule number vs. position along one midbody from a late telophase PtK cell fixed under either condition. The data are directly comparable to those obtained from midbodies of other unlysed cells (7, 22–24). The equivalence of the graphs, given the variability seen between normal cells, shows that the ends of the MTs situated at the midregion of the midbody, as marked both by the darkly staining material and by the peak in MT number, have not elongated during lysis in hook-forming buffer. The MTs seen on either side of the midregion are therefore preexisting MTs, not the products of MT elongation. Fig. 4a shows a single cross

section through a PtK midbody  $\sim 2$   $\mu\text{m}$  from the midregion. Almost every MT displays at least one hook, so we conclude that under our conditions, hooks will add to preexisting MTs.

### Polarity of Midbody MTs

We have sought evidence concerning MT polarity in PtK midbodies by serially sectioning two telophase cells and tabulating the number of hooks of each hand on a given section. By taking a micrograph of every twentieth section or so, we have clearly identified the approximate position of each section relative to the dark-staining material that marks the midregion of the midbody (Fig. 4b). Hooks are rare in this midregion, presumably because of an interference by the dark-staining material with the hook-forming process. Elsewhere in the midbody, however, hooks are common; along the spindle axis toward the midregion from one end, the hooks seen are predominantly curved counterclockwise (Fig. 4a). As one sections through the midregion to the far side, keeping the same direction of view, the hooks change their predominant sense (as in Fig. 4c). Our data on hook handedness at different positions of the PtK midbodies are summarized in Table I. They show that by this assay, the polarity of midbody MTs is uniform in any one place. On the two sides of the midregion, however, the polarities are opposite. Applying the “right-hand rule” specified above, we see that the plus ends of the midbody MTs are at the zone of overlap. Therefore, the polarity of the midbody MTs on each side of the midregion is the same as that of the astral MTs seen earlier in division on the same side of the spindle equator (13).

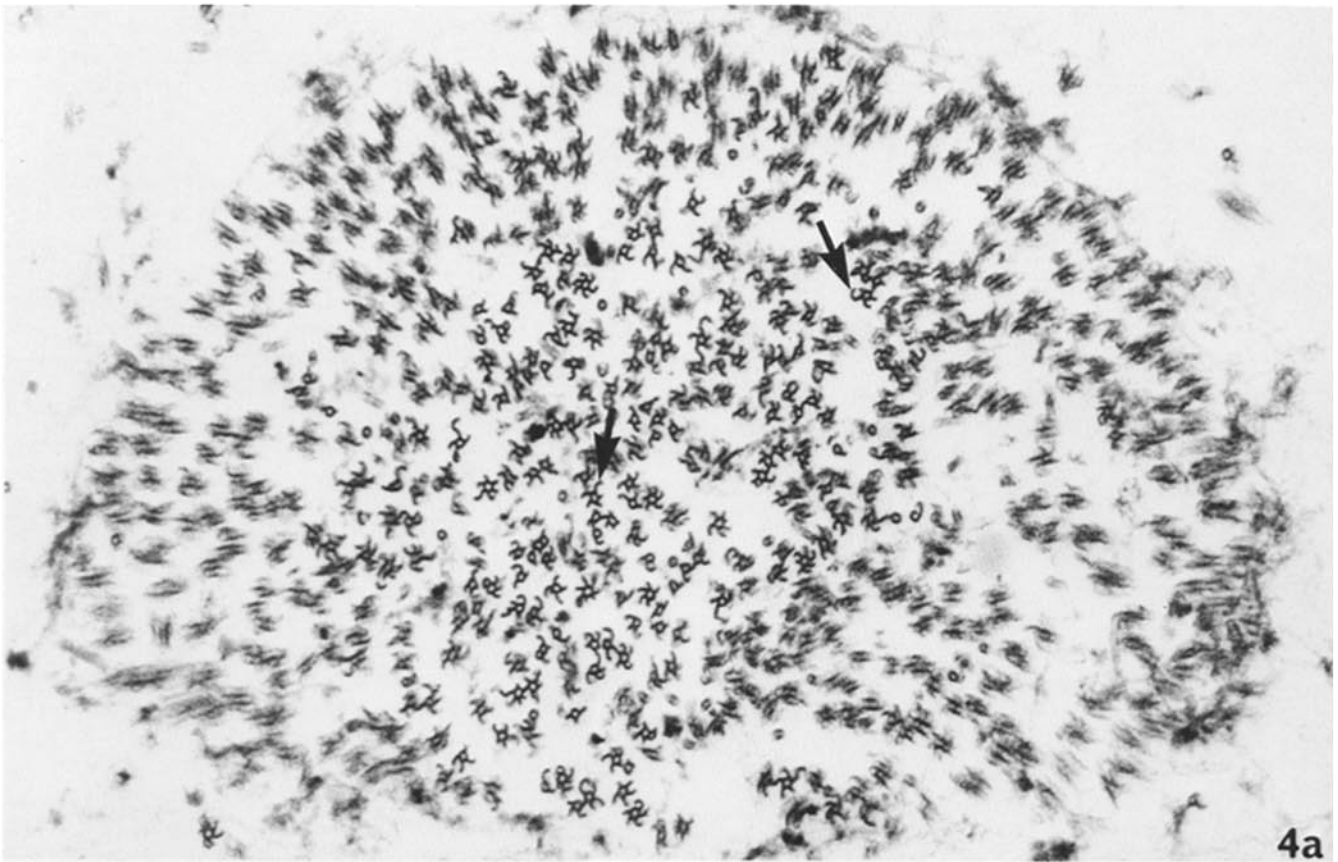
The midbodies of HeLa cells contain more MTs than those of PtK, and the courses that they follow are somewhat less divergent (Fig. 4c). We have therefore used HeLa cells for the bulk of our MT polarity data collection. Five telophase HeLa cells treated to form hooks have been serially sectioned, and the frequencies of hook handedness have been tabulated at different positions relative to the midregion. The hooks seen are qualitatively similar to those found in PtK cells. The relative numbers of HeLa cell hooks of each hand, presented in Table II, provide convincing evidence that the polarities of HeLa midbody MTs are the same as those found in PtK cells.

### Polarity of Phragmoplast MTs

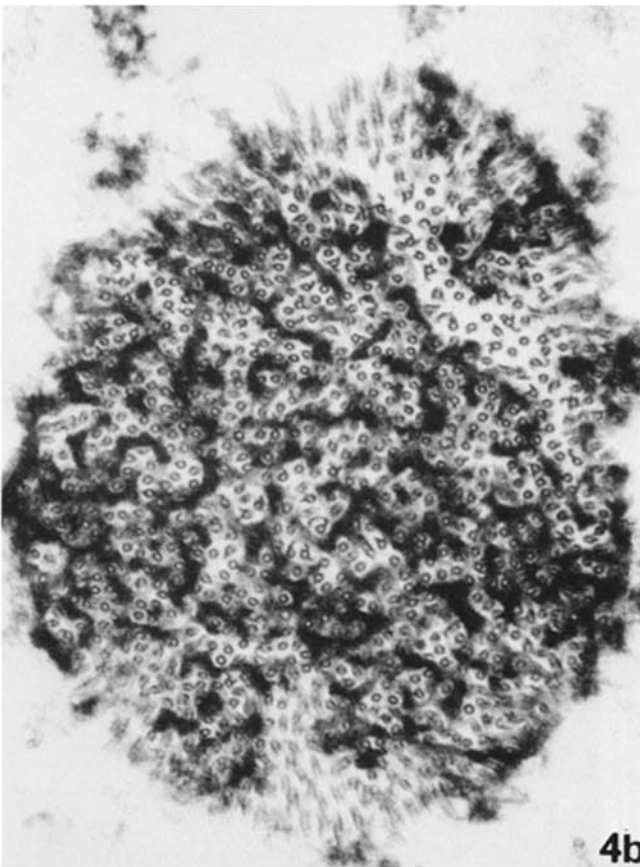
Fig. 5a and b show cross sections of a phragmoplast from a lysed cell of *Haemanthus* endosperm: Fig. 5a shows a section cut while approaching the cell plate; Fig. 5b is on the far side. As with the midbody the predominant hook hand switches as one passes the midregion of the structure. Table III presents data from two cells relating hook handedness to position on the phragmoplast. It is evident that the polarities of phragmoplast MTs as seen with this assay are the same as those of the midbody: the plus ends of the MTs are overlapping in the middle of the structure.

### DISCUSSION

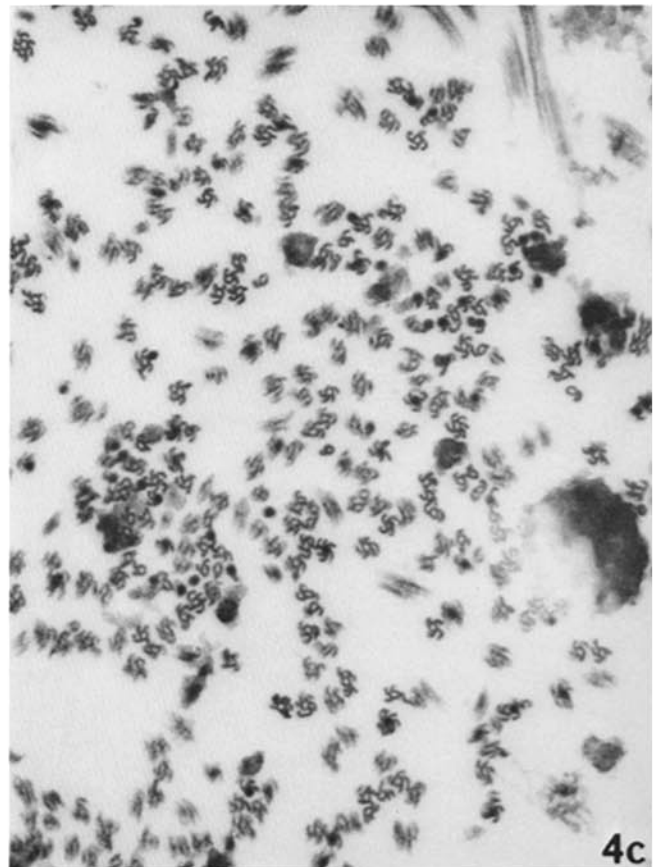
The tabulated frequencies of hook handedness for both midbody and phragmoplast MTs display a convincing consistency, supporting the assertion that our method is a dependable determination of MT polarity. The data suggest that all MTs on one side of the midbody have a single polarity, while those on the other side have the opposite orientation. Because MT number is known to be higher in the midregion of the midbody than on either side, the totality of available facts supports the



4a



4b



4c

FIGURE 4 Cross sections through a PtK<sub>1</sub> (a and b) and a HeLa (c) cell midbody treated with the hook-forming mixture. (a) When looking towards the center of the midbody mostly counterclockwise hooks are found. Sometimes a clockwise one can be seen on an MT which has also counterclockwise hooks attached to it (arrows).  $\times 51,000$ . (b) The midregion of the same midbody as in a. The electron-dense material present in this area seems to interfere with the formation of hooks, because only a very few can be seen in the regions where that material is common.  $\times 46,000$ . (c) HeLa cell midbody. The cross section was taken after passing through the midregion. Outward from the midbody, mainly clockwise hooks are displayed.  $\times 46,000$ .

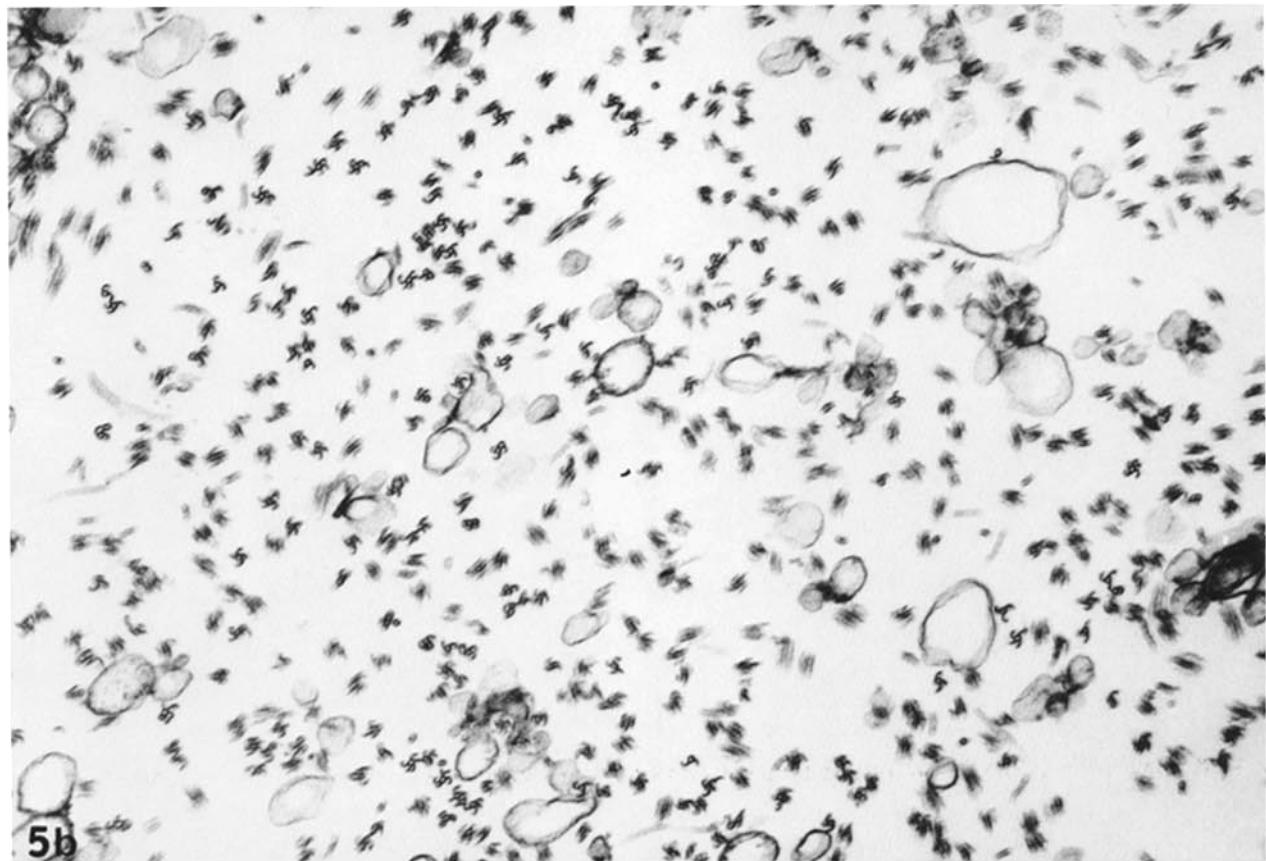
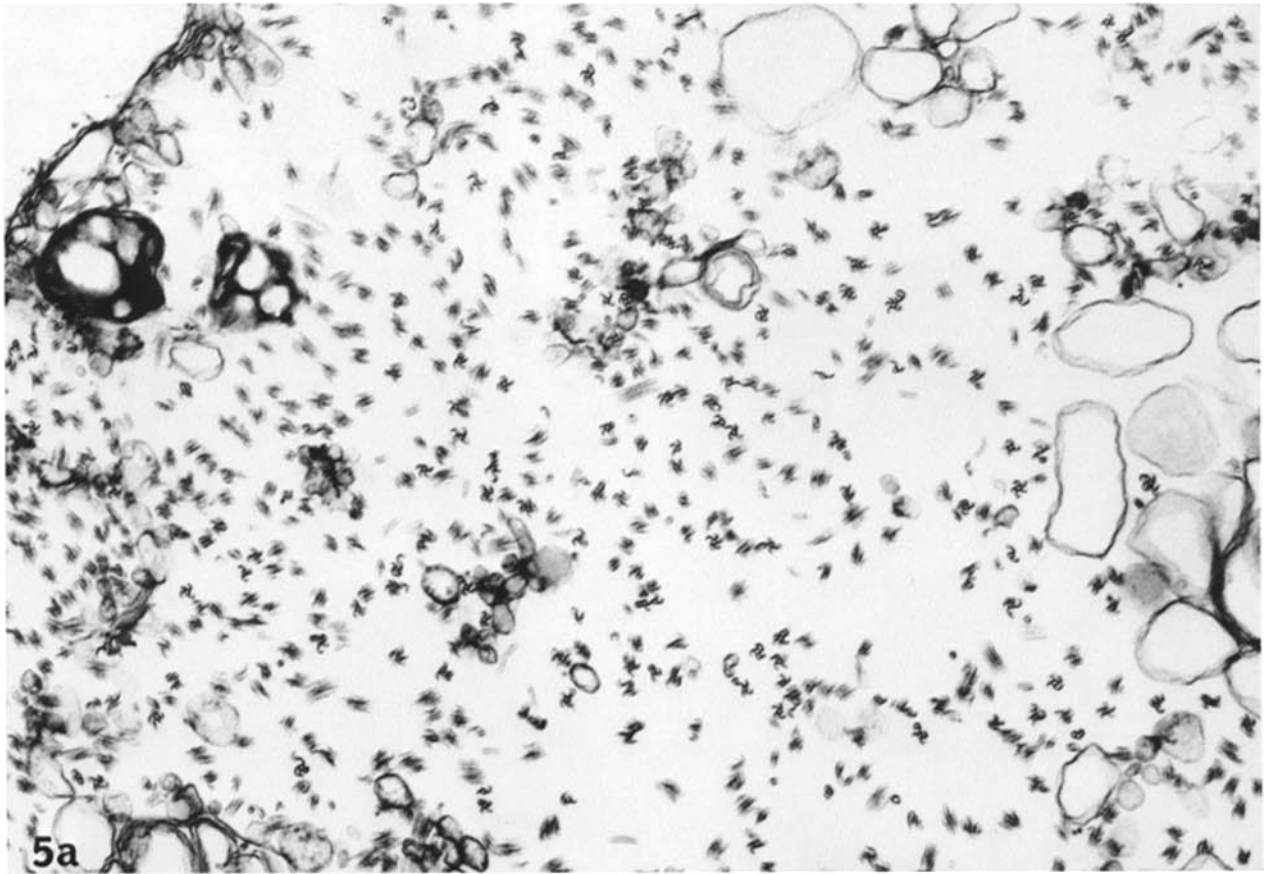


FIGURE 5 Part of the phragmoplast of a *Haemanthus* endosperm cell in cross section, lysed as described in Materials and Methods. Approaching the cell plate (a) one sees mainly counterclockwise hooks, while sections on the far side of the forming cell plate reveal predominantly clockwise hooks (b).  $\times 36,000$ .

TABLE I  
Number of Hooks on PtK<sub>1</sub> Cell Midbody MTs

Cell	Counter-clock-wise		Clock-wise		Total
	hooks	%	hooks	%	
a <sub>i</sub>	357	96.0	15	4.0	372
a <sub>o</sub>	36	5.1	668	94.9	704
b <sub>i</sub>	157	86.3	25	13.7	182
b <sub>o</sub>	8	9.1	80	90.9	88

Data from two cells, a and b. The subscripts i and o indicate the region of the midbody under examination: i implies sections taken on the way in toward the midregion, o implies sections cut on the way out.

TABLE II  
Number of Hooks on HeLa Cell Midbody MTs

Cell	Counter-clock-wise		Clock-wise		Total
	hooks	%	hooks	%	
a <sub>i</sub>	195	97.5	5	2.5	200
a <sub>o</sub>	3	2.7	107	97.3	110
b <sub>i</sub>	163	94.2	10	5.8	173
b <sub>o</sub>	15	8.8	155	91.2	170
c <sub>i</sub>	132	96.4	5	3.6	137
c <sub>o</sub>	1	1.6	60	98.4	61
d <sub>o</sub>	64	2.7	2,342	97.3	2,406
e <sub>o</sub>	17	7.2	219	92.8	236

Data from five cells, a, b, c, d, and e. The subscripts i and o are as defined in the legend to Table I.

TABLE III  
Number of hooks on Phragmoplast MTs of *Haemanthus*

Cell	Counter-clock-wise		Clock-wise		Total
	hooks	%	hooks	%	
a <sub>i</sub>	995	96.3	38	3.7	1,033
a <sub>o</sub>	31	3.6	832	96.4	863
b <sub>i</sub>	292	97.3	8	2.7	300
b <sub>o</sub>	19	2.1	882	97.9	901

Data from two cells, a and b. The subscripts i and o are as defined in the legend to Table I.

concept that the midbody is constructed from the interdigitation of two antiparallel families of MTs. The identity of the polarity of the astral and the midbody MTs in each half spindle is consistent with the hypothesis that the midbody arises through the interdigitation of aster MTs. We imagine that at some time during mitosis a fraction of the astral MTs from opposite poles bind to one another to form the bundles seen as interzone fibers at anaphase, and that these bundles ultimately form the midbody itself.

Inspection of the data on hook handedness shows, however, that not all hooks in one region of the midbody go the same way, raising the question, are there MTs present whose polarity is different from that of the majority of their neighbors, or can hooks of the opposite sense occasionally form? Several images in Fig. 4a show unambiguously that a single MT can have two hooks of opposite hand attached to its wall, so hooks of the wrong sense can definitely form. Heidemann and McIntosh (13) used two MT systems of well-defined polarity to test this method, and they found only ~90% of the hooks showing one handedness. On average, 93% of the hooks on midbody MTs

curve counterclockwise when one is looking toward the midzone, so the data conform to the hypothesis that all the MTs on one side of the midregion are of a single polarity. The possibility that a few are of different orientation cannot, however, be excluded.

Before we can be rigorous about excluding the existence of a few MTs of heterodox orientation, we will need to understand hook formation and the unusual wall junction it depends on better than we do. We are currently at work to characterize the hook-forming reaction, but substantial progress has already been made by two other groups (9, 16, 19). The current indications are that hooks are promoted both by high concentration of zwitterionic buffers and by the presence of small amounts of DMSO. MAPs are not necessary, and indeed, they co-assemble poorly with tubulin in hook-forming buffers (16). It will be interesting to learn whether the abnormal wall junction of our experiments is related either to the formation of B subfibers in cilia and flagella or to the one case in which large numbers of hooks have been found in vivo (32).

We have been able to learn the polarity of a hook relative to the MT it decorates. Several cases have been found in which a hook forms upon a hook. In all such cases, the handedness of the second hook is the same as that of the first, suggesting that the polarity of the protofilaments in the hook is the same as that of the MT itself.

The observed polarity of the midbody MTs can be related to some models for mitosis in which MT polarity is considered. Our observations, together with those of Heidemann and McIntosh (13) are consistent with the models of Nicklas (27) and of Margolis et al. (21). They are inconsistent with the model of McIntosh et al. (25) in which interdigitating astral MTs are anticipated, but it is predicted that they would slide into an arrangement with polarities opposite to those we observe (Fig. 6). This model should for this and other reasons (28) be discarded, although the ideas of MT polarity and specific interactions between antiparallel MTs upon which it is based are still likely to be important to mitotic mechanism.

Our observations on the phragmoplast must be regarded as preliminary. While many MTs and hooks have been scored for polarity, the phragmoplast appears to be a more complex

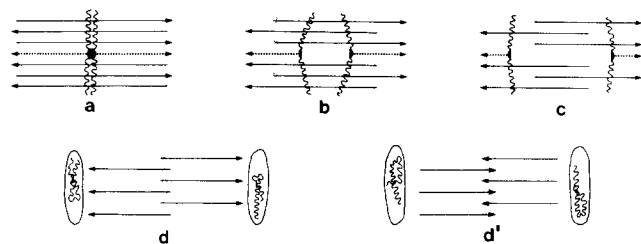


FIGURE 6 MT polarity in metaphase (a), anaphase (b and c), and telophase (d) as proposed by McIntosh et al. (25) in their model for mitosis. MTs are represented by lines; the kinetochore MTs by dotted lines. The stipling indicates the material that is, according to the model, transferred from the poles to the midregion of the midbody. The polarity of MTs with respect to their organizing centers is indicated by arrowheads. The results from the work of Bergen et al. (4) have shown that the fast-growing (plus) ends of aster MTs are distal to the organizing center, meaning that the arrowhead depicts the plus end of these MTs. In telophase (d) the polarity of the interzonal MTs, proposed by McIntosh et al. (25), is opposite to the one we found in this paper (d'): the MTs are oriented with their plus (fast-growing) ends embedded in the osmiophilic material at the midregion of the midbodies.

structure than the midbody, and only two cells have been successfully processed. We include these preliminary data here because of their striking similarity to corresponding data from the midbody. The equivalence of the MT polarities supports the idea that the two structures arise by a common mechanism (15). The phragmoplast, however, is capable of lateral growth, especially in cambium cells. This growth appears to involve the initiation of new MTs from the edge of the growing cell plate. A microtubule-organizing capacity for the phragmoplast is further suggested by the ultraviolet microbeam irradiations performed by Inoué (17). If phragmoplast MTs are initiated at the cell plate, then they are upside down with respect to their organizing center when compared to astral and flagellar MTs. We plan an extensive study of this organelle in different stages of formation to explore this enigmatic possibility.

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#### REFERENCES

- Allen, C., and G. G. Borisy. 1974. Structural polarity and directional growth of microtubules of *Chlamydomonas flagella*. *J. Mol. Biol.* 90:381-402.
- Amos, L. 1979. Structure of microtubules. In *Microtubules*. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., London. 1-64.
- Bergen, L. G., and G. G. Borisy. 1980. Head-to-tail polymerization of microtubules in vitro. *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.
- Borisy, G. G. 1978. Polarity of microtubules of the mitotic spindle. *J. Mol. Biol.* 124:565-570.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-253.
- Brinkley, B. R., and J. Cartwright. 1971. Ultrastructural analysis of mitotic spindle elongation in mammalian cells in vitro. *J. Cell Biol.* 50:416-431.
- Buck, R. C., and J. M. Tisdale. 1962. The fine structure of the midbody of the rat erythroblast. *J. Cell Biol.* 13:109-115.
- Burton, P. R., and R. H. Himes. 1978. Electron microscope studies of pH effects on assembly of tubulin free of associated proteins. *J. Cell Biol.* 77:120-133.
- Dentler, W. L., S. Granett, G. B. Witman, and J. L. Rosenbaum. 1974. Directionality of brain microtubule assembly in vitro. *Proc. Natl. Acad. Sci. U. S. A.* 71:1710-1714.
- Euteneuer, U., J. Bereiter-Hahn, and M. Schliwa. 1977. Microfilaments in the spindle of *Xenopus laevis* tadpole heart cells. *Cytobiologie*. 15:169-173.
- 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. W. 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.
- Hepler, P. K., and W. T. Jackson. 1968. Microtubules and early stages of cell-plate formation in the endosperm of *Haemaphys katherinae*. *J. Cell Biol.* 38:437-446.
- Himes, P. H., P. R. Burton, and J. M. Gaito. 1977. Dimethyl sulfoxide-induced self-assembly of tubulin lacking associated proteins. *J. Biol. Chem.* 252:6222-6228.
- Inoué, S. 1964. Organization and function of the mitotic spindle. In *Primitive Motile Systems in Cell Biology*. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 549-598.
- Lambert, A. M., and A. S. Bajer. 1972. Dynamics of spindle fibers and microtubules during anaphase and phragmoplast formation. *Chromosoma (Berl.)* 39:101-144.
- Mandelkow, E.-M., and E. Mandelkow. 1979. Junctions between microtubule walls. *J. Mol. Biol.* 129:135-148.
- Margolis, R. L., and L. Wilson. 1978. Opposite end assembly and disassembly of microtubules at steady state in vitro. *Cell*. 13:1-8.
- Margolis, R. L., L. Wilson, and B. I. Kiefer. 1978. Mitotic mechanism based on intrinsic microtubule behaviour. *Nature (Lond.)* 272:450-452.
- McIntosh, J. R., W. Z. Cande, and J. A. Snyder. 1975. Structure and physiology of the mammalian mitotic spindle. In *Molecules and Cell Movement*. S. Inoué and R. E. Stephens, editors. Raven Press, New York. 31-76.
- McIntosh, J. R., W. Z. Cande, J. A. Snyder and K. Vanderslice. 1975. Studies on the mechanism of mitosis. *Ann. N. Y. Acad. Sci.* 253:407-427.
- McIntosh, J. R., and S. C. Landis. 1971. The distribution of spindle microtubules during mitosis in cultured human cells. *J. Cell Biol.* 49:468-497.
- McIntosh, J. R., P. K. Hepler, and D. G. Van Wie. 1969. Model for mitosis. *Nature (Lond.)* 224:659-663.
- Mullins, J. M., and J. J. Bieseke. 1977. Terminal phase of cytokinesis in D-98S cells. *J. Cell Biol.* 73:672-684.
- Nicklas, R. B. 1971. Mitosis. In *Advances in Cell Biology*. D. M. Prescott, L. Goldstein, and E. McConkey, editors. Appleton-Century-Crofts, New York. 225-297.
- Nicklas, R. B. 1977. Chromosome movement: facts and hypotheses. In *Mitosis, Facts and Questions*. M. Little, N. Paweletz, C. Petzelt, H. Ponstingl, D. Schroeter, and H.-P. Zimmermann, editors. Springer-Verlag, Berlin. 150-166.
- Paweletz, N. 1967. Zur Funktion des "Fleming-Körper" bei der Teilung tierischer Zellen. *Naturwissenschaften*. 54:533-535.
- Shelanski, M. L., F. Gaskin, and C. R. Cantor. 1973. Microtubule assembly in the absence of added nucleotides. *Proc. Natl. Acad. Sci. U. S. A.* 70:765-768.
- Summers, K., and M. W. Kirschner. 1979. Characteristics of the polar assembly and disassembly of microtubules observed in vitro by darkfield light microscopy. *J. Cell Biol.* 83:205-217.
- Turner, F. R. 1972. Modified microtubules in the testis of the water strider, *Gerris remigis* (Say). *J. Cell Biol.* 53:263-270.
- Warner, F. D., and D. R. Mitchell. 1978. Structural conformations of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. *J. Cell Biol.* 76:261-277.
- Weingarten, M. D., M. M. Suter, D. R. Littman, and M. W. Kirschner. 1974. Properties of the depolymerization products of microtubules from mammalian brain. *Biochemistry*. 13:5529-5537.