

Characterization and Dynamics of Cytoplasmic F-Actin in Higher Plant Endosperm Cells during Interphase, Mitosis, and Cytokinesis

Anne-Catherine Schmit and Anne-Marie Lambert

Laboratoire de Biologie Cellulaire Végétale, Centre National de la Recherche Scientifique UA 1182, Université Louis Pasteur, Institut de Botanique, F-67083 Strasbourg-Cedex, France

Abstract. We have identified an F-actin cytoskeletal network that remains throughout interphase, mitosis, and cytokinesis of higher plant endosperm cells. Fluorescent labeling was obtained using actin monoclonal antibodies and/or rhodamine-phalloidin. Video-enhanced microscopy and ultrastructural observations of immunogold-labeled preparations illustrated microfilament-microtubule co-distribution and interactions. Actin was also identified in cell crude extract with Western blotting. During interphase, microfilament and microtubule arrays formed two distinct networks that intermingled. At the onset of mitosis, when microtubules rearranged into the mitotic spindle, microfilaments were redistributed to the cell cortex, while few microfilaments remained in the spindle. During mitosis, the cortical actin network remained as an elastic cage around the mitotic apparatus and was stretched parallel to the

spindle axis during poleward movement of chromosomes. This suggested the presence of dynamic cross-links that rearrange when they are submitted to slow and regular mitotic forces. At the poles, the regular network is maintained. After midanaphase, new, short microfilaments invaded the equator when interzonal vesicles were transported along the phragmoplast microtubules. Colchicine did not affect actin distribution, and cytochalasin B or D did not inhibit chromosome transport. Our data on endosperm cells suggested that plant cytoplasmic actin has an important role in the cell cortex integrity and in the structural dynamics of the poorly understood cytoplasm-mitotic spindle interface. F-actin may contribute to the regulatory mechanisms of microtubule-dependent or guided transport of vesicles during mitosis and cytokinesis in higher plant cells.

IN spite of a spectacular explosion of information, mostly about the microtubular system (Seagull and Heath, 1980; Wick et al., 1981; De Mey et al., 1982; Bajer and Mole-Bajer, 1982; 1986; Schmit et al., 1983, 1985; Kilmartin and Adams, 1984), the F-actin cytoskeleton of higher plants remains poorly understood. Long actin bundles have been identified in elongated cells during cytoplasmic streaming, as, for example, during pollen tube growth (Condeelis 1974; Perdue and Parthasarthy 1985; Pierson et al., 1986) or in vascular (Pesacreta et al., 1982) and epidermal (Parthasarathy, 1985) cells. This organization is comparable to the actin cables well documented in characean algae (review of Allen and Allen, 1978; Williamson, 1980; Nothnagel et al., 1981) or in fungi (Hoch and Staples, 1983). In higher plants, however, there is still no evidence for any actomyosin mechanism of cytoplasmic movement, as was assumed in *Chara* or *Nitella* (Kato and Tonomura, 1977; Sheetz and Spudich, 1983). Rhodamine-phalloidin as a specific probe for F-actin (Wieland, 1977; Wulf et al., 1979; Verderame et al., 1980), enabled microfilament strands to be identified in the cytoplasm of interphase cells in meristems (Metcalf et al., 1984; Clayton and Lloyd, 1985; Seagull et al., 1986, 1987) and staminal hairs (Tiwari et al., 1984). In these cells, during mitosis, these cytoplasmic microfilaments were no longer detected,

while diffuse staining reappeared at the equator during cytokinesis (Clayton and Lloyd, 1985; Seagull et al., 1987; Tiwari et al., 1984). Recent data also indicate that actin is present in the preprophase band and disappears thereafter (Palevitz, 1987). This preprophase band, typical of the onset of mitosis in meristematic cells (Wick et al., 1981; Wick and Duniec, 1984; for review see Gunning and Wick, 1985), was absent in our material.

The question of whether interphasic cytoplasmic F-actin persisted during mitosis initiation and/or was rearranged in the cell cortex around the mitotic apparatus remained unanswered. No studies have reported F-actin dynamics in the cortical cytoplasm of higher plant cells during the mitotic process. However, earlier data, which is still debated, suggested that actin might be located in the spindle of higher plant (Forer and Jackson, 1975, 1976) and animal cells (for review see Sanger, 1975; Schroeder, 1976) observed after heavy meromyosin or fluorescent-probe labeling. Recently, a spindle-like staining pattern of microfilaments has been observed in allium meristematic cells during mitosis (Seagull et al., 1987).

In this report we present original evidence of a well-organized F-actin cytoskeletal network, as a permanent and dynamic component of the cell cortex in the plant en-

dosperm, throughout the cell cycle. Double-labeling of the same cells with tubulin- and actin-specific probes revealed the spatial interactions between microtubules and microfilaments. During the interphase-mitosis transition, the previously described actin network (Schmit et al., 1985) was rearranged around the microtubule arrays that formed the future mitotic apparatus. During chromosome movement this actin network surrounded the mitotic spindle as a loose deformable cage which seemed to be "stretched" parallel to mitotic forces. Only rarely were filaments seen in the spindle. Detailed reorganization and assembly of actin filaments at the equator during cytokinesis were also observed, which corroborates our previous data (Schmit and Lambert, 1985a). Wall-free endosperm cells were used for this study. They are now frequently used for immunocytochemical investigation of the microtubular cytoskeleton (Bajer and Mole-Bajer, 1982, 1986; De Mey et al., 1982; Schmit et al., 1983, 1985; Vantard et al., 1985). Our data suggest that actin plays an important role in the structural integrity and physiology of the higher plant cell cortex during interphase, mitosis, and cytokinesis. Whether actin bundles are involved in the transport and/or saltatory motion of vesicles on the spindle surface or in the phragmoplast dynamics, remains unknown. Further investigations are now needed to identify and detect the potential activity of plant actin-binding and actin-cross-linking proteins in the mechanisms of microfilament-microtubule motility.

Materials and Methods

Preparation of Cells

Controls and Drug Treatment. Living mitotic endosperm cells of *Haemanthus katherinae* Bak. were prepared as previously described (Mole-Bajer and Bajer, 1968; Schmit et al., 1983; Bajer and Mole-Bajer, 1986). During drug experiments, cell preparations were perfused with colchicine (E. Merck, Darmstadt, Federal Republic of Germany) or vinblastine sulphate (Velbé, Eli Lilly Laboratories, Fegersheim, France), at a concentration of 50–80 μ M for 5–30 min. For cytochalasin treatment, cytochalasin B or D (Sigma Chemical Co., St. Louis, MO) was applied to cells at a final concentration of 2–10 μ M for 30 min. Stock solutions were made in DMSO so as not to exceed 1% DMSO in final concentration. During perfusion of the drugs chromosome movement was constantly monitored on individual cells by phase-contrast microscopy.

Fixation and Permeabilization. Cell preparations were fixed and permeabilized as described elsewhere (De Mey et al., 1982; Schmit et al., 1983). Glutaraldehyde or paraformaldehyde were used as fixatives and no difference was detected. Remaining glutaraldehyde was reduced by incubation for 30 min in NaBH₄ (0.5 mg/ml in 0.1 M phosphate buffer, pH 6.9) at room temperature.

Fluorescence Microscopy

F-Actin Labeling. After fixation-permeabilization procedures, cells were incubated in rhodamine- or fluorescein-phalloidin (R-415 and R-432; Molecular Probes Inc., Junction City, OR) at a concentration of 0.2–0.7 μ g/ml in Tris-buffered saline (TBS: 10 mM Tris, 0.15 M NaCl, pH 7.6) for 60 min at 37°C. Each cell preparation was incubated in 80–200 μ l of this solution. Some cell preparations were double stained with rhodamine-phalloidin and antibodies to actin in a two-step incubation procedure. Fixed and permeabilized cells were incubated first with monoclonal anti-actin IgM (350; Amersham, Les-Ulis, France) overnight at 37°C. After washing, incubation was continued in a mixture of rhodamine-phalloidin (0.7 μ g/ml in TBS) and fluorescein-tagged sheep anti-mouse IgM (Pasteur Institute, Paris, France) for 1 h at 37°C. After washing, mounting was done with Gelvatol, as described elsewhere (Langanger et al., 1983). As a control, fluorescein- or rhodamine-phalloidin staining of fixed and permeabilized cells was inhibited by preincubation with unlabeled phalloidin (Boehringer Mannheim,

Mannheim, FRG) for 1 h at room temperature at a concentration of 200 μ g/ml. No fluorescence was detected.

Microtubule and F-Actin Double Staining. Microtubule and F-actin distribution were analyzed simultaneously in the same cells with double staining using anti-tubulin antibodies labeled with fluorescein secondary antibody and rhodamine-phalloidin. Affinity-purified rabbit antibodies against mammalian tubulin (De Mey et al., 1982) or monoclonal anti- α -tubulin (356; Amersham) were used. Fixed and permeabilized preparations were incubated overnight at room temperature and then processed in a mixture of rhodamine-phalloidin (1 mg/ml) and fluorescein-labeled goat anti-rabbit IgG (Nordic Immunology, Tilburg, The Netherlands) for 1–2 h at 37°C. Mounting medium was the same as above. Some preparations were only stained for tubulin as described elsewhere (Schmit et al., 1983). All stained preparations were examined with a Leitz Orthoplan microscope equipped for epifluorescence, with an HBO 100/W2 lamp as light source and an oil fluorescence objective of 63 (NA, 1.30 Leitz).

Micrography. Pictures were taken with a Wild-Leitz Vario-Orthomat camera using Kodak Ektachrome ASA 125, OR Tri-X Pan ASA 400 film.

Immunogold-Silver Staining (IGSS). In some cases, fluorescent secondary antibodies were substituted for 5 nm gold particle-labeled IgG (Janssen Pharmaceutica, Beerse, Belgium), diluted 1/10 in TBS-BSA buffer for 5 h at 4°C. IGSS procedures outlined in the Janssen kit were followed as described elsewhere (De Mey et al., 1982; Bajer et al., 1986). After washing, cell preparations were dehydrated and mounted in Permount (Fisher, Fair Lawn, NJ).

Electron Microscopy. After dehydration immunogold labeled preparations were flat-embedded in epon-araldite (Merck), as previously described (Lambert and Bajer, 1977; Vantard et al., 1985). Serial sections were made with a Reichert UMU2 Ultramicrotome (Reichert Scientific Instruments, Buffalo, NY), stained with uranyl acetate/lead citrate, and observed with a Philips 410 transmission electron microscope at 80 kV.

Video-enhanced Light Microscopy

A Zeiss universal microscope was equipped with a differential interference contrast Nomarski system using Zeiss planapo 63/1.40 oil and phase-contrast Ph 3 Apo 40/1.00 oil objectives. An objective of 12.5 was placed under a TV camera (WV 1850/G; Panasonic, Secaucus, NJ) attached to mobile mounts. A 550-nm interference filter was used for all observations in transmitted light microscopy, and enabled selective visualization of gold/silver particles. Micrographs were taken directly from the TV screen of a monitor (PVM 122 CE; Sony, Clichy, France).

Haemanthus Crude Extract Preparation

Endosperm cells were isolated from young ovules of *Haemanthus katherinae* Bak. and homogenized with a mortar in cold extraction buffer: 200 mM Pipes (Sigma Chemical Co.), pH 6.9, 4 mM EGTA (Sigma Chemical Co.), 2 mM MgCl₂, 4 mM dithiothreitol (Aldrich, Strasbourg, France), 40 mM diethylthiocarbamic acid (Sigma Chemical Co.), 20 μ M leupeptin hemisulphate (Sigma Chemical Co.), 20 μ M pepstatin (Sigma Chemical Co.), 1 mM GTP (lithium salt) (Boehringer Mannheim), using 1 g of ovular liquid per milliliter of buffer.

SDS-PAGE

SDS-PAGE of the homogenate was performed according to Laemmli (1970), using 0.75-mm-thick mini-slab gels with an acrylamide concentration of 7.5% at pH 8.8. Gels were run at constant current (10 mA) at room temperature and stained for 90 min with 0.25% Coomassie Blue R250 in methanol/acetic acid.

Immunoblotting

After electrophoresis, the gel was equilibrated in cold transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) for 30 min and electrotransferred onto nitrocellulose paper (0.45 mm), according to Towbin et al. (1979), with a trans-blot electrophoretic transfer cell (Bio-Rad Laboratories, Richmond, CA) at 4°C for 2 h at 150 mA. Nonspecific binding sites were saturated with 5% BSA for 30 min at 37°C. All subsequent steps were carried out at room temperature. Incubation in the first antibody (anti-tubulin or anti-actin) was done in TBS enriched with 0.1% BSA and 1% normal goat serum for 2 h. After rinsing, the second antibody was applied. We used goat

1. *Abbreviation used in this paper:* IGSS, immunogold-silver staining.

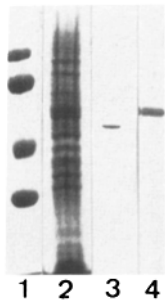


Figure 1. Immunoblotting analysis of cytoskeletal proteins recognized by anti-actin and anti-tubulin monoclonal antibodies. (Lanes 1 and 2) Coomassie Blue-stained gel; (lane 1) molecular mass markers at 94, 68, 45, and 29 kD; (lane 2) cell homogenate corresponding to the contents of 1 ovule; (lanes 3 and 4) corresponding to nitrocellulose immunoblots; (lane 3) anti-actin/IGSS; (lane 4) anti- α tubulin/IGSS.

anti-mouse IgG and IgM labeled with 20 nm gold particles at 1/100 overnight. Higher sensitivity was obtained by silver precipitation on the gold particles, as described by Moeremans et al. (1984).

Results

Controls of Actin Staining

The specificity of monoclonal anti-actin antibodies against endosperm actin was tested by Western blotting on crude extracts of *Haemanthus* endosperm cells (Fig. 1). One band at ~ 43 kD (Fig. 1, lane 3) was recognized. Double labeling with actin antibodies and phalloidin indicated an identical staining pattern in permeabilized cells (Fig. 4).

Actin Distribution in Interphase Endosperm Cells

A regular and loose network of thin actin bundles was present throughout the cytoplasm of interphase cells. Tubulin-actin double staining revealed the respective spatial distributions of intermingling microtubules and microfilaments (Fig. 2). Long microfilament bundles ran parallel to the cell membrane. Microfilaments were often perpendicular to microtubule arrays. We never detected, in interphase cells, the thick rigid actin cables resembling the stress fibers we found with the same techniques in 3T3 or HeLa cells.

Mitosis

The F-actin network was observed in the cell cortex of endosperm cells during all stages of mitosis as a permanent, deformable cage around the mitotic spindle (Figs. 3–9). As

the chromosomes began to condense at the onset of mitosis, the cortical cytoplasm was progressively emptied of microtubules. Microtubule density increased in the perinuclear region to form the bipolar prophase spindle. At that time, the cortical microfilaments were apparently reorganized. The open spaces observed between crisscrossing bundles were thought to form a mesh or a window, as indicated in Fig. 8 *b*. Some hundred measurements of such meshes per cell were made at different levels, i.e., in the interzone and the polar regions, on four to five cells at each stage of prophase, metaphase, and anaphase (Fig. 8, *c–e*). Within the depth of focus these crisscrossed bundles may be either in contact or in close vicinity. These measurements, along the pole to pole axis ($\pm 30\%$ deviation), gave an image of changes in the orientation and distribution of microfilament bundles during chromosome movements. During mitosis F-actin bundles became aligned more and more parallel to the spindle axis, reflected by an increase in the mesh size, while at the polar region they became tighter (Figs. 3 and 5). Some long thin filaments were detected at the spindle focus, as shown in Fig. 4 *d*. Those bundles were distributed between the poles as in metaphase, with some short filaments converging at the polar regions. Double labeling using rhodamine-phalloidin and monoclonal antibodies to actin indicated similar staining, as seen in Fig. 4, *b* and *c*. Some differences may be due to slight changes in focus. These data, compared with IGSS using video-enhanced microscopy with epi-illumination (Fig. 6 *d*), confirmed the fluorescence results. The images made after immunogold staining suggested microfilament-microtubule interactions, but the limits to the depth of focus did not enable us to establish whether these bundles were actually in contact or merely adjacent at the intersections. An example is given in Fig. 6 *a*, for late anaphase. Gold particles were specifically distributed around the thin microfilament bundles. No particles were seen on microtubules. One end of an actin bundle was often seen in the near vicinity of another bundle or microtubule, in an end to side or Y-shaped connection, closely parallel or juxtaposed to microtubules. Microfilament bundle length often reached 10–20 μm . In cells treated in vivo with microtubule inhibitors (Fig. 7), such as vinblastine (10^{-4} M), the F-actin network persisted (Fig. 7 *c*), while the spindle microtubules disassembled. Un-

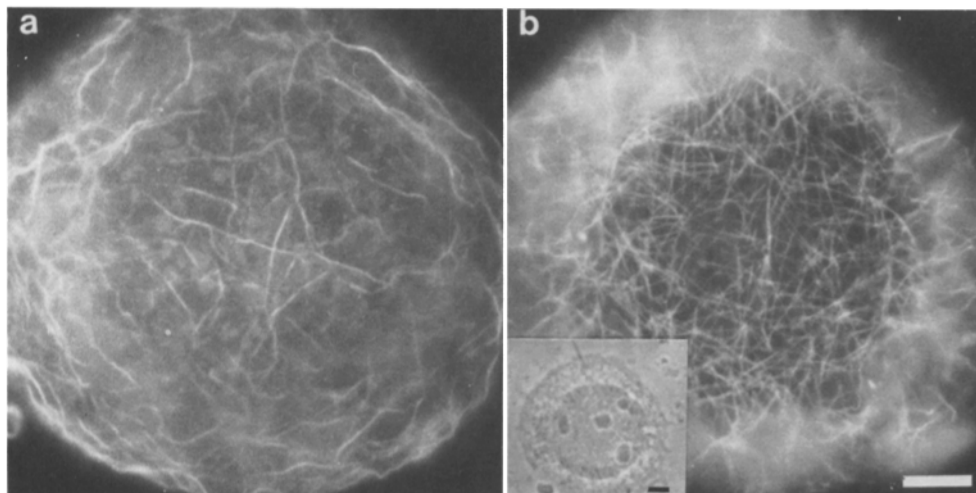


Figure 2. Interphase F-actin (*a*) and microtubule (*b*) distribution identified in the same *Haemanthus* endosperm cell by double fluorescence staining. (*b*) Microtubule arrays revealed by using polyclonal antibodies to brain tubulin/FITC; (*a*) F-actin meshwork, in the cell cortex, labeled by using rhodamine-phalloidin; (*inset*) phase contrast of the cell. Microtubules and microfilament bundles form two distinct networks that intermingle. Some microtubules run parallel to microfilaments. Bars, 10 μm .

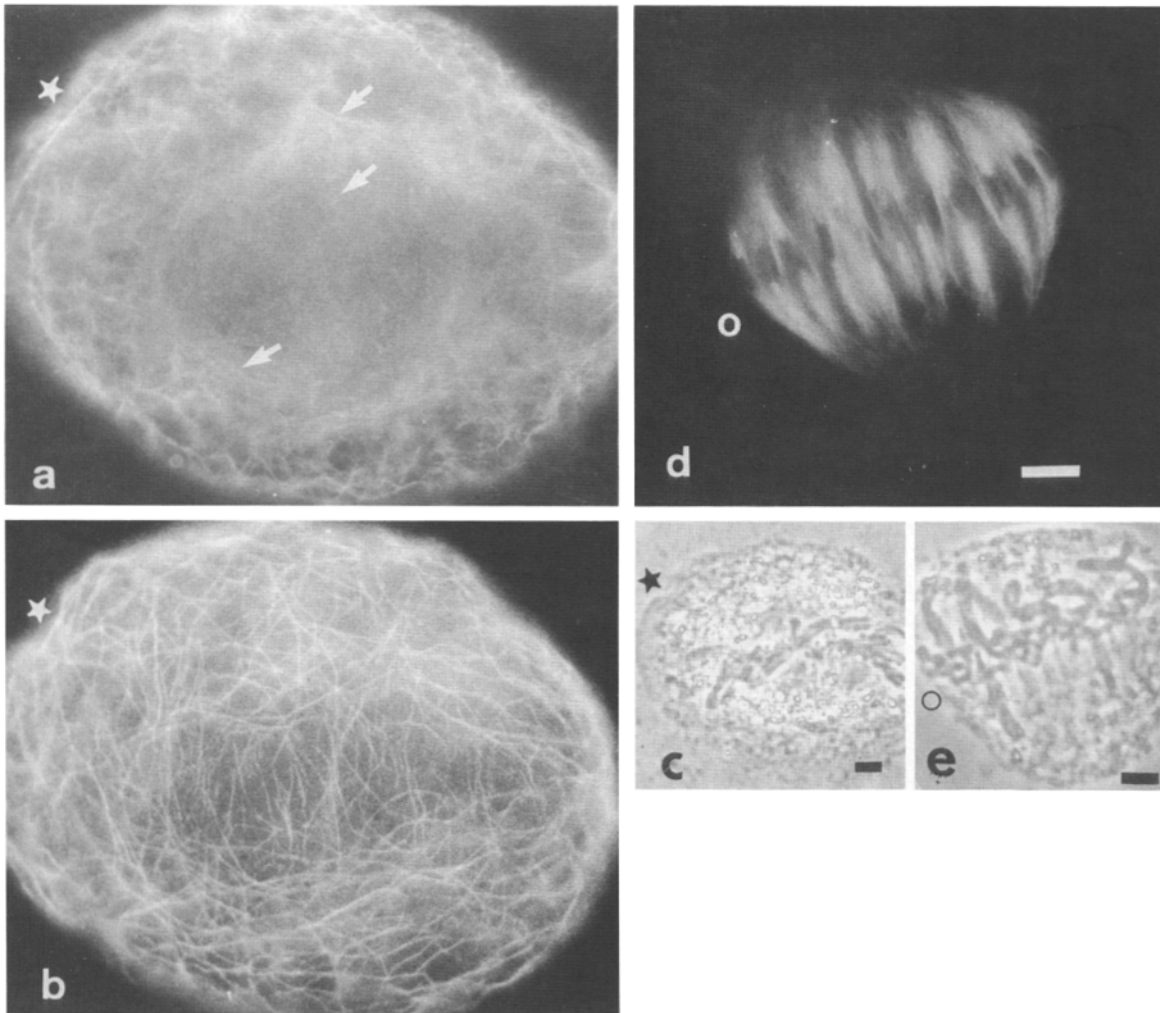


Figure 3. Actin (*a* and *b*) and tubulin (*d*) immunofluorescence in late metaphase *Haemanthus* endosperm cells at the onset of anaphase (cf. *c* and *e* in phase contrast). (*a* and *b*) Actin distribution at two focal planes of the same cell; (*a*) inside the spindle; (*b*) in the cell cortex. The F-actin network remains during mitosis as a loose deformable cage around the mitotic spindle. At the polar regions, microfilaments form a dense network, while on the spindle surface the actin filaments are aligned parallel to mitotic forces. Inside the spindle, only rare microfilaments are detected (*arrows in a*). (*d*) Spindle microtubules labeled with brain tubulin antibodies are observed on a focal plane similar to that of the actin-stained cell in *a*. Bar, 10 μm .

der these conditions anaphase movement was stopped instantaneously while the kinetochores moved back by as much as 2–4 μm (Fig. 7, *a* and *b*). At that time nonkinetochore microtubules were disassembled, while kinetochore fibers remained intact as we reported previously. A comparison of Fig. 7 (treated cell) with Fig. 5 (control) reveals differences in the actin distribution. When the chromosome movement was arrested and polar microtubules depolymerized, a loose network configuration with comparable meshes was observed both at the pole and around the spindle area. During cytochalasin B and D treatments in anaphase, chromosome movements were not inhibited. Even at a concentration of 10 $\mu\text{g/ml}$ cytochalasin D, chromosome velocity was, as for controls, between 1 and 2 $\mu\text{m/min}$. F-actin distribution was highly disorganized. The network configuration was no longer present and only a few thick bundles were detected in the cytoplasm.

Late Anaphase-Telophase Cytokinesis

When kinetochores reached the polar regions, kinetochore fibers were disassembled and new polar microtubules invaded the interzone, as we reported previously (Schmit et al., 1983). These new polar microtubules progressively formed the phragmoplast (Fig. 9 *b*). At that time, the cortical actin network remained as a loose basket around the anaphase-telophase spindle. However, a new dense population of short microfilaments appeared at the equatorial region, which was not yet invaded by polar microtubules (Fig. 9 *c*). Those microfilaments were closely associated with one another and ran parallel to the spindle axis. They formed a wreath just beneath the cortex. This actin wreath appeared when both the chromosome arms and the microtubule ends swung to the spindle surface in late anaphase. When the cell plate was formed, microtubules were denser at the equator

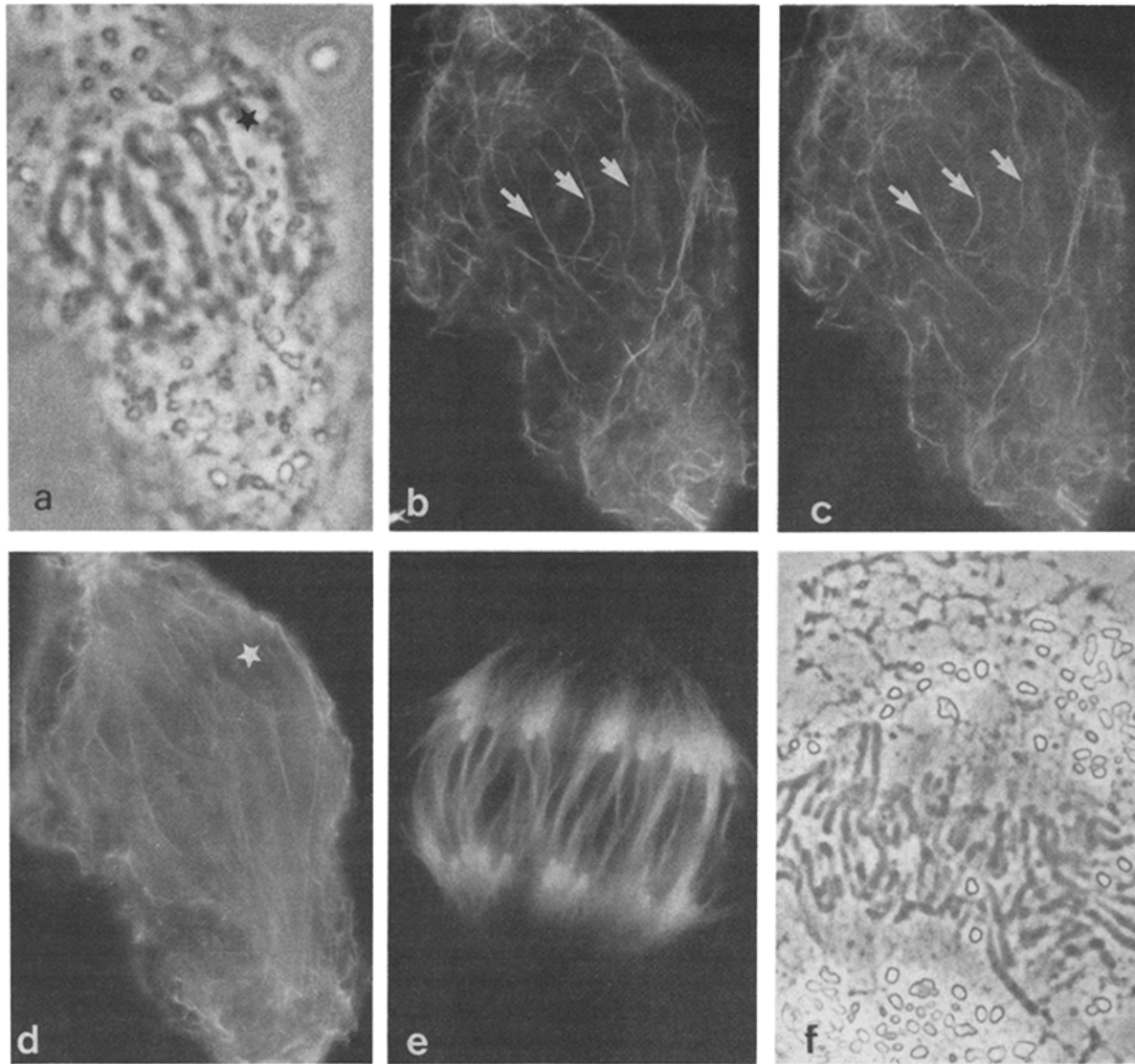


Figure 4. Actin (*b–d*) and tubulin (*e*) fluorescence in mid-anaphase *Haemanthus* endosperm cells. (*b*) Rhodamine-phalloidin staining and (*c* and *d*) anti-actin labeling using monoclonal antibodies in the same cell (phase contrast in *a*). (*b* and *c*) Same focus on the cell cortex, just beneath the plasma membrane. The same actin microfilaments are detected with both phalloidin (*b*) and antibodies (*c*) to actin (*arrows*). (*d*) Focal plane at the spindle. Thin fluorescent bundles are detected between the two poles and are parallel to both kinetochore and nonkinetochore fibers (cf. chromosome distribution seen in *a*). (*e*) Typical spindle configuration at a similar stage of anaphase (*f*), after staining with brain tubulin antibodies. Bar, 10 μm .

and the typical ring-shaped phragmoplast was formed, superimposed on the wreath-shaped actin organization. At the polar regions, around daughter nuclei, cytoplasmic microtubules were reorganized and radiated towards the cell membrane to progressively form the interphase microtubule meshwork. The F-actin bundles again formed a loose cage resembling that previously observed in interphase.

Discussion

Interphase Network

The F-actin network of endosperm cells appears as a well-defined highly organized cytoskeleton which differs from the

actin strands illustrated in allium root-tip cells (Clayton and Lloyd, 1985), tradescantia staminal hairs (Tiwari et al., 1984), or in coleoptile epiderm (Parthasarathy, 1985). It may resemble the actin organization recently reported in other meristematic cells (Seagull et al., 1987). The endosperm actin network also differs strikingly from animal stress fibers (for review see Aubin, 1981) but recalls the highly branched filament network of lung macrophages (Hartwig and Shevlin, 1986). Endosperm F-actin bundles intersect at different angles and their spatial distribution, both in interphase and in mitosis, recalls some morphological and molecular properties of actin gels carried out *in vitro* with added actin-binding proteins (Sato et al., 1985; Pollard and Cooper, 1986). As long actin bundles are running parallel to the cell membrane,

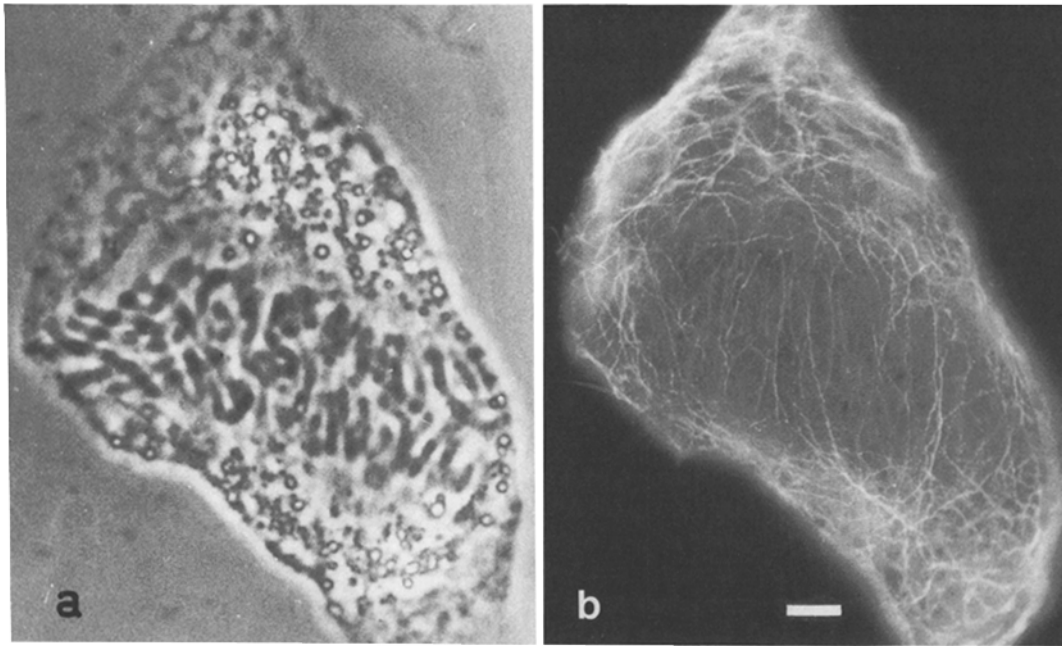
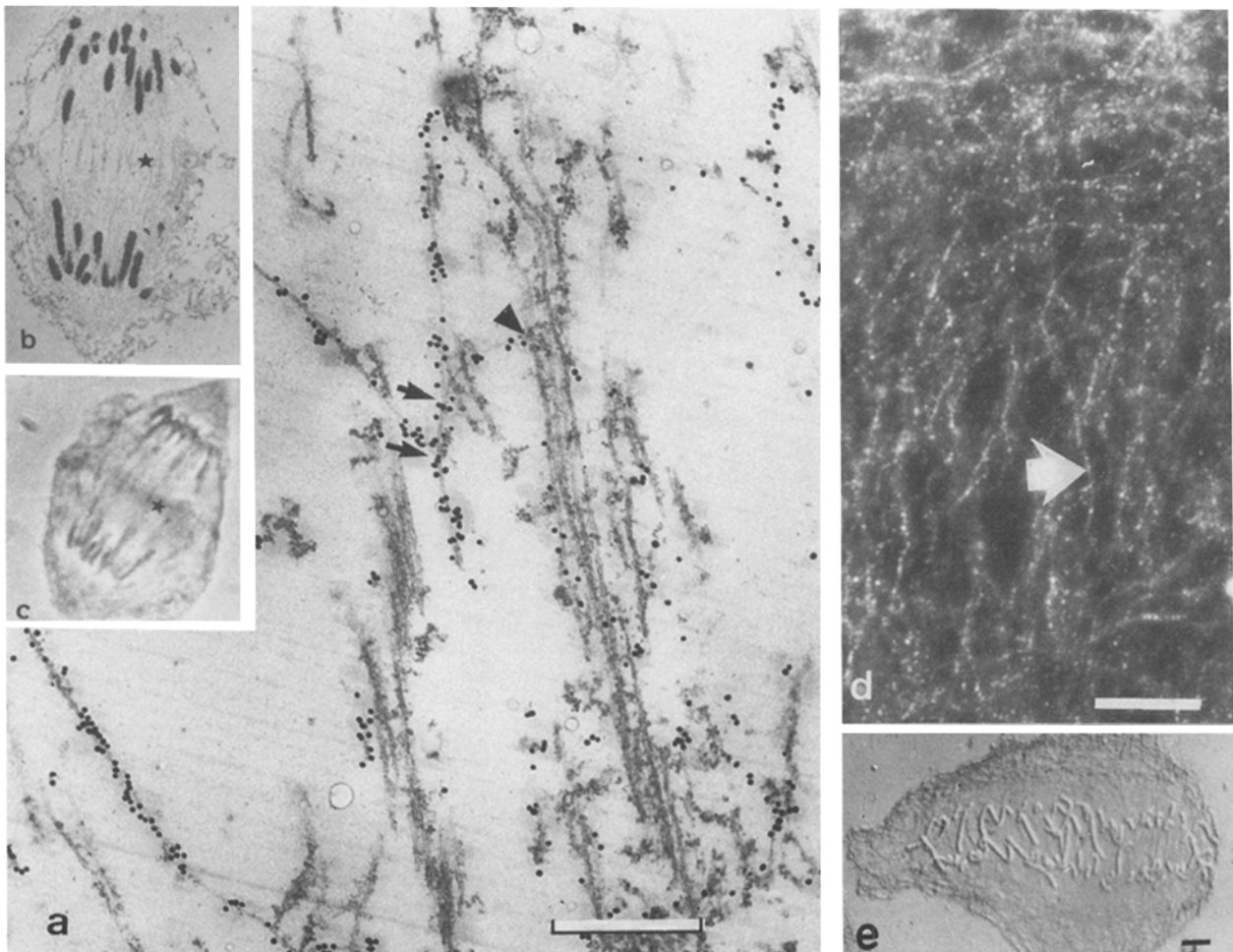


Figure 5. F-actin immunocytochemical labeling in midanaphase *Haemanthus* endosperm cells, using actin monoclonal antibodies (*b*). (*a*) Phase contrast. The cortical F-actin cage around the mitotic spindle is increasingly stretched parallel to the spindle axis, while the meshwork tightens up at the poles. Comparison with Fig. 3 suggests that F-actin arrays are continuously rearranged under the effect of mitotic forces (cf. Fig. 7). This may involve actin cross-linking proteins yet to be identified in this cell model. Bar, 10 μ m.



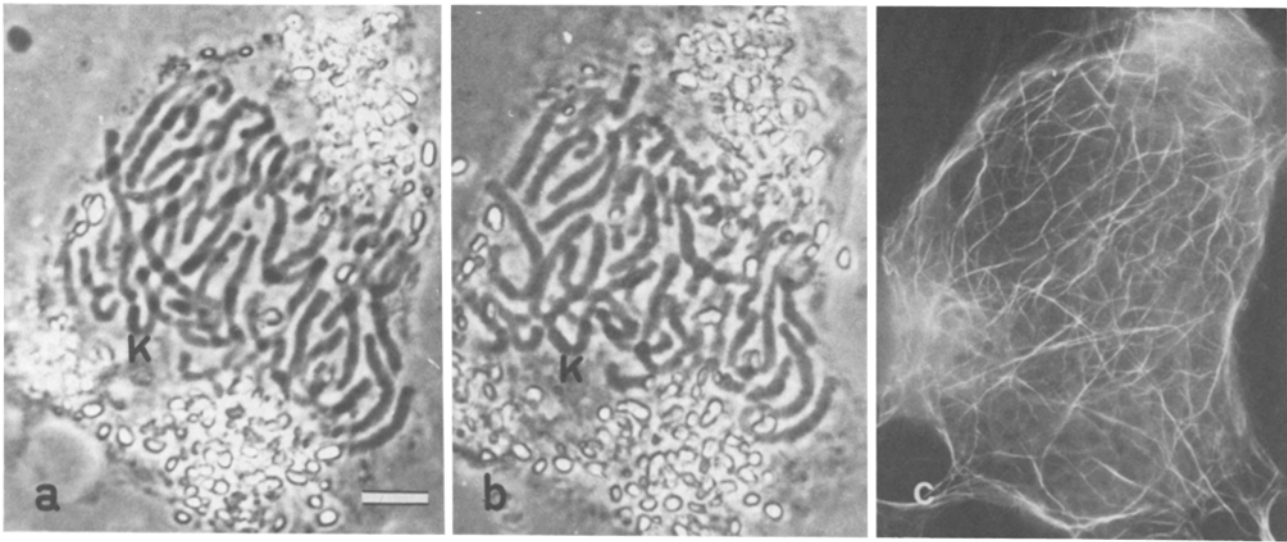


Figure 7. Actin distribution in a vinblastine-treated *Haemanthus* endosperm cell in anaphase. The living cell 1 min after vinblastine 10^{-4} M perfusion (a) and at 20 min after fixation (b). During treatment the chromosome movement is arrested and kinetochores (K) are moving backwards a distance of up to 4 μm . (c) The fixed cell observed after fluorescein phalloidin staining. Comparison with Fig. 4 at a similar stage of anaphase shows drastic changes: when chromosome movement is arrested, the F-actin cage is no longer aligned and microfilament bundles become homogeneously distributed without particular concentration at the poles. Kinetochore microtubules are already disassembled, as reported previously (see text). Bar, 10 μm .

the question is whether or not, in higher plant cells, microfilaments terminate at the plasma membrane, or are linked to it, as described in animal cells (Pollard and Korn, 1973; for review see Schliwa, 1981). No plant proteins associated with cytoplasmic F-actin have been identified so far. Microtubules penetrate the actin network and often run parallel to actin filaments, as also described for fungi (Hoch and Staples, 1983), suggesting structural interactions. These interactions could be mediated by microtubule-associated proteins, as reported in vitro (Griffith and Pollard, 1982; Pollard et al., 1984). Plant microtubule-associated proteins, however, have yet to be characterized in higher plants.

Persistence and Dynamics of the Cortical Actin Network around the Mitotic Spindle

In endosperm cells, the actin network persists throughout the mitosis process as a deformable cage around the mitotic spindle (Schmit and Lambert, 1985b, 1986). Our data are the first report of a permanent and dynamic actin cytoskeleton in the cortical cytoplasm of dividing higher plant cells. In various meristematic cells a majority of the cortical actin bundles seen during interphase with rhodamine-labeled phalloidin was no longer detected during cell division. It has been suggested that these microfilaments disassembled at the onset of mitosis (Tiware et al., 1984; Clayton and Lloyd, 1985;

Seagull et al., 1987). However, actin was detected in the preprophase band (Palevitz, 1987). We identified the cortical actin in mitotic endosperm cells by using actin monoclonal antibodies and rhodamine-phalloidin. Antibodies gave the finest resolution with immunofluorescence or immunogold staining. The specificity of the antibody labeling was demonstrated by Western blotting and on the ultrastructure with gold particles (Fig. 6 a). Our data indicate that F-actin filaments, like microtubule arrays, are permanent and dynamic components which are constantly rearranged and/or reorganized during mitosis and cytokinesis. One of the main questions is whether the microfilaments are redistributed as a result of an active independent stretching process, or as a consequence of microtubule bipolar redistribution into the mitotic spindle. Close observation of the different stages of prophase, which lasts 40 to 50 min in endosperm cells, shows that microtubules and F-actin rearrange in a complementary fashion. Our data do not yet reveal which cytoskeletal element, microtubules or microfilaments, reorganizes first and induces the future mitotic polarity at the onset of cell division. However, our observations do indicate that the spatial distribution of microtubules and microfilaments is coordinately regulated during mitosis. In this context, some evidence was recently given that actin is involved in centrosome positioning and motility in animal cells (Euteneuer and Schliwa, 1985). During anaphase chromosome

Figure 6. Details of anaphase–telophase in *Haemanthus* endosperm cells. (a) Electron microscopy of 20 nm immunogold staining using actin monoclonal antibodies. Thin F-actin bundles are selectively labeled with gold particles. No staining is seen on microtubules. Microfilaments are seen both on the surface and inside the phragmoplast. They intersect at variable angles (arrows), and often run parallel to microtubules or seem to connect end to side with them (arrowheads). (b) The section at low magnification. (c) The cell in phase contrast after immunolabeling. Detection of actin IGSS with video-enhanced microscopy. (d) Detail of the cortex in an anaphase cell observed in epillumination: gold particles are visible along a thin microfilament bundle (arrow), as in electron microscopy. b: phase contrast. Bars: (a) 0.5 μm ; (d and e) 10 μm .

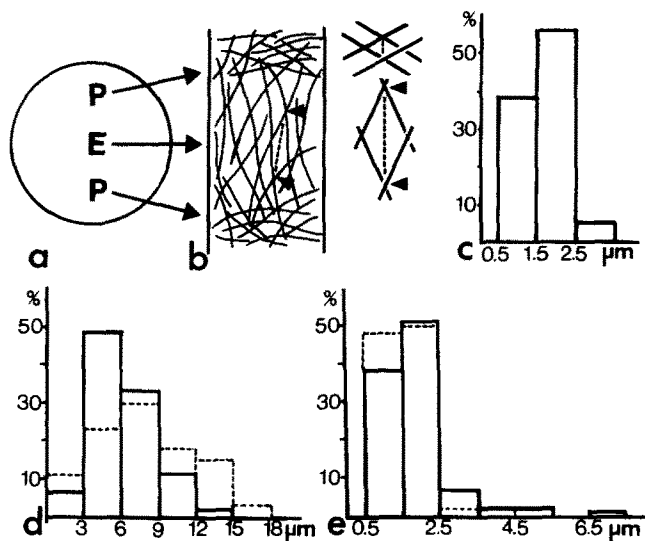


Figure 8. Measurements of progressive changes in the mutual alignment of microfilament bundles during mitosis. As an indicator, the distance between crisscrossed bundles as seen in *b* (dotted line between two arrowheads), was measured parallel to the pole to pole axis. This was considered a network mesh. As indicated on the schematic representation (*b*), microfilament bundles may be in actual contact or in close vicinity at these converging points. (*a*) Representation of a cell. *P*, polar region; *E*, interzone close to the equator. (*b*) Schematic drawing of the F-actin cage around the mitotic spindle, with different microfilament spatial arrangements at the poles and the interzone. Details on the right indicate changes in mesh size parallel to the spindle axis. (*c*) Average mesh size in prophase. No significant variations were detected between *P* and *E* regions. (*d*) Average mesh size in the interzone during metaphase (solid line) and anaphase (dotted line). Increases in mesh size indicated changes in microfilament orientation. They became progressively parallel to the spindle axis, particularly during poleward chromosome movement. (*e*) Average mesh size at the polar regions, in metaphase (solid line) and anaphase (dotted line). In metaphase and prophase the mesh size is comparable. The meshes became tighter in anaphase.

movements the F-actin network close to the spindle surface is highly deformed. Mesh size increases constantly in the same axis as the spindle elongation. In endosperm cells, anaphase transport of chromosomes lasts an average of 20–30 min. Therefore, slow mitotic forces are applied regularly over a long time and may force the F-actin bundles to rearrange on the spindle surface, forming an elastic basket. This could be explained by dynamic cross-links that could be displaced and/or assemble/disassemble in such mechanical conditions, as suggested *in vitro* by Sato et al. 1985). This would result in an increase in the mesh size of the actin network, as we describe in this report. However, we cannot rule out that such structural changes might be related to passive clearing of transverse microfilaments from the midzone during the mitotic process. Differences in the distribution of actin cross-linking proteins (α -actinin for example) at the cortex and the spindle surface of the actin network may be responsible for variable mechanical properties. Local cytoplasmic calcium gradients in mitotic cells (Izant, 1983; Keith et al., 1985; Vantard et al., 1985) might also be involved in the regulation of these events. In higher plant cells this elastic cage around the mitotic spindle may play an important role in the control of spindle shape and elongation, as well as its

orientation. It is suggested that actin bundles, associated or not with other as yet unidentified proteins are involved in fast transport as well as saltatory motion of vesicles, often observed on the spindle surface and in the cortex of endosperm cells.

Actin in the Mitotic Apparatus

Electron microscopic observations of glycerinated *Haemaphysalis* cells indicate the presence of actin filaments in the kinetochore fibers (Forer and Jackson, 1979). Similar conclusions have been drawn for the mitotic spindles of animal cells (Sanger, 1975; Cande et al., 1977; Sanger and Sanger, 1980). These data are still debated because the preparative techniques used might have induced changes in microfilament distribution (Schroeder, 1976). They suggest, however, that actin has an active role in chromosome movement. In our observations rare and thin actin bundles were sometimes detected between the poles, at the spindle level, and in the polar regions. These bundles were only revealed by monoclonal antibodies to actin. With phallotoxins, we, like other authors, failed to detect them in both plant (Pesacreta et al., 1982; Clayton and Lloyd, 1985) and animal (Barak, 1981; Forer, 1984) cells. However, a spindlelike actin pattern has recently been described in allium mitotic cells (Seagull et al., 1987). These different responses suggest that if actin is present in the spindle *in vivo*, it may be as a different isoform from cytoplasmic actin. Our data on endosperm cells do not rule out actin as an active component of the mitotic spindle. Using improved methods to preserve actin filaments for electron microscopy, Pollard et al. (1984) found no long actin filaments associated to the mitotic spindle of HeLa cells, whereas a large number of short, actinlike filaments were detected. In endosperm cells, as in animal cells, perfusing cytochalasin B and D at a concentration of 10 $\mu\text{g}/\text{ml}$ did not affect chromosome transport, as has also been reported for other mitotic plant cells (Palevitz, 1980). So the potential role of actin in chromosome movement remains open and calls for further investigation.

Late Anaphase-Telophase Cytokinesis

It seems that actin is involved both in animal and plant cytokinesis, although the mechanisms are different. During the cleavage furrow in animal cells the microfilaments run perpendicular to the spindle axis (for review see Schroeder, 1976), whereas in endosperm cells, we detected an equatorial wreath of short microfilaments parallel to the spindle axis. Our data suggest that a new population of short actin bundles assembles in the equatorial region of the mitotic spindle when the kinetochores reach the pole and when polar microtubules progressively invade this interzone (Schmit and Lambert, 1985a). However, it cannot be ruled out that microfilaments may be broken and pushed passively towards the equator by elongating polar microtubules. Our present results indicate that this equatorial actin precedes the complete development of the phragmoplast ring, and might be considered as the first marker of the cell plate. In meristematic cells an intense actin staining was reported in the phragmoplast region (Clayton and Lloyd, 1985; Seagull et al., 1987). It is also noteworthy that actin has recently been described in the preprophase band, which is in alignment with the future cell plate (Palevitz, 1987). Actin and associated proteins could help provide an active force for the mi-

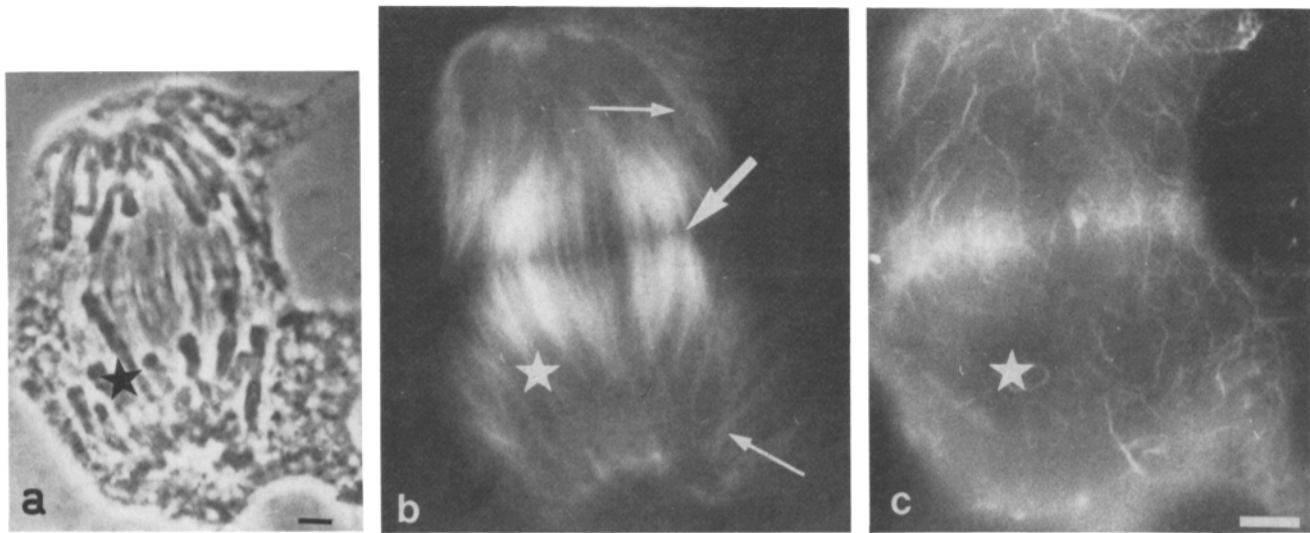


Figure 9. Late anaphase–telophase *Haemanthus* endosperm cells. Tubulin (*b*) and actin (*c*) fluorescence double labeling. (*a*) The cell in phase contrast. (*b*) New polar microtubules assembled in late anaphase (*thin arrows*). They invade the interzone, leading to phragmoplast formation (*thick arrow*). (*c*) Microfilaments are mostly concentrated at the equator. Numerous short microfilaments run parallel to the spindle axis, forming a wreath that heralds the future phragmoplast ring. Bar, 10 μm .

cro-tubule-dependent transport of vesicles towards the equator during cell plate formation. Whether myosin is involved in the nonkinetochore movement of particles, as suggested by the transport of myosin-coated beads (Sheetz and Spudich, 1983) remains unresolved. In vitro, electron microscopy observations suggested microfilament–microtubule interactions (Griffith and Pollard, 1982). During the late anaphase of endosperm cells, both chromosome arms and interzonal microtubules are moving sideways, as if drawn by the cell cortex (for review see Bajer and Mole-Bajer, 1982, 1986). These events are surprisingly concomittant with the wreath of actin at the equator. Further investigations are needed to define the cross-links, their nature and function, and the role of actin in all these cytokinetic dynamics. Our data enable us to postulate that actin, in association with microtubules, may play an important role in the structural integrity of the cell cortex, in the dynamics of vesicle transport on the spindle surface, and also in the establishment of the morphogenetic programs of higher plant cells.

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Note Added in Proof. Since this paper was accepted for publication, another article reported the presence of an actin network throughout the cell cycle of carrot cells in culture (Traas, J. A., J. H. Doonan, D. J. Rawlins, P. J. Shaw, J. Watts, and C. W. Lloyd. 1987. *J. Cell Biol.* 105:387–395).

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