An Actin Network Is Present in the Cytoplasm throughout the Cell Cycle of Carrot Cells and Associates with the Dividing Nucleus

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Abstract. We have studied the F-actin network in cycling suspension culture cells of carrot (Daucus carota L.) using rhodaminyl lysine phallotoxin (RLP). In addition to conventional fixation with formaldehyde, we have used two different nonfixation methods before adding RLP: extracting cells in a stabilizing buffer; inducing transient pores in the plasma membrane with pulses of direct current (electroporation). These alternative methods for introducing RLP revealed additional features of the actin network not seen in aldehyde-fixed cells. The three-dimensional organization of this network in nonflattened cells was demonstrated by projecting stereopairs derived from through-focal series of computer-enhanced images.

F-actin is present in interphase cells in four interconnected configurations: a meshwork surrounding the nucleus; thick cables in transvacuolar strands and deep in the cytoplasm; a finer network of bundles within the cortical cytoplasm; even finer filaments that run in ordered transverse array around the cell periphery.

The actin network is organized differently during division but it does not disappear as do the cortical microtubules. RLP stains a central filamentous cortical band as the chromatin begins to condense (preprophase); it stains the mitotic spindle (as recently shown by Seagull et al. [Seagull, R. W., M. Falconer, and C. A. Weerdenburg, 1987, J. Cell Biol., 104:995-1004] for aldehyde fixed suspension cells) and the cytokinetic apparatus (as shown by Clayton, L., and C. W. Lloyd, 1985, Exp. Cell Res., 156:231-238). However, it is now shown that an additional network of F-actin persists in the cytoplasm throughout division associating in turn with the preprophase band, the mitotic spindle, and the cytokinetic phragmoplast.

Actin has been identified as a component of the plant cell's cytoskeleton by several methods. Palevitz et al., (1974) used heavy meromyosin to characterize filaments in the alga Nitella. Forer and Jackson (1976) also used heavy meromyosin to decorate filaments in the cytoplasm of the higher plant Haemanthus. More recently, the use of fluorescent phallotoxins and, to a lesser extent, antibodies have provided an extensive description of the actin network in a range of plant cells (Barak et al., 1980; Pesacreta et al., 1982; Parthasarathy et al., 1985; Menzel and Schliwa, 1986).

From these studies it can be concluded that plant cells possess an extensive network of actin-containing bundles during interphase. These are most probably involved in cytoplasmic streaming (reviewed in Williamson, 1979; Tiwari et al., 1984; Lloyd, 1987; see also Menzel and Schliwa, 1986; Adams and Pollard, 1986). A variety of EM techniques indicate that thin filaments and filament bundles occur at the cell cortex, often in close association with the cortical microtubules (Tiwari et al., 1984; Traas et al., 1985; Brown, 1985; Hawes, 1985; Hepler, 1985). These thin cortical filaments are suspected as being F-actin but this has not yet been confirmed.

As for cell division there is both EM and light microscopical evidence for actin in the spindles of different plant cells (Forer and Jackson, 1976, 1979; Forer et al., 1979; Seagull et al., 1986). The presence of F-actin in the phragmoplast has also been reported by several authors (Clayton and Lloyd, 1985; Gunning and Wick, 1985; Derksen et al., 1986). However, it is not known whether F-actin is present in other parts of the cell during cell division.

In this report we describe the changes in the actin network occurring during the cycle of carrot suspension culture cells using rhodaminyl lysine phallotoxin (RLP) (Wulf et al., 1979). It has been shown that actin filaments can be especially sensitive to aldehyde fixation (Heuser and Kirschner, 1980; Lehrer, 1981) and it is possible that the full extent of the actin network is not revealed in aldehyde-fixed cells. In view of the possible artifacts caused by formaldehyde, we have developed alternative methods that avoid fixation. These methods allow us to conclude that an actin network, which is very fine in places, is present in the cytoplasm throughout the entire division cycle, and that actin co-

1. Abbreviations used in this paper: DAPI, diamidino-2-phenylindole; RLP, rhodaminyl lysine phallotoxin.

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distributes with all four of the higher plant cell’s microtubular arrays.

**Materials and Methods**

**Culture Methods**

Cell suspension cultures of carrot (Daucus carota L.) were maintained in Murashige and Skoog’s medium, containing 3% (wt/vol) sucrose, 5% (vol/vol) coconut milk, 0.5 mg/liter kinetin, and 1 mg/liter 2,4-D as described previously (Lloyd et al., 1980). The cells were used 3-7 d after sub-culturing when they were actively dividing.

Tradescantia plants were grown under greenhouse conditions. Fully developed flower buds were cut from the plants; stamenal hair cells were isolated from these buds under the dissection microscope and prepared for fluorescence microscopy. Also, epidermal peels from the leaf base of onion plants (Allium cepa L.) were used.

**Staining of F-Actin**

Different procedures for localizing F-actin were compared.

**Fixation with Formaldehyde.** Cells were fixed for 15 min in 3% (wt/vol) freshly prepared formaldehyde in stabilizing buffer; 100 mM Pipes buffer, 10 mM EGTA, 5 mM MgSO4, pH 6.9. The cells were then washed in the same buffer and stained in suspension with RLP (kind gift of Prof. T. Wieland, Heidelberg, FRG) at a concentration of 10^{-6} M in stabilizing buffer. Diamidino-2-phenylindole (DAPI) (Sigma Chemical Co., Poole, Dorset, UK) was used to stain nuclear DNA at a concentration of 0.2 μg/ml. After 30 min cells were washed in buffer and examined immediately in the microscope. Pictures were taken using a Zeiss camera, on Ilford FP4 film.

**Procedures Avoiding Fixation with Aldehyde.** Two different nonfixation procedures were used to localize F-actin. (a) Extraction: Cells were made permeable with final concentrations of 0.01-0.05% (vol/vol) NP-40 (British Drug Houses (B. D. H.), Poole, Dorset, UK) and 5% vol/vol DMSO in microtubule stabilizing buffer. Mannitol was used at different concentrations as an osmoticum. Usually, equal amounts of extraction buffer and cell suspension were mixed. To limit the possibility of artifacts, RLP was added to the extraction buffer at a final concentration of 10^{-7} M, which is 10-fold lower than had been used for fixed cells. The stained cells were mounted and examined immediately. (b) Electroporation: RLP was also introduced into the cytoplasm by electroporation. With this technique, pulses of direct current are used to open pores transiently in the plasma membrane. This technique is normally used for introducing viruses into protoplasts or for protoplast fusion (e.g., Watts and King, 1984; Watts et al., 1987). Prior treatment with cellulase was not necessary for the passage of low molecular weight RLP into living cells. 40 μl of cell suspension in 4% (wt/vol) mannitol were mixed with 10 μl RLP in Pipes buffer (final concentration 10^{-5} M RLP, 10 mM Pipes) and 10 μl of a DAPI solution (final concentration 0.05 μg/ml) in a 60-μl electroporation chamber. The resistance of the solution was 2,000Ω. The cells were electroporated by a pulse of 1 kV/cm provided by discharging a 5 μF capacitor through the chamber with an inter-electrode distance of 4 mm. The pulse had a rapid rise-time (<0.5 μs) and an exponential decay with a half-life of ∼5 μs (Watts et al., 1987). Cells were mounted on slides and examined immediately with a Zeiss fluorescence microscope.

**Drug Treatments**

Cells were incubated with 20 μM cytochalasin D (Sigma Chemical Co.) for 2 h in order to perturb the actin network. Microtubules were depolymerized with the organophosphoric herbicide cremart (Sumitomo Chemical Co., Osaka, Japan) at 30 μM for 2 h. After drug treatment the cells were extracted and stained as described above.

**Video Microscopy**

For computer video-enhanced microscopy a low light level (ISIT) camera and camera-linked framestore were used. Data acquisition by multiple video frame averaging and microscope fine-focus stepping were controlled through the host VAX 11/750 computer. Through-focus data sets were stored onto computer disc and typically consisted of 20-40 sections each 512 × 512 pixels separated by intervals of 0.5 μm in the vertical direction. The hardware and software will be described in more detail elsewhere (Lloyd et al., 1987). Deblurring of the data stacks was accomplished using a simple nearest neighbor algorithm (Weinstein and Castelmann, 1979; Agard, 1984). Stereo pairs were produced by computation from the deblurred section stacks by projecting groups of consecutive sections onto a monitor screen from two different angles to correspond to the left and right views. The deblurring and projecting programs were written by Dr. David Agard, University of California at San Francisco. Pictures were taken directly from the monitor screen.

**Results**

**Fixation**

After fixation in formaldehyde actin cables were observed in some but not all cells (Fig. 1). Where present, the actin network consisted of bundles dispersed throughout the cyto-
plasm; these bundles being fragmented in some cases. Randomly oriented strands were observed near the cell cortex, while the larger cables extended deeper into the cytoplasm, often associated with the nucleus (Fig. 1, A and B). In dividing cells, actin was detected in the phragmoplast as a bright and diffusely fluorescent disc (Fig. 1 C). Actin was not detected at other stages of cell division. To check whether some classes of F-actin had been made undetectable by the washing steps (between fixation and addition of RLP), RLP (10⁻⁷ M) was added together with the fixative. The same F-actin patterns were observed.

**Extraction**

A more extensive actin network was observed by avoiding aldehyde fixation, including staining of a preprophase band, the spindle, a cytoplasmic network during division, and fine transverse cortical filaments in interphase (Figs. 2, 3, and 4).

Actin arrays were observed in all cells 3-5 min after extraction. Compared with fixed cells the extracted cells did not bleach appreciably and their microfilaments appeared to be extremely stable as suggested by lack of the fragmentation sometimes seen with aldehyde. Although NP-40, DMSO, and mannitol preserved the actin network over a range of concentrations, optimal results were obtained at 0.01% (vol/vol) NP-40, 5% (vol/vol) DMSO, and 0.1 M mannitol. The extraction procedure did not cause any obvious collapse of the cytoskeleton as judged from the stereo pairs made from such cells (Fig. 4).

**Control Experiments**

To test the possibility that RLP might induce artificial actin polymerization in unfixed cells, two control experiments were performed: First, RLP was added at a final concentration of 10⁻⁴ M which is a 100-fold lower than the lowest concentration known to affect actin polymerisation (Paulin-Levasseur and Gicquaud, 1981). Although the staining was weak, the same patterns as with 10⁻⁷ M were observed (data not shown).

Second, we extracted soluble proteins from the cells before staining. Cell walls were first permeabilized for 10 min in 2% cellulase (Onozuka R-10; Yakult Honsha Co., Tokyo, Japan) and 2% driselase (Sigma Chemical Co.) before being extracted in the extraction buffer. Actin has been confirmed to be one of the proteins extracted under these conditions (Hussey, 1987). After the extraction of soluble protein, the addition of RLP gave the same results as when RLP was added simultaneously with the extraction buffer.

To test the possibility that the extraction procedure induced the formation of additional actin filaments the following controls were performed: First, cells were extracted before being fixed and stained. These cells showed the same actin patterns as unextracted, fixed cells (Fig. 2 F). Second, RLP was incorporated into cells using electroporation, bypassing the need for DMSO and detergent. A few minutes after electroporation the actin networks were brightly labeled. The distribution of actin in interphase and in dividing cells was identical to that found in extracted cells (Fig. 5, A-D). In general, 60-90% of the cells were stained.

To show that the observed patterns were due to specific binding of phalloidin, unlabeled phalloidin (10⁻⁵ M) was added to controls for 10 min. After this treatment the actin networks could no longer be stained with RLP (Fig. 2 E).

**Computer-enhanced Video Microscopy**

To appreciate the complex three-dimensional distribution of the actin networks in these large, nonflattened cells, computer-enhanced video microscopy was used (Fig. 4). Best results were obtained when the optical sections were taken at focus level intervals of 0.5 µm. In general, only part of the optical sections were projected for the stereo pairs in order to obtain optimal resolution. Although the theoretical resolution of the system lies around 100 nm, the actual resolution was lower, among other things, due to the low levels of light intensity used to avoid photobleaching.

**Distribution of Actin in Extracted Interphase Cells**

In extracted interphase cells a complex network of F-actin was observed throughout the cytoplasm (Fig. 2, A-D and Fig. 4 A). Larger bundles could be observed deep in the cytoplasm and in transvacuolar strands, and this is clearly demonstrated by the stereo pairs of Fig. 4 A. In the cell cortex the network consisted of a finer meshwork of randomly oriented filament bundles (Fig. 2 A and Fig. 4 A). In addition to this network, a population of very fine, transversely oriented elements, which were never seen in aldehyde-fixed cells, were observed within a shallow focal section very close to or associated with the membrane (Fig. 2, B-D). These filaments were sometimes barely distinguishable, or apparently absent but in other cases were much more prominent (cf. Fig. 2 C and 2 D), especially in older cultures (7 d after subculturing).

In all cases the nucleus was surrounded by a network of strands that was continuous with the rest of the actin network (Fig. 4 A). Large, transvacuolar strands were often seen linking the nucleus to the cell cortex.

To check whether the presence of the transverse arrays is a common feature of the actin system in plant cells, we also stained actin in *Tradescantia* staminal hair cells and onion epidermal cells. Both cell types showed transverse arrays of RLP-staining filaments (Fig. 2, G and H).

**Distribution of Actin Filaments in Extracted Dividing Cells**

During mitosis most of the large actin cables and transvacuolar strands disappear. However, a meshwork of bundles remains throughout the cytoplasm (Fig. 3, A-K and Fig. 4, B-C) and no dividing cell was seen without cytoplasmic F-actin. During preprophase (characterized by the chromatin condensation pattern) a transverse band of microfilaments was observed around the nucleus. This band of transverse filaments was sometimes barely visible within the cortical network, but in many cells it was very prominent (Fig. 3 A). Such a band was also present in a number of cells that in the absence of chromatin condensation, were judged to be in interphase. The band was also observed in some early metaphase cells (Fig. 3 C) but was always absent in later stages. During metaphase and anaphase, F-actin was also observed in the spindle. In addition, a web of actin-bundles linked the spindle to the cortex (Fig. 3, E-H and Fig. 4 B).
Figure 2. Extracted cells. (A) Group of carrot cells showing extensive actin networks. Bundles can be seen inside the cytoplasm, connecting the nucleus to the cortex. Finer networks can be observed in the cortical cytoskeleton. n, Nucleus. (B–D) Transverse actin arrays in the
Figure 3. Extracted, mitotic carrot cells demonstrating the persistence of the actin network throughout division. (A) A transverse cortical band of actin (arrows) around a prophase nucleus. Note the actin filaments elsewhere in the cell. (B) DAPI staining of the same cell showing the condensed chromatin. (C) A band of actin (arrows) around the nucleus of an early metaphase cell. Again, note that F-actin persists elsewhere in the cytoplasm at a time when cytoplasmic microtubules are known to be absent. (D) DAPI staining of the same cell. (E) Metaphase spindle (arrowhead) brightly stained with RLP. Note that cytoplasmic filament bundles are connected to the spindle (arrows). (Inset) Another spindle, showing that the staining has a filamentous pattern. (F) DAPI staining of the same cell. (G) Cortical view of a metaphase cell showing the filamentous network that remains at the cell periphery. (H) Internal view of the same cell, showing the spindle embedded in a fine network connected to the cortex. (I) Cytokinesis, cortical view, showing the actin network. A part of the phragmoplast (arrows) is still present. (J) Internal view of the same cell, note concentration of filaments surrounding the phragmoplast. (K) DAPI staining of the same cell. Bars, 10 μm.

cortex of carrot cells (arrows). In most cells the filaments are extremely fine and are difficult to record (B and C). In other cells (D) they are more prominent. (E) Cells treated with 10⁻⁵ M phalloidin before staining. After staining with RLP only a faint background is observed. (F) Extracted cells, that are fixed in formaldehyde. Few, if any, actin bundles are observed. (G) Focussing upon the cortex of a sheet of onion epidermal cells reveals fine transverse RLP-staining filaments (arrows). (H) Tradescantia staminal hair cell demonstrating the fine transverse actin arrays (arrows). Bars: (A-F) 10 μm; (G and H) 20 μm.
Figure 4. Stereo pairs of carrot cells. About 30 computer-enhanced optical sections per cell were taken but only some were projected to obtain the stereopairs. This resulted in thick optical sections of ~10 μm. (A) Interphase cell. The small arrow marks the position of the nucleus, which was not projected. Thick cables extended from the nuclear network to the cortex. Finer bundles can be seen in the cortical cytoplasm. The very fine transverse filaments illustrated in Fig. 2 were observed in the microscope, but were not resolved by the ISIT camera. (B) Metaphase cell, showing actin networks throughout the cytoplasm. The arrow marks the position of the spindle, surrounded by a fine network of bundles. Inset, spindle of the same cell, at a different focus level. (C) Telophase cell, showing the cytoplasmic actin network. The bundles are connected to the phragmoplast which is brightly labeled.

Figure 5. Electroporated cells contain the same extensive actin network as extracted cells. (A) Cortical networks showing fine transverse bundles. (B) Cortical view of a telophase cell with cortical actin networks. (C) Internal view of the same cell showing the phragmoplast. (D) DAPI staining of the same cell. Bars, 10 μm.
During telophase the phragmoplast stained brightly, and again a network was present in the cytoplasm (Fig. 3, I-K and Fig. 4 C).

**Drug Treatments**

When cells were treated with 20 μM cytochalasin D the actin network was degraded very rapidly. The finer networks disappeared within 10 min during which time the thicker cables became considerably shorter. After 35 min most of the network had disappeared. Only brightly fluorescent spots or small rods were visible, close to the plasma membrane and the nuclear envelope (Fig. 6, A and B). When the cells were allowed to recover in medium without cytochalasin D, and then stained with RLP, the integral actin system was reconstituted within 10 min (not shown).

The transverse, cortical distribution of the finest RLP-stained elements mirrors the known distribution of cortical microtubules. The latter were found to be depolymerized by the herbicide cremart at 30 μM, but under these conditions, the cortical RLP-staining elements did not disappear, although their distribution became more irregular and no transverse patterns were seen (Fig. 6, C and D).

**Discussion**

**Methods**

When carrot cells were fixed with formaldehyde, RLP staining was confined to larger bundles of F-actin in interphase and to the phragmoplast in mitotic cells. This agrees with previous findings (Clayton and Lloyd, 1985; Gunning and Wick, 1985; Derksen et al., 1986; Seagull et al., 1987). However, when unfixed cells were permeabilized in extraction buffer, a more extensive staining pattern was observed. In particular, fine filaments were seen in the cortex of interphase cells. The preprophase band and the mitotic spindle were stained, and cytoplasmic filament-bundles could be observed throughout the division cycle.

Clearly, either some components of the actin network are sensitive to fixation with aldehyde, or else the additional features observed in unfixed, extracted cells are artifacts. Different observations suggest that the results obtained using nonfixation methods are not artifactual. (a) Pretreatment with unlabeled phalloidin abolishes the specific patterns observed with RLP-staining. (b) RLP was used in concentrations as low as 10⁻⁸ M, which is 10⁻²-10⁻³ M lower than the minimal concentration affecting actin polymerization in animal cells (Wehland and Weber, 1981; Paulin-Levasseur and Gicquaud, 1981; Kersken et al., 1986, and references therein). (c) G-actin was extracted before staining. This is likely to reduce, if not remove, the risk of artifactual polymerization by phalloidin. Still, the same patterns were observed after a 10-min extraction as when RLP and detergent were added simultaneously. (d) The concentrations of DMSO (5% was used in most of our experiments) and detergent do not affect the actin organization in animal cells (Osborn and Weber, 1980, 1982; Small, 1981). (e) Perhaps the most satisfactory control is the use of electroporation. This avoids the use of detergent and DMSO, yet the same RLP-staining patterns are obtained.

The above observations imply that the F-actin network might be more extensive than has been described for aldehyde-fixed cells. That actin can be sensitive to fixation has been reported, particularly as far as the effects of glutaraldehyde and osmium fixation for electron microscopy are concerned. Both fixatives have been reported to damage actin

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Figure 6. Drug treatments. (A and B) Treatment with cytochalasin D. After 25 min the bundles have disappeared and only bright dots or small rods remain. These are present throughout the cytoplasm, often associated with the nuclei (n) (arrows). (C and D) Treatment with the antimicrotubule herbicide cremart affects the organization of the cortical actin skeleton. F-actin is not depolymerized by this treatment but its distribution becomes more irregular. Cortical, transverse microfilament bundles are never observed. All panels represent extracted cells. Bars, 10 μm.
filaments (e.g., Gicquaud et al., 1980; Lehrer, 1981; Heuser and Kirschner, 1980; Seagull and Heath, 1979; Maupin and Pollard, 1983). Formaldehyde is widely used for fluorescence microscopy and allows staining of actin bundles in both animal (e.g., Osborn and Weber, 1982) and plant cells (Pesacreta et al., 1982; Parthasarathy et al., 1985). However, some reports, especially on plant cells, mention negative aspects of formaldehyde fixation. For instance, Parthasarathy et al. (1985) found that long fixation in higher concentrations of formaldehyde diminished the quality of the results. Moreover, these investigators were unable to detect F-actin in a number of cell types and they attributed this to their preparatory methods (see also Seagull et al., 1987). Similarly, we find that fixation in formaldehyde diminishes the extent to which the actin network can be stained in carrot cells by RLP.

Although the possibility cannot be excluded that some changes are introduced at the ultrastructural level, the nonfixation methods presented here at least provide a useful counterview to be set alongside the existing images of aldehyde-fixed cells.

The Organization of F-Actin During Interphase

The actin network in carrot cells is organized in different, interconnected arrays during interphase. Some of these have been reported for a wide range of plant cell types (Parthasarathy et al., 1985; Perdue and Parthasarathy, 1985; Derksen et al., 1986; Seagull et al., 1987). In *Alfalfa* and *Vicia* suspension culture cells, Seagull et al. (1987) report the presence of three distinct arrays: (a) a cortical meshwork of fine, randomly oriented filament bundles; (b) large cables deeper in the cytoplasm; (c) a nuclear basket of microfilaments extending into the cytoplasmic strands. These are present in carrot, but according to present observations there is a fourth component in the cortical cytoplasm: a highly ordered array of transverse actin filaments, which is parallel to the known transverse alignment of microtubules (Lloyd et al., 1979, 1980; Hawes, 1985). This fine component was not only observed in carrot cells, but we also detected it in onion epidermal cells and in *Tradescantia* stamenal hair cells. The occurrence of fine filaments in the cortex is not without precedence. A variety of EM studies established their presence but has not characterized them. These filaments are closely associated with, often parallel to, cortical microtubules and this has been seen by freeze etching (Brown, 1985), by freeze substitution (Tiwari et al., 1984; Hepler, 1985), dry cleaving (Traas et al., 1985; Hawes, 1985), and in carrot suspension cells (of the kind used here) by freeze slam/deep etch (Hawes and Martin, 1986). In view of the RLP staining it is proposed that the thin filaments running alongside cortical microtubules might be composed of F-actin.

Our results using the antimicrotubule herbicide cremastin show that depolymerization of microtubules does alter the arrangement of the cortical RLP-staining filaments. From this it would appear that the organization of F-actin can be influenced by microtubules. Using a monoclonal anti–actin antibody, Menzel and Schliwa (1986) came to a similar conclusion. They found that F-actin and microtubules shared the same distribution in the green alga *Bryopsis*; they also found that perturbing one system caused the rearrangement of the other.

The parallelism of the F-actin pattern with all of the microtubular arrays suggests some interaction. It is known for animal cells that actin filaments and microtubules can interact via microtubule-associated proteins (Griffith and Pollard, 1982; Sattilaro et al., 1981; see Pollard et al., 1984 for review) but this remains to be established for plants.

As for the function of the thin cortical filaments, Heath and Seagull (1982) have already suggested that such filaments could be involved in moving cellulose synthesizing complexes along the plasma membrane. In addition, cortical microtubules are known to form helices of variable pitch (Lloyd, 1984; Traas et al., 1984; Roberts et al., 1985). Microtubule–microtubule interaction has been cited (Lloyd, 1984) as a possible means of altering the pitch of the cortical microtubule array, but experiments are under way to see if actin filaments could play a part.

Division

As the chromatin begins to condense, the even distribution of cortical microtubules characteristic of interphase becomes replaced by the more concentrated arrangement of the preprophase band. At this stage, RLP stains a similar perinuclear band at the cortex. It appears from fluorescence microscopy that actin could be present amongst the band microtubules as small bundles, although the intense fluorescence of this zone could be obscuring additional, more diffuse arrangements.

Our results indicate that F-actin is present in the spindle. As in animal cells (e.g., Cande et al. 1977; Aubin et al., 1979) there is still debate about both the function and the presence of F-actin in the plant cell spindle. Several studies have been unable to detect actin in the plant cell's spindle (Clayton and Lloyd, 1985; Derksen et al., 1986; Gunning and Wick, 1985). However, studying *Haemanthus* endosperm cells, Forer and Jackson (1979) identified actin-containing filaments in the spindle using heavy meromyosin labeling. More recently, Seagull et al. (1987), reported the presence of spindle-associated actin in *Alfalfa* and *Vicia* suspension cells using conventional formaldehyde fixation in combination with phalloidin staining.

Considering the evidence as a whole, the idea that actin is present in the spindle seems no less likely than the possibility that some classes of actin filaments are especially sensitive to fixation (see also, Pollard et al., 1984). More pertinent to cellular morphogenesis in plants are the findings that actin networks appear to be present in the cytoplasm throughout the division cycle. One of the unexplained features of the microtubule cycle concerns the way in which the “memory” of the preprophase band (which foretells the position of the future division site, but disappears by metaphase) persists until cytokinesis (e.g., Wick and Duniec, 1983, 1984). At cytokinesis, the centrifugally growing phragmoplast deposits a cell plate that eventually fuses with maternal side walls at the site previously marked by the preprophase band (see Hepler, 1985; and Lloyd, 1987 for discussion). There is no evidence that the memory is retained by intermediate filament-like proteins, since it has been shown that a conserved intermediate filament epitope in plant cells also disappears from the cytoplasm during mitosis (Dawson et al., 1985). The F-actin network does, however, persist in the cytoplasm throughout division and, as indicated by the three-dimensional reconstructions, forms a cage around the dividing nucleus. The phragmoplast, too, is associated with the RLP-staining network. As the only filamentous system so far known to endure mitosis and cytokinesis, the possibility
should be strongly considered that the actin network is involved in positioning the division plane. This is consistent with the findings of Palevitz (1980) that cytochalasin B blocks normal rotation of the spindle/phragmoplast axis in guard mother cells of Allium.

We would like to thank P. Hussey for helpful discussions on the extraction conditions. We thank Drs. D. Agard and J. Sedat for generously sharing their programs with us, and the Gatsby Foundation for a grant to cover the costs of part of the equipment used. We thank Patricia Phillips and Andrew Davies for secretarial and photographic assistance.

This study was supported by the Agricultural and Food Research Council via a grant-in-aid to the John Innes Institute. J. A. Traas was supported by a European Molecular Biological Organization long-term Fellowship; J. H. Dooman by the Science and Engineering Research Council; D. Rawlins, P. J. Shaw, and J. Watts by the Agricultural and Food Research Council; C. W. Lloyd by The Royal Society.

Received for publication 24 November 1986, and in revised form 19 February 1987.

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