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. 1 a).

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. 1 b). 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 MgCl₂, 1 mM EGTA, 1 mM GTP, and 8 mM glycerol (30, 34). An MTP pellet was resuspended in 0.5 M PIPES, pH 6.9, 1 mM MgCl₂, 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 supernatant, 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 H₂O, dried, and sterilized with ultraviolet light. PtK₂ (Spray-Mate, dry lubricant, 3 M Co., St. Paul, Minn.) was embedded between 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. Handeness 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 incuba-

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**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-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.
section through a PtK midbody ~2 μm 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...
Figure 4 Cross sections through a PtK (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). × 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. × 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. × 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). × 36,000.
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.

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

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

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 interdigititation 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 sense attached to it, as in the midbody in Fig. 6b, and that these bundles ultimately form the midbody itself.

The observed polarity of the midbody MTs can be related to some models for mitosis in which MT polarity is considered.

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 model of McIntosh et al. (25) in which interdigitating astral MTs are anticipated, but it is predicted that MTs 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 concept that the midbody is constructed from the interdigititation 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 interdigititation 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.

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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
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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