Reorganization of Microtubules in Endosperm Cells and Cell Fragments of the Higher Plant *Haemanthus* In Vivo

A. S. Bajer and J. Molè-Bajer

Department of Biology, University of Oregon, Eugene, Oregon 97403

Abstract. The reorganization of the microtubular meshwork was studied in intact *Haemanthus* endosperm cells and cell fragments (cytoplasts). This higher plant tissue is devoid of a known microtubule organizing organelle. Observations on living cells were correlated with microtubule arrangements visualized with the immunogold method. In small fragments, reorganization did not proceed. In medium and large sized fragments, microtubular converging centers formed first. Then these converging centers reorganized into either closed bushy microtubular spiral or chromosome-free cytoplasmic spindles/phragmoplasts. Therefore, the final shape of organized microtubular structures, including spindle shaped, was determined by the initial size of the cell fragments and could be achieved without chromosomes or centrioles. Converging centers elongate due to the formation of additional structures resembling microtubular fir trees. These structures were observed at the pole of the microtubular converging center in anucleate fragments, accessory phragmoplasts in nucleated cells, and in the polar region of the mitotic spindle during anaphase. Therefore, during anaphase pronounced assembly of new microtubules occurs at the polar region of acentriolar spindles. Moreover, statistical analysis demonstrated that during the first two-thirds of anaphase, when chromosomes move with an approximately constant speed, kinetochore fibers shorten, while the length of the kinetochore fiber complex remains constant due to the simultaneous elongation of their integral parts (microtubular fir trees). The half-spindle shortens only during the last one-third of anaphase. These data contradict the presently prevailing view that chromosome-to-pole movements in acentriolar spindles of higher plants are concurrent with the shortening of the half-spindle, the self-reorganizing property of higher plant microtubules (tubulin) in vivo. It may be specific for cells without centrosomes and may be superimposed also on other microtubule-related processes.

OBSERVATIONS of microtubule reorganization in a new experimental system, anucleate cell fragments in *Haemanthus* endosperm (7), have provided evidence for a previously unknown property of higher plant microtubules (MTs)[1] in vivo: the tendency of an initially irregular MT meshwork to reorganize itself until a very stable structure is formed. Such reorganization may be an intrinsic property of higher plant MTs, as it has not been observed in animal tissue culture (28). This is a sequential reorganization that follows always the same steps and is triggered merely by removing endosperm from the ovule. The final result may be the formation of chromosome-free spindles/phragmoplasts, or spirals that may close and form rings. Further observations of MT reorganization have revealed the presence of unexpected MT configurations, appearing under specific conditions, whose morphological structure often resembles that of fir trees (35). These MT converging centers, with the shape of

microtubular fir trees (MTFTs), are of special interest because they were also identified as components of the higher plant acentriolar spindle and may be directly related to the spindle function. These unexpected observations revise our understanding of anaphase, emphasizing the role of the polar region in spindle elongation. Data reported earlier (9, 35) suggest that a functional MT fiber has a complex organization: it consists of MTs terminating in the kinetochore at the one end, and extends to the pole as a more difficult to define MT organization, often seen as an MTFT. Relating these observations of MT behavior in vivo to known properties of MTs in vitro suggests a role for MTFTs during anaphase and leads to a new concept of spindle elongation.

Inoué and Ritter (24) suggested that in typical standard anaphase the kinetochore-to-pole movements are accompanied by shortening of half-spindles, and polar separation is accompanied by an active elongation of the interzone (anaphase A and B). However, our observations show that in the higher plant *Haemanthus* the half-spindle does not shorten during the first two-thirds of anaphase, i.e., anaphase B occurs before anaphase A.

---

[1] *Abbreviations used in this paper:* DIC, differential interference contrast; IGS, immunogold stain; KF, kinetochore fiber; KFC, kinetochore fiber complex; MT, microtubule; MTFT, microtubular fir tree; NA, numerical aperture.

The present observations extend the data on the behavior of birefringent chromosomal fibers (21) to the whole half-spindle by including reorganization within the polar region. We conclude that during anaphase the kinetochore fiber (KF) shortens, while the entire kinetochore fiber complex (KFC) elongates due to new MT assembly in the polar region. Moreover, observation of MT reorganization in the absence of chromosomes demonstrates how some intrinsic properties of higher plant MTs might be expressed during mitosis.

Materials and Methods

The endosperm sandwich technique (7) was modified; coverslips were coated with 0.5% phytagar (Gibco, Grand Island, NY) with 3.5% glucose, and cells were covered with 1% gelrite (40) with 3.5% glucose.

The following MT reorganization in vivo from early stages was unsuccessful because these processes were completed before the preparation was mounted for the light microscopic observations. The final stage, however, formation of the cell plates within the pharagmoplasts, can be easily followed. This work is based, therefore, on precisely timed fixation after preparation and correlation of these data with observations in vivo. The minimum time after preparation we were able to fix the cells using the sandwich technique was 60 ± 10 s. The moment the ovule was opened was counted as 0, and the time in seconds was measured until the fixative covered the whole preparation.

Methods for low temperature shocks, which partly disassemble MTs, and recovery after the treatment, were described previously (7).

The immunogold stain (IGS) method for MTs (14) was used as described (15). Monospecific affinity column purified rabbit antitubulin antibody against dog brain tubulin was used as a primary antibody. The technique, using 15- and 20-nm IGS, does not stain cells that died during endosperm preparation (7, 15). The endosperm cells are free-floating and during IGS processing ~30% of large cells are lost, as well as perhaps more than 90% of cell fragments, which prevents meaningful statistics. Improper handling results in 100% loss. The use of polylysine-coated slides to improve cell attachment was not successful due to decreased cell survival.

The maximum absorption of immunogold is at 520 nm after dehydration and embedding in permount (Fisher Scientific Co., Pittsburg, PA, or similar medium). The micrographs of cells treated with IGS were taken with a narrow band interference filter (520 nm) using a Zeiss 63× Plan-Apo 1.4 numerical aperture (NA) objective, a Zeiss condenser of 1.4 NA (Carl Zeiss, Inc., Thornwood, NY), and a Xenon 75-W arc lamp. Measurements (Tables I–III) were made with a measuring eyepiece. Kodak negative film, type 2415, processed in Kodak HC 110 developer (dilution B), was used. The dehydration and mounting (in Permount) procedure may result in a slight (5%) shrinkage of the spindle, which we assume is uniform for all cells.

Our image enhancement system is based on rectified differential interference contrast (DIC) (21) as described elsewhere (36). This system permits us to analyze both water medium mounted and dehydrated preparations with stained chromosomes. It permits also elimination of cells not stained properly due to insufficient immunogold penetration.

Both in cytoplasts and in postmitotic phragmoplasts, the cell plate is not stained due to a dense accumulation of vesicles around MTs which prevents antibody penetration. At least some interzonal MTs are trapped within the cell plate, while most polar MTs terminate within this structure. The vesicles are not seen with standard optics in dehydrated preparations but can be visualized with an image enhancement system (Fig. 3d). The relation of polar MTs to interzonal and their role in the cell plate formation is beyond the scope of this paper.

MT arrangement after IGS in about 80 cells in different stages of mitosis was compared with the distribution of birefringence in the same cell in vivo. This is the first report where the same endosperm cells, in a living state and after glutaraldehyde fixation followed by immunoprocessing, are shown. The time between recording in vivo and fixation for IGS was 1–4 min. A Leitz polarizing microscope (E. Leitz, Inc., Rockleigh, NJ) was used in conjunction with strain-free rectified Nikon optics (Nikon Inc., Garden City, NY) (40× objective, 0.65 NA with 16-mm condensor, 0.52 NA and 100× objective, 1.25 NA with 8-mm condensor, 1.15 NA; micrographs included were taken with 40× objective) and a Leitz 1/30 λ compensator. The light source was an HBO 100-W mercury arc lamp. The illumination was filtered through a liquid $CuSO$ filter, two Zeiss Calfex heat reflecting filters, and a green 550-nm multilayer high transmission interference filter (Baird Corp., Bedford, MA, nominal half band width 7 nm) with its accessory high and low cut-off filters.

Statistical analysis of the measurements was performed using the SPSSX statistical package on an IBM 431 computer (IBM Corp., Danbury, CT). Unless indicated otherwise, all fixations were made at room temperature, 22°–24°C.

Terminology

MT organization seen in IGS processed cells does not correspond precisely to the organization observed either with an electron microscope or with birefringent pattern in the living cells detected with a polarizing microscope, DIC, etc. This is due to specifics of immunocolloidal gold reaction with MTs combined with its visualization in the light microscope. The problem is beyond the scope of this paper. Therefore spindle components as seen in IGS-processed cells require definitions and comparison to the existing terminology.

Chromosomal Fiber. The chromosomal fiber is the birefringent fiber connected to a chromosome (5), detected in living cells with a polarizing microscope. Images of birefringent fibers depend on extinction of the polarizing microscope and orientation of the spindle. The fibers in flattened cells often show weak birefringence and do not correspond precisely (area and shape wise) to MT distribution in the same cells after IGS processing. Figs. 5 and 7 illustrate these differences and the measured parameters (see also Fig. 14). The birefringent chromosomal fibers in Fig. 7 are shorter than the half-spindle. The length of chromosomal fibers in vivo cannot be well defined (cf. Figs. 5 and 7).

KF and KFC. Our use of the term KF is somewhat different in meaning from that used previously (27). The latter (27) corresponds to kinetochore/centromere-associated MTs (41) and is only a few μm long. We define KF as densely stained with the IGS part of the chromosomal fiber associated with the kinetochore region. The fiber is several μm long and is longer than the chromosomal fiber detected under 45° orientation with crossed polarizers in a polarizing microscope. It should be distinguished from the broader term KFC, which with other often skewed MTs (which are not associated with the kinetochore region), form the entire KFC (9, 35). As the length of chromosomal fibers in vivo, the measurements of 576 KFs in IGS preparations could not be done as precisely as that of the half-spindle. A precise comparison of birefringent chromosomal fiber with MT distribution within KF is beyond the scope of the present work.

The KFC includes the portion of the fiber that penetrates the polar region of the spindle and includes a less defined structure shaped often as an MTFT. KFC extends all the way to the spindle periphery and is usually longer than the

---

Figure 1. Self-reorganization and rearrangement of MTs in cytoplasts of *Haemanthus katherinae.* (*a*) Irregular mesh of MTs in a large cytoplast a few minutes after preparation. (*b*) Early stages of reorganization of MTs in a large cytoplast 20 min after the preparation was made. Several MT converging centers with few MTs are seen (arrows). (*c–d*) Small cytoplasts with irregularly arranged MTs. Very few MTs are present. 1½ h after preparation. (*e–f*) Medium-sized spherical cytoplasts 30 min after preparation. *e* shows an advanced stage of the formation in a circular structure: the elongating head of the cone has nearly entered the tail. Since there was no more space for growth, the tail curved and both ends of the structure interconnected, forming a spiral or a ring. *e* is an earlier stage of development than *f*. (*g–h*) Chromosome-free spindles, structures similar, both structurally and functionally, to mitotic spindles. Granules of undetermined origin have been transported toward the poles, where they form pseudo-nuclei (thin arrows). MTs radiating from the poles probably correspond to polar MTs during mitosis. 1½ h after preparation. *g* is an earlier stage of development than *h*, where a cell plate has already been formed. Thick arrows show polar MTs. (*i*) Multiple chromosome-free spindles in the cytoplasm of an interphase cell at different stages of their development. Configurations of converging centers are complex and may indicate that phragmoplasts, which are the final stage, may form in diverse ways. 1 h after preparation. (*j*) A typical MT converging center formed at room temperature during recovery from low temperature shock (5°C). (*k*) A growing MT converging center 25 min after preparation. (*l*) Multiple chromosome-free spindles before cell plate formation in a very large cytoplast, 1 h after preparation. Bar, 10 μm.

Bajer and Molè-Bajer *Reorganization of Plant Microtubules*
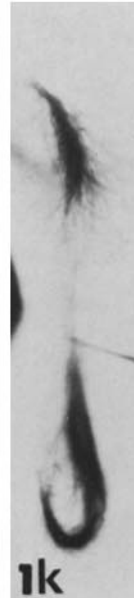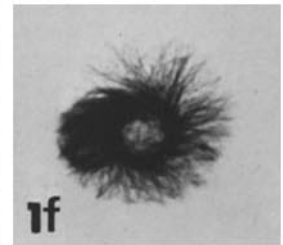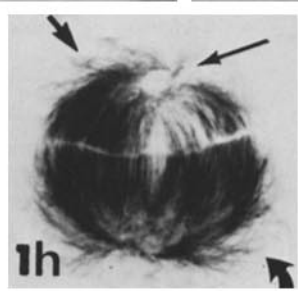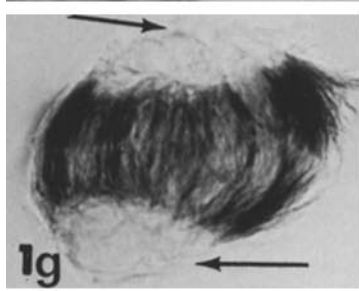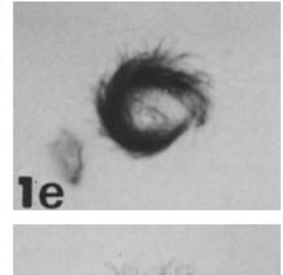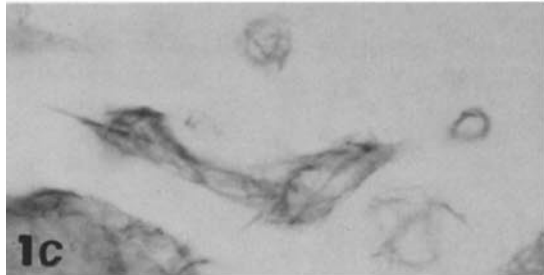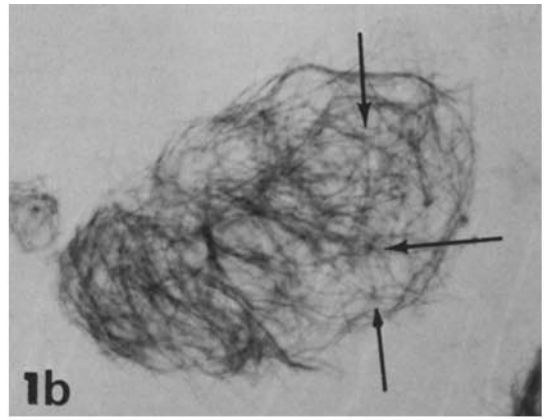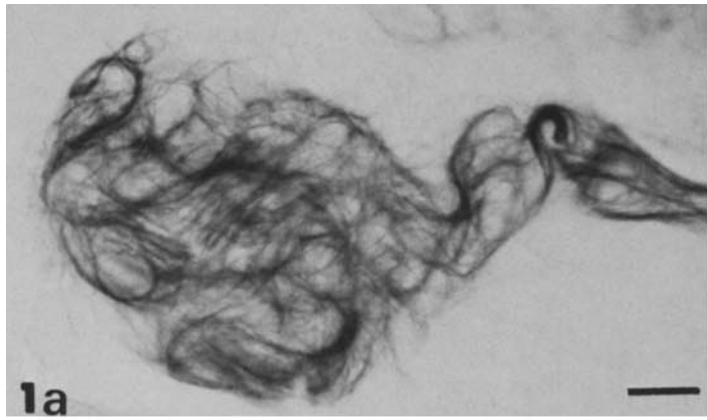
KF (see Fig. 7 for additional explanation). It should be stressed that such differentiation is detected only in IGS-processed cells and has not been detected in vivo. The KF in flattened cells may be arranged under an angle to the spindle axis.

Half-spindle and Interzone. The whole MT flattened cone-like structure in front of kinetochores and the surrounding cytoplasm is a half-spindle. It includes the polar region with the irregular arrangements of MTs (which can only be seen after immunoprocessing). Time-lapse films of *Haemanthus* endosperm taken under a polarizing microscope suggest that poorly defined ends of chromosomal fibers do not penetrate the polar region.

The length of the half-spindle in IGS preparations was measured from the kinetochores to the border of the polar region along its long axis and does not include asters. Therefore the half-spindle may be shorter than tilted KFCs located at the spindle periphery and is the measure of the KFCs located on the spindle axis. These measurements depend on the light microscope resolution and contain little error.

Interzone. Interzone is the region between the separating chromosome groups in anaphase. Therefore the metaphase spindle is composed from two half-spindles only, while anaphase consists of two half-spindles and an interzone. The interzone transforms into a phragmoplast during the anaphase-telophase transition.

Pole and Polar Region. In unflattened cells MTs of the half spindle terminate at the relatively small pointed area called the pole, and in flattened ones in the broad, polar region that is often well defined due to the presence of birefringent granules at the spindle periphery. The broad polar region may also be interpreted as multiple poles.

Materials

Endosperm. Free cells and syncytia of young endosperm have no cellulose cell walls but have the ability to form such walls between sister and non-sister nuclei. Endosperm of *Haemanthus* is a triploid tissue ($n = 9$). All cells reproduced have 27 chromosomes. Due to the size, the spindle can be sectioned optically. Such sections can contain only few chromosomes and make a false impression of lower chromosome number (e.g., Fig. 6, *a* and *b*). The main difference between the endosperm and cellular higher plant tissues is the lack in the former of the preprophase band of MTs (45).

According to our knowledge it has never been reported that *Haemanthus* endosperm has two extreme populations of cells and mitotic spindles: large and very small (lengths ratios up to 1:4). The origin of the small cells is unknown, and they cannot be flattened in our hands. Additionally, there is a considerable variation in the size of the cells within each population and in different ovules. In this and previously published work on endosperm, mostly large cells partly flattened were studied (see exception in reference 17). The present work includes also medium-sized cells. Therefore some values are somewhat lower than previously reported.

Intact Cells. Endosperm cells of *Haemanthus katherinae* (South African globe or blood lily) have the largest known mitotic spindle (40–60-μm long in metaphase). There is considerable variation in the organization of the spindle pole, or the whole polar region. Polar regions are often not well defined, and multiple subpoles may be seen. However, they gradually fuse during anaphase, and multipolar divisions are rare (below 5%). The preparatory technique always results in a partial flattening of large and medium-sized cells. Cells flatten due to the action of the surface tension and not mechanical compression. The spindle becomes wider, but the length does not seem to be affected. The spindle can be further dissociated by flattening, or occasionally during preparation into separate functional units containing few, or even one chromosome, which proceed normally through mitosis and cytokinesis in over 95% of cells.

Cytoplasts. Anucleate cell fragments (cytoplasts) containing MTs (7) arise both from interphase and from mitotic cells, as demonstrated by occasionally persisting partial connections. The number of cytoplasts varies from zero in old ovules to a few hundred in young ones. MTs in anucleate cell fragments retain their ability to assemble–disassemble and rearrange for several hours. The majority of cytoplasts is lost during the IGS processing, which prevents meaningful statistics.

Results

Chromosome-free Spindles

Reorganization of MTs in Cytoplasts. In the slides fixed 1–5 min after preparation, irregular arrays of MTs are found in all cytoplasts, regardless of their size (Fig. 1). No other MT configurations were ever found in controls, i.e., in preparations not treated with drugs. A strict relationship exists be-

tween the size and shape of cytoplasts and the mode of their future MT reorganization: MTs in small and large fragments behave quite differently. No reorganization is observed in very small fragments (Fig. 1, *c* and *d*), even several hours after preparation. The rate of MT reorganization can be experimentally modified by such factors as temperature and other conditions influencing critical concentration of tubulin (work in progress). The time scale of rearrangement described here applies to our standard preparatory conditions at room temperature.

Irregular arrays of MTs in large size fragments are rarely seen after 10–15 min, and only exceptionally 2 h after the preparation. Regular arrays are occasionally seen as early as 5 min after preparation (Fig. 1 *b*). Intermediate configurations between such arrays and MT cones indicate that they originate from regular arrays.

Two most characteristic configurations found in small cytoplasts are cones and rings. Cones with MTs converging toward one center are seen in medium and large sized cytoplasts (Fig. 1, *j* and *k*) on slides fixed between 8 and 45 min after endosperm preparation. The considerable loss of cytoplasts during the IGS procedure prevents statistical analysis. The rings are not found 8 min after preparation and appear only later. Several intermediate configurations demonstrate that converging centers are unstable intermediates in spherical cytoplasts. They transform into bushy spirals or rings, a first type of configuration that is very stable, and do not reorganize any further. This transformation occurs through the formation of MTFTs. In large and not spherical cytoplasts the MT cones transform into spindle/phragmoplasts. This is indicated by the low number or absence of cones in preparations fixed after 1–2 h. Several intermediate stages between an MT cone and a closed ring were observed. The first stage is the formation of MTFTs, which may be seen as soon as 10 min after preparation. The MTFT subsequently elongates (Fig. 1 *k*). A closed ring is formed when the head enters the tail (Fig. 1, *e* and *f*). A considerable asynchrony is seen in both the formation of MT cones and closed rings.

Large converging centers are very seldom observed after 2 h and transform into cytoplasmic spindles/phragmoplasts, with cell plate (Fig. 1, *g* and *h*). They have been called (7) cytoplasmic chromosome-free spindles. We could not distinguish among the variety of MT configurations, which might be intermediates between the large converging centers and the spindles (Fig. 1, *i* and *l*). There is also a considerable asynchrony of reorganization. In most cytoplasts the process is completed (cell plate formed) in about 20–45 min. Very large fragments always develop multiple, remarkably uniform sized spindles/phragmoplasts (Fig. 1, *i* and *l*) with cell plates. In single phragmoplasts a small incomplete aster may occasionally develop at one or two poles (cf. Fig. 1 *h*). We have never observed a single spindle/phragmoplast longer than a metaphase spindle (40–60 μm) or shorter than a metaphase half-spindle. MT converging centers may also occasionally give rise to asymmetrical spindles/phragmoplasts. We do not know yet whether or in what conditions these asymmetrical phragmoplasts, after several hours, finally transform into symmetrical ones (work in progress).

The nonkinetochore transport (7, 27), also called passive transport (5) or particle and/or state movements (1), within these structures is the same as in the mitotic spindle. It distributes inclusions (such as starch grains, etc.) randomly
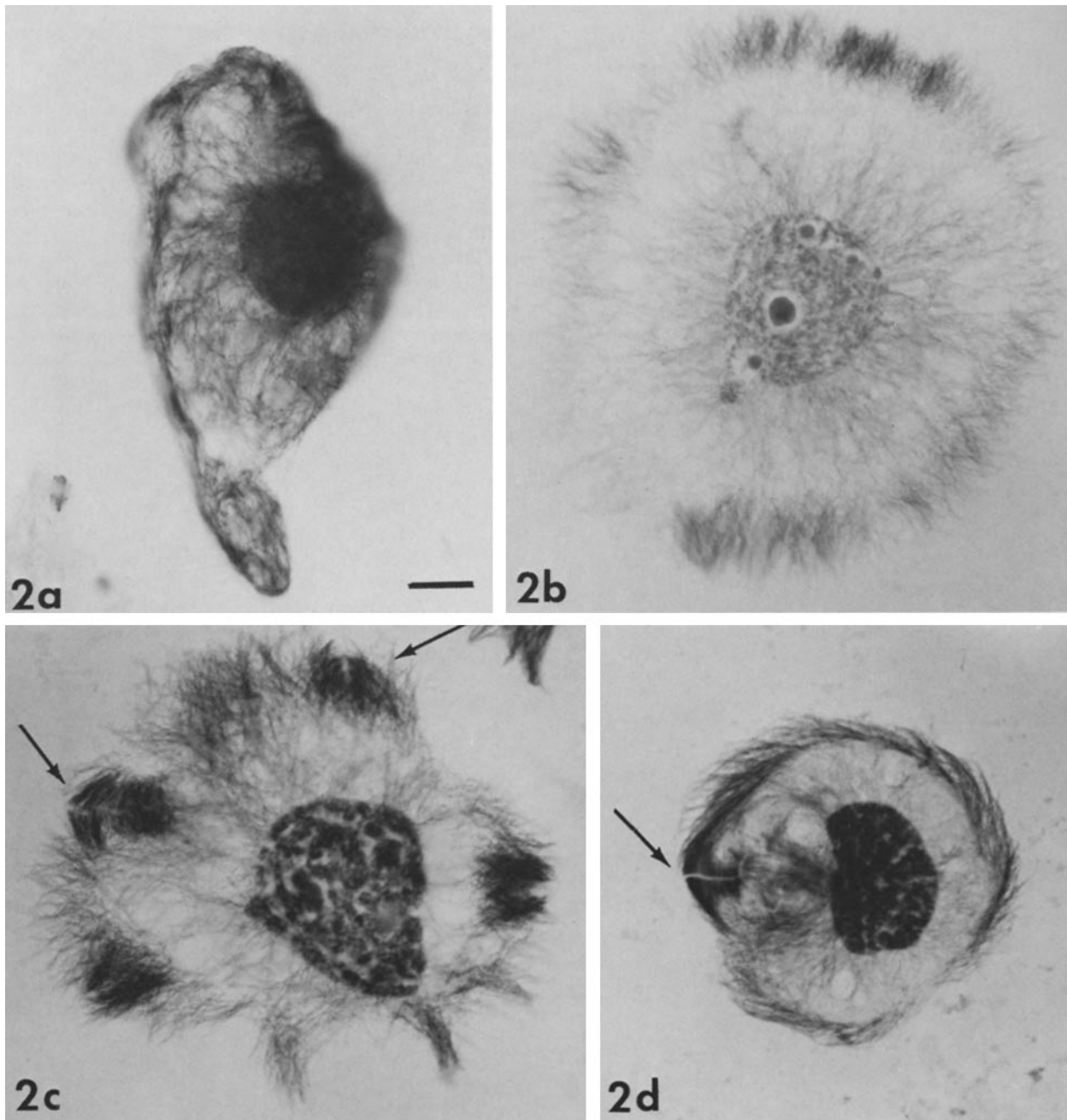
Figure 2. MT arrangements in interphase cells. (*a*) A typical interphase cell with an irregular mesh of MTs in the cytoplasm a few minutes after preparation (*b*) An interphase cell 30 min after preparation. The arrangement of MTs is changed. There is a dense ring of short MTs at the cell periphery. Some MTs are still located around the nucleus. Note the regular shape of the cell. (*c*) Similar rearrangement of MTs as in *b*. Dense bundles of short MTs are located at the cell periphery, while only a few MTs are left in the center of the cell. Note the irregular outline of the cell. Two small phragmoplasts are formed at the cell periphery (arrows), and dense patches might represent earlier stages of the formation of cytoplasmic phragmoplasts. *c* is a more advanced reorganization than *b*. (*d*) Further reorganization of MTs at the cell periphery. A fir tree with side branches predominantly located on one side (outer) forms a long tail encircling the cell. A small phragmoplast (arrow) is formed, where both ends of the tail meet. Bar, 10 μm.

toward the two poles, where they accumulate and form pseudo-nuclei (Fig. 1, *g* and *h*). The transport appears before the cell plate is seen and continues for several hours after its appearance.

Nucleated Cells and Syncytia. As in cytoplasts, MTs form a uniform meshwork in intact interphase and early prophase cells immediately after the preparation (Fig. 2*a*). Only occasionally is this meshwork somewhat denser around the nu-

cleus. In less than 5% of the cells, part of this meshwork transforms into cytoplasmic spindles/phragmoplasts within ~30 min.

The events essentially follow the same pattern in most interphase and late telophase cells (Fig. 2, *a–d* and Fig. 3, *a* and *f*). The uniform distribution of MTs found in interphase and early telophase gradually differentiates. The region around the nucleus is gradually depleted of MTs, and a dense
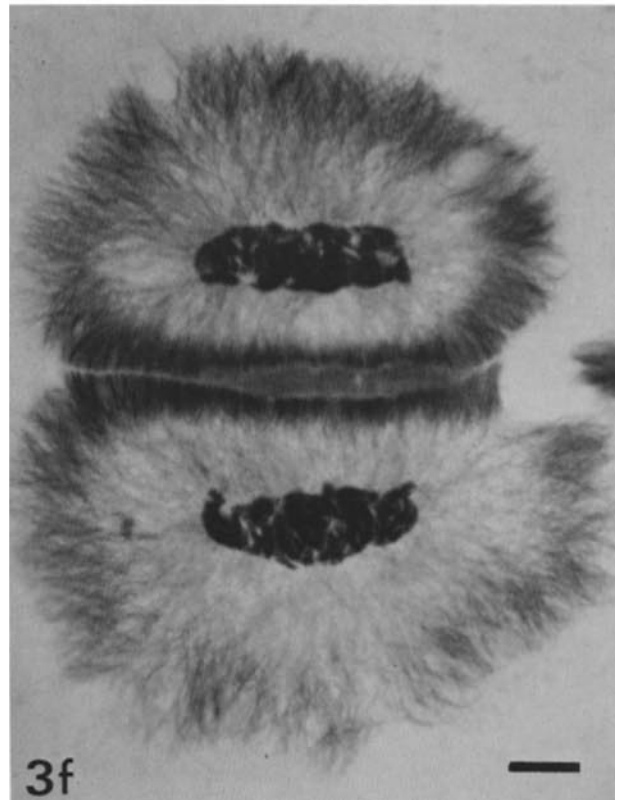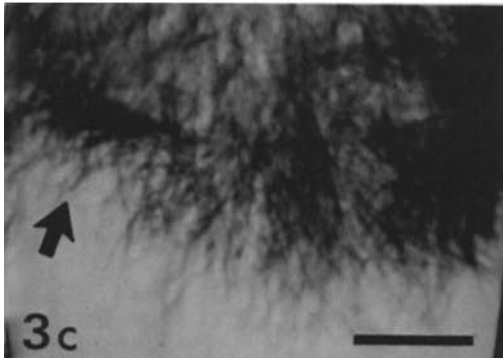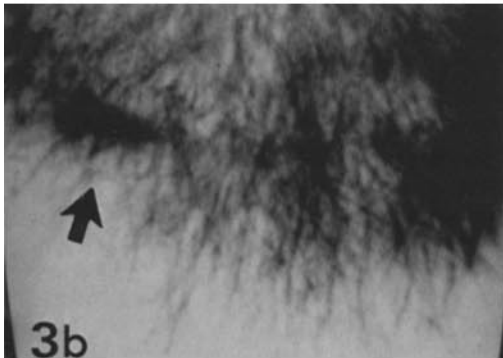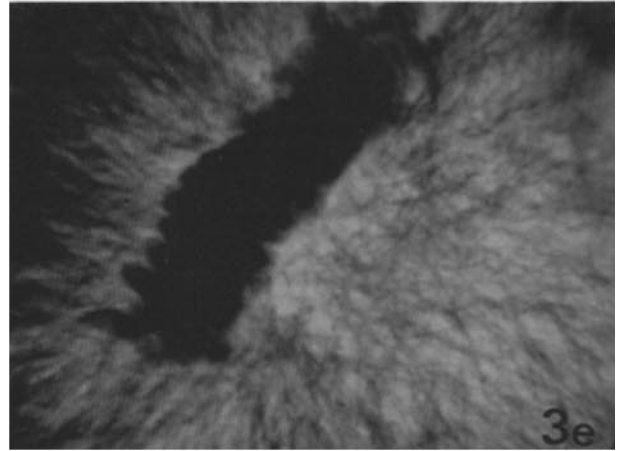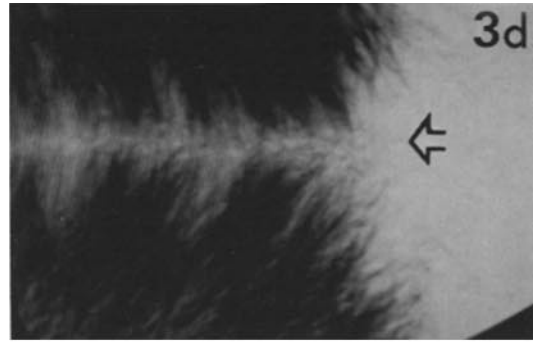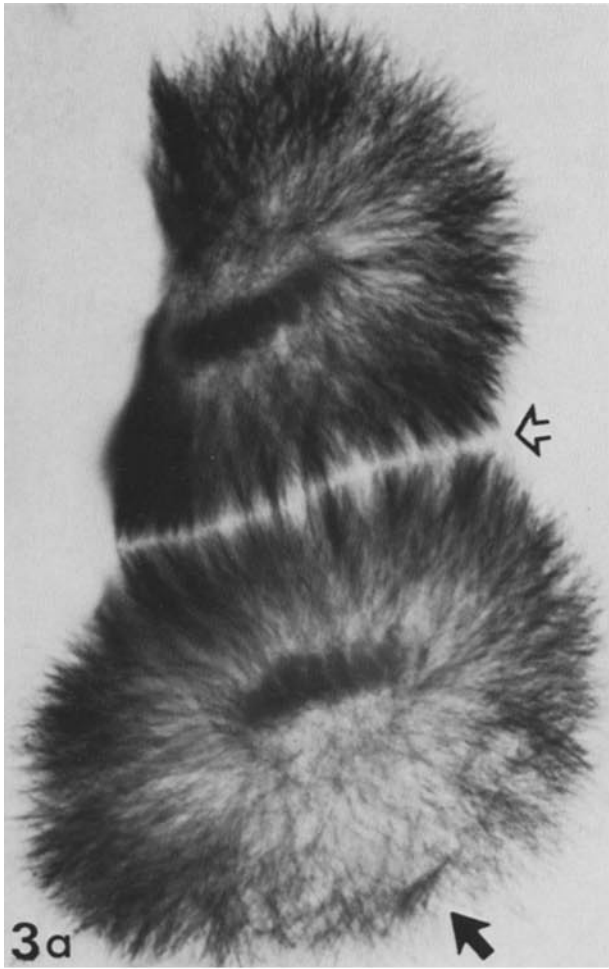
band of MTs arranged perpendicularly to the cell periphery is formed (Fig. 2, *b* and *c* and Fig. 3*f*). This accumulation forms either a continuous band or separate patches. A continuous band is usually observed in cells with a regular shape (Figs. 2*b* and 3*f*), while patches are observed in cells with an irregular shape (Fig. 2*c*) and longer time after preparation. Thus, during the progress of telophase MTs grow outward from the nuclear region toward the cell periphery and eventually, the nuclear region becomes depleted of MTs (see also figures in reference 7.) Similar processes are seen in interphase cells (Fig. 2*b*).

MT patches at the cell periphery often undergo further transformations. When two MT bundles curve and their ends become opposed, a phragmoplast forms. It usually arises within 30–45 min at room temperature (Fig. 2, *c* and *d*). The early stages of such cytoplasmic spindle/phragmoplast formation are difficult to recognize after IGS procedure. This precludes in vivo predetermination of the position in the cell at which the cytoplasmic spindles/phragmoplasts will form. Consequently, attempts to follow the reorganization of the MT bundle from very early stages in vivo have been unsuccessful, although fully developed cytoplasmic phragmoplasts are easily detected in the living cells (see figures in reference 7).

Mitotic Spindle/Phragmoplast

The Formation of KFC in Late Prometaphase–Metaphase.
The formation of KFs at the onset of prometaphase was not followed. Our observations begin in mid-prometaphase. The fir tree organization of individual KFCs is gradually established in late prometaphase. MTs attached to the kinetochore represent the trunk, and wispy non-kinetochore MTs are the branches (polar MTs in later stages of anaphase). The bending of the branches toward the cell equator, at angles up to 45°, is a pronounced feature of metaphase and some late prometaphase spindles, especially in flattened cells (Fig. 4, *a* and *b*). The branches, which are often S-shaped with the distal ends pointing toward the equator, fuse with adjacent KFC. Individual KFC also may be twisted. However, this has been seen only in 12 out of hundreds of observed cases. A right-handed twist (looking from the kinetochore to the pole) was observed in 9 of these fibers.

The length of the KF after IGS corresponds approximately to the length of the chromosomal fiber as seen in the polarizing microscope in the living cell (Fig. 5, *a* and *b*). The region between the end of the chromosomal fiber seen in vivo with the polarizing microscope (22, 25) and the outline of the spindle occupied by MTs, i.e., the polar termination of the KFC as seen after IGS, is not resolved by the polarizing microscope. The difference is especially striking in spindles with a low number of MTs (Fig. 5).

Anaphase. The analysis of KFCs during anaphase without an image enhancement system is possible only in very flat cells. In the majority of cells only the branches, or polar MTs, are seen at the periphery of half-spindles as a hairy outline (Fig. 6*a*).

Such organization of whole half-spindles becomes increasingly pronounced during the progress of anaphase (Fig. 6*d*). The polar MTs (branches of MTFTs), which form abundantly in anaphase (15), penetrate the interzonal region after mid-anaphase in an asynchronous manner and associate laterally with the interzonal MTs often present around the trailing chromosome arms.

The number and spatial organization of the interzone MTs vary markedly in different cells. Some cells contain large numbers of MTs while others are practically devoid of them by the end of anaphase (compare Fig. 8*c*, and Fig. 6, *a* and *d*). As a rule the number is low in unflattened cells. In cells fixed less than 5 min after preparation, the number of interzonal MTs is often lower than in any figure reproduced here, and the number of polar MTs is very high, implying that such relations exist in the ovules in situ. In both extreme cases anaphase progresses in a normal fashion; we have, however, never seen a control anaphase without abundant polar MTs. The arching and bending of adjacent polar and interzonal MTs is often variable (Figs. 6*b* and 7*c*), demonstrating that such bends do not result from the shrinkage during the IGS procedure unless the shrinkage is non-uniform for different MTs, which we feel is unlikely.

Anaphase Elongation. The direct measurements of separation of the diffuse polar regions of the acentriolar spindle in *Haemanthus* endosperm in vivo cannot be done precisely because the border between the MT-occupied region and the cytoplasm in most cells is not sharp. The granules around the spindle allow such measurements in some cells (Fig. 9) and the data seem to be not fully compatible with those derived from IGS preparations. The results of indirect measurements are given in Figs. 10 and 11.

Histograms (Fig. 10) showing the length distribution of half spindles (centrally located KFC) of individual cells at different stages of anaphase reveal an unexpected pattern. There is an increase in the length of the half-spindle between metaphase and early anaphase. This increase may be even larger than our measurements indicate due to inclusion in the measurements of metaphase and late prometaphase spindles, which cannot be distinguished in IGS preparations. The measurements in anaphase demonstrate that the length of the half-spindle remains unchanged until mid-anaphase, when the half-spindle rapidly shortens. The length distribution of KFs shows a continuous shortening, indicating that the half-spindle does not change in length or may even elongate at the time when the KF proper shortens.

Figure 3. MT reorganization in telophase. (*a*) Mid-telophase. Newly formed polar MTs form big asters. The phragmoplast is well developed and the cell plate (white line) is formed. MTs within these two structures are evenly distributed. (*b–c*) Small regions of the same cell (thick arrow in *a*) with video enhancement. MTFT in the lower aster on two different optical sections. The MTFT is located at the end of a long MT array composed from very few MTs. These long MTs transverse the whole aster. (*d*) Cell plate (open arrow in *a*) in video enhancement. Dense accumulation of small granules prevents penetration of immunogold. These granules are not detected without an enhancement system. Some MTs cross the cell plate. (*e*) Lower aster in video enhancement. MTs criss-cross and terminate in broad area and form irregular mesh. (*f*) Late telophase (approximately 1½ h after preparation). The reorganization of MTs follows the same pattern as in interphase cells. There is a dense ring of short MTs at the cell periphery, while MTs around the nuclei disassemble. Both cells are stained with 15-nm immunogold, dehydrated, and mounted in permount. Bar (*a* and *f*), 10 μm. Bar (*b–d*) (video enhancement), 10 μm.
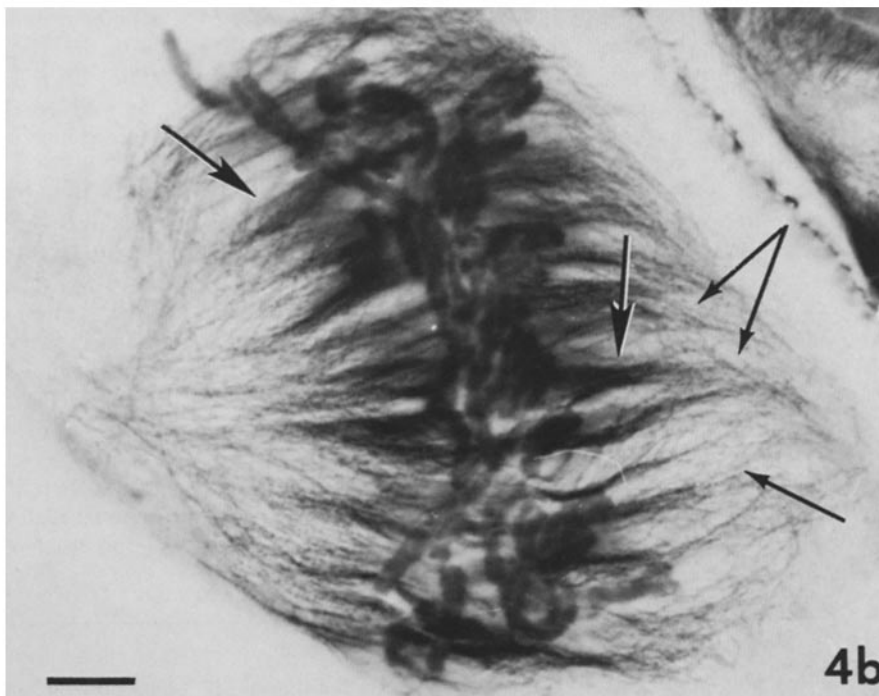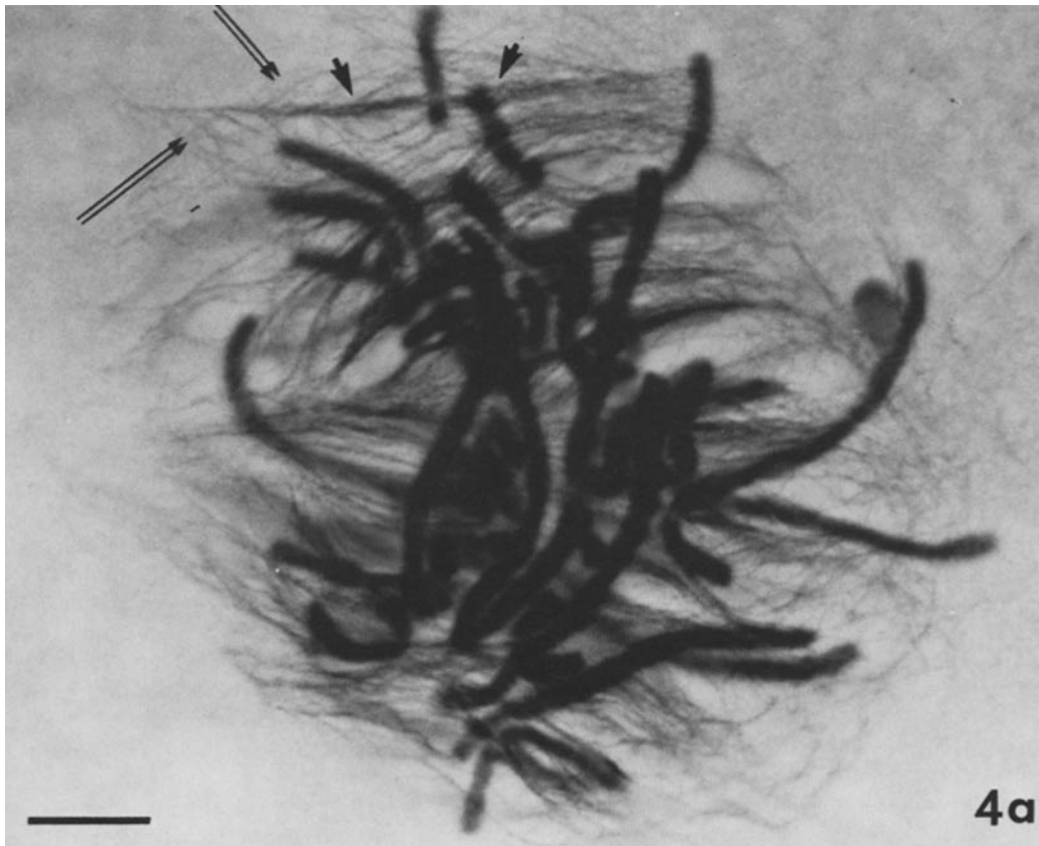
Figure 4. KF in prometaphase. (*a*) Late prometaphase; half-flattened cell, some KFC with chromosomes are very well seen. Structural details of MTFTs are seen; the trunk (KF) and the side branches are marked with short and double thin arrows, respectively. (*b*) Late prometaphase. The spindle is more compact than in *a*, but MTFTs are still seen (arrows). The darkly stained part of KFC corresponds approximately to the flaming birefringent chromosomal fiber (25) as detected in a polarizing microscope (large arrows). Such a spindle shortens before the start of anaphase. Bars, 10 μm.
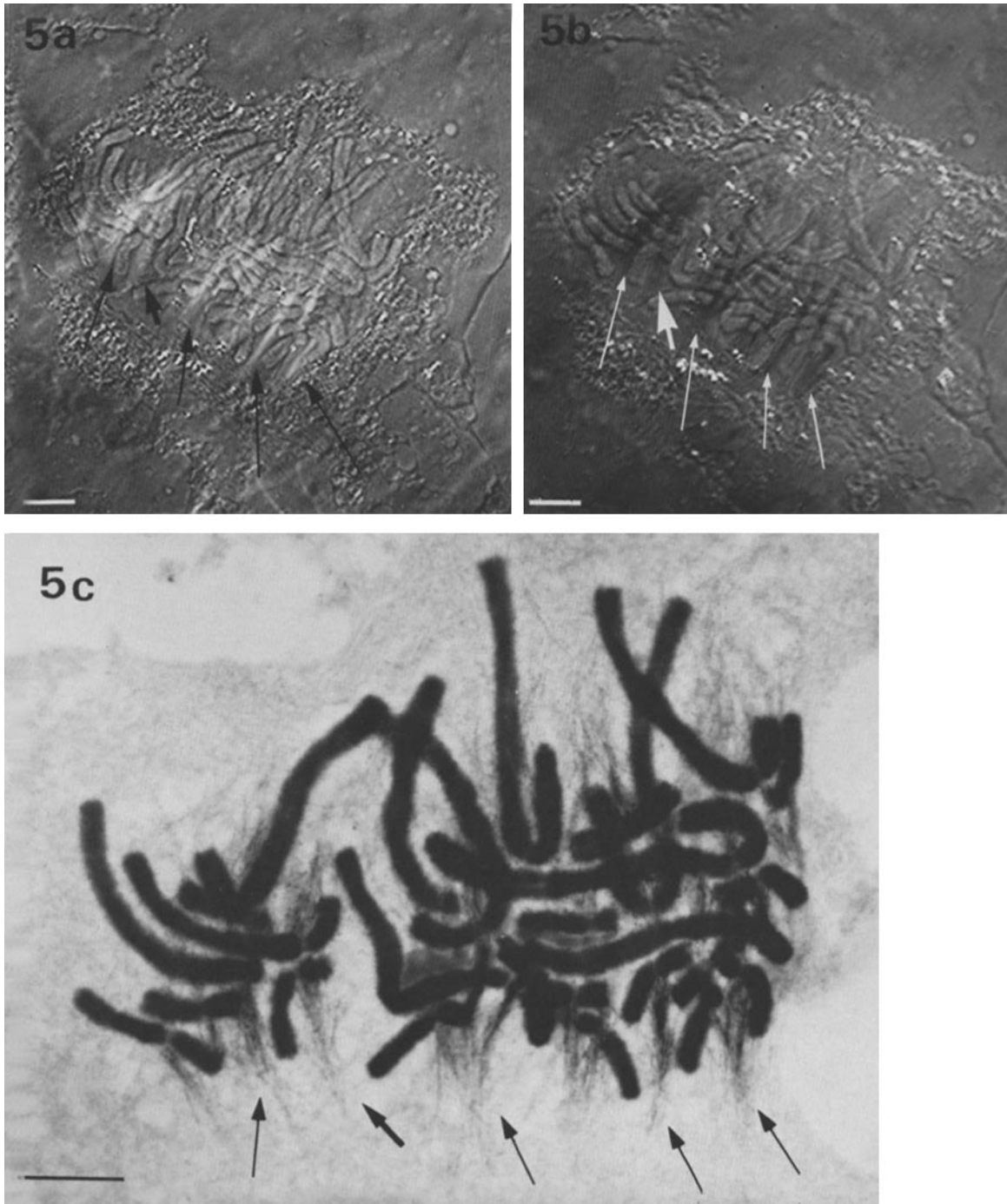
Figure 5. Organization of late prometaphase spindle. The same cell in vivo in polarized light and after IGS. (*a* and *b*) Living cell in white and black compensation, respectively. Some chromosomal fibers are seen as thin birefringent structures (arrows). This is a half-flattened cell. The same fibers (arrows) seen in IGS (*c*) have much more complex structure. KFC with MTFT organization marked by thick short arrows (black in *a* and white in *b*). The KF with MTFT is hardly detected in the polarizing microscope. Comparison of *a* and *b* shows cracks in agar. (*b*) 1 min after *a*; (*c*) 1½ min after *b*. Bars, 10 μm.

Statistical analysis on a larger number of cells (Tables I–III) leads to the same conclusions. The difference between the means of groups for each set of measurements, the length of the KF at metaphase, early anaphase, mid-anaphase, and late anaphase, the length of the KFC at the same four stages, and the distance between the chromosome groups at early, mid-, and late anaphase, are all statistically highly significant ($P \leqslant 0.001$). The only exception is the two-tail probability of 0.46 between the measurements for early anaphase and mid-anaphase, since the length of the KFC at these two stages practically does not change.

The data are summarized in Fig. 11. The graphs show that the KF shortens at a constant rate from metaphase to the end of mid-anaphase, when chromosomes move with an approximately constant speed (Fig. 9). The KFC elongates at the metaphase/anaphase transition (see Discussion) and, while
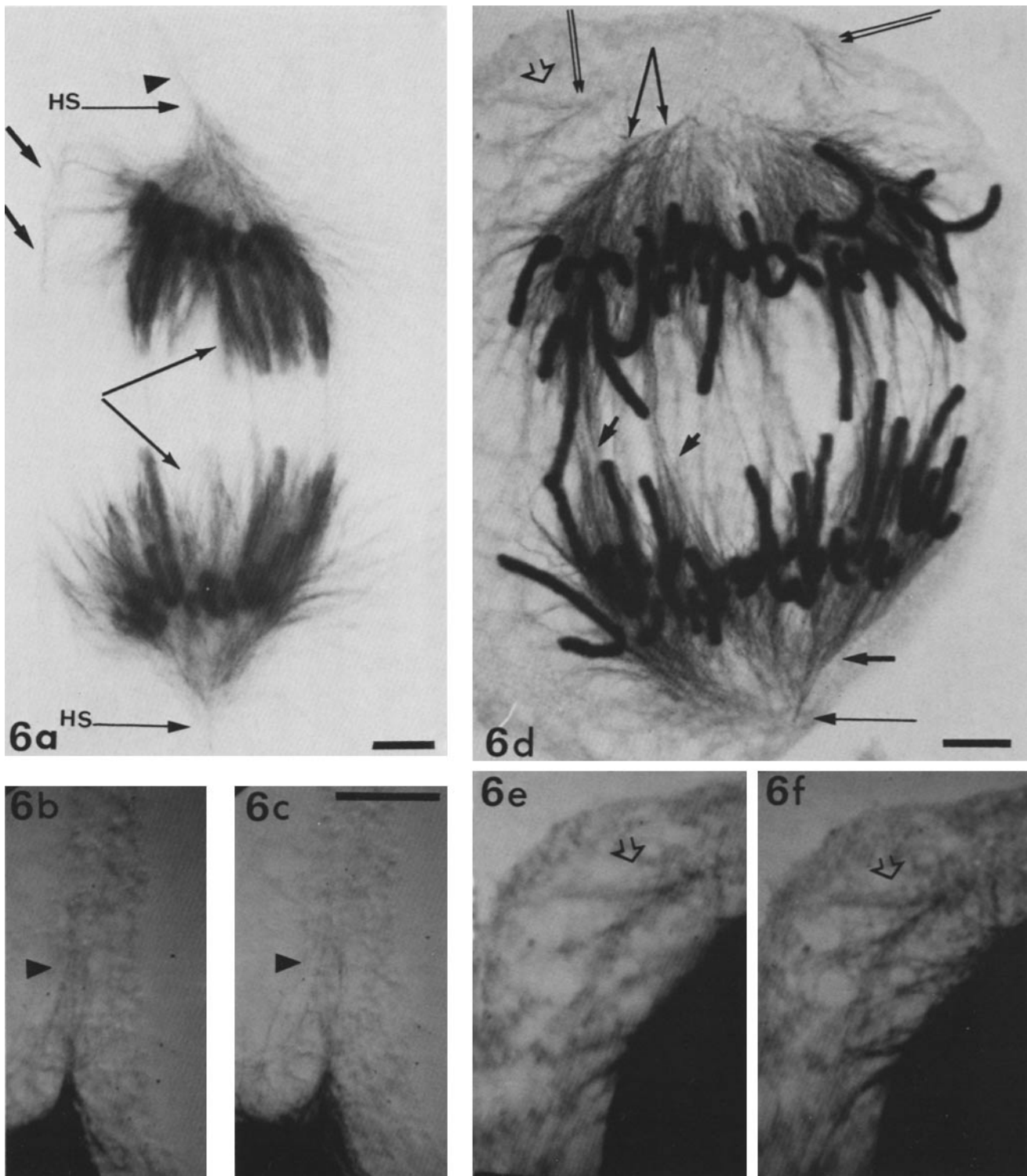
Figure 6. KFCs in anaphase. (*a*) Unflattened cell. The half spindles are very compact and details of organization of the KFC are not seen. No measurements of length of the KF are possible, while the length of the half-spindles can be measured precisely (arrows, *HS*). The bushy outlines of the half-spindles, which are the side branches of MTFTs in flattened cells, are pronounced. These MTs (branches) finally form asters in telophase. Distal segments of some side branches (short arrows) are bent under 90° and follow the outlines of the cell periphery. At this stage, the polar MTs have already grown beyond the kinetochores and stretch into the interzone between the trailing chromosome arms (forked arrow). There are very few MTs left in the interzone. (*b* and *c*) Video enhancement of the distal regions of the upper pole on two optical levels. Few MTs are located within cytoplasmic strand not visible without an enhancement system. (*d*) Slightly flattened cell. KFCs are well seen (forked arrow). The formation of polar MTs is less advanced than in *a* and most of those which grow into the interzone still do not reach beyond the kinetochores. Their elongation is asynchronous. In some regions of the interzone they grow along and between the trailing chromosome arms where they associate with remnants of interzonal MTs (short thick arrows). Some MTFTs are not associated with the spindle (double arrows). They are composed from few MTs not terminating in one point as seen in *e* and *f* (two optical sections in video enhancement). Such unassociated MTFTs may also terminate very close to each other and may form massive arrays. Both cells are stained with 15-nm immunogold, dehydrated, and mounted in permount. Bars: (*a* and *d*) 10 μm; bar on *c* (also for *b*, *e*, and *f*) 10 μm.
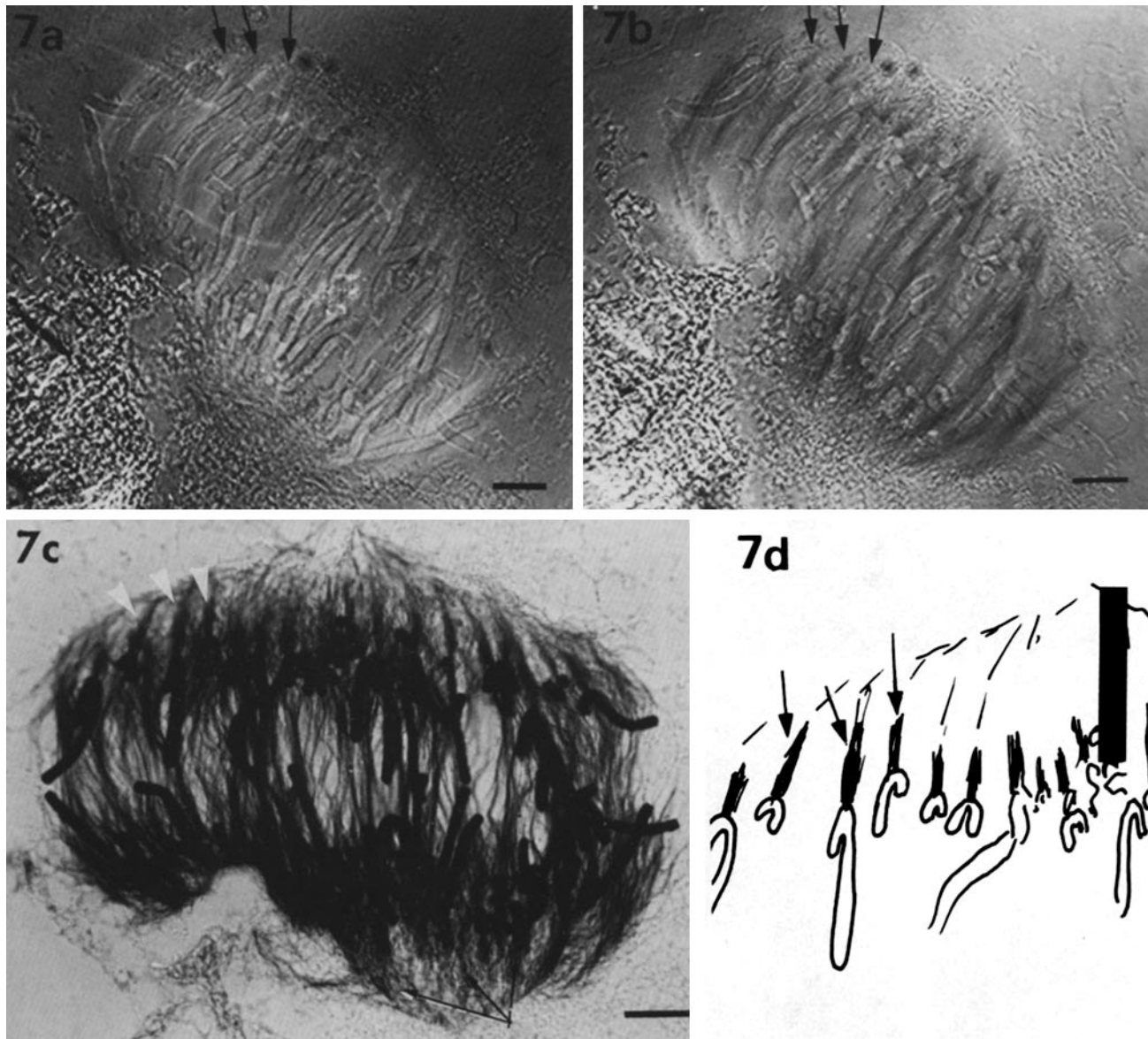
Figure 7. Late anaphase spindle organization in polarized light in living cell and after IGS. In the upper half-spindle of this flattened cell all 27 KFCs are distinguished after IGS and over 20 chromosomal fibers are seen in vivo. (*a*) Black and (*b*) white compensation. Chromosomal fibers seen as short birefringent stubs (arrows) slightly converging toward the poles. They approximately correspond to KFs in *c* (the same cell after IGS) and on the schematic drawing in *d*. The distal portions of KFCs are not detected with the polarized light. Several chromosomal fibers (some are marked by thin black arrows in *a–b*) and the same fibers seen as KFs (some marked by white triangles) are seen. Comparison of *a* and *d* shows similarities and differences between KFs, chromosomal fibers, and KFCs. The length of KFs and chromosomal fibers cannot be measured precisely. The latter are somewhat shorter because their polar end is less defined than those of KFs. Additionally the length of KF is somewhat longer on black and white micrographs than on IGS preparations observed directly due to contrast enhancement (see Materials and Methods). The thick bar in *d* is the length of the half-spindle. White lines across the cell in *a* are cracks in agar. (*b*) 1 min after *a*; (*c*) 2 min after *b*. Bars, 10 μm.

KF shortens, the length of the KFC (which in our definition is the actual half-spindle length) remains unchanged during the first two-thirds of anaphase. Thus, during this period of anaphase the shortening of the KF is slower than chromosome movement during anaphase and is not concurrent with shortening of the half-spindle. The rapid shortening of the half-spindle occurs only in the last one-third of anaphase, when the half-spindle breaks down and the phragmoplast and astral (polar) MTs are formed abundantly.

To study the relationship between the change in length of

polar MTs in mid-and late anaphase and chromosome movement, we plotted the length of the interzonal segments of polar MTs (measured from the kinetochores to their distal ends) against the distance between the chromosome groups in microns (Fig. 12). X-axis corresponds to the average time of anaphase in minutes (the average speed of anaphase chromosomes in *Haemanthus* is 1 μm/min). Therefore the measurements provide average values for the elongation of polar MTs in anaphase. Since the measurements could be done only on a limited number of cells, with an especially suitable

Figure 8. Anaphase–telophase transition. The same cell in polarized light in vivo (*a–b*) and after IGS. This is the stage when trailing chromosome arms begin to contract, marking the start of telophase. The interzonal segments of polar MTs (measured from the kinetochores to their distal ends in the interzone) are clearly seen. Chromosomal fibers are no longer detected (*a–b*) and the remnants of the half-spindle transform into an astral organization (cf. Fig. 13). (*d*) Higher magnification of *c*, illustrating waviness of MTs in the interzone. The comparison of living cells with those after dehydration never showed a shrinkage in excess of 7% of their length. Assuming that the shrinkage is 7 ± 2 μm, it is about 2× too low to explain the observed waviness. This suggests that some polar MTs elongate faster than others, which is consistent with some observations in vivo (stretching of trailing chromosome arms in anaphase). Arrows mark short remnants of chromosomal fibers. (*b*) 1 min after *a*; (*c*) 3 min after *b*. Bars, 10 μm.
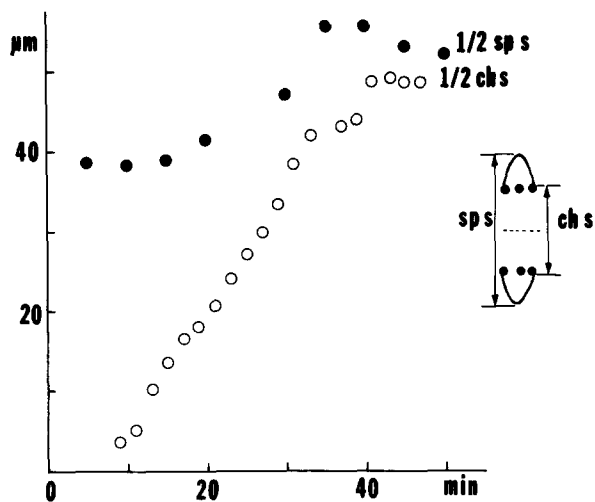
274

Figure 9. Elongation of the spindle in vivo. The half distance between sister kinetochores (chs) and spindle poles (sps) during anaphase was plotted against the time. The spindle poles were defined as the border between the clear spindle and coarse cytoplasm. Measurements were made from time-lapse film taken in DIC Nomarski. The measurements indicate that the spindle poles separate only in later stages of anaphase (~30 min), which is not consistent with data derived from IGS cells. This controversy may perhaps result from the difficulties of measurements on living cells which do not allow us to determine where MTs terminate. Therefore such measurements cannot be used to determine separation of the polar regions (spindle poles) in the acentriolar spindle.
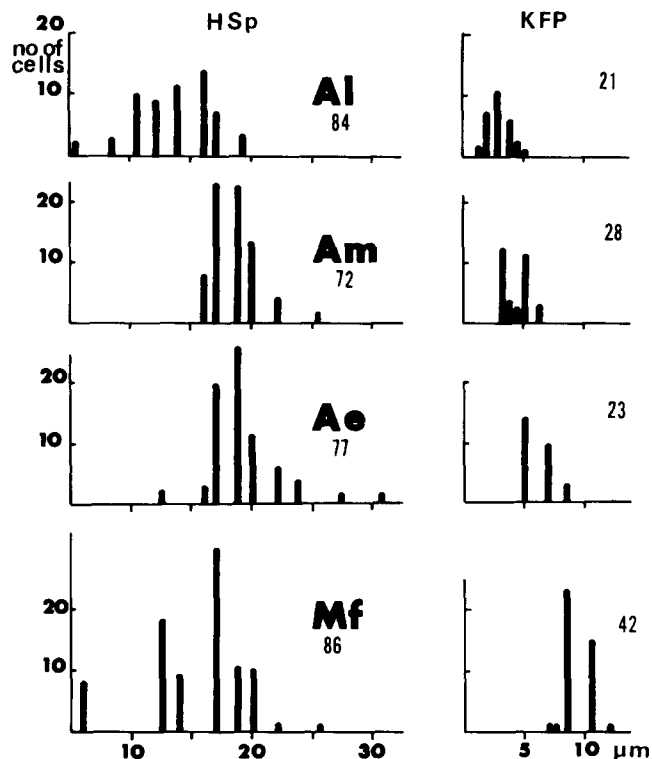

Figure 10. Changes in lengths of half-spindle and KFs during anaphase. The histograms show that half-spindles (HSp) do not shorten until late anaphase, while KFs decrease in length during the progress of anaphase. Definitions of anaphase stages are on Fig. 11.

configuration of polar MTs (e. g. Fig. 8, a–c), no statistical analysis was performed, and the numerical values are probably an underestimation of the MT elongation rate in the living cells (see Discussion). The distance between chromosome groups reflects the time and stage of anaphase and permits us to estimate the rate of polar MT elongation. This rate, in the range of 2 μm/min, is faster than chromosome movement at these stages.

Anaphase-Telophase Transition. At the onset of telophase chromosomes stop their migration and trailing chromosome arms contract. A great variability exists in the appearance and growth of polar MTs that elongate toward the equator and may or may not form aster-like structures (7, 8). In very flat and thin cells this process may start in mid-anaphase (Fig. 13). The development of a polar system always follows the same pattern. MTs grow away from the telophase chromosome group in a centrifugal direction until they reach the cell periphery. MTs, elongating toward the equator especially in the first stage of growth (mid-anaphase), are mostly straight and uniformly distributed (Fig. 3, a and b). The criss-crossing begins in this stage and is increasingly common in asters and phragmoplast where long, usually slightly curved MTs may cross the entire structure. This may indicate that these MTs elongated after the phragmoplast was already formed. In contrast to polar MTs growing toward the cell equator, the distal ends of those forming an aster do not show a tendency to a lateral association. Adjacent MTs have nearly identical lengths on the light microscopic level. This pattern of uniform distribution invariably reorganizes within 30–60 min.

The Development of Phragmoplast and Post-mitotic MTFTs. When the laterally growing post-mitotic or cyto-plasmic phragmoplast reaches the cell periphery, MTFT(s) are often formed. Newly formed MTFTs grow along the cell periphery orthogonal to the cell plate. They resemble hairy tails and, within 60–90 min after preparation, may form gently twisting arched spirals up to 80 μm in length. These tail structures have been observed both in symmetrical (branches on two sides) and in asymmetrical (branches on one side) forms. Even though an entire tail structure may be arched and twisted, its constituent MT strands are usually straight. Similar formation of MTFTs is observed in phragmoplasts which form in the cytoplasm of interphase cells (Fig. 2d). Therefore MTFTs, which basically are growing converging centers, appear at the distal (from the cell plate) regions of the phragmoplasts only in medium and larger cytoplasts and in intact cells. This demonstrates that processes in the living cell require a large tubulin pool and/or unknown associated components.

Occasionally, MTFTs are also formed in the cytoplasm at the polar region of the spindle and may be arranged at an angle to the long axis of the spindle (33). They may be associated with very long single or few MTs detected with image enhancement system (Fig. 3c). Such long MTs are often found around the cell periphery and may form within the aster, where they are often arranged under the angle to the majority of astral MTs. Such MTFTs are of different size, with a dense MT cone as a trunk, and may not even be associated with the half-spindle (Fig. 6, d and f). Thus, the polar region of the spindle is often composed of multiple MTFTs: some are components of KF, and some are not associated with the mitotic spindle.

Discussion

The kinetochore/centromere (41) and centriole–centrosome complex in vitro and probably in vivo modify some properties of MTs while facilitating the nucleation step (13). Activity of these structures yielded abundant information concerning MT nucleation centers in the living cell (11). MT turnover in
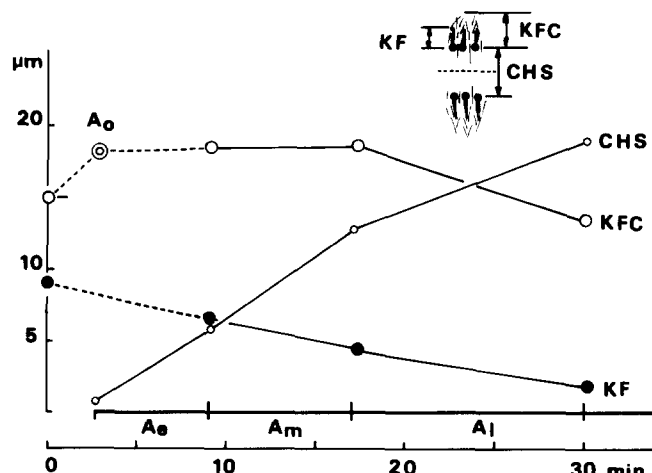

Figure 11. Elongation of the half-spindle and shortening of KFs during anaphase. We measured the length of the kinetochore fiber proper, K/CF, and the distance between the kinetochores of one chromosome group and the equator in late metaphase, early-, mid-, and late anaphase (medium values based on 1,727 spindles) and plotted it against the stage of anaphase. The stages of anaphase were classified on the basis of the distance between the separating chromosome groups. Small class of cells (see Materials and Methods) and measurements of the start of anaphase (A_o) were excluded from these measurements. Since there is a variation in cell size even among medium and large cells, some measurements of chromosome separation for different stages of anaphase overlap. The stages of anaphase correspond to the average time of anaphase, which was the average duration of anaphase in 25 cells measured from time-lapse films. Chromosome separation of <5 μm was defined as the start of anaphase (A_o), 5–18 μm as early anaphase (A_e), 18–34 μm as mid-anaphase (A_m), and 34–61 μm as late anaphase (A_l). *CHS,* half the distance between kinetochores of anaphase chromosome groups. Double circle for A_o is the mean of 40 measurements not included in statistical analysis (Tables I–III). Since metaphase in *Haemanthus* lasts up to 1 h, and there is an indistiguishable transition between prometaphase and metaphase, we do not know how long before the start of anaphase the metaphase measurements were taken. This and especially sudden jump apart of kinetochores at the onset of anaphase may account partly for the abrupt change of length of the spindle between metaphase and the start of anaphase (cf. Discussion).

endosperm occurs both in nucleated cells and in anucleate fragments (cytoplasts) in the absence of such discrete organizing organelles. Cytoplasts are the simplest known cytoplasmic unit where structural MT transformations can be followed and their time sequence deduced.

Nucleation and Self-Reorganization/Rearrangement of MTs

We do not know whether cytoplasts exist in intact ovules and, if present, whether they undergo similar reorganizations as after preparation. The final MT transformations (small phragmoplasts and spiral rings) were never seen a few minutes after preparation. This demonstrates that a rapid reorganization of MTs in cytoplasts is triggered in an unknown manner, perhaps due to specific properties of higher plant MTs. Animal MTs in cytoplasts with and without centrosomes do not reorganize in a similar manner (28). We believe, however, that our data may be related to formation of aster-like structures and tubulin gelation in vitro (44) and perhaps unusually rapid MT assembly–disassembly of some MTs (32).

The first organized MT configuration in cytoplasts is a cone or converging center. The two final transformations are a closed bushy ring or a phragmoplast.

MT converging centers, the simplest ordered arrangement of MTs formed spontaneously, are not restricted to endosperm. They are common in plant and animal cells, both in controls and in experimental conditions when assembly is initiated. They appear in endosperm after a rapid recovery from the low temperature shock (7) or under the influence of taxol (Fig. 1 *m* in reference 34). They have been observed in the root of the fern *Azolla* (20) and in *Haemanthus* endosperm after various experimental treatments (7, 35) that change the critical concentration of tubulin. A high temperature shock produces such converging centers in the streak stage of sea urchin eggs (cf. 19, 39) and taxol in animal cytoplasts (28).

Spontaneous formation of MT converging centers, which in endosperm cytoplasts are unstable intermediates, must involve several steps. The irregular meshwork always present immediately after preparation in large anucleate fragments is composed of intermingled long MTs. The final structures (e.g., multiple phragmoplasts) are composed from shorter MTs. Therefore formation of an organized structure must involve either breaking of MTs or, more likely, their partial disassembly. The nucleation step in vitro is thermodynamically more difficult than the elongation. Only 8 subunits are necessary to form a seed (42) in vitro. However, in very small cytoplasmic fragments, the MT meshwork does not reorganize

Table I. Length of the KFC in μm at Metaphase and during the Progress of Anaphase

Variable	No. of cases	Mean	Standard deviation	Standard error	Pooled variance estimate		
					T value	Degrees of freedom	Probability
Metaphase	284	15.1	3.8	0.2	−9.0	442	$P \leq 0.0001$
E-anaphase	160	18.4	3.4	0.3			
E-anaphase	160	18.4	3.4	0.3	−0.7	333	$P \leq 0.400$
M-anaphase	175	18.6	2.5	0.2			
M-anaphase	175	18.6	2.5	0.2	18.3	366	$P \leq 0.0001$
L-anaphase	193	13.2	3.1	0.2			

Stages of anaphase are defined on Fig. 11. Explanation in text. 812 cells. E-, M-, and L-anaphase, early, mid-, and late anaphase.

Table II. Distance between Chromosome Groups in μm at Metaphase and during the Progress of Anaphase

Variable	No. of cases	Mean	Standard deviation	Standard error	T value	Degrees of freedom	Probability
E-anaphase	104	11.9	5.6	0.5	−19.5	219	$P \leqslant 0.0001$
M-anaphase	117	26.5	5.7	0.5			
M-anaphase	117	26.5	5.7	0.5	−14.9	233	$P \leqslant 0.0001$
L-anaphase	118	39.1	7.2	0.7			

Stages of anaphase are defined on Fig. 11. Explanation in text. 339 cells. E-, M-, and L-anaphase, early, mid-,and late anaphase.

Table III. The Length of the KF Proper in μm at Metaphase and during the Progress of Anaphase

Variable	No. of cases	Mean	Standard deviation	Standard error	T value	Degrees of freedom	Probability
Metaphase	133	9.2	1.0	0.1	2.9	256	$P \leqslant 0.0001$
E-anaphase	125	6.3	1.2	0.1			
E-anaphase	125	6.3	1.2	0.1	18.7	303	$P \leqslant 0.0001$
M-anaphase	180	4.2	0.8	0.0			
M-anaphase	180	4.2	0.8	0.0	15.1	316	$P \leqslant 0.0001$
L-anaphase	138	2.8	0.9	0.1			

Stages of anaphase are defined on Fig. 11. Explanation in text. 576 cells. E-, M-, and L-anaphase, early, mid-, and late anaphase.
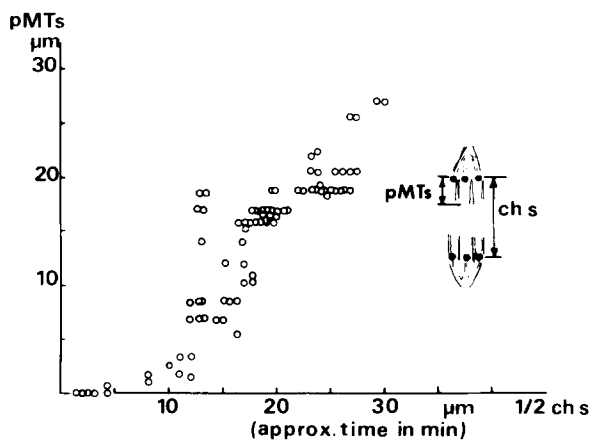

Figure 12. Elongation of polar MTs in anaphase. The length of the interzonal portion of polar MTs plotted against the distance of one chromosome group from the equator (½ chs). Since chromosomes at this stage of anaphase migrate with the speed of ~1 μm/min, the length in μm corresponds approximately to time in minutes (cf. Figs. 10–11). The length of polar MT, i.e., their interzonal segments, was measured from the kinetochores to their ends in the interzone. It should be stressed that terminations of polar MTs are clearly seen only in ~10% of the cells and prevent large number of measurements. Measurements were made on 85 individual cells.

even if it contains over 30,000 tubulin dimers (assuming ~1,700 dimers/1 μm of MT [33]). This suggests that a threshold concentration of tubulin pool, accessory proteins, number of free MT ends, etc., are needed in vivo for the MT reorganization to proceed and to form a specific structure.

The second MT configuration, which is usually formed by large converging center(s), is the cytoplasmic spindle, which invariably transforms into a phragmoplast with a cell plate. Several MT configurations might be interpreted as intermediates between the converging center and the phragmoplast. Work in progress may determine whether phragmoplasts originate in two different ways, i.e., by fusion of two MT cones and/or internal reorganization. A phragmoplast, however, is

always the final structure. This MT organization may therefore be related to intrinsic properties of MTs, either alone or with superimposed regulatory factors.

Our data do not exclude the existence of hypothetical polar organizers (diffuse centrosomes; 31). We believe, however, that the polar reorganization may also be determined without their existence by an increase of local concentration of tubulin in minute domains by a continuous assembly/disassembly of MT free ends, perhaps modified by free Ca^{2+} (29), calmodulin (41), and lateral association of polymerized MTs.

The transformation and rearrangement of MTs in endosperm, both in cytoplasts and mitotic spindles, may be related to the thermodynamically optimal polymer length in vivo and specific properties of (−) ends of MTs. Since the polymer size in vitro is limited and determined by specific conditions (37), the maximum length of cytoplasmic spindles/phragmoplasts may correspond to the optimal length of polymer (MTs) in vivo, similarly as suggested for cytoplasmic MTs (12). This assumption would also explain why in certain conditions existing MTs do not elongate and new MTs and/or MTFTs are formed instead. Such configurations deserve special attention. Because they are found both in interphase and in mitosis, they are of general occurrence and may represent a basic unit in MT turnover in plant endosperm. The problem is more complex, however, because both MTFTs and single MTs form within the wide polar region and cytoplasm in anaphase/telophase and elongate in all directions. If converging ends of MTs are stabilized by capping, then the overall critical concentration decreases and assembly/disassembly is determined by the properties of (+) ends. Thus specific molecular conditions, especially critical concentration, in minute cell domains must be precisely regulated and determine formation of MTFTs vs. single MT and the direction of their growth. Thus our data are consistent with the model of MT nucleation proposed by De Brabander (13).

The Structural Polarity and Elongation of MTs. The structural polarity of cytoplasmic phragmoplasts and MTFTs is not known. The polarity of the mitotic spindle and phrag-

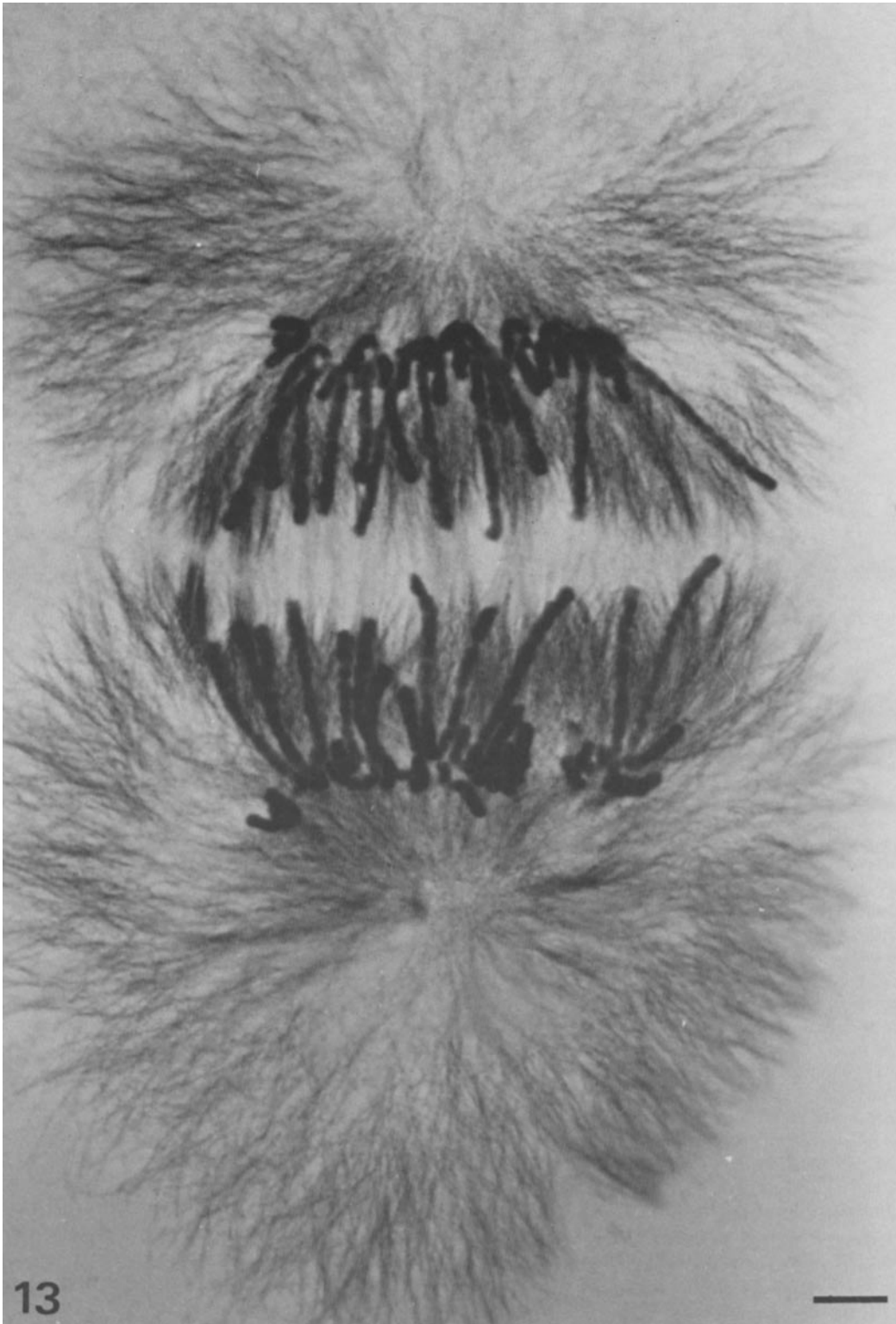Bajer and Molè-Bajer *Reorganization of Plant Microtubules*

Figure 13. Asters in mid-anaphase. Very flattened cell. There is asynchrony in aster development and they form between late mid- to late anaphase (cf. with Fig. 3). This particular cell has developed exceptionally large asters at mid-anaphase (cf. Fig. 6*b*), which is reproduced at the same magnification. The asters extend between the trailing chromosome arms into the interzone and converge toward a small defined polar region. Note criss-crossing of MTs within aster. MT arrangement and orientation strongly suggest that their proximal to the nucleus ends terminate over a wide area and not in well defined domain. Such arrangements are difficult to explain by discrete organelle type nucleating centers. Bar, 10 µm.
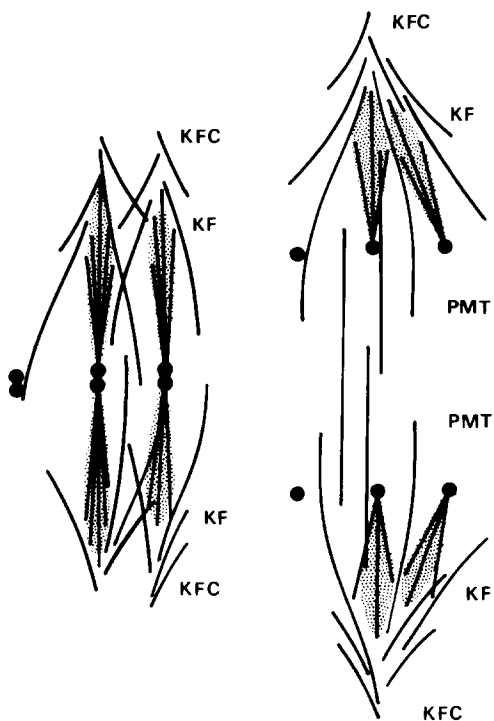
Figure 14. Schematic drawing of spindle organization in metaphase and anaphase. Black circles, kinetochores. Only two KFC (dotted) and KF are drawn. The length of the KF is the distance from the kinetochore approximately to the end of regularly arranged MTs. The length of KFC is the distance from kinetochores to the ends of polar regions. Due to increasing tilt of the peripheral KFCs during anaphase, only the length of the central KF corresponds to the length of the half-spindle. The MTs of one KFC intermingle with MTs from neighboring complexes. MTs from the side branches of one complex may become terminate within the KF of another complex (34). During anaphase new MTFTs side branches are formed. The number of kinetochore MTs decreases during anaphase but due to formation of new MTs in the polar region (MTFTs) the KFC (half-spindle) does not shorten. Concurrently with shortening of the KFC, the MTs of the side branches of MTFTs elongate toward the equator (*PMT*, polar MTs). This highly simplified explanation reflects the lack of our understanding of the basic structure and function of the mitotic spindle. Thus, it is not known how interzonal MTs elongate and whether interzonal MTs seen in anaphase are polar MTs or remnants of interpolar MTs in metaphase. Moreover, it may be even questioned whether the latter exist at all in metaphase.

moplast of *Haemanthus* has been determined (16) on the basis of hook formation. The fast (+) end of over 90% of MTs is distal to the poles. Cytoplasmic MTFTs often fuse with the phragmoplast proper. We assume therefore that the polarity of MTs is the same in both types of structures. Experimental confirmation is required, however, because the hook technique does not distinguish between native MTs and those that might be additionally formed.

Anaphase Assembly. The formation of polar MTs in the acentriolar *Haemanthus* spindle (9) involves MT assembly. Therefore unknown controls in the polar region must promote the nucleation and elongation of astral (polar) MTs concurrently with shortening of kinetochore MTs. The elongation may be the result of on/off rates at the (+) and (−) MT ends combined with local controls, which may turn out to be of primary importance. The few long or single MTs (our methods do not permit us to distinguish between a single and

few MTs) detected often as extensions of the poles (Fig. 6*a*) may be MTs elongating at their (−) ends. Such polar organization is often pronounced when nucleation is inhibited but mitosis not arrested permanently (35).

Our technique does not permit a quantification of tubulin. However, the comparison of the sizes of asters, often seen by mid-anaphase (Fig. 13), and half-spindles (Fig. 6) suggests that *Haemanthus* asters and phragmoplasts together may contain more polymerized tubulin than the half-spindles at any stage. Thus, disassembly of the half-spindle is not sufficient to account for the formation of polar MTs, suggesting in turn that additional tubulin becomes competent to polymerize in anaphase–telophase. Asters in cytoplasmic phragmoplasts are always small or nonexistent, suggesting that chromosomes may be involved in regulation of the polymerizable tubulin pool, as suggested by Lambert (30).

Spindle Elongation. Centriole/centrosome complex and asters, if present, allow for little variation in the organization of the polar region. If present and detected in the light microscope, they also allow precise measurement of half-spindle length in vivo.

Acentriolar spindles of higher plants lack a known MT organizing organelle and their poles are usually not well defined. This prevents precise measurements of spindle length in vivo. The only organization that can be followed in vivo based on conventional (i.e., without image processing system), polarizing microscopy, and DIC, is that of the chromosomal fibers. It is impossible, however, to determine where they terminate. Thus, the only method to measure the entire half-spindle length in plants is an indirect method using immunolabeling of MTs. If chromosomal fibers are considered to be the measure of half-spindle length, then the acentriolar half-spindle shortens gradually during anaphase (22, 23). However, if the length of the KFC is measured, then the half-spindle length remains constant throughout a considerable part of anaphase and shortens only toward the end of chromosome migration (cf. schematic drawing, Fig. 14). A probable cause of the increase in length is the sudden jump apart of chromosomes at the onset of anaphase, which for *Haemanthus* is ~4 μm (30); it corresponds well to an increase of spindle length at metaphase/anaphase transition, reported here. These two sets of data obtained with different methodologies (polarizing microscope and IGS) are therefore complementary.

Previous observations (9, 35) document that the assembly of new MTs occurs during anaphase. The present data demonstrate that during the first two-thirds of anaphase, when KF shorten, the length of the half-spindle remains constant. This indicates that the polar region, i.e., the region between poorly defined ends of KFs and the spindle periphery, or the ends of KFCs, elongate concurrently with shortening of KF. We also assume that the shape of the cell and the spindle may influence the direction of new MT growth. This partly explains the asynchrony of their formation and why they are pronounced in flattened cells and all syncytia. Moreover, a considerable variation of spindle elongation is expected in higher plant endosperm, as well as the existence of well developed polar MTs and small asters in long unflattened cells. Our observations are consistent with these assumptions, but the basic function of the half-spindle remains unknown (Fig. 14).

Our attempts to measure directly the elongation rate of polar MTs in living cells, using time-lapse films made with a

high extinction polarizing microscope, but without image processing, were not conclusive due to the weak birefringence (see Fig. 8). Previous observations on living cells indicated, however, that chromosomes move faster than the spindle fibers decrease in length (2). Indirect measurements of polar MTs on IGS preparations suggest that their elongation is faster (2 μm/min) than chromosome movement which in anaphase is 1–1.5 μm/min. Although they are indirect, they are the only data available on the average rate of polar MT elongation. The actual rate of at least some MTs in vivo is probably higher. The rates are consistent, however, with our work on the transport of granules (in progress). These data and the analysis of particle movements suggest that some MTs in the living cells may elongate much faster than average, as implied by the work in vitro (31). The speed of kinetochore migration in anaphase is ~1 μm/min, while some acentric chromosome fragments move to the equator in anaphase 8 μm/min (4), and the speed of 4 μm/min is very common. These fragments are most likely pushed by the elongating MTs, as are backward-moving chromosomes in taxol-treated cells (8, 34). This suggests that the elongation of polar MTs toward the equator may exert a considerable pushing force and may even break chromosomes (3). Such a force may contribute to chromosome movement and perhaps may be sufficient to push the half-spindles against the cytoplasm and account for spindle elongation (35).

MTFT and Spindle Organization. The basic question is whether MTFT spindle organization is the result of specific optical properties of immunogold as visualized by the light microscope (superimposing of optical images, etc.), increasing divergence of KFC during anaphase (18), preparatory methods (e.g., flattening of cells), etc. Extensive discussion of these problems is beyond the scope of this paper and will be presented elsewhere (10). These data demonstrate the existence of fir tree-like structure of numerous, but not all, individual KFC, both in flattened and unflattened spindles in controls. However, MTFT spindle organization of all KFC is very clear when the number of MTs is reduced, e.g., by low temperature (6°C) which does not arrest mitosis permanently, and chromosome segregation is completed (36). Similarly, when the assembly of branches of the MTFTs is inhibited by plant MT-specific drugs at physiological concentrations (micro- and nanomolar range), anaphase is instantly arrested (9, 35). Thus, the whole functional spindle from prometaphase to late anaphase seems to be composed from associated MTFTs. Such organization may be essential for chromosome transport. It remained undetected until the introduction of IGS techniques and was demonstrated in the unflattened cells with the image enhancement system (10). The density of MT arrangement does not allow its easy detection with conventional light microscopy. The skew arrangement of MTs around the KFC in an umbrella-like fashion was practically impossible to detect in a polarizing microscope as the optical signal of the superimposed branches of the KFC cancel each other. The northern light phenomenon or flaming chromosomal fiber observed in *Haemanthus* (22) and in the meiotic spindle of grasshoppers (25) is most likely the result of continuous lateral rearrangements due to the formation of MTFTs. We conclude, therefore, that MTFT organization of KFC(s) may be necessary for the fiber(s) to function. We should caution, however, that the splaying of kinetochore fibers observed in vivo (18) in *Haemanthus* is difficult to distinguish

from the formation of MT fir trees in unflattened cells. We hope that work in progress (10) may permit both better understanding of these structures and their role in mitosis. We believe also that such organization is probably of wide occurrence and is not restricted to the acentriolar spindle of the endosperm tissue. Spindles of other organisms contain large percentages of MTs that are skew to the long axis of the spindle. They have been demonstrated by electron microscopic analysis of crane fly spermatocytes (17), and the data of Schaap and Forer (38) are consistent with such organization. This suggests that the basic structure of the KFC is the same in both the acentriolar spindle of *Haemanthus* and the centriolar spindle of crane fly.

We conclude that the MTFT is a newly described structural and functional unit of MT organization in *Haemanthus* endosperm. It occurs in cell fragments and intact cells during interphase and mitosis. The reorganization of MTs in cell fragments reflects the intrinsic properties of MTs in vivo, which occur both in interphase and mitosis. Reorganization during anaphase leads to pronounced assembly of new polar MTs in front of disassembling KFs. Polar MTs elongating toward the equator may push half-spindles apart and contribute to polar separation.

References

1. Allen, R. D., A. Bajer, and J. LaFountain. 1969. Poleward migration of particles or states in spindle fiber filaments during mitosis in *Haemanthus. J. Cell Biol.* 43:4a. (Abstr.)
2. Bajer, A. 1961. A note on the behaviour of spindle fibers at mitosis. *Chromosoma (Berl.).* 12:64–71.
3. Bajer, A. 1983. Pushing by elongating microtubules produces spontaneous chromosome fragments in *Haemanthus* endosperm. *J. Cell Biol.* 97:191.
4. Bajer, A. S., and J. Molè-Bajer. 1963. Cine analysis of some aspects of mitosis in endosperm. *In* Cinematography in Cell Biology. 357–409.
5. Bajer, A. S., and J. Molè-Bajer. 1972. Spindle dynamics and chromosome movements. *Int. Rev. Cytol. Suppl.* 3:1–271.
6. Bajer, A., and J. Molè-Bajer. 1979. Anaphase—stage unknown. *In* Cell Motility: Molecules and Organization. University of Tokyo Press, Tokyo. 569–591.
7. Bajer, A. S., and J. Molè-Bajer. 1982. Asters, poles and transport properties within spindle-like microtubule arrays. *Cold Spring Harbor Symp. Quant. Biol.* 46:263–283.
8. Bajer, A. S., C. Cypher, J. Molè-Bajer, and H. H. Harrison. 1982. Taxol-induced anaphase reversal: evidence that elongating microtubules can exert a pushing force in living cells. *Proc. Natl. Acad. Sci. USA.* 79:6569–6573.
9. Bajer, A. S., and J. Molè-Bajer. 1985. Drugs with colchicine-like effects that specifically disassemble plant but not animal microtubules. *Ann. N.Y. Acad. Sci.* In press.
10. Bajer, A. S., J. Molè-Bajer, and S. Inoué. 1985. Three-dimensional distribution of microtubules in *Haemanthus* endosperm cells. *J. Cell Biol.* 101

(5, Pt. 2):146a. (Abstr.)

11. Brinkley, B. R. 1985. Microtubule organizing centers. *Annu. Rev. Cell Biol.* 1:19–224.

12. Brinkley, B. R., S. M. Cox, D. A. Pepper, L. Wible, S. L. Brenner, and R. L. Pardue. 1981. Tubulin assembly sites and the organization of cytoplasmic microtubules in cultured mammalian cells. *J. Cell Biol.* 90:554–562.

13. De Brabander, M. 1982. A model for the microtubule organizing activity of the centrosomes and kinetochores in mammalian cells. *Cell Biol. Int. Rep.* 6:901–915.

14. De Mey, J. M., M. Moeremans, G. Geuens, R. Nuydens, and M. De Brabander. 1981. High resolution light and electron microscopic localization of tubulin with the IGS (immuno-gold staining) method. *Cell Biol. Int. Rep.* 5:889–899.

15. De Mey, J., A. M. Lambert, A. S. Bajer, M. Moeremans, and M. De Brabander. 1982. Visualization of microtubules in interphase and mitotic plant cells of *Haemanthus* endosperm with the immuno-gold staining (IGS) method. *Proc. Natl. Acad. Sci. USA.* 79:1898–1902.

16. Euteneuer, U., and J. R. McIntosh. 1980. Polarity of midbody and phragmoplast microtubules. *J. Cell Biol.* 87:509–515.

17. Fuge, H. 1977. Ultrastructure of the mitotic spindle. *Int. Rev. Cytol. Suppl.* 6:1–58.

18. Hard, R., and R. D. Allen. 1977. Behaviour of kinetochore fibers in *Haemanthus katherinae* during anaphase movements of chromosomes. *J. Cell Sci.* 27:47–56.

19. Harris, P., M. Osborn, and K. Weber. 1980. Distribution of tubulin-containing structures in the egg of sea-urchin *Strongylocentrotus purpuratus* from fertilization through first cleavage. *J. Cell Biol.* 84:668–679.

20. Gunning, B. E. S., A. R. Hardham, and J. E. Hughes. 1978. Evidence of initiation of microtubules in discrete regions of the cell cortex in *Azolla* root-tip cells, and a hypothesis on the development of cortical arrays of microtubules. *Planta (Berl.).* 143:161–179.

21. Inoué, S. 1981. Video image processing greatly enhances contrast, quality, and speed in polarization-based microscopy. *J. Cell Biol.* 89:346–356.

22. Inoué, S., and A. Bajer. 1961. Birefringence of endosperm. *Chromosoma (Berl.).* 12:43–63.

23. Inoué, S., J. Fuseler, E. D. Salmon, and G. W. Ellis. 1975. Functional organization of mitotic microtubules. *Biophys. J.* 15:725–744.

24. Inoué, S., and H. Ritter, Jr. 1978. Mitosis in Barbulanympha. II. Dynamics of two stages of anaphase, nuclear morphogenesis and cytokinesis. *J. Cell Biol.* 77:655–684.

25. Izutsu, K., H. Sato, H. Hakabyashi, and N. Aoki. 1977. The behavior of spindle fibres and movement of chromosomes in dividing grasshopper spermatocytes. *Cell Struct. Funct.* 2:119–133.

26. Jensen, C. G. 1982. Dynamics of spindle microtubule organization: kinetochore fiber microtubules of plant endosperm. *J. Cell Biol.* 92:540–559.

27. Jensen, C., and A. S. Bajer. 1973. Spindle dynamics and arrangement of microtubules. *Chromosoma (Berl.).* 44:73–89.

28. Karsenti, E., S. Kobayashi, T. Mitchison, and M. Kirschner. 1984. Role of the centrosome in organizing the interphase microtubule array: properties of cytoplasts containing or lacking centrosomes. *J. Cell Biol.* 98:1763–1776.

29. Keith, C. H., A. S. Bajer, R. Ratan, F. R. Maxfield, and M. L. Shelanski. 1985. Calcium and calmodulin in the regulation of the microtubular cytoskeleton. *Ann. N.Y. Acad. Sci.* In press.

30. Lambert, A-M. 1980. The role of chromosomes in anaphase trigger and nuclear envelope activity in spindle formation. *Chromosoma (Berl.).* 76:295–308.

31. Mazia, D. 1984. Centrosomes and mitotic poles. *Exp. Cell Res.* 153:1–15.

32. Mitchison, T., and M. Kirschner. 1984. Microtubule assembly nucleated by isolated centrosomes. *Nature (Lond.).* 312:232–237.

33. Mitchison, T., and M. Kirschner. 1984. Dynamic instability of microtubule growth. *Nature (Lond.).* 312:237–242.

34. Molè-Bajer, J., and A. Bajer. 1983. The action of taxol on mitosis. Modification of microtubule arrangements of the mitotic spindle. *J. Cell Biol.* 96:527–540.

35. Molè-Bajer, J., and A. S. Bajer. 1985. Pinnate organization of the mitotic spindle in endosperm of a higher plant Haemanthus demonstrated by experimental disassembly of microtubules. *In* Yamada Conference on Motility 1984. Tokyo Univ. Press. 429–442.

36. Molè-Bajer, J., M. Vantard, P. C. Hewitt, and A. S. Bajer. 1985. Some aspects of the organization of kinetochore fibre complex during anaphase in acentriolar spindle of the higher plant *Haemanthus. In* Microtubule and Microtubule Inhibitors. M. De Brabander and J. De Mey, editors. Elsevier/North Holland-Biomedical Press, Amsterdam. In press.

37. Oosawa, F., and S. Asakura. 1975. Thermodynamics of the Polymerization of Protein. Academic Press, Inc., NY. 204 pp.

38. Schaap, C. J., and A. Forer. 1984. Video digitizer analysis of birefringence along the lengths of single chromosomal spindle fibres. *J. Cell Sci.* 65:41–60.

39. Schroeder, T. E., and D. A. Battaglia. 1985. "Spiral asters" and cytoplasmic rotation in sea urchin eggs: induction in *Strongylocentrotus purpuratus* eggs by elevated temperature. *J. Cell Biol.* 100:1056–1062.

40. Shungu, D., M. Valiant, V. Tutlane, E. Weinberg, B. Weissberger, L. Koupal, H. Gadebush, and E. Stapley. 1983. Gelrite as an agar substitute in bacteriological media. *Appl. Environ. Microbiol.* 46:840–845.

41. Valdivia, M., and B. R. Brinkley. 1985. Biochemical studies of the kinetochore/centromere of mammalian chromosomes. *In* Molecular Biology of Cytoskeleton. G. Borisy, D. Cleveland, and D. Murphy, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 79–86.

42. Vantard, M., A-M. Lambert, J. De Mey, P. Picquot, and L. J. Van Eldik. 1985. Characterization and immunocytochemical distribution of calmodulin in higher plant endosperm cells: localization in the mitotic apparatus. *J. Cell Biol.* 101:488–499.

43. Voter, A. W., and H. P. Erickson. 1984. The kinetics of microtubule assembly. *J. Biol. Chem.* 259:10430–10438.

44. Weisenberg, R. C., and C. Cianci. 1984. ATP-induced gelation-contraction of microtubules assembled in vitro. *J. Cell Biol.* 99:1527–1533.

45. Wick, M. S. M., R. W. Seagull, M. Osborn, K. Weber, and B. E. S. Gunning. 1981. Immunofluorescence microscopy of organized microtubule arrays in structurally stabilized meristematic plant cells. *J. Cell Biol.* 89:685–690.