Microfilaments: Dynamic Arrays in Higher Plant Cells

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Abstract. By using fluorescently labeled phalloidin we have examined, at the light microscope level, the three-dimensional distribution and reorganization of actin-like microfilaments (mfs) during plant cell cycle and differentiation. At interphase, mfs are organized into three distinct yet interconnected arrays: (*a*) fine peripheral networks close to the plasma membrane; (*b*) large axially oriented cables in the subcortical region; (*c*) a nuclear "basket" of mfs extending into the transvacuolar strands. All these arrays, beginning with the peripheral network, disappear at the onset of mitosis and reappear, beginning with the nuclear basket, after cytokinesis. During mitotic and cytokinetic events, mfs are associated with the spindle and phragmoplast. Actin staining in the spindle is localized between the chromosomes and the spindle poles and changes in a functionally specific manner. The nuclear region appears to be the center for mf organization and/or initiation. During differentiation from rapid cell division to cell elongation, mf arrays switch from an axial to a transverse orientation, thus paralleling the microtubules. This change in orientation reflects a shift in the direction of cytoplasmic streaming. These observations show for the first time that actin-like mfs form intricate and dynamic arrays in plant cells which may be involved in many as yet undescribed cell functions.

THE components, organization, and dynamics of actin microfilament (mf)[1] arrays during animal cell growth and development have been extensively studied. While the many specifics are far too detailed for discussion here, changes in the organization of mfs have been documented during cell migration (Gabbiani et al., 1984), cell spreading (Kaiho and Sato, 1978; Connolly et al., 1981), changes in cell shape (Aubin et al., 1983), and cell differentiation (Wang and Taylor, 1979; Scher et al., 1983; Nagata and Ichikawa, 1985). From studies such as these we know that mfs in animal cells form very dynamic, three-dimensional networks which interact with many cell components to modulate numerous cell processes.

In plant cells, the study of actin-like mfs has been restricted primarily to an analysis of their role in cytoplasmic streaming. Using the large internodal cells of the algae *Nitella* and *Chara*, much information has been accumulated concerning the chemical nature of filament bundles, the organization of filaments within the bundles and the regulation of streaming (for review see Hepler and Palevitz, 1974; Tiwari et al., 1984; Jackson 1982). There have been a few reports which describe actin mfs that are not involved in streaming. Using decoration with heavy meromyosin, Forer and Jackson (1979) and Forer et al. (1979) reported actin filaments in the mitotic spindle of *Haemanthus*. Microfilaments have also been reported associated with the cortical microtubules (Seagull and Heath, 1980; Mueller and Brown, 1982; Traas, 1984; Brown 1985). No chemical evidence (i.e., binding with heavy meromyosin) exists to prove that these are indeed actin.

The study of actin mf arrays in plant cells has been facilitated by the use of fluorescently labeled phallotoxins. These probes specifically bind filamentous actin (Wulf et al., 1979). Their use has greatly increased our knowledge of the three-dimensional organization of actin arrays in plant cells (Parthasarathy et al., 1985; Parthasarathy 1985; Clayton and Lloyd, 1985; Pesacreta et al., 1982). We now know that actin arrays form complex patterns throughout the cytoplasm of many highly elongate plant cells (Parthasarathy et al., 1985). The dynamics involved in establishing these arrays however remains totally unknown. Clayton and Lloyd (1985) have examined the various actin configurations found during the cell cycle but have made no attempt to describe in detail the reorganization of mf arrays.

Using cycling plant cell suspension cultures, we have monitored in detail the reorganization of mf arrays as the cells progress from interphase, through mitosis and cytokinesis, back to the interphase configuration. In addition, by inducing a conversion in growth kinetics from cell division to cell elongation, we have described the changes in mf arrays which accompany this form of differentiation.

Our results confirm that mf arrays in plant cells are highly complex and dynamic. They consist of several interconnected arrays which can be distinguished using organization and location within the cell as criteria. The observed reorganization of mfs during the cell cycle supports the hypothesis that the nuclear region may play a significant role in nucleating and/or organizing actin networks.

1. *Abbreviations used in this paper*: 2,4-D, 2,4-dichlorophenoxyacetic acid; GA, gibberellic acid; mf, microfilament; Rd-phal, rhodamine-conjugated phalloidin.

Materials and Methods

Culture Methods

Alfalfa (*Medicago sativa* L.) cell suspension cultures were maintained in B5 medium (Gamborg et al., 1968) supplemented with 1 mg/liter 2,4-dichlorophenoxyacetic acid (2,4-D), and regularly subcultured at 3.5-d intervals, 10 ml of suspension culture in 50 ml of fresh medium. Cultures were maintained at 26–28°C with an 8-h dark/16-h light photoperiod on rotary shakers at 160 rpm. *Vicia hajastana* cultures were maintained under the same conditions but were subcultured into Eriksson's medium (Eriksson, 1965) containing 0.5 mg/liter 2,4-D.

Induction of Cell Differentiation

When maintained under the above conditions, the cell cultures consist primarily of small, rapidly dividing cells. By washing the cultures three times (centrifuging at 500 rpm for 4 min) with the respective hormone-free medium, the 2,4-D can be removed and replaced by gibberellic acid (GA). Parameters of cell division and growth are altered (Simmonds et al., 1983) so that both Alfalfa and *Vicia* cultures differentiate into large, elongated, nondividing cells.

Microfilament and Microtubule Staining

F-actin localization was carried out according to the basic staining method of Parthasarathy (1985). Cells grown in the presence of 2,4-D were layered onto coverslips that had been previously cleaned with chromic acid, rinsed, and layered with poly-L-lysine (Sigma Chemical Co., St. Louis, MO) (800 kD; 1 mg/ml in water). Cells were fixed in 1.5% paraformaldehyde in 0.1 M potassium phosphate buffer (PO_4 buffer), pH 7.0 for 15 min, rinsed twice in PO_4 buffer (3 min each rinse), then stained with 3.3 μM rhodamine-conjugated phalloidin (Rd-phal) (Molecular Probes, Inc., Junction City, OR) in 0.1 M PO_4 buffer for 20 min, followed by two rinses in PO_4 buffer (3 min each) and then 2 min in Hoechst No. 33258 (1 μg/ml in buffer) to stain nuclear material. Cells were rinsed in PO_4 buffer for 3 min and then mounted in glycerol/PBS (1:1) containing 0.1% phenylene diamine. Large cells, grown in the presence of GA for 3 or more days were stained as above with the addition of 0.02% Triton X-100 to the fixative. The level of autofluorescence and degree of nonspecificity of the Rd-Phal binding were determined. Some cells were prepared for microscopy but only stained with Hoechst. Competition experiments were done by inserting into the standard protocol a 1-h incubation in 3.3 mM phalloidin just before staining with Rd-Phal.

Double staining of microtubules and microfilaments in Alfalfa cells was carried out by layering cells onto coverslips as described above. Both 2,4-D and GA cells were fixed in 3% paraformaldehyde in 0.1 M Pipes buffer containing 0.02% Triton X-100 for 1 h. Cells were rinsed three times (3 min each) in Pipes, then enzyme treated using 1% cellulase (Worthington Biochemical Co., Mississauga, Ontario, Canada), 1% pectinase (ICN Nutritional Biochemicals, Cleveland, OH), 1% hemi-cellulase (Corning Biosystems, Corning, NY) for Alfalfa or 0.5% cellulase, 1.0% hemi-cellulase, 0.5% pectinase (for *Vicia*) in Pipes buffer for 10 min followed by three rinses (3 min each). Cells were postfixed in 1% glutaraldehyde in PBS for 8 min followed by two rinses in 0.1% $NaBH_4$ (3 min each rinse) and two rinses in PBS. Rat monoclonal antibody to yeast tubulin (Cedarlane Laboratories, Hornby, Ontario) was layered on coverslips for 45 min. Coverslips were then washed in PBS three times and incubated for 45 min with fluorescein-conjugated secondary antibody (rabbit anti-rat, Miles Laboratories, Rexdale, Ontario). Cells were then stained with Rd-Phal (as above) for 20 min followed by staining DNA with Hoechst No. 33258 (1 μg/ml in PBS) for 2 min. After rinsing two times (3 min each) with PBS, coverslips were mounted in glycerol/PBS containing phenylene diamine.

Cells were examined immediately after staining using a Zeiss photomicroscope fitted with epifluorescence optics. A neuofluor 40× oil immersion lens (NA 0.9) was used for routine observations and photography. Experiments were repeated at least three times with counts being done on a minimum of 300 cells per experiment. Photographs were made on Ilford XP1-400 film.

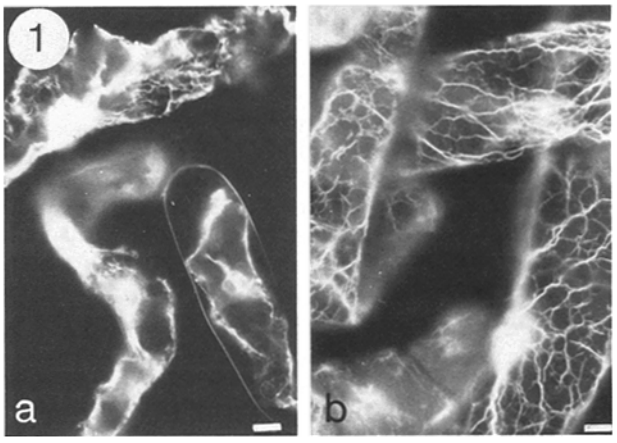

Figure 1. (*a*) Actin staining of elongated cells using standard procedure. Most cells show severe shrinkage. (*b*) Actin staining of mfs in elongated cells permeabilized with 0.02% Triton X-100 during fixation. Bars, 10 μm.

Results

Actin Microfilament Staining of Cultured Plant Cells

When grown in the presence of 2,4-D, both Alfalfa and *Vicia* suspension cultures show coupled growth and division and consist primarily of relatively small (40–80 μm in length), rapidly dividing cells. Both cultures show virtually identical mf rearrangements during the cell cycle therefore all illustrations are representative of events in both Alfalfa and *Vicia*.

Using a modification of Parthasarathy's procedure (1985), we were able to obtain staining of mf patterns in all cells growing in medium containing 2,4-D. However, cells cultured with GA differentiate into highly elongated cells with a very large central vacuole. When these cells are stained with the above procedure, >80% of the cells are severely shrunken (Fig. 1 *a*) although actin staining is visible. By permeabilizing the plasma membrane with a small amount of Triton X-100 in the fixative, we are able to reduce the shrinkage seen in very large cells without disrupting mf staining. Approximately 90% of cells fixed in this manner show little or no shrinkage (Fig. 1 *b*).

These culture cells exhibit minimal autofluorescence when viewed using the filter combinations used to reveal rhodamine fluorescence (Fig. 2 *h*). Competition experiments using unconjugated phalloidin before staining with Rd-Phal greatly reduced the level of Rd-Phal binding in cells. When cells pretreated with phalloidin are photographed at exposure times equal to the longest time of normally prepared cells, no filamentous staining was evident and fluorescence was greatly reduced (see, e.g., Figs. 4 *m* and 5 *j*).

The staining of mf arrays in plant cells using Rd-Phal is somewhat labile. If cells are observed immediately after staining, images such as those reported here are readily observed. If however cells are observed 24 h after staining they exhibit a diffuse fluorescence throughout the cytoplasm and vacuoles (data not shown). Fine fibrillar arrays in the cortical cytoplasm and spindles are no longer detected. The increase in diffuse fluorescence and apparent loss of fine arrays of filaments is not related to photobleaching of the fluorochrome since cells stored in total darkness and not observed previously are also different in this way.

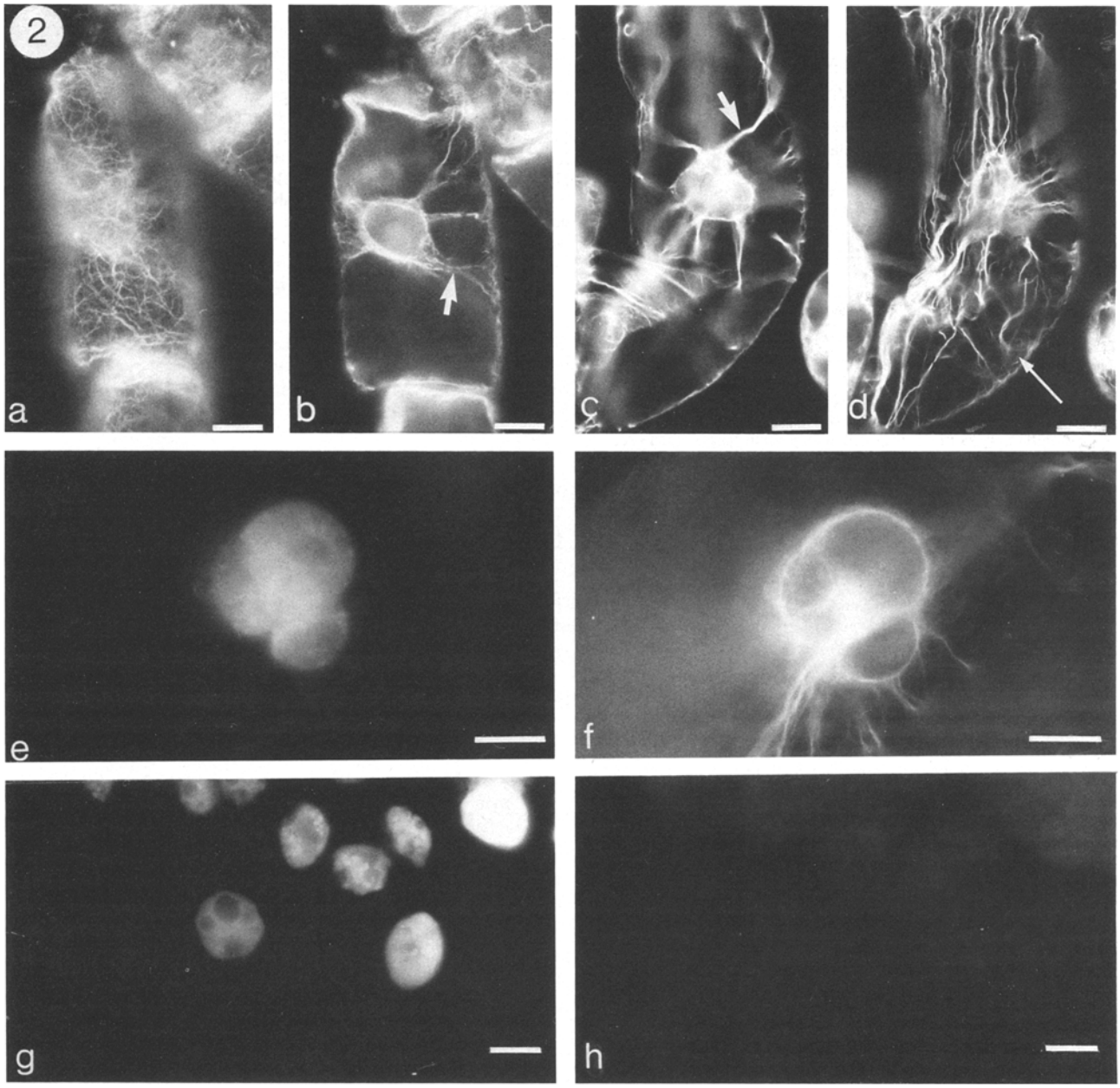

Figure 2. Interphase mf arrays in small (*a* and *b*) and elongate (*c* and *d*) cells. In a small cell: (*a*) randomly arrayed cortical mfs are seen, and no subcortical cables are seen; and (*b*) median focal plane shows the nuclear basket and transvacuolar strands (*arrow*). In a larger cell: (*c*) the nuclear basket and transvacuolar strands (*arrow*) continue evident; and (*d*) subcortical cables form a prominent feature. The fine cortical network is visible only where the focal plane permits (*long arrow*). (*e*) Hoechst stain of a multi-lobed nucleus. (*f*) Microfilaments of the nuclear basket follow the contours of the nuclear lobes (compare with *e*) and extend outward into the cytoplasm to merge with the cables of the transvacuolar strands (*arrow*). (*g* and *h*) Control cells prepared using standard staining procedures except that Rd-phal was not applied. (*g*) Hoechst stain reveals nuclei of several cells. (*h*) Minimal autofluorescence evident when these cells were photographed using exposure times equivalent to Rd-phal–stained cells (i.e., 20 s). Bars, 10 μm.

Spatial Arrangement of Microfilament Arrays during Interphase

Microfilament arrays of cultured plant cells can be divided spatially into three regions: (*a*) a fine network of randomly arrayed mfs found in the cortical cytoplasm immediately adjacent to the plasma membrane (Fig. 2 *a*); (*b*) bundles of mfs found in the subcortical region of the cytoplasm adjacent to the central vacuole, a prominent feature in plant cells as in Fig. 2 *d* (these bundles may be ordered either parallel to the long axis of the cell or transverse to the long axis [see Fig. 6, *b* and *c*]); (*c*) the "basket" of mfs that surrounds the nucleus and extends into the transvacuolar strands of cytoplasm con-

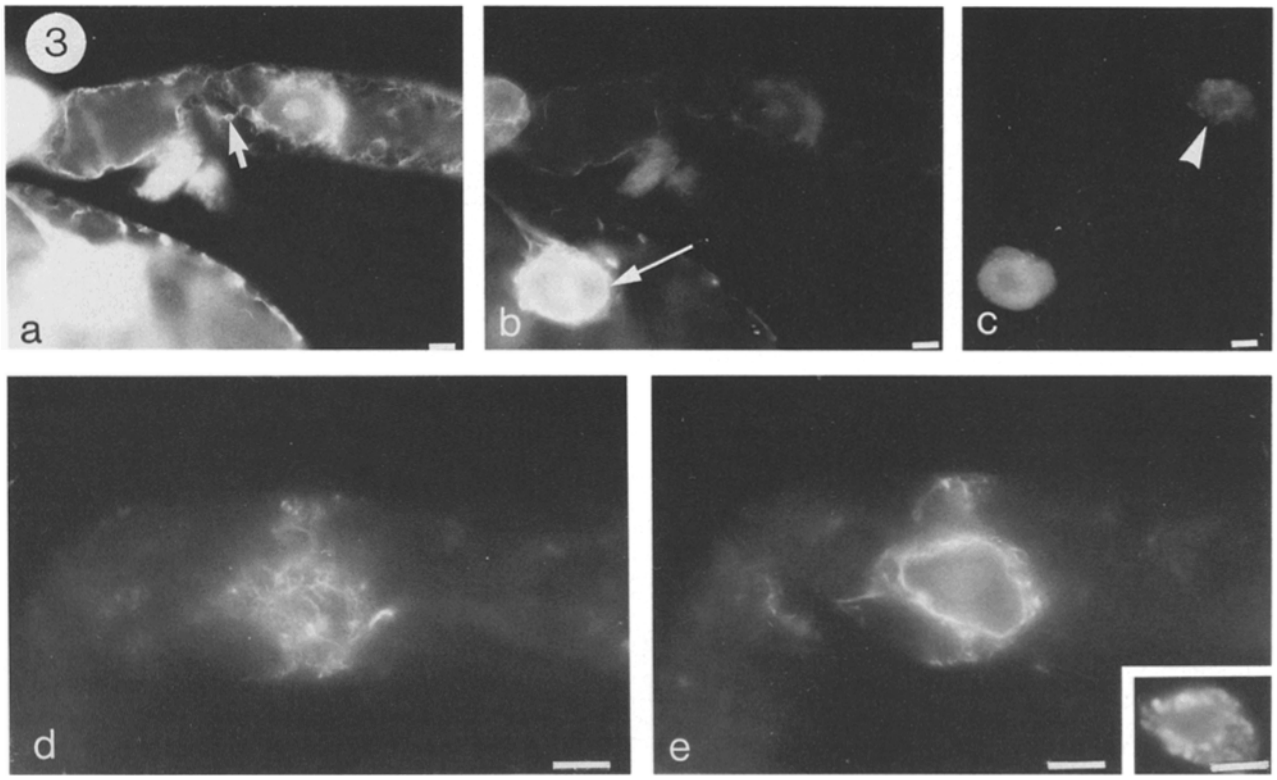

Figure 3. During prophase, interphase mf arrays disappear. (*a*) Cortical and subcortical networks as well as transvacuolar strands (*arrow*) break up. The nuclear basket loses the distinct outline of interphase (compare with Fig. 2 *b*). *a* is printed to enhance the mf arrays still present in a prophase cell. *b* is printed to show nuclear basket of the adjacent interphase cell (*long arrow*). Note faint staining of mf arrays in the prophase cell above. (*c*) Hoechst stain to show chromosome condensation in the prophase cell (*arrowhead*). (*d* and *e*) Detail of nuclear region in a prophase cell. (*d*) Surface focal phase of nucleus shows that most mfs are gone except those associated with the nucleus. (*e*) Transvacuolar strands break up and the nuclear basket, shown in optical cross section, becomes diffuse. (*Inset*) Hoechst stain of nucleus. Bars, 10 μm.

necting the nucleus with the subcortical and cortical layers of cytoplasm (Fig. 2, *b* and *c*). The mfs of the nuclear "basket" may be associated with the nuclear envelope as they can be seen to follow the indentations and depressions of a lobed nucleus (Fig. 2, *f* and *g*). These three mf patterns form a continuous network in the cell. The spatial separation between cortical and subcortical layers can be difficult to detect, particularly in large cells with a thin cytoplasmic layer between the plasma membrane and vacuolar membrane.

Continuous plant cultures have two types of cells: small, isodiametric, rapidly dividing cells which grow in files as well as a subpopulation of larger, cylindrical shaped cells which grow in files or as isolated cells.

During interphase, the mf pattern in small cells consist of randomly arrayed cortical mfs (Fig. 2 *a*) which merge directly into the cables of the transvacuolar strands (Fig. 2 *b*). Subcortical bundles of mfs are absent from these small cells. Axial cytoplasmic streaming, usually associated with such bundles, is not seen in these cells (data not shown).

The interphase pattern of mf arrays in the subpopulation of larger cells is somewhat different. The cortical layer of cytoplasm shows only a very fine network (Fig. 2 *d*). In the subcortical region, large bundles of brilliantly staining mfs make up a complex pattern extending the length of the cell. These cables merge with those of the transvacuolar strands and come to a focal point at the nucleus (Fig. 2, *c* and *d*). When living cells of a similar size are observed with phase-contrast microscopy, they exhibit rapid and complex streaming in patterns which approximate the staining patterns of the mf cables.

A focal plane showing the surface of the nucleus in either large or small cells shows numerous mfs encircling the nucleus to form an intricate, fibrillar "basket" of mfs that extends to, or originates from, the nuclear envelope (Fig. 2). These mfs of the nuclear region then merge with the brightly staining bundles of the transvacuolar strands (Fig. 2, *b* and *c*).

Prophase Microfilament Disappearance

Both Alfalfa and *Vicia* cultures show a complex redistribution of mfs during mitosis and cytokinesis. Determination of the stage of mitosis is accomplished by staining the condensing chromatin with Hoechst No. 33258. Although mitosis in plant cells is not easily synchronized, it is possible to assign a probable sequence to these events by correlating chromatin condensation and mf distribution in large numbers of cells.

As prophase proceeds, the mf networks typical of interphase fade into a dimly staining pattern which virtually disappears by the end of prophase (compare Figs. 2 *d* and 3 *b*). In early prophase (as shown by Hoechst staining in Fig. 3

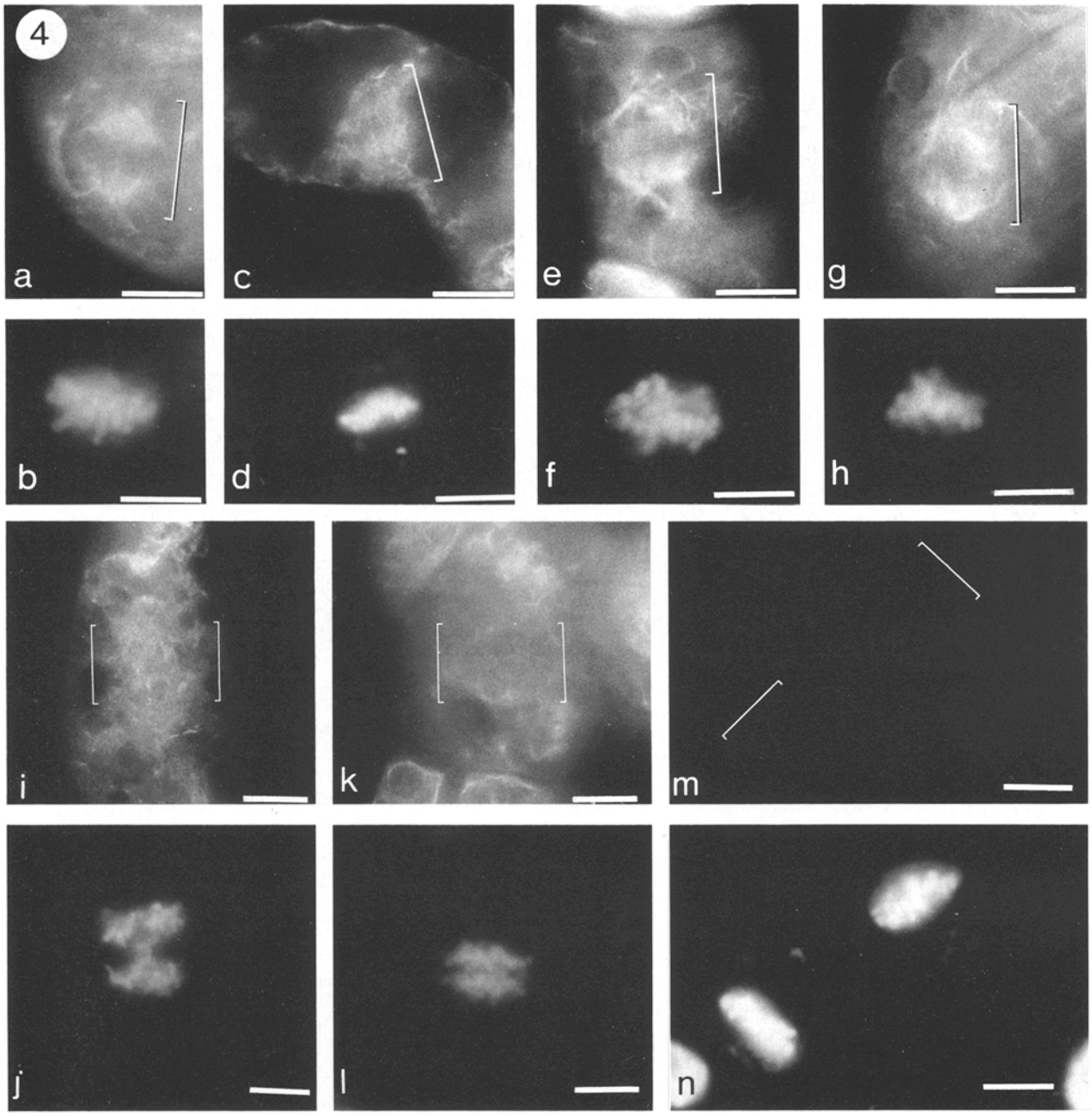

Figure 4. During metaphase, a distinct spindle-like staining pattern (*bracket*) can be seen in most cells (*a*, *c*, *e*, and *g*) having chromosomes arranged in a metaphase plate as shown by Hoechst staining (*b*, *d*, *f*, and *h*). During anaphase (*i–l*) very faint fibrillar staining (bracketed areas in *i* and *k*) is associated with the spindles. (*j* and *l*) Hoechst stained chromosomes show separation of daughter nuclei. (*m* and *n*) Competition experiments indicate that metaphase cell preincubated with phalloidin before staining exhibits no fluorescence above autofluorescence levels (*m*). Hoescht staining shows two cells in metaphase (*n*). Bar, 10 μm.

c, and the inset in Fig. 3 *e*), the fine cortical network and subcortical cables are disassembled (Fig. 3 *a*). Thereafter the cables of the transvacuolar strands break up and the nuclear basket loses its fibrillar stain to become a diffuse glow (Fig. 3 *a*). The intensity of staining diminishes markedly during prophase. Fig. 3 *a* shows the remaining mf networks. Note that the interphase nucleus of the cell at the lower left is brilliant compared to the prophase nucleus. When this same micrograph is printed to bring out the detail of the interphase nucleus (i.e., with the same exposure as in Fig. 2, *a–d*) the staining of the prophase cell is nearly invisible (Fig. 3 *b*).

When examined in greater detail (Fig. 3, *d* and *e*), the cortical and subcortical networks of a prophase cell virtually disappear except in the area of the nucleus (Fig. 3 *d*). The nuclear basket breaks up into short, diffuse filaments (Fig. 3 *e*) and the transvacuolar strands are visible only a short distance from the nucleus (Fig. 3 *e*). Hoechst staining of the nucleus (inset in Fig. 3 *e*) indicates the cell is in mid-prophase.

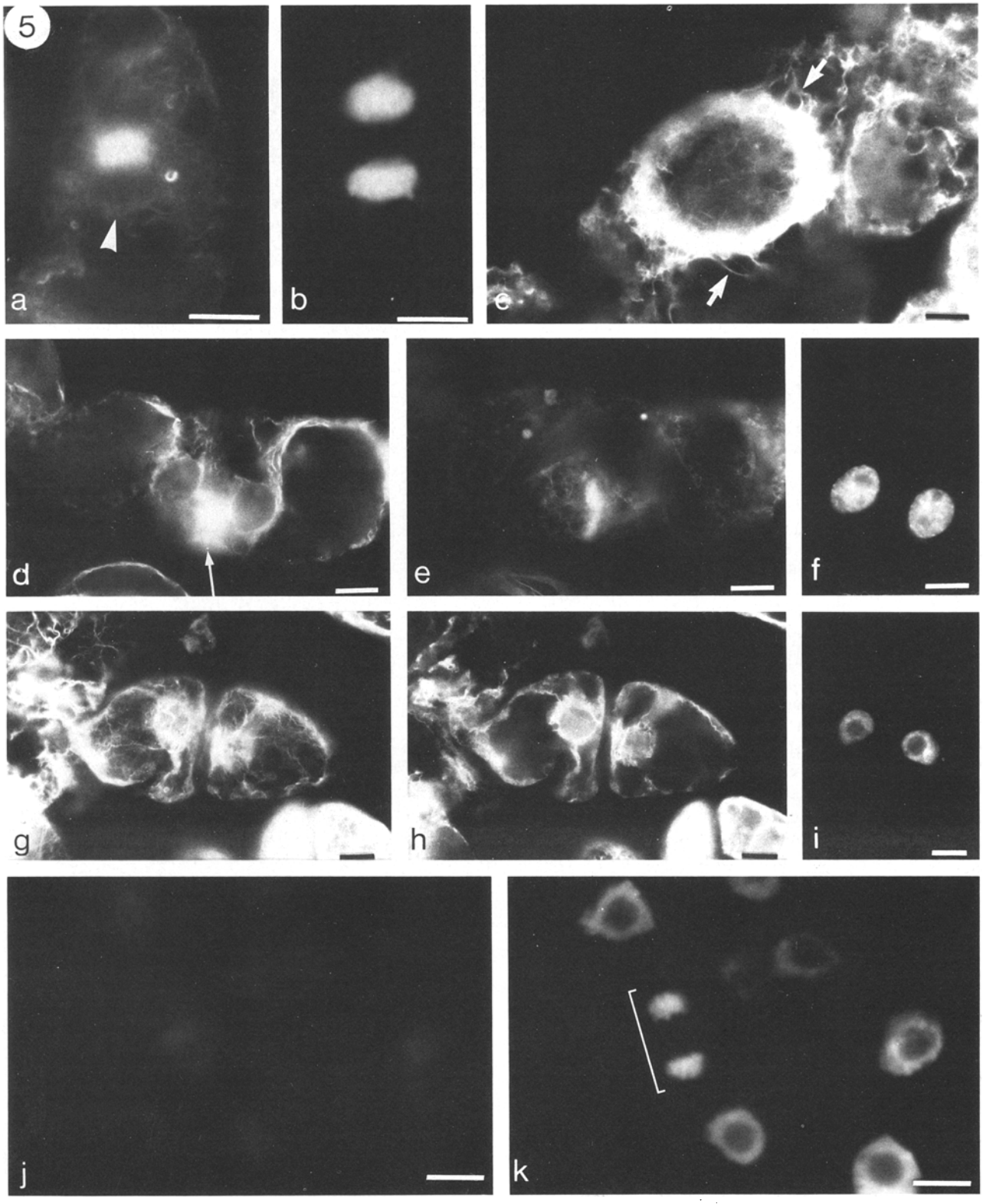

Figure 5. (*a*) Early telophase. Formation of phragmoplast (which guides cell wall deposition). Faint stain of reforming nuclear baskets can be detected (*arrowhead*). (*b*) Hoechst stain of daughter nuclei. (*c*) Surface view of a well-developed phragmoplast extending toward the cell walls. Microfilaments radiate from phragmoplast into the cytoplasm (*arrows*) and may aid in moving wall material to the newly forming cell plate. (*d*) Phragmoplast mfs (*long arrow*) disappear in late telophase. Both nuclear baskets and cables of the transvacuolar strands have returned. (*e*) Focal plane at cell surface reveals interphase cortical mf network has not yet reestablished. (*f*) Hoechst stain of decondensing chromatin. By early G1 (*g–i*) all mf patterns have returned. (*g*) Cortical region displays random mf pattern typical of small cells. (*h*) Nuclear baskets and transvacuolar strands are present. (*i*) Chromatin decondensation is complete. Competition experiments with phalloidin result in cells which have minimal fluorescence due to rhodamine (*j*) yet are easily detected with Hoechst stain (*k*). Note decondensing daughter nuclei (*bracket*). Bars, 10 μm.

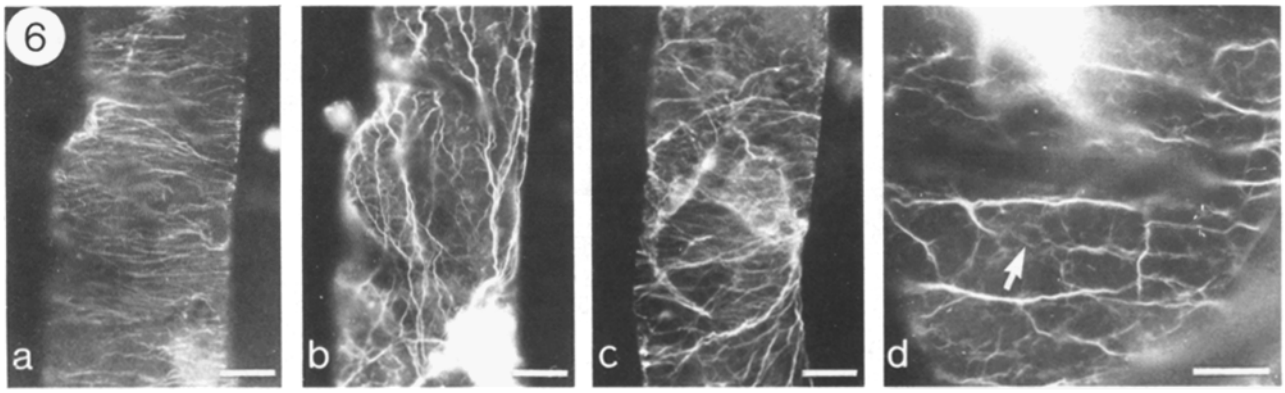

Figure 6. Microtubule and microfilament patterns in differentiated cells. (*a*) The microtubules of elongating, nondividing cells are oriented transverse to the long axis. (*b*) As the cells begin to elongate, the mf pattern in most cells parallels the long. (*c*) As elongation continues, the mf orientation changes to transverse. (*d*) The subcortical cables of elongated cells are parallel to the microtubule array but the fine, cortical network (*arrow*) continues to display a somewhat random configuration. Bars, 10 μm.

Microfilaments in Metaphase/Anaphase

During metaphase and anaphase, few or no mfs are visible in the cortical cytoplasm and no bundles of mfs can be found in either the subcortical cytoplasm or in the transvacuolar strands (Fig. 4). Fibrillar staining is seen in the area occupied by the spindle (Fig. 4, *a*, *c*, *e*, and *g*). When the layer of cytoplasm surrounding the spindle region is relatively thin, the mf stain can be easily detected and appears as discrete fibers. It is not possible to ascertain if these are individual mfs. When the spindle is located deep within the cytoplasm, diffuse fluorescence of the cytoplasm may obscure any spindle stain (not shown).

Spindle staining during anaphase follows the same restrictions. If the spindle is located in the center of the cell surrounded by cytoplasm, it is difficult to detect mf staining above the diffuse cellular fluorescence. If the spindle is positioned near the cell surface, short mfs can be detected between the separating chromosome masses and the spindle poles (Fig. 4, *i* and *k*). Hoechst stain of the chromosome masses is seen in Fig. 4, *j* and *l*.

When phalloidin (not conjugated with the fluorochrome rhodamine) is applied to fixed cells to bind the active sites followed by application of rhodamine-conjugated phalloidin, no staining of any mf arrays can be detected. There is no staining of any spindle-like structure in these preparations (Fig. 4 *m*).

Microfilament Repolymerization during Telophase

Telophase is marked by the appearance of brilliant staining in the phragmoplast (Fig. 5, *a* and *c*), the structure which is instrumental in guiding the deposition of the new cell wall between the daughter cells.

Cytoplasmic arrays of mfs return during telophase. The nuclear "basket" reappears, faintly at first, to surround each daughter nucleus as the phragmoplast forms. Microfilaments can be seen arising from the phragmoplast and spreading into the cytoplasm (Fig. 5 *c*). Later in telophase (as shown by comparing Hoechst stain of the nuclei in Fig. 5, *b* and *f*), the mf bundles and/or cables of the transvacuolar strands return. These cables may originate from the area of the nuclear membrane or from the nuclear "basket" (Fig. 5 *d*). Often, but not always, the last mf array to be reinstated is that seen in the cortical cytoplasm (Fig. 5 *e*). In many cells, complete interphase of mf arrays do not appear until early G1 (Fig. 5, *g* and *h*).

Microfilament Redistribution during Cell Differentiation

Plant cells regularly subcultured in the presence of 2.4-D are thought to be analagous to undifferentiated cells found in the meristematic regions of plants. However, when these same cells are grown without 2,4-D, but in the presence of GA, they undergo differentiation into elongated, nondividing cells. Presumably these large cells are similar to cells seen in the elongating and/or differentiated regions of the intact plant.

The cytoskeleton and, in particular, the microtubules are known to play a role in differentiation of plant cells (Falconer and Seagull, 1985*a*, *b*). For this reason we examined the interphase arrays of microtubules in combination with the interphase arrays of actin mfs in both rapidly cycling cells and differentiated, nondividing cells by double staining of both actin mfs and microtubules.

Microtubule arrays change during cell differentiation. In small, rapidly cycling cells, cortical microtubules are predominantly found in random arrays (Simmonds et al., 1983). When these rapidly cycling cells are transferred to medium containing only GA, division virtually ceases and the cells elongate. Microtubule arrays in these elongated cells, as in elongated cells in plants, reorient from random to highly organized, transverse arrays (Simmonds et al., 1983).

The mf arrays also change during culture in GA-containing medium. After 2 d growth in the presence of GA, the cells are elongated with microtubules transverse to the long axis of the cell (Fig. 6 *a*). The mf cables in 75% of such cells are parallel to the long axis with a focus at the nucleus (Fig. 6 *b*). However, 15% of the cell population has mfs oriented approximately at right angles to the length of the cell (Fig. 6 *c*). The remaining 10% continue to have randomly oriented cortical mfs.

After a 3-d culture in GA, the percentage of cells with mfs transverse to the long axis of the cell has increased from 15 to 54%. Thus mf cables show an increasing tendency to be aligned with the microtubule orientation as differentiation proceeds. The fine network of cortical mfs continues to display a random organization in these elongated cells (Fig. 6 *d*).

Streaming patterns in cultured cells change during differentiation in a manner which is consistent with the observed pattern of mf reorientation. In 2,4-D cells, streaming is concentrated in the subcortical and transvacuolar strands and the motion is either random or parallel to the long axis of the cell. As cells elongate, a portion of the streaming pattern may continue to parallel the long axis of the cell but additionally streaming may now also encircle the cell. The streaming patterns in all cells are capable of reforming with great rapidity as can be seen using phase-contrast optics (data not shown).

Discussion

It is now apparent that actin mfs are an integral part of the plant cytoskeleton and as such may be expected to be involved in a variety of functions including streaming, mitosis/cytokinesis, and differentiation. In cultured higher plant cells, both the cell cycle and differentiation are accompanied by extensive redistribution of mfs.

Microfilament Dynamics during Interphase

The architecture of plant cells forces the observer constantly to be aware of the three-dimensional relationships of mfs within the cytoplasm. Interphase arrays of mfs can be categorized into three distinct yet contiguous networks within the cell (Fig. 2): (*a*) a meshwork of fine, randomly organized filaments located close to the plasma membrane; (*b*) large bundles or cables of filaments in the subcortical cytoplasm; (*c*) a "nuclear basket" of filaments which extends into the transvacuolar strands.

The existence of a cortical network of actin-like mfs (in addition to the cables of filaments involved in cytoplasmic streaming) has been proposed by numerous researchers. Microfilaments, in association with the cortical arrays of microtubules, were first observed by Seagull and Heath (1979, 1980) in developing radish root hairs. Since then microfilament-like structures have been found associated with microtubules and the plasma membrane in *Azolla* (Heath and Seagull, 1982), *Zea* (Seagull 1983), *Lepidium* (Traas, 1984), *Equisetum* (Traas et al., 1985), and *Psilotum* (Brown 1985). Because of this association with microtubules and the proximity to the plasma membrane, cortical arrays of mfs have been proposed to function in the deposition of wall material (Seagull and Heath, 1980; Heath and Seagull, 1982; Mueller and Brown, 1982). Our observations support and extend those of Parthasarathy (1985) showing that an extensive array of anastomosing mfs, closely associated with the plasmalemma, does exist. The precise function(s) of this array has yet to be tested.

The formation of mf interphase arrays begins during cytokinesis. As chromosome masses in the daughter nuclei decondense, the nuclear basket of mfs is constituted (Fig. 6). Next, cables of mfs appear, initially in the transvacuolar strands and then in the subcortical regions. Finally, the fine meshwork typical of the cortical region is seen in late telophase or early G1. The reverse process occurs during prophase with mfs disappearing first in the cortical and subcortical regions and finally at the nuclear basket (Fig. 2 and 3).

It has been previously reported that cytoplasmic streaming ceases at the onset of mitosis (Jones et al., 1960; Wada et al., 1982; Mineyuki et al., 1984). The observed depolymerization of mf arrays during prophase indicates for the first time the mechanism by which this might be accomplished. The pattern of disassembly is also consistent with the observed accumulation of cytoplasm around the nucleus at the onset of mitosis (Wada et al., 1982; Jones et al., 1960; Mineyuki et al., 1984). It seems reasonable to suggest that movement of cytoplasm may be progressively restricted to the nuclear region (the last region to contain intact mf arrays).

The rapid redistribution of actin indicates that the mf networks are exceedingly labile. Patterns of mf assembly/disassembly indicate that the nucleus or the nuclear region may be important in controlling mf turnover and organization. Control of actin filament assembly and organization via actin-associated proteins has been well documented in in vitro systems (Pollard et al., 1982; Sheterline 1983). It remains to be seen how these observations will relate to the in vivo state in plant cell systems.

Microfilament Reorientation during Cell Differentiation

By controlling the hormonal environment of cell cultures it is possible to have either small, nondifferentiating, rapidly cycling cells or large, elongated, noncycling differentiated cells. In small nondifferentiating cells the interphase array of mfs consists of the nuclear basket, transvacuolar strands, and a fine cortical network of random filaments (Fig. 2). At this stage, little or no streaming is evident. As cells enlarge, axially oriented cables of mfs develop in the subcortical cytoplasm. The distribution and orientation of filaments closely approximates the streaming patterns at this time. The formation of large cables from fine mfs implies the function of an actin-binding protein during cell enlargement. While such proteins are well documented in animal systems (DeRosier and Tilney, 1982; Pollard et al., 1982; Sheterline 1983) their existence in plants has yet to be shown.

As cell enlargement continues, there is an abrupt shift of the subcortical cables (Fig. 6) from an axial to a transverse orientation. The other mf arrays remain unaltered. The mechanism for this reorientation remains unknown. We are currently investigating the relationship between cell age, cell size, and mf cable orientation.

Microfilament Redistribution during Division

Our observations of positive and specific binding of rhodamine-conjugated phalloidin in most plant spindles supports but does not prove that actin-like mfs are functional components of spindles. The inability to detect actin staining in some spindles is strongly correlated with those cells having high nonfibrillar staining in the nuclear regions. The increased diffuse staining is cell cycle dependent (found in cells in mitosis) and may be due to the large amounts of nonfilamentous actin present in the cell as a result of the depolymerization of interphase arrays. While phalloidin does not specifically bind G-actin, it has been proposed (Wieland, 1977) that small oligomers of G-actin (2–3 monomers) can bind phalloidin. It is possible that within the cytoplasm numerous oligomers of actin may exist during mitosis and this could result in the observed diffuse staining.

The specificity of the Rd-Phal staining was tested using a preincubation of the cells in unlabeled phalloidin. Neither filamentous arrays nor diffuse fluorescence patterns, above

the normal autofluorescent levels, were detected in cells pretreated with phalloidin (Fig. 4 *m*). The minimal cell preparation used in these studies reduces the likelihood that the actin observed in the spindles is the result of artifactual staining. In addition, the actin staining pattern changes in a functionally specific manner, with the staining fibers becoming shorter between metaphase and anaphase (Fig. 4). This stage-specific change in mf staining is consistent with actin functioning between chromosomes and spindles pole (Forer et al., 1979; Forer, 1982; Forer, 1985).

Our observations are in apparent contradiction to those of Barak et al. (1981) who reported a lack of spindle-specific actin staining in rat kangaroo cells using nitrobenzoxadiazole–phallacidin. The difference in observations may result from differences in tissue preparation. Barak et al. (1981) extracted cells with acetone before staining, whereas we do not. We have found in plant cell systems that extraction (using glycerol) or prolonged fixation (using paraformaldehyde at higher concentrations or for longer times) results in mf arrays which are less extensive and poorly stained (our unpublished observations).

Ours is not the first study which details mf redistribution during the cell cycle in plants, however it is the first to report spindle-specific actin staining. Neither Clayton and Lloyd (1985) studying onion or Derksen et al. (1985) studying *Equisetum* report any actin localization in the spindle. The difference in results between this study and those previous may stem from minor yet critical modification to the preparative procedures. Of crucial importance is the use of phosphate buffer throughout the procedure (fixation and staining). The substitution of Pipes buffer for phosphate during fixation in our system produces images very similar to those described by Clayton and Lloyd (1985) (i.e., no spindle staining, faint arrays in the cell cortex). Conversely applying our techniques to onion root meristems produces observations very similar to those described for the Alfalfa and *Vicia* culture systems including spindle-specific actin staining (our unpublished results).

We have provided evidence that actin-like mfs form extensive and dynamic arrays in plant cells. The changes observed in mfs during cell growth and differentiation indicate that within plant cells there are complex mechanisms for coordinating actin organization and function in a developmentally regulated manner. We have yet to fully document the extent to which mfs are involved in plant cell growth. The existence of actin-associated proteins, with the exception of myosin (Vahey et al., 1982) remains to be verified in higher plants. Observations such as those reported here strongly imply that such proteins must be functioning.

We thank Dr. Daniel Brown of Agriculture Canada for a gift of the alfalfa cell cultures used in this work.

This work was funded by a Natural Sciences and Engineering Council of Canada grant to R. W. Seagull.

Received for publication 2 October 1986, and in revised form 2 December 1986.

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