Development 102, 211–221 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

The role of F-actin in determining the division plane of carrot suspension cells. Drug studies

CLIVE W. LLOYD and JAN A. TRAAS

Department of Cell Biology, John Innes Institute and AFRC Institute of Plant Science Research, Colney Lane, Norwich NR4 7UH, UK

Summary

Following the report that a network of F-actin is associated with the nucleus throughout the division cycle, we have examined the involvement of F-actin in determining the division plane of carrot suspension cells. This was achieved by treating cells with drugs and then staining the unfixed cells with rhodaminyl lysine phallotoxin in detergent extraction buffer.

In interphase, actin cables radiate from the nucleus but at the cortex become more or less transversely arranged in the pattern already known for cortical microtubules. Concentration of the cortical F-actin into a band at preprophase draws most of the nucleus-associated actin into a transvacuolar disc, thereby forming the phragmosome within which mitosis and cytokinesis occur.

In addition to this transversely aligned structure, F-actin is also associated with the spindle poles during mitosis but these filaments tend to align at right angles to the phragmosomal actin. F-actin therefore defines transverse and longitudinal vectors as division approaches. Depolymerization of F-actin with cytochalasin D can cause the spindle axis to reorientate such that the pole–pole axis comes to lie, abnormally, parallel with the phragmosome.

The cytokinetic apparatus (the phragmoplast) develops centrifugally within the phragmosome. There has been considerable speculation on the nature of the elements that guide the phragmoplast to the cortical site previously occupied by the preprophase band of microtubules. This study demonstrates that F-actin bridges the leading margin of the outgrowing phragmoplast to the opposing cortex. Radial actin strands therefore provide a 'memory' of the predetermined division plane whose perimeter had been marked at preprophase by a band composed of microtubules and F-actin.

This relationship was perturbed with the herbicide, chloroisopropylphenyl carbamate. Preprophase bands of actin appear to form normally in herbicide-treated cells. However, cytokinesis does not occur within this predicted plane since the drug perturbs the mitotic spindle, forming three nuclei which become separated by Y-shaped, actin-containing phragmoplasts. Cytoplasmic actin strands connect the edges of the phragmoplast to the cortex. It is suggested that the irregular distribution of F-actin, which radiates from the herbicide-altered mitotic apparatus, provides alternative paths for outgrowth of the abnormal phragmoplasts.

Caffeine is known to cause failure of cell plate formation. But apart from inducing cytoplasmic 'starbursts' of F-actin in interphase cells it does not appear to have any effect on F-actin-containing division structures.

It is concluded that the formation of a transvacuolar phragmosome, spindle alignment and the 'correct' outgrowth of a planar cytokinetic apparatus to the predetermined boundary of the division site all involve F-actin.

Key words: F-actin, plane of division, cytokinesis, carrot cells.

Introduction

The precision with which a new cell plate is aligned across a dividing plant cell determines the spatial relationship between the daughters and, ultimately, the organization of cells into tissues.

There has been a long-lasting search for the underlying rules governing the placement of the cross wall.

Early investigators (see Gunning, 1982, for review) considered that the cross wall would be subjected to physical forces much in the way that the meeting points of liquid films are determined in clusters of soap bubbles.

Sinnott & Bloch (1940, 1941) argued against the liquid film hypothesis. They observed that an anastomosis of cytoplasmic strands, quite unlike a liquid film, formed across vacuolated cells at prophase. This structure was termed the phragmosome and is notable in that it forms in the plane to be assumed later by the cell plate.

Another structure also anticipates the division plane. The preprophase band (PPB) is a cortical belt of microtubules which marks the site where the outgrowing cell plate will fuse with the parental side walls (Pickett-Heaps & Northcote, 1966; Palevitz & Hepler, 1974; Gunning, Hardham & Hughes, 1978). The phragmosome and the PPB should therefore define the same plane. This was confirmed by Venverloo *et al.* (1980) who located a band of microtubules at the junction between the phragmosome and the cortical cytoplasm in large, vacuolated epidermal cells of *Nautilocalyx*.

A puzzling feature of this prepositioning of the division site is that the PPB microtubules disappear by metaphase, yet some imprint would be expected to remain until the division plane is made actual during cytokinesis. The imprint does not appear to take the form of microtubule nucleation sites since these have been detected, immunologically, around the nucleus and not in the cortex of onion cells (Clayton *et al.* 1985). Neither does an intermediate filament antigen specify the cortical site. Although detected at the PPB site, the epitope disappears along with band microtubules during mitosis (Dawson *et al.* 1985).

An alternative way of framing the problem has been to look for structures that might tether the dividing nucleus to the cortex. This places more emphasis on radial rather than circumferential elements of the division plane. Ota (1961) displaced dividing nuclei by centrifugation. While the nucleus recovered its normal position, he observed that the growing edges of the cell plate curved towards the division site to which they appeared to be attracted by a pulling force. Similarly, Gunning & Wick (1985) observed that young phragmoplasts, which were displaced or tilted when the cell was punctured by a needle, turned their margins to the 'correct' division site. A further example of the interaction between the edge of the phragmoplast and the cortical division site comes from Palevitz's (1986) work on guard mother cells (GMCs). A tilted mitotic apparatus gives rise to a tilted phragmoplast, which does not initially conform to the PPB site. But as the phragmoplast grows out, its edges apparently interact with the former PPB zone to yield a sigmoidal cell plate which finally straightens out into the predicted plane. This illustrates both that spindle alignment does not necessarily correlate with the eventual division plane, and that the PPB-imprinted cortical site exerts an influence capable of correcting misaligned cell plates (see also Gunning, 1982; Galatis *et al.* 1984).

Attempts have been made to identify cytoskeletal elements between the edges of the phragmoplast and the cortical division site. Inhibiting realignment of the skewed phragmoplast in GMCs by phalloidin and cytochalasin B (Palevitz, 1980) implies a role for microfilaments. Bundles of thin filaments have been observed, by electron microscopy, to be components of the highly extended phragmosomes which form across cambial cells of *Fraxinus* (Goosen-deRoo *et al.* 1984) although their composition is unknown.

Initial attempts at detecting F-actin, with labelled phallotoxin, between phragmoplast and cortex were unsuccessful (Clayton & Lloyd, 1985; Lloyd *et al.* 1985; Gunning & Wick, 1985) although the phragmoplast itself could be stained. However, to avoid the potentially damaging effects of aldehyde fixation, we (Traas *et al.* 1987) recently used two methods for introducing rhodamine phalloidin into unfixed carrot suspension cells: mild detergent extraction and electroporation. Both revealed an extensive network of F-actin which codistributes generally, but not exclusively, with the four microtubular arrays formed by cycling cells. In interphase (Parthasarathy, 1985; Parthasarathy *et al.* 1985; Clayton & Lloyd, 1985; Seagull *et al.* 1987) thick F-actin cables form across the vacuole and in the cortical cytoplasm, but finer elements have been seen at the cortex, parallel to the known transverse alignment of cortical microtubules (Seagull *et al.* 1987; Traas *et al.* 1987). At preprophase, the cortical F-actin forms a transverse band, corresponding to the PPB microtubules. This has been observed in aldehyde-fixed onion cells (Palevitz, 1987) but we have observed that in unfixed carrot suspension cells the preprophase band of F-actin is part of a more pervasive cytoplasmic network which associates with the nucleus throughout mitosis and cytokinesis (Traas *et al.* 1987).

In that paper, it was suggested that this network could be involved in setting the division plane. In the present study, this issue has been investigated further. In particular, we tried to determine whether F-actin connected the margin of the growing phragmoplast to the cortex. Actin filaments do bridge phragmoplast to cortex and the results of drug perturbation are described.

Materials and methods

Carrot suspension cells were maintained as described by Lloyd *et al.* (1980). The cells were taken 1–4 days after subculture, when they were actively dividing. Cells were 'permeabilized' as described in Traas *et al.* (1987). Briefly, equal volumes of cell suspension and extraction buffer (microtubule-stabilizing buffer, 5% (v/v) dimethylsulphoxide (DMSO), 0·025% (v/v) Nonidet P40, 100 mM mannitol) were mixed then made 10^{-6} M with rhodaminyl lysine phallotoxin (RLP, a kind gift Prof. Th. Wieland). Diamidino 2-phenylindole (DAPI) was added to 0·2 $\mu g\,ml^{-1}$ in order to stain DNA.

The following controls were outlined in that previous paper. (1) Staining with rhodaminyl lysine phallotoxin (RLP) is still detectable at 10^{-8} M which is 100-fold lower than is known to stimulate actin polymerization. (2) After extracting soluble proteins with detergent, the addition of RLP produces the same staining patterns, implying that they are not produced artefactually from G-actin. (3) The patterns are abolished by prior incubation with unlabelled phalloidin. (4) The patterns are, generally, cytochalasin sensitive. (5) Staining is not abolished by drugs which selectively depolymerize microtubules. (6) RLP introduced into cells by electroporation produces similar patterns as when cells are extracted with detergent/DMSO.

In this paper, all results were obtained by adding RLP to the extraction buffer.

Caffeine (Sigma Chemical Co., Poole, Dorset) was added to 0·1% (w/v) by adding solid directly to cell suspension. Chloroisopropyl phenyl carbamate (CIPC, Sigma) was added to 30 μM from a 3 mM stock in DMSO. Controls received 1% (v/v) DMSO. Cytochalasin D (Sigma) was added to 10 μM from a 1 mM stock. When added together, cytochalasin D was dissolved in stock CIPC/DMSO in order to keep the final DMSO concentration to 1% (v/v).

Results

Actin in the division plane of control cells

During interphase there appears to be no great concentration of F-actin to anticipate a future division plane. In cylindrical carrot suspension cells, F-actin is arranged more or less transversely around the cortex although other orientations are possible in this region (Fig. 1A). At a deeper level of focus, longitudinal bundles can be seen to be associated with the nucleus (Fig. 1B). In cells with a larger circumference the noncortical, nucleus-associated actin filaments are not as markedly aligned to the cell's long axis. In long, bloated cells longitudinal nucleus-associated bundles can still be discerned (Fig. 1C) but in isodiametric cells (Fig. 1D) the F-actin radiates from the nucleus in several directions. At preprophase to prophase the cortical F-actin concentrates in a transverse band, as has been described (Traas *et al.* 1987; Palevitz, 1987). F-actin is still present in distal parts of the cortex, connected with, but not as organized as the band actin (Fig. 1E). At this stage, through-focussing indicates a meshwork of F-actin occupying the central region of the belt defined by the cortical preprophase band of actin. Actin is therefore a major component of the transvacuolar phragmosome.

At metaphase, F-actin can be seen between nucleus and cortex, continuing the plane defined by the metaphase plate (Fig. 2A). The pole–pole axis of the mitotic spindle is therefore orientated at right angles to the phragmosomal plane. At the cortex, the F-actin meshwork persists but appears randomly organized. There is no evidence for the persistence through metaphase of the cortical band of F-actin which formed at preprophase. The radial F-actin within the presumptive division plane is not the only class of nucleus-associated actin stained with RLP. Actin cables also associate with the spindle poles giving the appearance of horns (Fig. 2B). This F-actin curves away from the mitotic apparatus to the cortex where it tends to become aligned along the cell's long axis. During telophase, the F-actin that radiates from the spindle poles is especially prominent (Fig. 2C,D).

The phragmoplast develops within the phragmosome which, in terms of F-actin, comprises a complicated meshwork. Fig. 3A,B illustrate something of the complexity of this central, transvacuolar raft of filaments within which the more brightly staining phragmoplast is situated. The phragmoplast often develops eccentrically. In narrow cylindrical cells, the short distance between the outer margin of this apparatus and the cortex can be seen to be bridged by short F-actin filaments within the transverse phragmosomal plane (Fig. 3B–D). In highly vacuolated cells with a larger circumference, there is a greater gap between phragmoplast and cortex and the connecting strands are more conspicuous (Fig. 3E,F).

CIPC-treated cells

In onion root tip cells CIPC was shown to induce tripolar spindles which are followed by three-way phragmoplasts (Clayton & Lloyd, 1984). The complex cytokineses were not predicted by PPB microtubules. In view of this drug-induced dislocation between PPB site and the paths taken by the phragmoplast, CIPC was added to carrot suspension cells in an attempt to produce a similar effect in these vacuolated cells. In particular, we wished to see whether all limbs of three-way phragmoplasts stained with RLP, and that F-actin extended out from their margins.

After 4–6 h treatment with CIPC the interphase cortical actin is not as well ordered in transverse arrays as are controls. Central, cortical bands of F-actin do, however, form at preprophase (Fig. 4A).

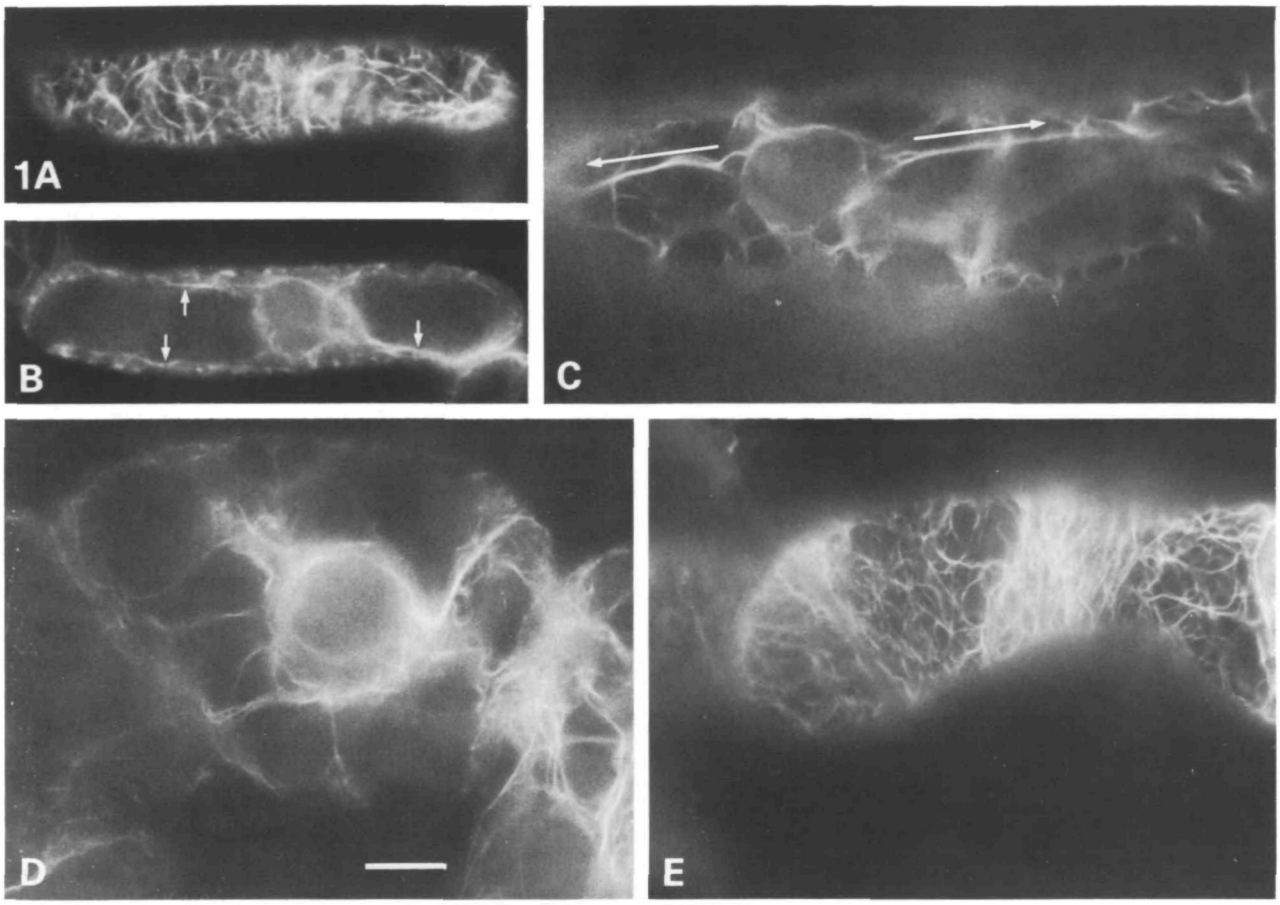


Fig. 1. F-actin during interphase and preprophase. In elongated cells (A) actin filaments tend to be orientated transversely at the cortex, although other alignments can be discerned. In the same cell, at a deeper level of focussing (B), filaments associated with the nucleus are seen to run along the long axis in the subcortical region (arrows). In a subclass of bloated cells, the nucleus is still associated with axially aligned subcortical filaments in the longer cells (arrows), although F-actin can now be seen to radiate in other directions (C). In isodiametric cells the tendency to axial alignment is lost (D). At preprophase (E) the cortical F-actin bunches-up to form a band but nonband actin becomes disorganized rather than depolymerized. Bar, 10 μm.

In initial experiments using 100 μM-CIPC, dividing cells were seen to be arrested over a 6 h period in a pro-metaphase-like configuration. Actin strands radiated from the nucleus (Fig. 4B,C) at a time when the chromatin consisted of a nuclear-envelope-free ball of chromosomes (Fig. 4C). The radial actin strands connected with a disorganized cortical meshwork (Fig. 4D). However, when the herbicide concentration was lowered to 30 μM, mitosis proceeded some 2–4 h later. No single clear-cut pattern could be discerned (e.g. each pole of a tripolar spindle associated with a single cytoplasmic bundle of actin) for nucleus-associated cytoplasmic actin. Instead, spindles were associated with a more diffuse meshwork which, by focussing, could be traced out to the disorganized cortical meshwork as in Fig. 4C,D. From approximately 3 h onwards in 30μM-CIPC, three-way (Fig. 4E–G) and less commonly four-way, (Fig. 4H,I) phragmoplasts could be stained with RLP. The angles between the three-limbed phragmoplasts are usually equal. A meshwork of filaments could be seen to extend beyond the confines of the brightly staining phragmoplast and to connect with the cortical F-actin.

CIPC treatment is also capable of inducing aberrant longitudinal phragmoplasts as shown in Fig. 4J.

Caffeine-treated cells

Caffeine inhibits cell plate formation (see Gunning, 1982). It does not, however, have any noticeable effect on cytokinetic F-actin since there was no difference between the RLP-staining of control and drug-treated cells. One prominent effect of 0·1% (w/v) caffeine is that it induces tangles of cytoplasmic F-actin, usually within interphase cells. These tangles or 'starbursts' bear no obvious relationship to the nucleus, often occurring tens of micrometres away (inset, Fig. 4J).

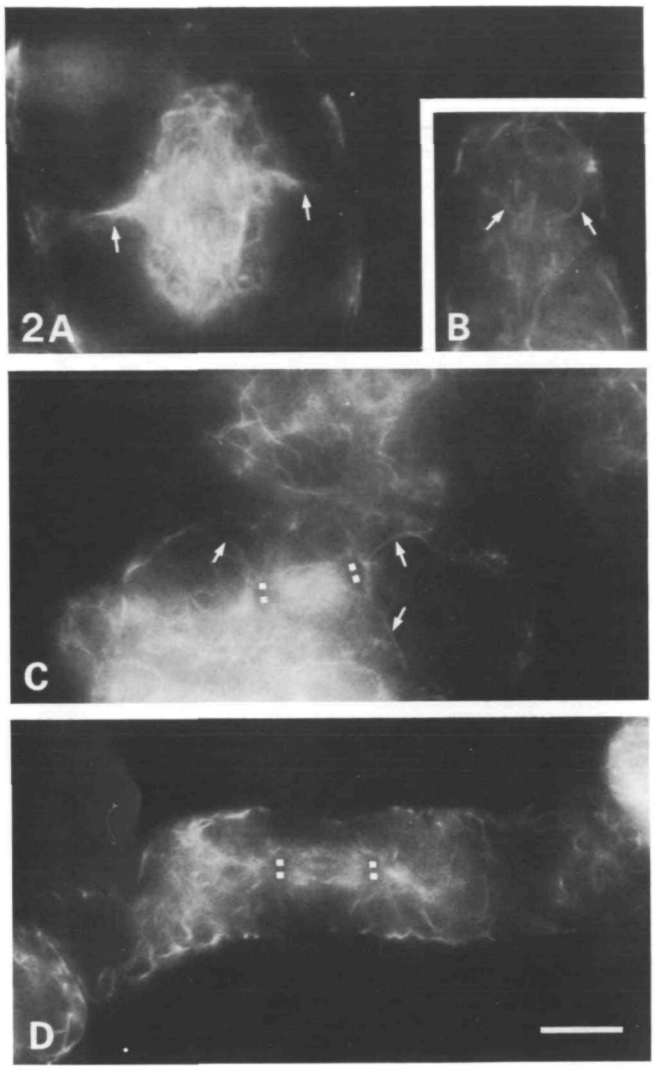


Fig. 2. Nucleus-associated actin filaments during mitosis. At metaphase (A), actin filaments cage the nucleus. Filaments also contribute to the phragmosome – forming strands that extend to plane of the metaphase plate, connecting nucleus to cortex (arrows). Another class of nucleus-associated actin filaments is associated with the spindle poles. These tend to curve around the vacuole towards the end walls. This is illustrated for metaphase (arrows) (B) and telophase (arrows) (C). At late telophase, radiation of F-actin from the spindle poles is sometimes particularly prominent (D). The positions of the telophase nuclei in C and D are indicated by double dots. Bar, 10 μm.

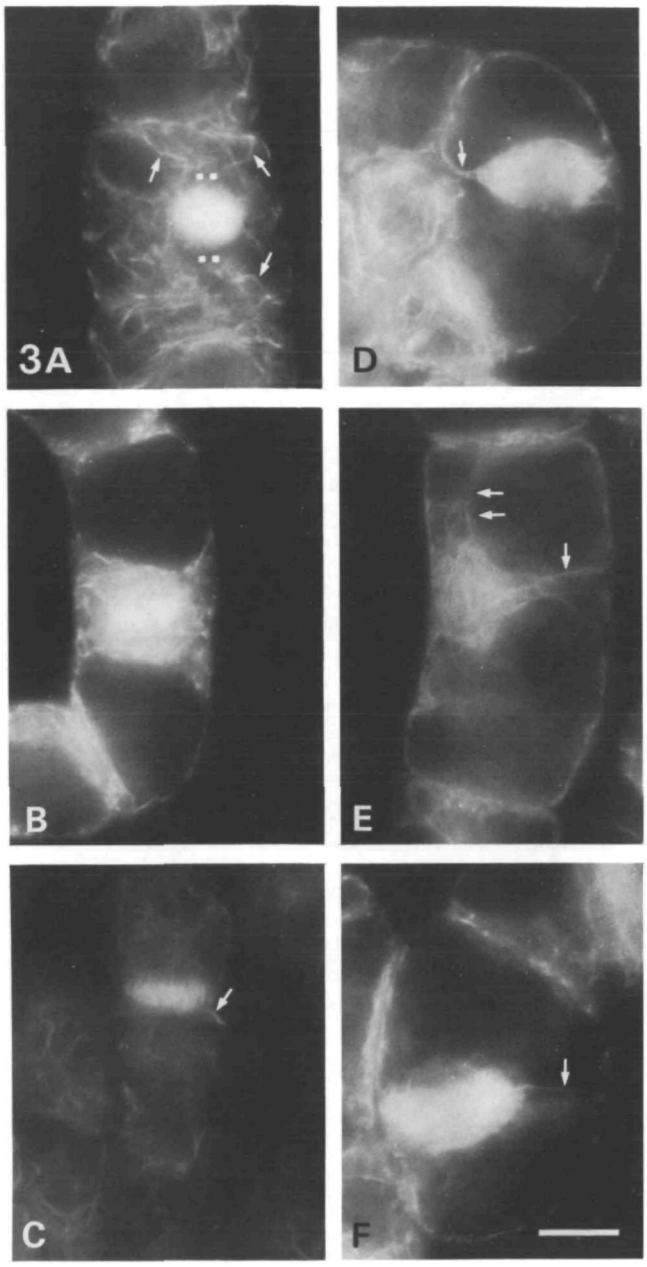
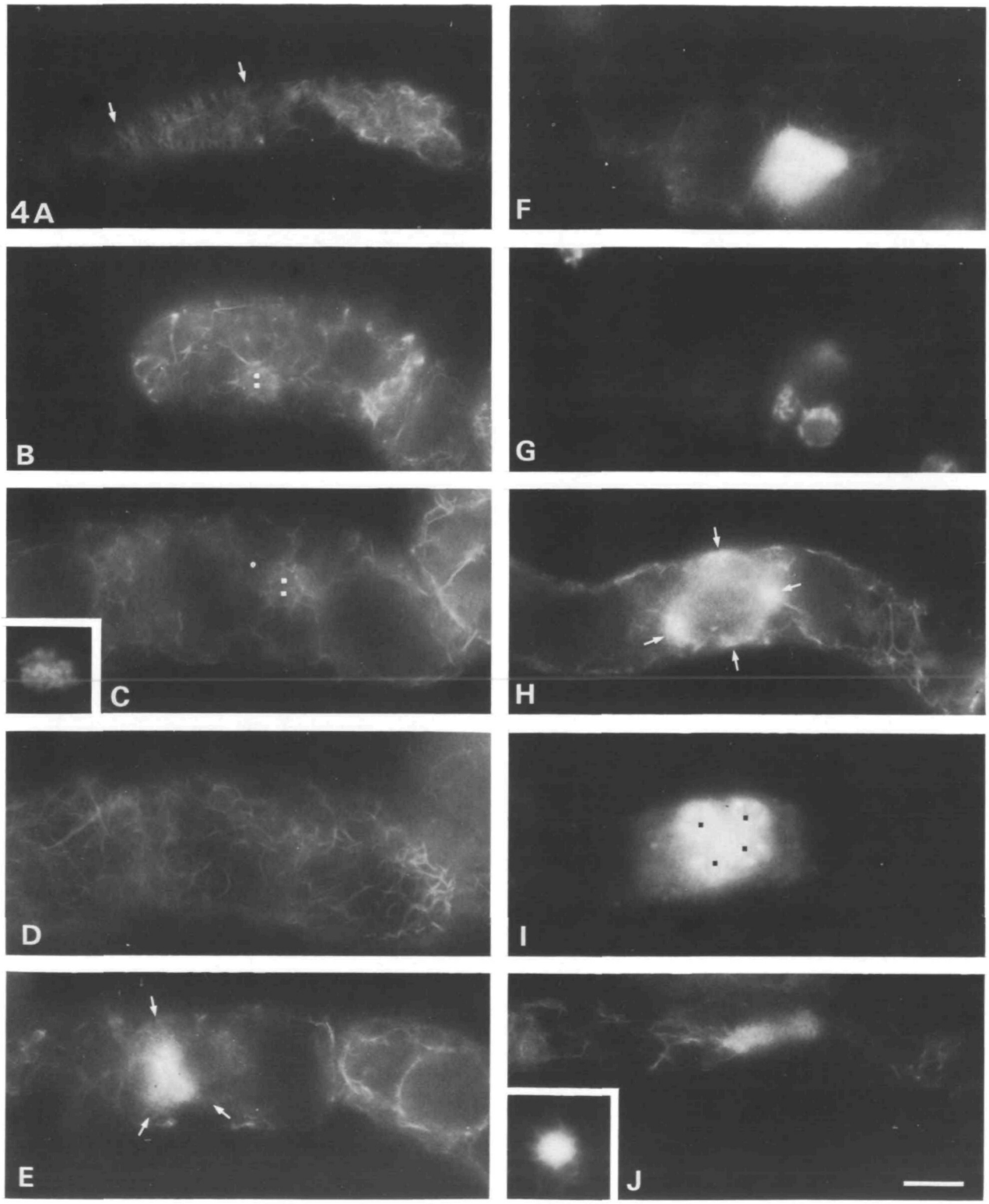


Fig. 3. F-actin during cytokinesis. The RLP-staining phragmoplast develops amongst a complex meshwork of transvacuolar F-actin (A,B). Pole-associated strands are arrowed in A and the positions of chromatin indicated by double dots. By through-focussing, F-actin can be seen to bridge the leading edge of the outgrowing phragmoplast to the opposite cortex (C,D). This is pronounced in cells with a greater gap between cytokinetic apparatus and side wall (single arrows in E,F). Note also the pole-associated actin (double arrows in E) aligned at right angles to the phragmosomal strand during early cytokinesis. Bar, 10 μm.

Cytochalasin D-treatment

10 μM-CD for 20 min fragmented most of the interphase actin cables although some cables are resistant for periods between 6 and 24 h. During preprophase, most nonband actin is fragmented by CD but the transverse cortical F-actin band is seen in many cells to be resistant (Fig. 5A). This resistant staining is not confined to the cortex of preprophase cells since it occurs across the vacuole i.e. within the phragmosome. RLP staining of the mitotic spindle is also resistant to a treatment with CD which fragments the

majority of extraspindle F-actin (Fig. 5B). The polar-associated actin filaments do not appear to be resistant to CD. In the subpopulation of bloated carrot cells (as opposed to the narrow cylindrical cells) spindles tilted at 45–90° to the anticipated division plane (which is invariably transverse to the long axis of elongated cells) can be observed in the presence of 10 μM-CD (Fig. 5B–D). Phragmoplast staining is also resistant to CD (Fig. 5E) as are actin filaments which extend, within the phragmosomal plane, from the margin of the phragmoplast to the opposing cortex (Fig. 5F).



Discussion

The redistribution of F-actin and microtubules during mitosis and cytokinesis is summarized in an explanatory diagram (Fig. 6).

Premitotic events

In the elongated, cylindrical carrot suspension cells, the cortical microtubules are known to occur in regular transverse arrays (Lloyd *et al.* 1979, 1980; Doonan *et al.* 1987). The cortical F-actin is also arranged transversely (Traas *et al.* 1987; present results). Actin bundles are also associated with the nucleus and, in cylindrical cells, with a relatively narrow diameter they are conspicuously longitudinal. Such longitudinal bundles run along the cortical cytoplasm beneath the transverse cortical elements or along transvacuolar strands. In wider nearly isodiametric cells, the nucleus-associated F-actin cables are not exclusively longitudinal; they radiate to various parts of the cortex and at this stage there is no preferred transverse alignment.

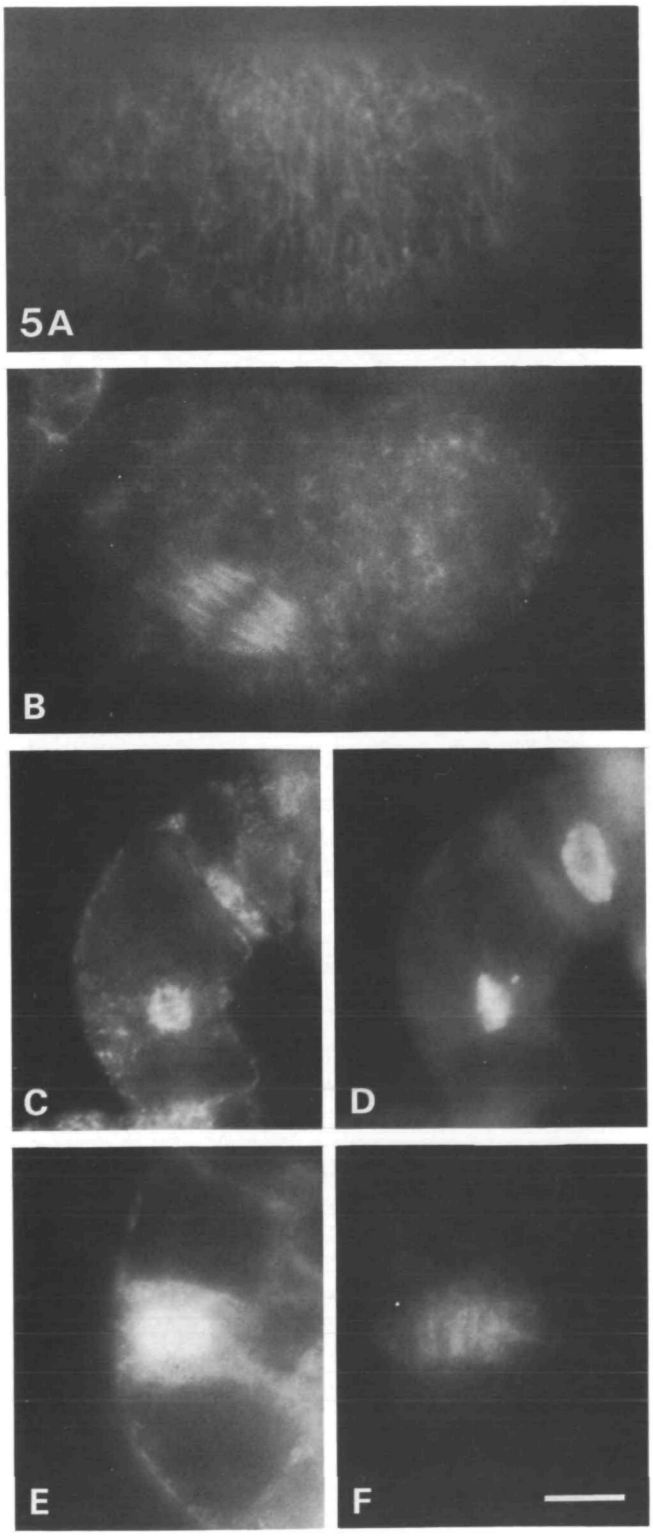


Fig. 4. The herbicide CIPC perturbs the organization of F-actin during cell division. CIPC induces formation of multipolar spindles. At preprophase (A), transversely oriented F-actin forms a preprophase band (between arrows) in the presence of 30 μM-CIPC. At 100 μM, the dividing nucleus is arrested at a pro–metaphase-like stage. At this stage, the F-actin that radiates from the nucleus (indicated by double dots) does not appear to have a preferred axis of alignment (B). This is also the case for cells treated with 30 μM-CIPC (C) except that cells proceed through division. The pro–metaphase-like ball of chromosomes, stained with DAPI, is illustrated in the inset to C. In the same cell, the radial actin strands can be traced to the cortex where the meshwork has a disorganized appearance (D). During cytokinesis, three-way phragmoplasts are formed (E), each limb of which connects to the cortex by a meshwork of F-actin. An early three-way phragmoplast is shown in F; the three nuclei of the corresponding DAPI image presented in G. A less-common four-way phragmoplast is shown in H. The actin pattern is three-dimensionally complex. At this focal plane, four edges of the cytokinetic apparatus are in focus (arrowed) and are continuous with the distal cytoplasmic meshwork. The four DAPI-stained nuclei (single dots) are shown in I. Rare, CIPC-induced abnormalities include phragmoplasts abnormally aligned parallel to the cell's long axis (J). Note the actin strands that extend from the edge of the phragmoplast. Caffeine causes starbursts or tangles of F-actin to form in the cytoplasm of interphase carrot cells (inset to J). Bar, 10 μm.

Fig 5. The effects of cytochalasin D upon F-actin. At 10 μM, CD causes the fragmentation of the majority of cytoplasmic actin filaments. Resistant structures include preprophase band actin (A), the mitotic spindle (B,C) and the phragmoplast (E,F). Pole-associated actin is destroyed by CD treatment (B,C). In larger cells, spindles are frequently seen rotated at 90° to the transverse, transvacuolar cytoplasm (C). The DAPI-stained metaphase plate is shown in (D), parallel to the cell's longer axis. Phragmoplasts can still be stained with RLP in the presence of 10 μM-CD (E,F). Remnants of the actin network can still be detected between phragmoplast and cortex (F). Bar, 10 μm.

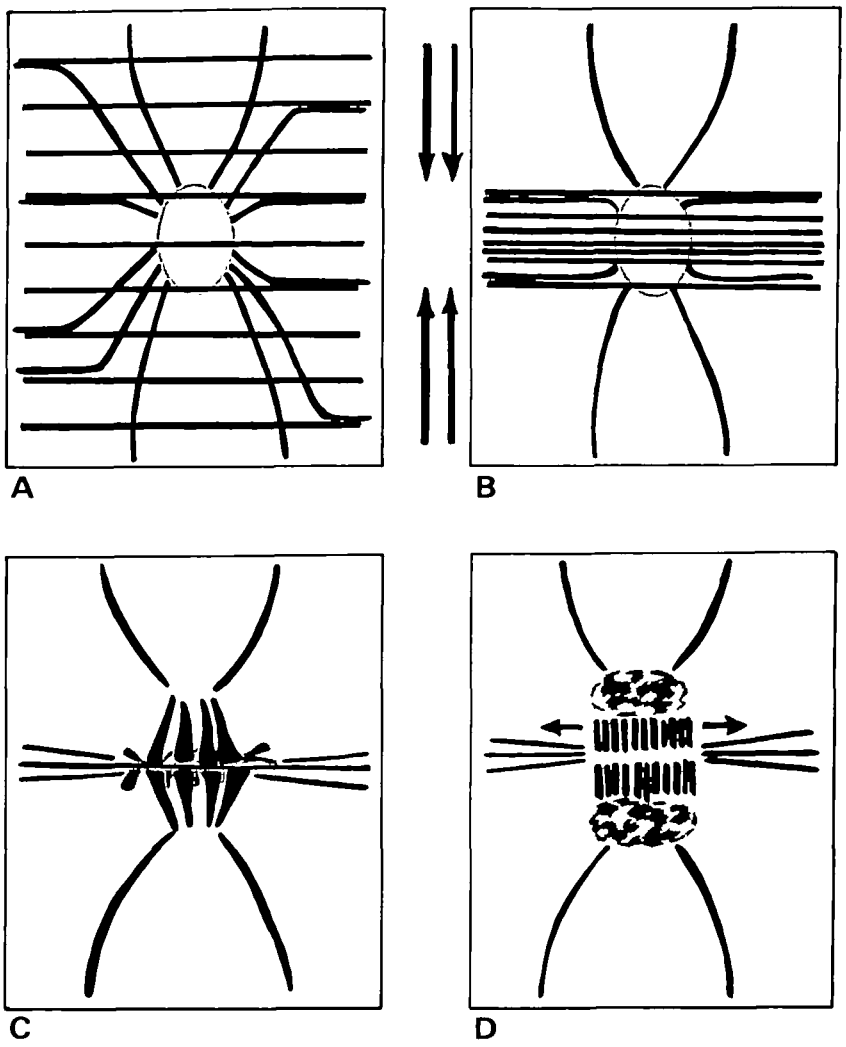


Fig. 6. (A). During interphase, F-actin strands (black) radiate from the nucleus to side walls and end walls. Some of the strands, particularly the finer cortical ones (see Traas *et al.* 1987), run parallel to the cortical microtubules, represented here as transverse grey lines. (B) At preprophase, cortical microtubules become concentrated in the PPB. F-actin that radiates from nucleus to side walls becomes similarly concentrated, perhaps by the sweeping action of the PPB microtubules as they bunch together. Nucleus to end wall F-actin remains to define a longitudinal axis at right angles to the transvacuolar phragmosomal actin. (C) By metaphase, the cortical microtubules have depolymerized but the transverse, phragmosomal F-actin remains to define the division plane. (D) Cytokinesis is brought about by a double ring of microtubules (the cytokinetic phragmoplast) which deposits the cell plate at its midline. This grows out centrifugally to the zone formerly occupied by PPB MTs, guided by phragmosomal F-actin which has remained throughout division in that plane.

During preprophase, however, (recognized by the pattern of chromatin condensation) both the cortical and the nucleus-associated actin cables become rearranged. Transverse cortical F-actin forms a band that encircles the nucleus (see also Traas *et al.* 1987). Non-band actin is also present at parts of the cortex distal to the nucleus but it loses its transverse order. It is during this phase that the phragmosome forms as a thick disc of actin filaments across the vacuole and around the nucleus. This series of observations is consistent with F-actin being concentrated into a disc by the act of forming a preprophase band of microtubules except that this phragmosome is transvacuolar and not just confined to the cortex. Whether the PPB is formed from newly polymerized tubulin or results from rearrangement of interphase microtubules there is evidence to suggest that 'bunching-up' is part of the process; that is, initially broad PPBs of MTs become tighter by prophase (Wick & Duniec, 1984; Doonan *et al.* 1987). That cortical microtubules influence the alignment of cortical F-actin, and not *vice-versa*, is supported by two lines of evidence. Palevitz (1987) has reported that PPBs of MTs form even though PPBs of actin are prevented from forming by cytochalasin B; we (Traas *et al.* 1987) have shown that cortical F-actin loses its transverse order when interphase microtubules are depolymerized by cremart. Bunching of cortical MTs into a preprophase band would, therefore, not only sweep parallel cortical F-actin into the band but also draw into the phragmosome any nucleus-associated, transvacuolar strands which radiate to the cortex within the greater band zone.

Cytoskeletal elements do appear to be stabilized within the band zone. In contrast to the randomized cortical actin outside the transverse PPB zone, actin within the band is resistant to aldehyde fixation (Palevitz, 1987) and to 10 μM-cytochalasin D (present observations). Another explanation, therefore, for the concentration of phragmosomal actin across the PPB zone is that strands radiating from the nucleus are offered more stable anchorage within the cortex of the band region than elsewhere along the side walls.

Having established the division plane, the preprophase band of actin disappears concomitantly with the depolymerization of PPB MTs. The phragmosomal actin (see below) remains. In terms of F-actin, it is therefore the radial and not the circumferential elements which retain the memory of the division site throughout division.

In carrot cells, the nucleus migrates from a position at one flank of the cell towards (but not necessarily at) the centre of the phragmosome. In this case, there is minimal movement but there are instances where the nucleus migrates over greater distances before dividing (see Gunning, 1982). Our work (Lloyd *et al.* 1987) has shown that when *Vicia* root hairs are treated with cremart in order to depolymerize MTs, the nucleus (which is also associated with actin cables) becomes free to migrate over tens of micrometres in a cytochalasin-sensitive manner. That is, maintenance of a particular position requires MTs but in their absence the nucleus is propelled to a new site by a process involving F-actin. In cells capable of division, the reorganization of cortical MTs prior to division, although not drug-induced, causes a similar shift in the balance between F-actin and MTs.

Migration induced by such a shift should tend to bring the nucleus to the plane within which the nucleus-to-cortex actin strands retain firm cortical anchorage i.e. within the PPB zone.

Once the division plane is set up there is evidence that F-actin-based cytoplasmic streaming ceases (Mineyuki *et al.* 1984; Ota, 1961). This may be part of the mechanism by which the division plane remains fixed after the preprophase band disappears – the nucleus becoming more resistant to displacement by centrifugation at this stage (Mineyuki & Furuya, 1980; see Gunning, 1982).

Spindle alignment

Elongated carrot suspension cells divide transversely to form chains of cells. Longitudinal 'T' divisions are only seen in shorter cells which divide to form clusters. In the former category, the mitotic spindle is aligned such that the metaphase plate is parallel to the phragmosome (i.e. the pole–pole axis is at right angles to the future division plane). Either the spindle aligns parallel to the longitudinal cell axis or by setting itself at right angles to the phragmosome. The RLP staining confirms that F-actin exists in both planes: actin forms a meshwork within the phragmosome and filaments associated with the spindle poles curve away along the cell's long axis. The latter filaments proceed to the ends of the cell or, in chains of cells, towards the shorter cross walls i.e. away from the PPB zone. Nucleus-associated actin filaments therefore delineate transverse as well as axial vectors during division. These two classes of filaments correspond to structures observed by Sinnott & Bloch (1940, 1941) in their light microscopical study of dividing vacuolate cells. In addition to the transvacuolar strands that coalesce to form the phragmosome by prophase, they also noted apical–basal strands running from the nucleus at right angles to the future division plane, giving the cytoplasm the appearance of a Maltese cross. Ota (1961) made similar observations, reporting that 'the spindle is held in its right position in the cell' by connection 'to some differentiated pattern in the cell cortex by the polar plasmic strands, the phragmosome and other cytoplasmic strands'. One set of observations particularly emphasizes the role of the 'polar plasmic strands' in stabilizing the spindle axis. Ota (1961) broke the strands by centrifugation, in which case the spindle pole swayed with a large amplitude and anaphase separation was slower than normal. The spindle pole was restabilized by a thickening cytoplasmic strand which swung from between spindle and side wall through 90° to reestablish the polar plasmic strand. In the larger, isodiametric carrot cells (in which there is greater latitude for spindle rotation), the entire spindle can be made to reorientate in the presence of CD such that the pole–pole axis is parallel to the phragmosomal cytoplasm and not at right angles to it. Unfortunately, CD treatment depolymerizes both pole-associated and (to a lesser extent) phragmosomal actin. Although this indicates that some form of actin is involved in spindle alignment it does not allow us to conclude which class. However, there are many observations in the literature (see Gunning, 1982) which indicate that the metaphase plate need not necessarily conform to the PPB/phragmosomal plane. This weakens the argument that phragmosomal actin provides the reference for spindle alignment. But further experiments, such as the laser ablation studies performed by Aist & Berns (1981) on *Fusarium*, are required in order to establish that polar-associated actin influences spindle alignment in higher plants.

Postmitotic events

Results of several experiments have stressed the presence of a physical connection between the margin of the phragmoplast and the predetermined division site at the cortex (e.g. Sinnott & Bloch, 1940, 1941; Ota, 1961; Gunning, 1982; Gunning & Wick, 1985). Inhibition of cell plate realignment by anti-actin drugs (Palevitz, 1980, 1986) and observation of microfilamentous bundles in EM sections of extended phragmosomes (Goosen-de Roo *et al.* 1984) have encouraged the idea that the physical connection would take the form of actin filaments. The present study now establishes that F-actin does connect the leading edge

of the phragmoplast to the opposing cortex. This is especially conspicuous in the bloated cells where F-actin spans a greater distance than in the narrow cylindrical cells.

The herbicide CIPC induces tripolar spindles succeeded by three-way phragmoplasts. In a previous anti-tubulin study of CIPC-treated onion root tip cells, it was demonstrated that PPB microtubules formed regular bands which could not, therefore, predict the paths to be taken later by supernumerary limbs of the phragmoplast (Clayton & Lloyd, 1985). Such three-, and sometimes four-way, phragmoplasts form in the larger, vacuolated CIPC-treated carrot suspension cells. All limbs of the phragmoplast stain with RLP, and F-actin undoubtedly continues out beyond the margins of the phragmoplast. However, this actin takes the form of a meshwork rather than the more clearly defined phragmoplast-cortex cables seen in controls. Preprophase actin bands occur in the presence of CIPC and so the formation of supernumerary cytokinetic limbs out of the plane of the band cannot be ascribed to lack of cortical imprinting. Re-adjustment of tilted phragmoplasts through at least 45° can be made in order to align the cell plate with the former PPB zone (Palevitz, 1986). Phragmoplasts are also sufficiently flexible that they can bend as they grow (Ota, 1961; Palevitz, 1986). So why aren't the limbs of three-way phragmoplasts re-adjusted during outgrowth to the former PPB zone? Lack of space is unlikely to be important since realignment of the forming cell plate can be performed successfully in *Allium* guard mother cells where the spindle almost fills the cell (Palevitz, 1986). One reason could be that the picture of phragmosomal (transverse) and pole-associated (longitudinal) F-actin observed in controls cannot be discerned in CIPC-treated cells. Unlike controls with bipolar spindles, CIPC-treated cells must form three or more spindle poles so that the pole-associated actin radiates out in different directions to those seen in controls. F-actin therefore defines additional radii along which aberrant limbs could develop during cytokinesis. The 'correction system' functions in control cells where cell plate and preprophase band are both planar. However, the introduction of a third spindle pole at metaphase sets up a nonplanar geometry irreconcilable with a planar division site formed by the premitotic bunching of cortical microtubules.

C.W.L. was supported by a Royal Society Senior Research Fellowship, J.A.T. by an EMBO long-term Fellowship. Pat Phillips is thanked for secretarial assistance and Andrew Davies for photographic assistance. We are grateful to Prof. Th. Wieland for the rhodamine phalloidin.

References

Aist, J. R. & Berns, M. W. (1981). Mechanics of chromosome separation during mitosis in *Fusarium* (Fungi imperfecti): New evidence from ultrastructural and laser microbeam experiments. *J. Cell Biol.* **91**, 446–458.

Clayton, L., Black, C. M. & Lloyd, C. W. (1985). Microtubule nucleating sites in higher plant cells identified by an auto-antibody against pericentriolar material. *J. Cell Biol.* **101**, 319–324.

Clayton, L. & Lloyd, C. W. (1984). The relationship between the division plane and spindle geometry in Allium cells treated with CIPC and griseofulvin: an anti-tubulin study. *Eur. J. Cell Biol.* **34**, 248–253.

Clayton, L. & Lloyd, C. W. (1985). Actin organization during the cell cycle in meristematic plant cells. Actin is present in the cytokinetic phragmoplast. *Expl Cell Res.* **156**, 231–238.

Dawson, P. J., Hulme, J. H. & Lloyd, C. W. (1985). Monoclonal antibody to intermediate filament antigen cross-reacts with higher plant cells. *J. Cell Biol.* **100**, 1793–1798.

Doonan, J. H., Cove, D. J., Corke, F. M. K. & Lloyd, C. W. (1987). Preprophase band of microtubules, absent from tip-growing moss filaments, arises in leafy shoots during transition to intercalary growth. *Cell Motility Cytoskel.* **7**, 138–153.

Galatis, B., Apostolakos, P. & Katsaros, C. (1984). Positional inconsistency between preprophase microtubule band and final cell plate arrangement during triangular subsidiary cell and atypical hair cell formation in two *Triticum* species. *Can. J. Bot.* **62**, 343–359.

Goosen-de Roo, L., Bakhuizen, R., van Spronsen, P. C. & Libbenga, K. R. (1984). The presence of extended phragmosomes containing cytoskeletal elements in fusiform cambial cells of *Fraxinus excelsior* L. *Protoplasma* **122**, 145–152.

Gunning, B. E. S. (1982). The cytokinetic apparatus: Its development and spatial regulation. In *The Cytoskeleton in Plant Growth and Development* (ed. C. W. Lloyd), pp. 229–292. New York, London: Academic Press.

Gunning, B. E. S., Hardham, A. R. & Hughes, J. E. (1978). Pre-prophase bands of microtubules in all categories of formative and proliferative cell division in *Azolla* roots. *Planta* **143**, 145–160.

Gunning, B. E. S. & Wick, S. M. (1985). Preprophase bands, phragmoplasts, and spatial control of cytokinesis. *J. Cell Sci. Suppl. 2*, 157–179.

Lloyd, C. W., Clayton, L., Dawson, P. J., Doonan, J. H., Hulme, J. S., Roberts, I. N. & Wells, B. (1985). The cytoskeleton underlying side walls and cross walls in plants: molecules and macromolecular assemblies. *J. Cell Sci. Suppl. 2*, 143–155.

Lloyd, C. W., Lowe, S. & Peace, G. (1980). The mode of action of 2,4-D in counteracting the elongation of carrot cells grown in culture. *J. Cell Sci.* **45**, 257–268.

Lloyd, C. W., Pearce, K. J., Rawlins, D. J., Ridge, R. W. & Shaw, P. J. (1987). Endoplasmic microtubules connect the advancing nucleus to the tip of legume root

hairs, but F-actin is involved in basipetal migration. *Cell Motility Cytoskel.* **8**, 27–36.

LLOYD, C. W., SLABAS, A. R., POWELL, A. J., MACDONALD, G. & BADLEY, R. A. (1979). Cytoplasmic microtubules of higher plant cells visualised with anti-tubulin antibodies. *Nature, Lond.* **279**, 239–241.

MINEYUKI, Y. & FURUYA, M. (1980). Effect of centrifugation on the development and timing of premitotic positioning of the nucleus in *Adiantum* protonemata. *Dev. Growth Differ.* **22**, 867–874.

MINEYUKI, Y., TAKAGI, M. & FURUYA, M. (1984). Changes in organelle movement in the nuclear region during the cell cycle of *Adiantum* protonemata. *Plant Cell Physiol.* **25**, 297–308.

OTA, T. (1961). The role of cytoplasm in cytokinesis of plant cells. *Cytologia* **26**, 428–447.

PALEVITZ, B. A. (1980). Comparative effects of cytochalasin B and phalloidin on motility and morphogenesis in *Allium*. *Can. J. Bot.* **58**, 773–785.

PALEVITZ, B. A. (1986). Division plane determination in guard mother cells of *Allium*: Video time lapse analysis of nuclear movements and phragmoplast rotation in the cortex. *Devl Biol.* **117**, 644–654.

PALEVITZ, B. A. (1987). Actin in the preprophase band of *Allium cepa*. *J. Cell Biol.* **104**, 1515–1519.

PALEVITZ, B. A. & HEPLER, P. K. (1974). The control of the plane of division during stomatal differentiation in *Allium*. I. Spindle reorientation. *Chromosoma* **46**, 297–326.

PARTHASARATHY, M. V. (1985). F-actin architecture in coleoptile epidermal cells. *Eur. J. Cell Biol.* **39**, 1–12.

PARTHASARATHY, M. V., PERDUE, T., WITZTUM, A. & ALVERNAZ, J. (1985). Actin network as a normal component of the cytoskeleton in many vascular plant cells. *Am. J. Bot.* **72**, 1318–1323.

PICKETT-HEAPS, J. D. & NORTHCOTE, D. H. (1966). Organization of microtubules and endoplasmic reticulum during mitosis and cytokinesis in wheat meristems. *J. Cell Sci.* **1**, 109–120.

SEAGULL, R. W., FALCONER, M. M. & WEERDENBURG, C. A. (1987). Microfilaments: Dynamic arrays in higher plant cells. *J. Cell Biol.* **104**, 995–1004.

SINNOTT, E. W. & BLOCH, R. (1940). Cytoplasmic behaviour during division of vacuolate plant cells. *Proc. natn. Acad. Sci. U.S.A.* **26**, 223–227.

SINNOTT, E. W. & BLOCH, R. (1941). Division in vacuolate plant cells. *Am. J. Bot.* **28**, 225–232.

TRAAS, J. A., DOONAN, J. H., RAWLINS, D. J., SHAW, P. J., WATTS, J. & LLOYD, C. W. (1987). An actin network is present in the cytoplasm throughout the cell cycle of carrot cells and associates with the dividing nucleus. *J. Cell Biol.* **105**, 387–395.

VENVERLOO, C. J., HOVENKAMP, P. H., WEEDA, A. J. & LIBBENGA, K. R. (1980). Cell division in *Nautilocalyx* explants. I. Phragmosome, preprophase band and plane of division. *Z. Pflanzenphysiol.* **150**, 161–174.

WICK, S. M. & DUNIEC, J. (1984). Immunofluorescence microscopy of tubulin and microtubule arrays in plant cells. II. Transition between the preprophase band and the mitotic spindle. *Protoplasma* **122**, 45–55.

(Accepted 2 October 1987)