Polarity of Spindle Microtubules in *Haemanthus* Endosperm

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**ABSTRACT** Structural polarities of mitotic spindle microtubules in the plant *Haemanthus katherinae* have been studied by lysing endosperm cells in solutions of neurotubulin under conditions that will decorate cellular microtubules with curved sheets of tubulin protofilaments. Microtubule polarity was observed at several positions in each cell by cutting serial thin sections perpendicular to the spindle axis. The majority of the microtubules present in a metaphase or anaphase half-spindle are oriented with their fast-growing or “plus” ends distal to the polar area. Near the polar ends of the spindle and up to about halfway between the kinetochores and the poles, the number of microtubules with opposite polarity is low: 8-20% in metaphase and 2-15% in anaphase cells. Direct examination of 10 kinetochore fibers shows that the majority of these microtubules, too, are oriented with their plus ends distal to the poles, as has been previously shown in animal cells. Sections from the region near the spindle equator reveal an increased fraction of microtubules with opposite polarity. Graphs of polarity vs. position along the spindle axis display a smooth transition from microtubules of one orientation near the first pole, through a region containing equal numbers of the two orientations, to a zone near the second pole where the opposite polarity predominates. We conclude that the spindle of endosperm cells is constructed from two sets of microtubules with opposite polarity that interdigitate near the spindle equator. The length of the zone of interdigitation shortens from metaphase through telophase, consistent with a model that states that during anaphase spindle elongation in *Haemanthus*, the interdigitating sets of microtubules are moved apart. We found no major changes in the distribution of microtubule polarity in the spindle interzone from anaphase to telophase when cells are engaged in phragmoplast formation. Therefore, the initiation and organization of new microtubules, thought to take place during phragmoplast assembly, must occur without significant alteration of the microtubule polarity distribution.

The structural polarity of spindle microtubules (MTs) has been considered an important factor in the mechanisms of mitotic chromosome movement; several models for mitosis are based upon specific, assumed polarities (40, 30, 34, 27, 32). Methods to identify MT polarity and thereby distinguish between these various assumptions have only recently been found. The first technique discovered used a difference in the rates of subunit addition at the two ends of a MT (13, 1, 8). Centrosomes and chromosomes were isolated and incubated in vitro with neurotubulin, treated so as to reduce its tendency to self-nucleate polymerization. Microtubules grew from both the centrosomes and the kinetochore regions of the chromosomes, elongating at rates consistent with the addition of subunits only at the fast-growing or “plus” end of the MTs (41, 7). The investigators in these studies assumed that the MT end associated with the initiating structure was blocked to subunit addition, and so inferred that the plus ends of both centrosome-associated and chromosome-associated MTs must be distal to their respective sites of initiation.

More recently two additional methods for identifying MT polarity have been found. These reveal the structural polarity of endogenous MTs by (a) decorating MTs with isolated dynein molecules (18, 42), or (b) decorating MTs with curved sheets of tubulin protofilaments, called hooks (19, 15, 16). The results so obtained for the structural polarity of spindle MTs in mammalian cells (19, 16) and sea urchin eggs (42) confirm the
kinetic polarity studies for MTs growing from centrosomes, but are inconsistent with the conclusions drawn from neurotubules initiated at kinetochores. Both decoration methods show that essentially all spindle MTs in animal cells, including those attached to kinetochores, are oriented with their plus ends distal to the spindle poles.

Cell division in higher plant cells is thought by some to be quite different in certain respects from cell division in animal cells (reviews in references 5, 6, 17). For example, spindle assembly begins with the formation of a clear zone around the cells (21, 4). Later in prophase, polar areas assembly begins with the formation of a clear zone around the cells (reviews in references 5, 6, 17). For example, spindle quite different in certain respects from cell division in animal plants relies upon the "phragmoplast," a MT-based organelle not found in animal cells (24, 2, 20). We therefore decided to apply the method of polarity-revealing hooks to plant spindle MTs. Many studies of higher plant mitosis have been carried out on endosperm cells of the African blood lily, Haemanthus katherinae. These cells contain beautiful spindles and obviate the difficulties imposed by the cellulose wall surrounding most plant cells. We have therefore used this material to determine the polarity of spindle MTs in a higher plant system.

MATERIALS AND METHODS

Tubulin Preparation

Microtubule protein (MTP) was prepared from bovine brain. After two cycles of temperature-dependent polymerization and depolymerization (38) the MTP was stored as a pellet at -70°C for subsequent use. To prepare the tubulin used in forming hooks, one or two pellets of the MTP were resuspended in 0.5 M piperazine-N,N'-bis(ethane sulfonate) (PIPES), pH 6.9, 1 mM MgCl2, 1 mM EGTA, and 1 mM GTP (henceforth called 0.5 PMEG) at 0°C. The MTP was then taken through another cycle in 0.5 PMEG and finally spun at 250,000 g for 2.5 h at 4°C (15). The resulting high speed supernatant, which is essentially free of high molecular weight proteins as judged by polyacrylamide gel electrophoresis (29), was frozen in aliquots of 0.5 ml and stored in liquid nitrogen. Protein concentrations were determined by the method of Bradford (9).

Preparation and Treatment of Cells

Endosperm cells were obtained from the immature fruits of Haemanthus katherinae Baker as described by Molé-Bajer and Bajer (33). The cells were allowed to settle in a moist chamber onto slides that had been coated with "Spraymate" (JM Co., St. Paul, MN) and a thin layer of 0.5% agarose in 3.5% glucose. The cells attach loosely to the agar as the liquid in which they were suspended in the fruit infiltrates the agar. After ~15 min the cells were carefully covered with about 150 µl of a mixture containing 2.5% dimethylsulfoxide, 0.04% saponin or 0.3% polyoxyethylene 20 ethyl ether (Brij 38), 0.5 PMEG, and 0.5 mg/ml tubulin prepared as described above. The preparation was then incubated in a moist chamber for 30 min at 37°C.

Electron Microscopy

Cells were fixed with 2% glutaraldehyde in 0.1 M PIPES, 1 mM MgCl2, and 1 mM ethylene glycol bis β-aminooethyl ether)-N,N',N"-tetraacetate (EGTA) for 30 min at room temperature, washed twice with 0.1 M cacodylate buffer, pH 7.0, and then postfixed in 1% OsO4 in the same buffer. Dehydration, including en bloc staining with uranyl acetate and phosphotungstic acid, and infiltration with plastic were carried out according to standard procedures (14). Cells were flat-embedded in Epon/Araldite between two slides coated with "Spraymate" and separated by chips of cover slips (16). After polymerization, one slide was removed. The plastic embedded cells stayed with the slide to which they were initially attached. Cells appropriate for cross-sectioning were selected under phase contrast optics. Cells were sectioned using an MT-2B ultramicrotome (Dupont Instruments-Sorvall Biomedical Div., Dupont Co., Newtown CT.) and observed in either a Philips EM 300, a JEOL 100C, or a JEOL 100S electron microscope. Handedness of the hooks was determined either from electron micrographs with a final magnification of 30,000-50,000 x or directly in the microscope. A correlation between MT structural polarity (as determined by the direction of hook curvature) and MT kinetic polarity (based on rates of assembly and disassembly) has previously been made, using aster and ciliary MTs (19); when the hooks curve clockwise, one is looking from the plus toward the minus end of the MT.

RESULTS

During incubation with the hook-forming solution, a high percentage of the endosperm cells detached from the agar and were subsequently lost. No effort was made, therefore, to follow particular cells through the course of mitosis, lysing them at a given stage of anaphase or phragmoplast formation. All cells included in this study were selected after embedding. Stage of mitosis was determined by spindle shape and chromosome distribution. It is noteworthy that most of our cells displayed an overall shape corresponding to the morphology seen in situ, where they are suspended in the endosperm liquid of the ovule. Examples of spindles from living and from lysed, plastic embedded Haemanthus endosperm cells are shown in Fig. 1. The chromosomes have been decondensed by lysis in 0.5 PMEG, but the stage of division can still be determined.

![Figure 1](image-url) Phase micrograph of a living (a) and a lysed (b) Haemanthus endosperm cell embedded in plastic (b). Neither cell is flattened. The molarity of the buffer causes chromosome decondensation so that the chromosomes are more difficult to see after lysis than in the living cell. Nonetheless, the stage of mitosis (late anaphase) is unambiguous. × 875.
**Metaphase**

Transverse sections show that in metaphase, each half-spindle is predominantly composed of MTs with the same polarity (Fig. 2). The majority of the MTs in one half-spindle is oriented with plus end distal to the nearest polar region. Table I shows the number and polarity of MTs scored at a region about halfway between the metaphase plate and the poles from six spindles. At this position, the fraction of MTs showing an opposite polarity (minus ends distal to the pole) varies from 6 to 20%. Between 100 and 1500 MTs were scored on these sections in each spindle. While this is only a fraction of the MTs in a metaphase cross section (23), the data show no correlation between number of MTs scored and percent hooks of one hand, suggesting that greater numbers would not change the polarity distributions seen. When hooks are scored on successive sections perpendicular to the metaphase spindle axis, a similar percentage of MTs with each polarity is seen from the region near the poles to a position about two-thirds of the way to the spindle equator (Fig. 3). As one approaches the metaphase plate, there is an increasing number of MTs with opposite polarity, reaching 50% near the spindle equator. In the second half-spindle, the distribution of MT polarities is the inverse of that in the first half (Fig. 3). Fig. 4 shows the distribution profiles of MT polarity along the axes of the six metaphase cells examined. All the cells reveal essentially the same pattern, although spindle length and MT number vary from cell to cell.

These data show that most of the MTs in each half-spindle are oriented with their plus ends distal to the poles, but they do not necessarily address the question of the polarity of the MTs attached to the kinetochore. This question cannot be answered unequivocally for metaphase cells in *Haemanthus*. The detergent mixture and incubation time used previously to identify kinetochore MT polarity in PtK1 cells (16) caused complete disruption of the *Haemanthus* spindles. After incubation in the gentle hook-forming conditions described above, the spindles were maintained, but even so, the kinetochores were difficult to recognize. Only in 10 cases was it possible to identify MT bundles right at the metaphase kinetochores. Fig. 5 a-d shows images from metaphase spindles lysed in hook-forming buffer of four kinetochores with MTs embedded in them. According to the curvature of the hooks, ~90% of the MTs in each kinetochore bundle are oriented with their plus ends at the kinetochores. (See the legend to Fig. 2 for a discussion of the interpretation of these hook images.)

**Figure 2.** A small region from a cross section of a metaphase endosperm spindle taken between the poles and the chromosomes at ~7 μm from the first pole (cell no. 4 in Fig. 4). About 15 such pictures are necessary to create a montage of the entire cross section. On this micrograph, we can score the direction of hook curvature on 23% of the MTs. Ninety-six percent of these are counterclockwise. Three MTs have hooks of both hands (0.4%). Some MTs (~12%) have no hooks at all. Some (~4%) have one or more hooks that have grown so long that they have curved around and closed on the MT wall to form a doublet or triplet MT, and convey no polarity information. Many (~400) are oblique, because the section was taken close to one pole. By using a gonemeter stage to reorient the section in the microscope, about half of the oblique MTs can be viewed so as to reveal their direction of hook curvature. The fraction of clockwise-curving hooks is approximately the same for any orientation of the section in the microscope and for any part of the section. The line of sight in this print is looking from the spindle pole toward the equator. The polarity rules described in Materials and Methods say that the predominance of counterclockwise-curving hooks places the plus end of most of these MTs away from the poles. × 59,600.
Anaphase

The polarity distribution of the spindle MTs in anaphase is quite similar to that described for metaphase. Again the spindle is comprised of two sets of MTs with opposite polarity, and these sets overlap at the equatorial region. Fig. 6 shows the distribution profiles of MT polarity for the seven anaphase cells studied. Comparison with the analogous curves from metaphase spindles (Fig. 4) reveals that the pole-to-chromosome region of most anaphase cells contains a lower percentage of MTs with opposite polarity (2-15%) than the comparable region of metaphase cells. In the zone between the kinetochores and the poles, 13 out of 14 half-spindles displayed a number of MTs with opposite polarity that was <10% of the total. In nine half-spindles it was even <6%. We have not traced MT bundles into anaphase kinetochores, but the preponderance of MTs oriented with their plus ends distal to the nearby pole sets a low value to the upper limit on the possible numbers of antiparallel kinetochore MTs in anaphase.

The transition between the two half-spindles in the equatorial region is more abrupt in anaphase than in metaphase cells. The increase in percentage of MTs with opposite polarity starts closer to the equator, so the rate of increase is higher than in metaphase, indicating that there is a time-dependent change in the structure of the zone of MT interdigitation. We have identified three convenient ways to describe these changes. All the polarity distributions show a flat portion in either half-spindle and a sigmoid curve near the equator. In the first method we extrapolate the plateaus parallel to the spindle axis and fit a straight line by eye to the points near the equator. The points of intersection of the plateaus with the line fit to the central points define two positions along the spindle axis (Fig. 7a). The axial distance between these points of intersection is one measure of the length of the zone of interdigitation. The second method is based on a reploting of the data. The predominant handedness of the hooks in each half of the spindle is called "correct," and other "wrong." The percent wrong hooks is plotted versus position in the spindle to obtain a graph such as Fig. 7b. The width at half height of the peak of wrong hooks at the equator of the spindle is our second estimate of the length of the zone of interdigitation. Table II shows the lengths of the zones of interdigitation for metaphase and anaphase cells as estimated in these two ways. Our third method for comparing the polarity distributions at different stages of mitosis combines the percent hooks of each handedness from all cells at a given stage, e.g., metaphase. All meta-

### Table I

**MT Polarity in Metaphase Cells**

| Cell No. | MTs with plus ends distal to the poles | MTs with plus ends proximal to the poles |
|----------|----------------------------------------|-----------------------------------------|
|          | No. counted | % of total | No. counted | % of total |
| 1        | h₁          | 575        | 79.8        | 146        | 20.2       |
|          | h₂          | 334        | 87.2        | 49         | 12.8       |
| 2        | h₁          | 375        | 83.7        | 73         | 16.3       |
|          | h₂          | 450        | 85.1        | 79         | 14.9       |
| 3        | h₁          | 643        | 90.6        | 67         | 9.4        |
|          | h₂          | 1028       | 83.6        | 201        | 16.4       |
| 4        | h₁          | 1181       | 94.3        | 74         | 5.9        |
|          | h₂          | 624        | 86.0        | 102        | 14.0       |
| 5        | h₁          | 433        | 79.2        | 114        | 20.8       |
|          | h₂          | 103        | 92.8        | 8          | 7.2        |
| 6        | h₁          | 415        | 88.3        | 55         | 11.7       |
|          | h₂          | 559        | 82.3        | 120        | 17.7       |

Cell number identifies the six different cells examined. h₁ and h₂ refer to the two distinct half-spindles in each cell. All cells were assayed about halfway between poles and metaphase plate.
phase polarity data collected in the region 0–1.2 µm from the axial position of polarity symmetry were treated as a single set and averaged. Equivalent sets were constructed every 1.2 µm from the spindle center, combining data from the two half-spindles and from all cells of equivalent stage. The resulting means and standard deviations for metaphase and anaphase are graphed as Fig. 8.

The distribution of *Haemanthus* spindle MTs in the plane of the cross section of lysed anaphase cells varies with position along the spindle axis. Throughout the interzone of most cells, the MTs are in clusters. In a majority of cells these bunches are distributed uniformly across the interzone cross section, though in a few they lie predominantly at the periphery of the spindle. The MTs between the kinetochores and the poles, on the other hand, are more evenly distributed: bunching of these MTs is rare, as soon as one moves away from the immediate vicinity of the kinetochore. For published pictures demonstrating these points, see reference 23 and Fig. 4 of reference 22. As described above, the unbunched MTs between the kinetochores and the poles are largely of one polarity, whereas the bunches in the

FIGURE 5 Four different kinetochores sectioned right at their surfaces, showing some MTs as they emerge from the dark-staining chromatin. The orientation of view in every case is toward the poles, and the majority of the visible hooks curve clockwise. The high fraction of clockwise-curving hooks among the nonkinetochore MTs shown here is not representative of this region of the spindle as a whole, and may be due to the small sample shown, to the nearby presence of another kinetochore, or to the location of these kinetochores some distance from the spindle equator. X 68,000.
The anaphase half-spindles are somewhat longer than those of metaphase (mean anaphase length is 29 ± 5 μm; mean metaphase length is 22 ± 6 μm [M ± SD]). The percentage of MTs with opposite polarity in each half spindle is slightly reduced. The axial length over which MT polarity changes at the spindle equator is a bit shorter in anaphase, but overall, the metaphase and anaphase distribution are similar.

**Phragmoplast Formation**

In *Haemanthus* endosperm, phragmoplast formation starts in mid-anaphase (2, 20, 25). Thus, the polarity data for the interzone MTs of anaphase cells (Fig. 6) also represent the early stages of phragmoplast formation. The five cells chosen to represent the later stages in the formation of the phragmoplast were all characterized by a distinct phase-dense line at the equator of the interzone (Fig. 9). In such cells the degree of MT organization within the phragmoplast is already high at the time of lysis, and an accumulation of vesicles at the cell plate has already taken place (20, 25). Distribution profiles of MT polarity along the length of the interzone in these cells are shown in Fig. 10. Comparison with Fig. 6 shows the similarity between the MT polarity distributions in the anaphase interzone and in the phragmoplast. There are, however, two distinct differences between the stages. The fraction of MTs with the wrong polarity found on either side of the cell plate is lower in telophase than in anaphase. Further, the equatorial transition between the two sets of MTs is even more abrupt at the later stage. A quantitative description of the axial length of the zone...
of interdigitation in the phragmoplast is included in Table II, and a summary comparison of the phragmoplast polarity distributions with the spindle at other stages is shown in Fig. 8. Note, however, that these data on polarity do not reflect the absolute number of MTs found as a function of position at different stages of division. We made no effort to count the total number of MTs in the lysed cells, because we felt that the experimental treatment used here might have altered the MT numbers in any given cross section from those in the living cell. Such data for *Haemanthus* fixed without lysis have been published (23).

In the lysed cells, the electron-dense material characteristic of the phragmoplast equator is usually found in those sections that contain MTs of both polarities (data not shown). This poorly defined material seems to be associated with small, ribbonlike structures that are probably remnants of the vesicles characteristic of the forming cell plate.

Although the distribution profiles for MT polarity from the interzone are quite similar for cells during anaphase and phragmoplast formation, the spatial arrangement of the MTs is different. In anaphase the majority of the interzone MTs are in bundles, but in the phragmoplast they are more or less evenly distributed (Fig. 11).

**DISCUSSION**

Our data on MT polarity in spindles from *Haemanthus* endosperm show that this mitotic apparatus, like others, is constructed from two sets of MTs with opposite polarity. Most MTs in each half-spindle are oriented with their plus ends distal to the nearest polar region. The number of MTs with the wrong polarity in each half-spindle increases as one approaches the equator, suggesting the existence of a zone of MT interdigitation in that region of the cell. The axial length over which the equatorial transition between MT polarities is spread changes gradually during the course of mitosis (Fig. 8), implying that the two half-spindles are moved apart after metaphase.

Our results are compatible with previous observations on division in *Haemanthus* endosperm. Light and electron microscopy studies (5, 6) have suggested that in most cases this spindle is formed by two sets of MTs entering the former nuclear region from the polar areas. Just before spindle formation, there is a fusiform cluster of MTs immediately surrounding the nucleus (the "clear zone," reference 6). These MTs disappear at about the time that the spindle forms. The polarity data show that whatever the pathway for formation of this plant spindle, most of the MTs finally associated with each pole have their plus ends distal to the nearest pole.

Some of the MTs that invade the former nuclear region during early prometaphase appear to become attached to the kinetochores; it has therefore been suggested that at least some kinetochore MTs are former polar MTs (4). If this suggestion were true, then these kinetochore MTs should possess the same polarity as the non-kinetochore MTs. Our data on kinetochore MT polarity are consistent with this interpretation of the observations on living cells. The limited, direct information on the structure of the individually recognized kinetochore MT bundles supports the assertion that the kinetochore MTs of *Haemanthus* are oriented with their plus ends distal to the pole. Further, if most kinetochore MTs in *Haemanthus* were oriented with their plus ends distal to the kinetochores, then one would expect a larger fraction of wrong hooks in each half-spindle. There are 27 chromosomes in the endosperm cells and an average of 83 MTs per kinetochore at metaphase (22), projecting an average of 2241 kinetochore MTs in each metaphase half-spindle. The average total number of MTs at metaphase is 3567 (23), suggesting that 63% of the MTs in each metaphase half-spindle should have the "wrong" polarity. While the absolute number of MTs in a spindle varies from cell to cell, this fraction seems so far from the observed average
of 12% wrong hooks that biological variation is unlikely to explain it. Some recent experimental work on spindle formation in animal cells supports the suggestion that kinetochores capture MTs initiated at the spindle poles (36, 37), while other studies suggest that kinetochores initiate MTs (11, 7, 41, 45). Our data certainly do not prove the case for kinetochore capture of polar MTs; they do suggest that if kinetochores initiate the majority of the MTs that are attached to them in *Haemanthus*, then these MTs are initiated upside down relative to polar MT initiation (see reference 16 for additional discussion).

MTs originating from each pole have been thought, on the basis of electron microscopy, to reach some distance into the opposite half of the forming spindle (3). Such interdigitation would explain our observation of MTs with both polarities close to the equatorial plane and account for the formation of a "zone of overlap."

The changes over time in the MT polarity distributions suggest that the MTs at the spindle equator become reorganized during the course of anaphase and phragmoplast formation: the length of the overlap decreases. Recent light microscopy observations of endosperm spindle structure using colloidal gold-staining with antitubulin have shown that in late anaphase and telophase, first the interzonal spindle, then the phragmoplast displays a region at its equator which becomes devoid of staining (12). These images imply that between mid and late anaphase, the *Haemanthus* spindle becomes two completely separate half-spindles that do not interdigitate. The midbody of telophase animal cells also contains a midregion that does not stain with antitubulin (10). In both cases, however, fine structure studies of these equatorial, nonstaining regions reveal many MTs surrounded by dense, amorphous material (2, 10, 20, 28, 31, 35). We infer that the immunological reactivity of this portion of the interzonal MTs is reduced by the dense, amorphous material, and that both plant and animal spindles contain an equatorial zone of interdigitation from metaphase through telophase.

The similarity in interzonal MT polarities between plant and animal cells suggests functional similarities between the spindles of these two groups of organisms. The significance of this similarity is, however, difficult to evaluate because of differ-
ences in the events during anaphase in the two groups. In animal cells, the interzone spindle appears to be the residue of those nonkinetochore MTs that interdigitated with others of their kind from the opposite pole. There is no evidence for an increase in MT number in the anaphase interzone of animal cells, though spindle elongation can be associated with MT growth. In higher plants, on the other hand, the interzone is initially formed as in animal cells, but then the number of MTs in the interzone increases during mid-to-late anaphase as the phragmoplast develops.

Phragmoplast formation is not well understood. Because a phragmoplast can form without any spindle present (24), and because it frequently broadens beyond the area of the spindle cross section (39), the structure has been thought to be composed predominantly of newly formed MTs. In the normal course of cell division, interzone MTs participate in phragmoplast formation, but the role of the edge of the cell plate as a MT-organizing center for new MTs has always been emphasized (20, 3). We noted here a redistribution of the MT spacings in the interzone between anaphase and phragmoplast stages. This could be related to the formation of new MTs, either initiated in the interzone or growing into the interzone from the poles, as indicated in some recent structural work (12). Regardless of the pathway, however, our data show that whenever the new MTs are initiated, they are incorporated into the phragmoplast in such a way that the distributions of MT polarity are not markedly affected. The true pathways of phragmoplast formation remain to be identified, but our polarity data impose limitations on the acceptable models.

The decrease in length of the zone of overlap in \textit{Haemanthus} is reminiscent of structural changes described in the central spindle of \textit{Dictamo vulgar} (26) and other diatoms (43, 44). In diatoms the MTs associated with each pole have been identified by serial section reconstruction. The distribution of MT positions along the metaphase spindle axis shows a certain amount of equatorial interdigitation between MTs from opposite poles. This zone of overlap decreases as the cell progresses through mitosis. Although the data of McDonald et al. (26) are presented differently, they reveal the same basic pattern as that presented here for \textit{Haemanthus}. The changes in MT distribution in the spindles of diatoms were interpreted as a strong indication for a sliding mechanism involved in pole separation (26, 44). In \textit{Haemanthus}, the formation of the phragmoplast complicates matters, so interpretation is less straightforward. Further, because of the methods used here, there are three possible explanations for the observed changes of MT distribution in the interzone: (a) During anaphase MTs coming from opposite poles move relative to one another along the spindle axis without major changes in length, thereby decreasing the extent of overlap. (b) The MTs that extend into the opposite half-spindle shorten by disassembly at their distal (plus) ends. (c) The observed changes are a subtle artifact of the methods used and do not reflect changes that occur in vivo.

In possibility \textit{a}, the movement might be either active or passive, but the data are interpreted as a relative motion of MTs. In possibility \textit{b}, spindle elongation would result from subunit addition at the minus MT ends located at the poles, whereas the decrease in overlap would derive from plus end subunit loss. In vitro assembly data suggest that such a combination is very unlikely (7, 41), but the possibility cannot be rigorously excluded. Possibility \textit{c} would most likely be based upon the idea that the hook-forming conditions cause MT elongation, thereby altering the length of the zone of overlap. One can imagine that the capacity of the interzonal MTs to elongate with exogenous tubulin varies with time in anaphase, so the observed overlap decrease would be due to altered MT extendability under lysis conditions. This possibility seems to us unlikely, but it cannot be excluded on current evidence. We think the most likely interpretation of the data is that the two half-spindles move apart during anaphase. This motion would contribute to spindle elongation and may be physiologically significant. Unfortunately, the mechanism for the movement is not revealed by the observations reported here.

The data on MT polarity in the metaphase and anaphase spindles of \textit{Haemanthus} endosperm cells show a striking correspondence to the comparable data from the animal cells studied so far (16, 18). In the animal systems, the majority of the MTs coming from a polar region have their plus ends distal to the pole; the majority of the MTs attached to the kinetochores are oriented with their plus ends at the kinetochores. Even the structural organization of the animal cell midbody corresponds strongly to the plant cell phragmoplast (15). Such correspondences suggest fundamental design similarities among spindles across a broad phylogenetic gamut. No polarity distribution profiles have yet been constructed for animal spindles, so a comparison of the time-dependent changes in spindle design (with respect to MT polarity) is not yet possible. It will be informative, indeed, to see whether a pattern similar to that described here for endosperm cells can be thought of as a universal property of spindle MT reorganization during mitotic function.

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