A role for preprophase bands of microtubules in maturation of new cell walls, and a general proposal on the function of preprophase band sites in cell division in higher plants

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Summary

Time-lapse video microscopy of dividing *Tradescantia* stamen hair cells that are undergoing cytokinesis has revealed that the maturation of the new cell wall is aided by factors at the site where the preprophase band of microtubules forms before mitosis. The wall changes from being fluid and wrinkled before it is inserted into the parental wall at the end of cytokinesis, to being stiff and flat by about 20 min after the time of attachment. This change occurs only if the new wall is inserted at the site formerly occupied by the preprophase band. The cell plate does not flatten when it is caused to insert elsewhere by drug treatments or by centrifugal displacement. If insertion at the correct site is delayed locally by centrifugation against the direction of expansion of the cell plate, then flattening is delayed at the same locality. In combination with a number of points from the literature of plant cell division, some of them very long-standing, our observations lead to a general proposal regarding the nature of the preprophase band site, its mode of action and timing of its operations, and how its role in spatial regulation of histogenesis is achieved.

Key words: cell plate, cytokinesis, microtubules, preprophase band, plant cell division cycle, *Tradescantia* stamen hair cell.

Introduction

Preprophase bands are microtubule arrays in higher plant cells that predict where new cell plates will be inserted into the parental walls at cytokinesis (for a recent review, see Baskin and Cande, 1990). They are thought to be involved in the positioning of cell plate insertion, but their effect must be indirect because they develop before prophase and disperse when the nuclear envelope breaks down at the beginning of mitosis. The site where they appear transiently is referred to as the division site (Gunning, 1982).

Vesicles associate with preprophase band microtubules, and cell walls at division sites are sometimes seen to be differentially thickened, indicating that localised wall deposition may have been promoted by the band (Packard and Stack, 1976; Galatis and Mitrakos, 1979; Galatis *et al.* 1982). It has often been suggested that the preprophase band leaves information in some as yet unidentified form at the division site, to guide the insertion of the cell plate after mitosis. One possibility is that material accumulated in the cell wall component of the division site may function in cell plate development. To investigate this possibility we have made observations on cell plate formation and maturation in *Tradescantia* stamen hair cells in relation to division sites (for micrographs of preprophase bands in stamen hair cells, see Busby and Gunning, 1980; Hepler, 1985). We emphasise a particular stage of cell plate maturation that has not hitherto received much attention. The main feature of interest is that this stage is completed if the cell plate attaches at the division site but not if attachment is displaced away from this site or if attachment is prevented by drug treatments. The observations enable us to re-interpret a number of properties of preprophase bands and cytokinesis, leading to a new synthesis concerning the nature and roles of the division site in cells of higher plants.

Materials and methods

Preparation of plant material

Stamen hair cells of *Tradescantia virginiana* were used throughout these experiments. The preparation method was described elsewhere (Mineyuki and Gunning, 1988). Briefly, the base of the bud was excised with a razor blade and the stamens were gently squeezed out into a few drops of Hepes–Ca buffer (20 mM Hepes, 20 mM KCl, 0.1 mM $CaCl_2$, pH 7.0) with 0.05 % Triton X-100. The anthers were then removed and the stamen filaments were mounted in the Hepes–Ca buffer and observed at approximately 21 °C.

Video microscopy

A Nomarski-rectifier microscope (Nippon Kogaku K.K.) with a

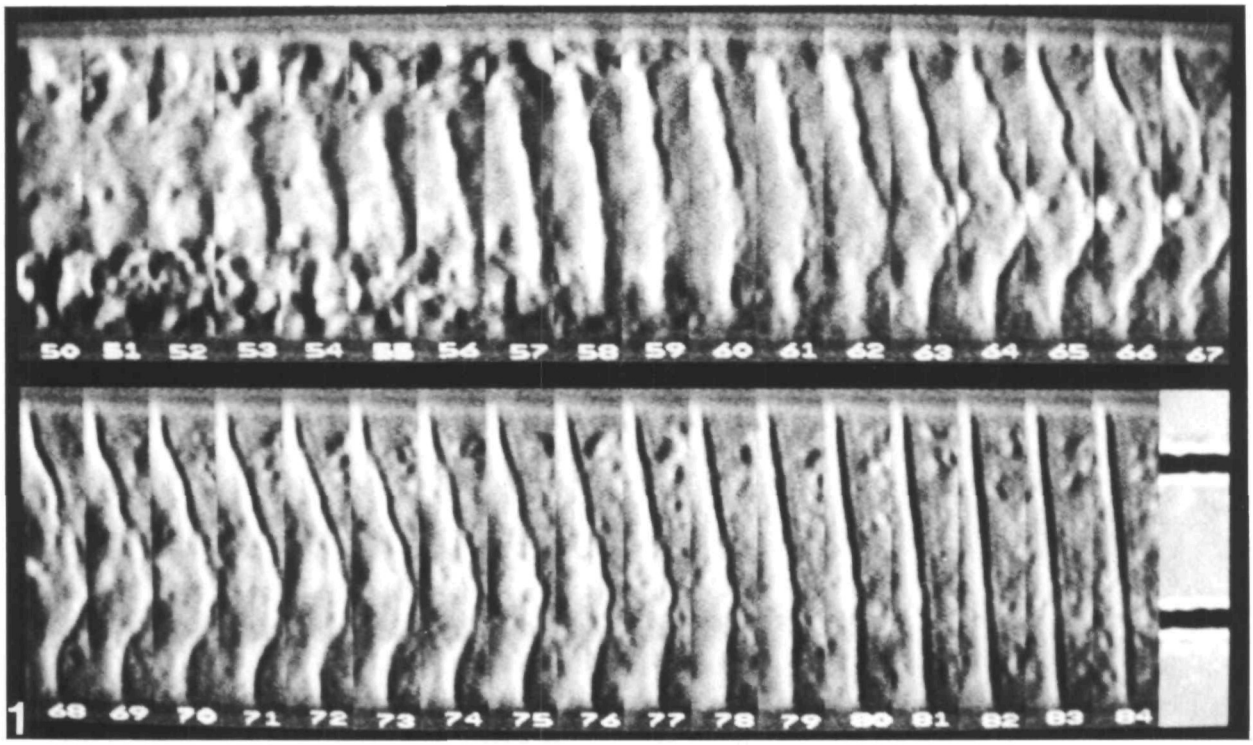


Fig. 1. Cell plate formation and flattening in a *Tradescantia* stamen hair cell. Images of the cell plate region were arranged from upper left to lower right according to time course. The number beneath the each image represents min after the preparation of samples for observation. Each interval in the last box is 10 μm.

compensator and high-intensity mercury arc illuminator was used to obtain high-resolution differential interference contrast images. The image-enhanced recording and dynamic image analysis procedures used have been described (Mineyuki and Gunning, 1988).

Centrifugal treatment

Stamen filaments with hairs were placed in a small volume of Hepes–Ca buffer in 1.5 ml capacity microcentrifuge tubes and these in turn were suspended in a 50 ml centrifuge tube and centrifuged using a table centrifuge at 2260 *g* for 15 min. The temperature was kept at less than 26 °C during centrifugation.

Chemical treatments

Stamen hairs were pretreated with 1.0 mg ml^{-1} pectinase (from *Rhizopus* species, Sigma Chemical Co., St Louis, MO, USA) in Hepes buffer (20 mM Hepes, 20 mM KCl, pH 7.0) for 30 min, then washed and mounted with Hepes–Ca buffer. The Hepes–Ca buffer was replaced with test substances dissolved in Hepes–Ca buffer by drawing the latter under the coverslip with a piece of filter paper. Dimethyl sulphoxide (DMSO) and methanol were used to bring some substances into solution (see Fig. 4, below) but at the concentrations used had no effect on the processes that were under observation.

Results

Cell plate flattening

The process of cell plate formation was continuously observed by image-enhanced videomicroscopy (Fig. 1). Cell plates appeared in the middle part of cells as irregular disks in profile view (51 min, in Fig. 1). The edges of the plates then began to extend centrifugally towards the parental cell walls. The position and shape of the cell plates varied in detail during phragmoplast development but in general they were wrinkled and retained their undulations for some time after they attached to the parental cell walls (65–70 min, in Fig. 1). The time of attachment could usually be pinpointed to within a few minutes, and most cell plates flattened within the next 15–20 min (10–35 min covered the extremes).

Cell volume changes are not required for the new wall to become flattened. This was demonstrated by digitally subtracting images recorded just after cell plate attachment (Fig. 2A) from images of the same cell recorded just after flattening had been completed (Fig. 2B). Because pixel grey scale values in the cell contents change in the intervening period, the resultant image still shows material within the cell. However, the parental cell walls essentially disappear (Fig. 2C), showing that they were in the same place and hence that the cell had not changed in volume.

Cell plate flattening is not affected by mounting the cells in media that are hypotonic or hypertonic relative to the normal mountant. Hepes–Ca buffer with more than 0.2 M mannitol caused plasmolysis in stamen hair cells, but neither 0.1 mM $CaCl_2$ in distilled water nor Hepes–Ca buffer with 0.1 M mannitol changed the timing of flattening relative to the standard Hepes–Ca buffer (Fig. 3). Mild plasmolysis is a simple way of demonstrating the alteration in the physical strength of the new cell wall that occurs at this time. Beforehand the cell plate deforms; afterwards it remains flat when the protoplast withdraws during plasmolysis.

Effects of drugs

Effects of various types of drugs that were known to inhibit mitosis, cytokinesis or cell wall formation were examined. Stamen hair cells were continually observed

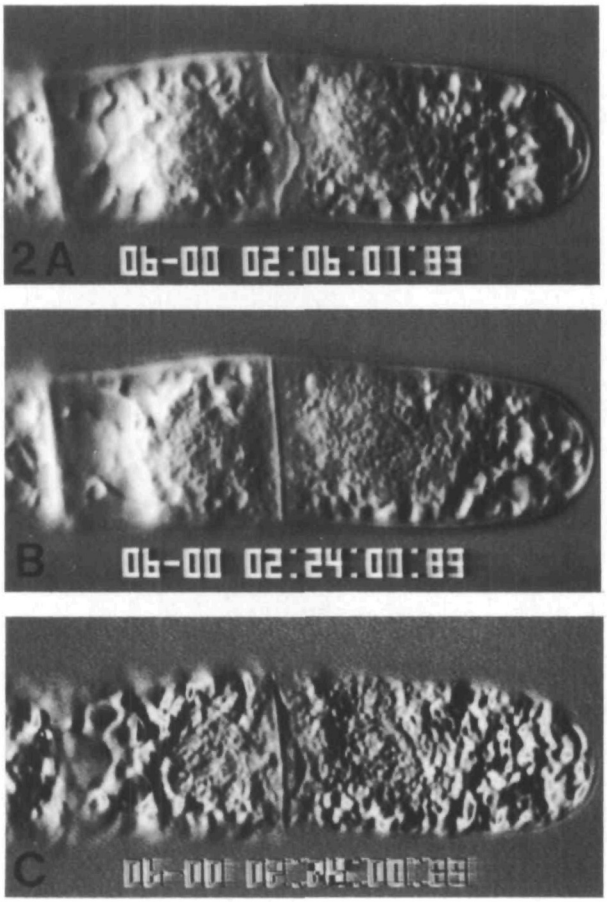


Fig. 2. Cell plate flattening. (A) Just after the cell plate has reached the cell wall; (B) end of cell plate flattening, taken 18 min after A; (C) image obtained from the subtraction of A from B. The outline of the cell wall has disappeared. The scale interval is 10 μm.

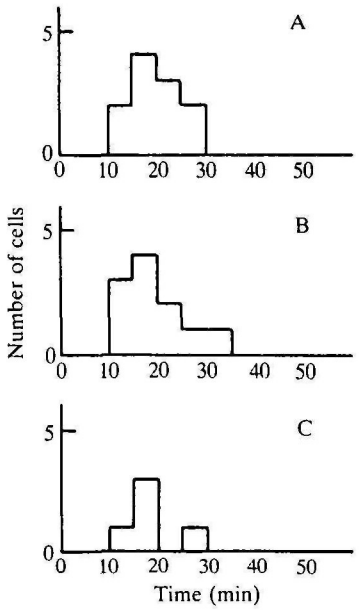


Fig. 3. Effect of media of various osmotic strengths on the duration of cell plate flattening. (A) Water with 0.1 mM $CaCl_2$; (B) Hepes–Ca buffer; (C) Hepes–Ca buffer with 0.1 M mannitol. Dividing cells were observed at 5 min intervals to determine the duration of cell plate flattening (e.g. interval between Fig. 2A and B).

under the microscope and then the standard buffer was quickly replaced by irrigating with buffers containing drugs either at the moment when cell plates first attached to the parental cell walls, or for more extended periods leading up to and including cytokinesis.

Oryzalin (3.5 μg ml^{-1}), 2,4-dinitrophenol (180 μg ml^{-1}) and 2-deoxy-D-glucose (160 μg ml^{-1}) all inhibited anaphase chromosome movement within a few minutes of their application (data not shown). However, neither these concentrations of drugs, nor 7 μg ml^{-1} oryzalin (Fig. 4A), nor 2,4-dinitrophenol combined with 2-deoxy-D-glucose (Fig. 4E), inhibited cell plate flattening when applied at the time of attachment. Caffeine (580 μg ml^{-1}) affected cell plate formation to produce binucleate cells if applied early enough, but neither 580 μg ml^{-1} nor 970 μg ml^{-1} caffeine affected the flattening of the new wall (data not shown here, but an equivalent experiment on a subapical cell in Fig. 4 of Bonsignore and Hepler (1985) clearly shows the same result). Newly formed cell plates stained well with 1 mg ml^{-1} Aniline Blue, but at this concentration cytoplasmic motions including streaming were totally inhibited and no progression of cell division was observed. In the presence of 50 μg ml^{-1} Aniline Blue, cytoplasmic motion was inhibited in 50 % of cells, but in the remaining 50 %, in which mitosis progressed, the new walls flattened in all cells observed (Fig. 4D). Progression of mitosis in 40 % of dividing cells observed was inhibited in the presence of 10 μg ml^{-1} monensin, but this concentration did not prevent wall flattening when applied at the time of attachment (Fig. 4F).

New walls flattened normally when 10 μg ml^{-1} or 20 μg ml^{-1} cytochalasin B were applied at the time of cell plate attachment (Fig. 4B). Cytoplasmic streaming almost stopped a few minutes after replacing the medium with 10 μg ml^{-1} cytochalasin B (data not shown), and cell plate formation was partially inhibited in prolonged treatments (also recorded for stamen hair cells by Gunning and Wick, 1985). In 23 cells out of 42 observed in the presence of 10 μg ml^{-1} cytochalasin B and in four cells out of seven observed in the presence of 20 μg ml^{-1} the complete periphery of the cell plate attached to the parental wall, and in all of these cases the walls matured normally (Fig. 5A, B). However, in another 22 cells the cell plates extended almost to the parental cell walls and the rate of cell plate extension then decreased. The cell plates did not become attached, except in restricted sites (Fig. 6A). In these cases the cell plates remained irregularly curved even 1 h later (Fig. 6B, C and D).

2,6-Dichlorobenzonitrile at 10 μg ml^{-1} or 20 μg ml^{-1} was non-inhibitory when applied just after the cell plate had attached to the parental cell walls (Fig. 4C). However, cell plate formation was inhibited and distorted cell plates formed in the extended presence of this inhibitor of cellulose synthesis. Normal cell plates were formed and flattened in only five cells out of 25 treated with this reagent at 20 μg ml^{-1} and in one cell out of three treated with 10 μg ml^{-1}. In another five cells treated with 20 μg ml^{-1}, cell plates with some gaps (e.g. arrow in Fig. 7E) developed. Despite their partially complete state, they flattened normally (Fig. 7). In another 15 cells treated with 20 μg ml^{-1} and in two cells treated with 10 μg ml^{-1}, large portions of the cell plates were sufficiently tenuous (or absent) to give no clear profile view

(Fig. 8B), and these did not become straight even 2 h after cytokinesis (Fig. 8D and E).

Effect of centrifugation

As described by Ota (1961), the mitotic apparatus of stamen hair cells remigrates towards its original position after longitudinal displacement by centrifugation. We have confirmed his pioneering work and have added information on later stages of development of the new wall. When metaphase or anaphase cells were centrifuged along the long axis of the hairs (Fig. 9A), cell plates developed in the phragmoplast during the recovery period (Fig. 9B). The edge of the developing cell plates extended towards the expected position on the parental cell walls during remigration. This produced U-shaped cell plates,

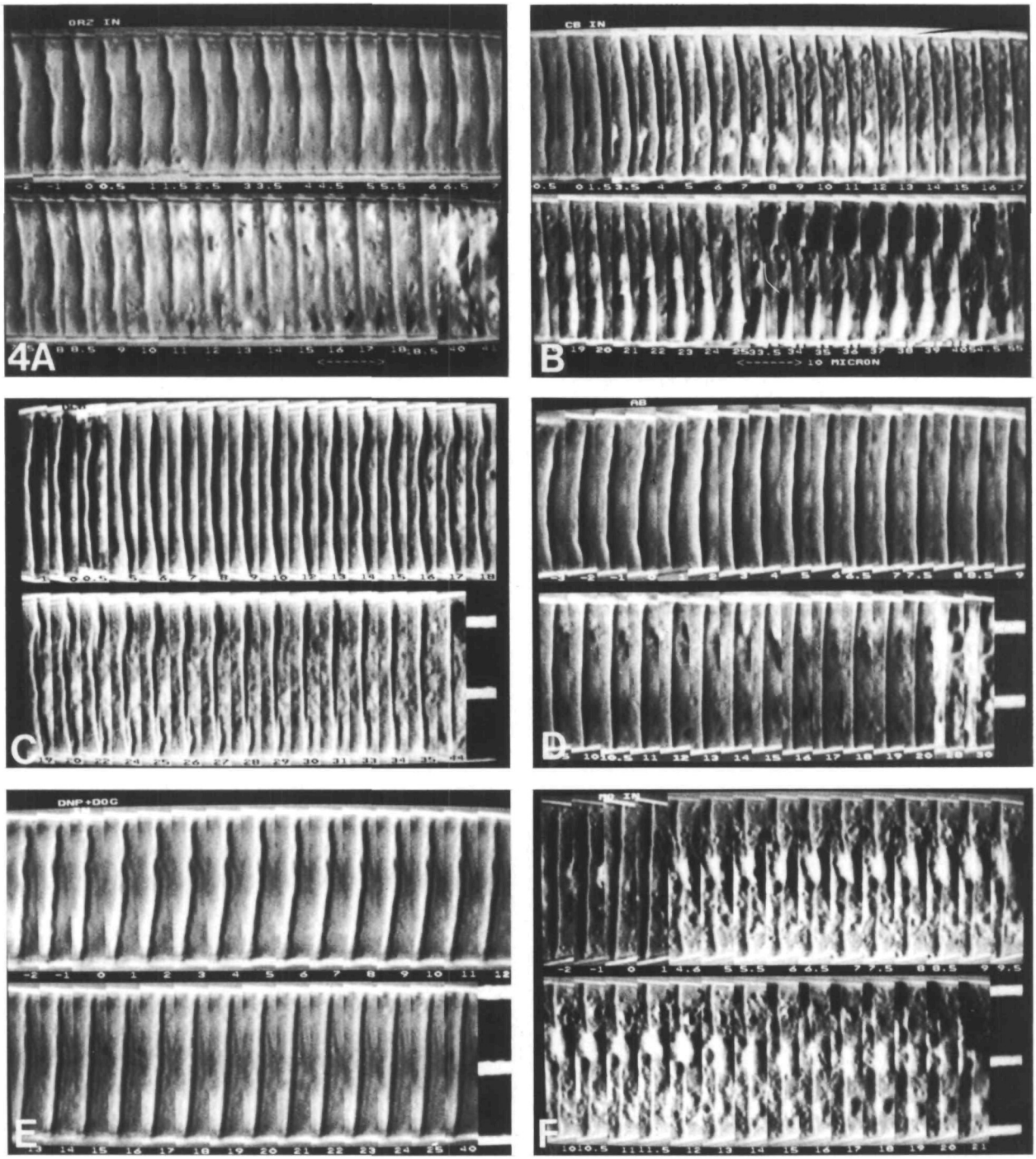
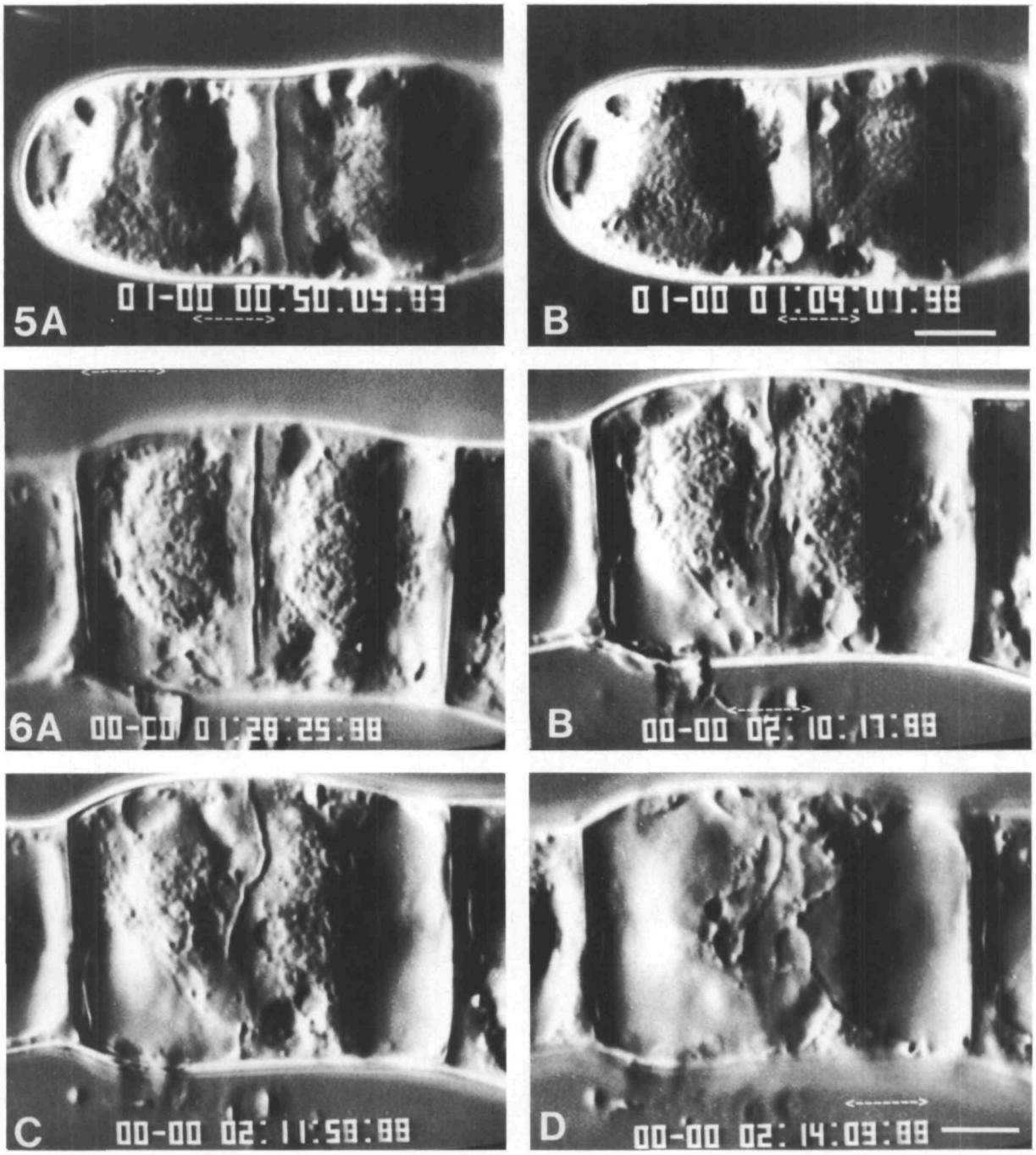


Fig. 4. Cell plate flattening in the presence of various chemical inhibitors. Hepes–Ca buffer was replaced by Hepes–Ca buffer containing dissolved inhibitors at zero time and observed continually. The number beneath each box shows min after the replacement of buffers. (A) 7 μg ml^{-1} oryzalin in 0.5 % DMSO; (B) 20 μg ml^{-1} cytochalasin B in 1 % DMSO; (C) 20 μg ml^{-1} 2,6-dichlorobenzonitrile; (D) 50 μg ml^{-1} aniline blue; (E) 184 μg ml^{-1} 2,4-dinitrophenol plus 164 μg ml^{-1} 2-deoxy-D-glucose in 0.1 % DMSO; (F) 7 μg ml^{-1} monensin with 0.1 % methanol. Bar, beneath images or each interval of the bar at lower right corner shows 10 μm scale.

which finally attached at the expected position (Fig. 9C). The U-shaped cell plates then gradually flattened and, finally, they were indistinguishable from controls (Fig. 9D).

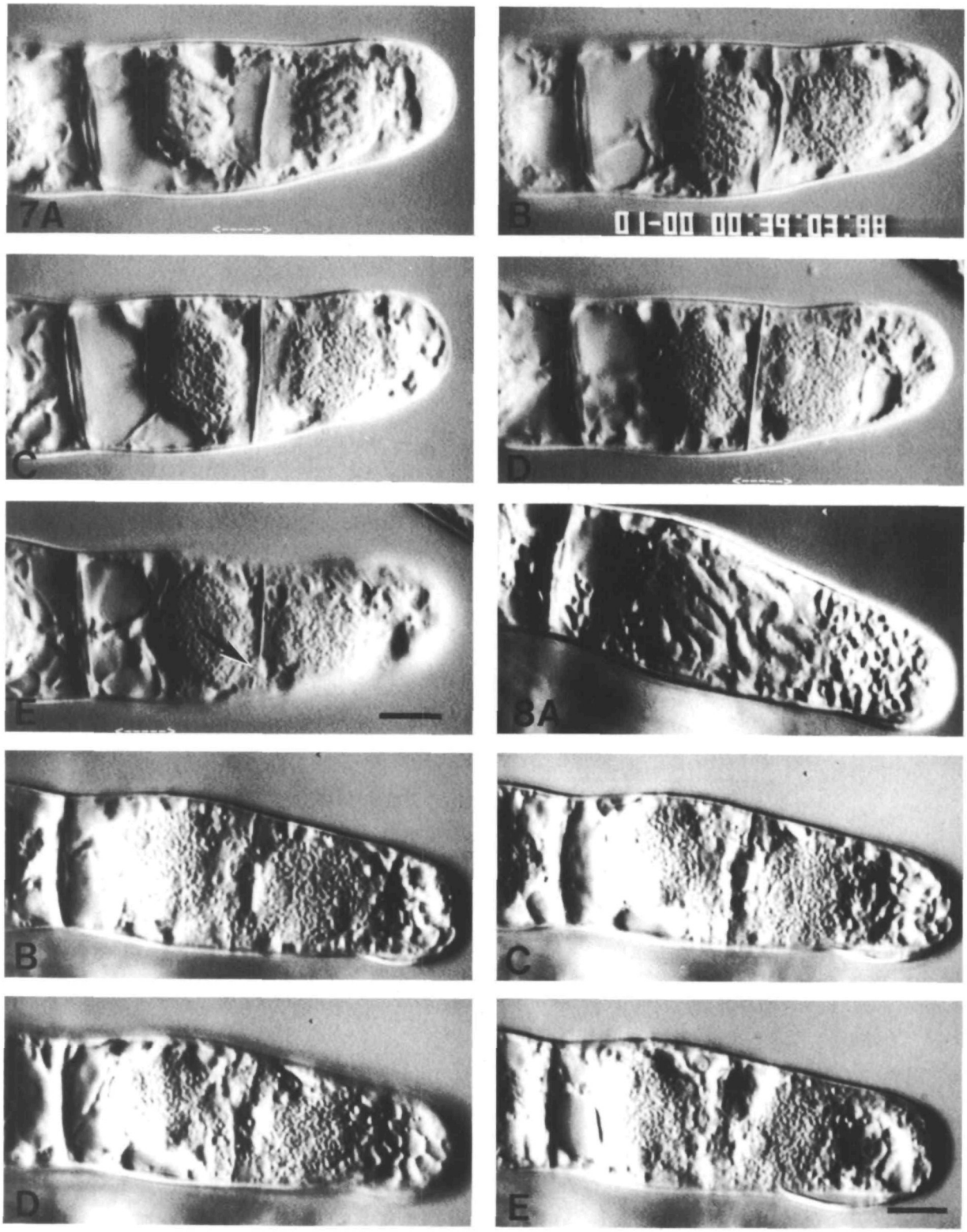
Phragmoplasts were displaced to one side of the cell when stamen hairs were centrifuged transversely. In these cases the centrifugal part of the cell plate attached to the parental cell wall at the expected position, but development of the centripetal region of the cell plate was modified (Fig. 10A). Many cytoplasmic strands radiated from this region of the displaced phragmoplast to the parental cell wall, and cell plate formation was considerably delayed (Fig. 10A, B). As a consequence, it could be seen that wall maturation can be a local phenomenon. The free, extending parts of the cell plates were not flat, yet flattening was already complete at the attached centrifugal side of the cell (Fig. 10B, C). All parts of the cell plates eventually became flattened within a few minutes of their attachment to the expected position on the parental cell walls (Fig. 10D).

When cells were centrifuged at a later stage of mitosis, cell plates could become attached to the parental cell walls



Figs 5 and 6. Effect of cytochalasin B on cell plate formation and flattening. Fig. 5(A) 50 min; (B) 1 h 9 min. Fig. 6(A) 1 h 28 min; (B) 2 h 10 min; (C) 2 h 12 min; (D) 2 h 14 min after the replacement of medium with 10 $\mu g\,ml^{-1}$ cytochalasin B in 0.5% DMSO. Fig. 6B, C and D are different planes of focus of the same cell. The complete edge of the cell plate attaches to the cell wall in Fig. 5, but not in Fig. 6. Bars, 10 μm.

while they were still being centrifuged. The site of attachment then varied according to the timing and the direction of centrifugation, and it was not always possible to tell whether the newly formed cell plates had attached at the expected position or not, because there is some variation even in normal stamen hair cells (and sometimes the new walls are slightly oblique; e.g. see Fig. 2B). In all but one of 40 centrifuged cells in which the cell plate



Figs 7 and 8. Effect of 2,6-dichlorobenzonitrile on cell plate formation and flattening. Fig. 7(A) 3 min; (B) 39 min; (C) 1 h 2 min; (D) 2 h 43 min; (E) 2 h 44 min. Fig. 8(A) 31 min; (B) 1 h 55 min; (C) 2 h 30 min; (D) 2 h 30 min; (E) 3 h 50 min after the replacement of the medium with 20 $\mu g\,ml^{-1}$ 2,6-dichlorobenzonitrile. Fig. 7D and E, and Fig. 8C and D are different planes of focus of the same cells, respectively. Bars, 10 μm.

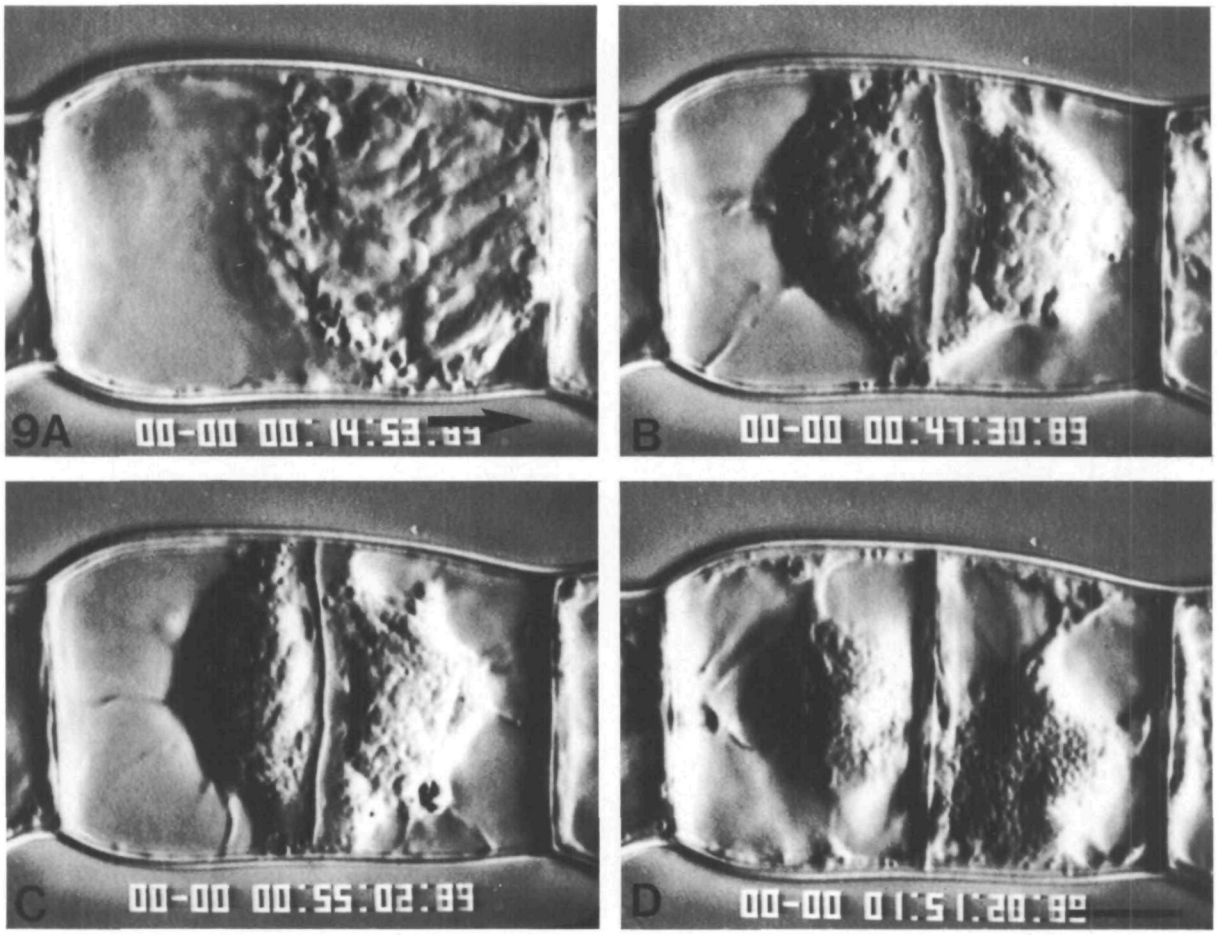


Fig. 9. Cell plate formation and flattening after centrifugation at 2260 *g* for 15 min. (A) 15 min; (B) 48 min; (C) 55 min; (D) 1 h 51 min after the end of centrifugation. Black arrow shows the direction of centrifugation. Bar, 10 μm.

attached at or very close to the expected position the cell plate became flattened as usual, whether the centrifugation had been longitudinal or transverse. However, in 18 centrifuged cells the cell plates became attached at positions that were clearly remote from the expected. In 16 of these cells the attachment sites were on the usual longitudinal walls and in two cells they included one of the transverse walls (e.g. Fig. 11A, B and Fig. 11C, D, respectively). In these 18 cells the cell plate remained curved, even several hours after centrifugation, and did not flatten (Fig. 11B and D).

Discussion

It is apparent from many cytological studies that cell plates and young cell walls are more wrinkled, fluid and deformable than older walls. The cell plate flattening process highlighted in this paper is an early event in wall maturation. It is very conspicuous in stamen hair cells and almost certainly is very widespread. There are few detailed time-lapse studies of cell plate growth in walled cells, but micrographs presented by Ball (1969) show exactly the same phenomenon in *Nicotiana* cells in tissue culture.

Wall flattening occurs after the phragmoplast has completed its role in cell plate development. It is not caused by the parental cell increasing its girth in the critical period to stretch the newly attached cell plate. It still happens if the turgor conditions are varied, and if inhibitors of energy metabolism (sufficient to block anaphase movements of chromosomes) are applied. If attachment of the cell plate to the 'correct' part of the parental wall is prevented by a physical means (centrifugation) flattening does not occur, but if the centrifugation merely introduces a local delay, then flattening is seen to start near regions of attachment, soon after it occurs, and irrespective of when it is achieved. Comparable phenomena occur when cell plate attachment is locally delayed by micropuncturing at the division site (Gunning and Wick, 1985). On the other hand, if centrifugation leads to attachment remote from the normal division site, then, as also shown for leaf epidermal and stomatal cells by Galatis *et al.* (1984), flattening does not follow.

Results of experiments with chemical agents are ambiguous because it is not clear whether a function of the parental division site is affected, or whether the cell plate is made unable to respond to the division site because an antecedent process such as phragmoplast development has been disrupted. The anti-actin drug cytochalasin B and the inhibitor of cellulose synthesis 2,6-dichlorobenzonitrile (Venverloo *et al.* 1984) have the most relevant effects. Malformed cell plates produced by prolonged treatments with these agents undergo flattening if they attach to the correct site but not if they do not attach, indicating that the phenomenon is more likely to be the

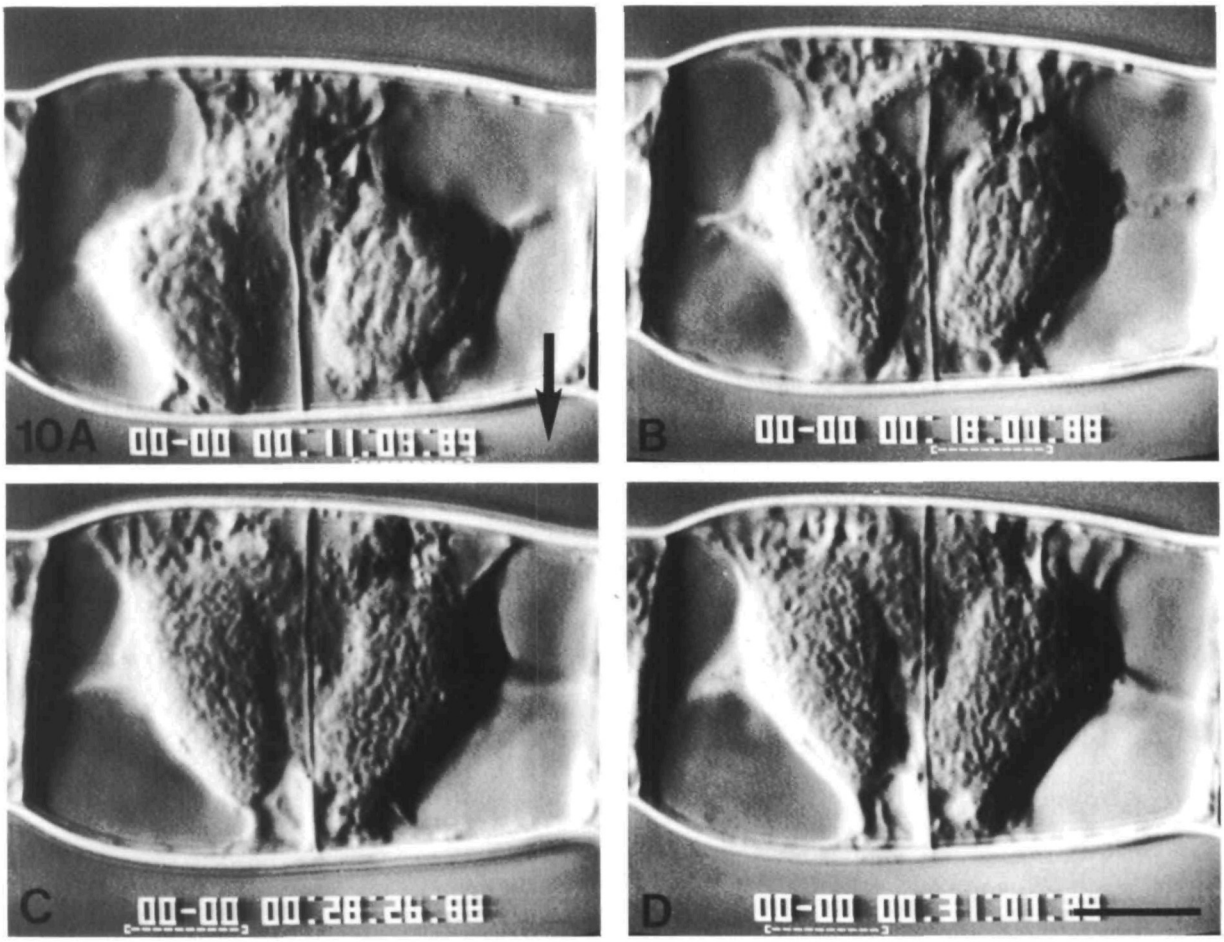


Fig. 10. Cell plate formation and flattening after centrifugation at 2260 *g* for 15 min. (A) 11 min; (B) 18 min; (C) 28 min; (D) 31 min after the end of centrifugation. Black arrow shows the direction of centrifugation. Bars, 10 μm.

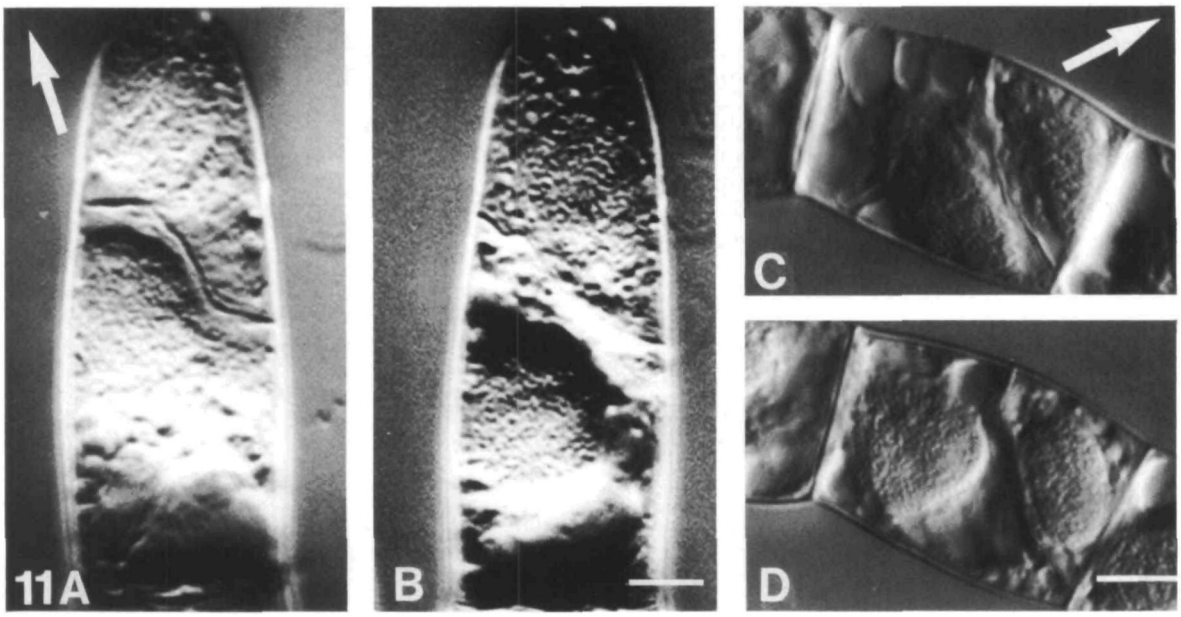


Fig. 11. Malformed cell plate after centrifugation at 2260 *g* for 15 min. (A and B) and (C and D) are the same cells, respectively. A. 22 min; B, 2 h 30 min; C, 44 min; D, 2 h 40 min after centrifugation. White arrows show direction of the centrifugation. Bars, 10 μm.

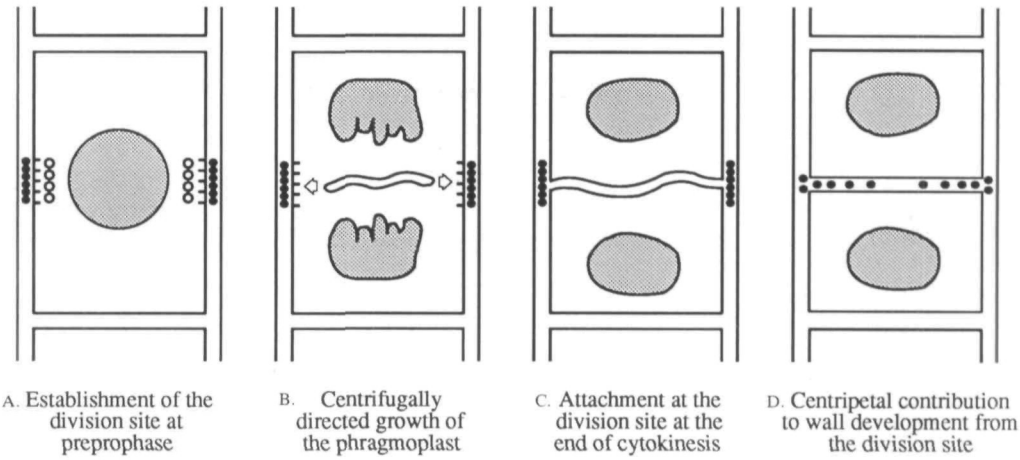


Fig. 12. Diagram to show the main features of a proposal concerning the nature and operation of the division site in dividing cells. The preprophase band of microtubules develops during the G_2 period of the cell cycle (Mineyuki *et al.* 1988*b*) at a particular position in response to internal or external polarizing influences: (A) The division site is then established at the preprophase band by: (1) deposition of insertion and maturation factors for the new wall, in the parental wall and/or plasma membrane (black dots); and (2) by providing a recognition system, perhaps at the inner face of the plasma membrane, or perhaps also including nearby gelled cytoplasm (Mineyuki *et al.* 1984), which will be a target for subsequent directed growth of the phragmoplast once it has reached a critical distance of a few μm. (B) The microtubules of the preprophase band disperse at late prophase, but the recognition system and the deposited factors remain at the division site. After mitosis the phragmoplast/cell plate expands centrifugally. (C) The edge of the cell plate is brought to the mid-line of the former preprophase band site and the new plasma membrane and cell wall become attached to the parental ones. (D) Finally, the new wall is flattened and strengthened by contributions emanating centripetally from the division site.

result of maturation events in the wall, as distinct from effects of the cytoskeleton on wall development after the phragmoplast has gone. Palevitz and Hepler (1974*b*) and Palevitz (1980) have also shown that malformed cell plates formed under the influence of cytochalasin B in onion guard mother cells do not become taut and flat if they attach to abnormal portions of parental wall. Similarly, aberrantly shaped and positioned cell plates are induced when preprophase cells of *Adiantum* protonemata (Mineyuki, 1987) and those of small leaf explants of *Nautilocalyx* are treated with colchicine or cytochalasin B (Venverloo and Libbenga, 1987), or 2,6-dichlorobenzonitrile (Venverloo *et al.* 1984). Malformed cell plates also arise when they are prevented from attaching at the expected site in *Adiantum* protonemata by manipulating the light regime (Mineyuki, 1987). This manipulation also prevents formation of the cortical microtubule bands that form in *Adiantum* apical cells at preprophase (Y. Mineyuki, T. Murata and M. Wada, unpublished).

The fact that cell plates do not flatten if they either fail to attach, or attach at a position remote from the site formerly occupied by the preprophase band, is a clear indication that this site has a role in maturation of the new wall. All of the results indicate that there is an interaction between the division site and the new wall that cannot take place until *after* attachment. These observations led to a far-reaching re-evaluation of the nature and roles of the division site, which we summarize in the remainder of this discussion.

The simplest interpretation (see diagram in Fig. 12) is that, after attachment, material that for convenience we term 'insertion and maturation factors' interacts with or passes into the cell plate from the cell wall, plasma membrane or (less likely) the cortical cytoplasm of the division site. The factors could take a number of forms. For example, they could be wall material or precursors, enzymes of wall synthesis or turnover, receptors for particular classes of Golgi vesicle or cellulose-synthesising complexes, or combinations of these. At any event, it is envisaged that they function in attaching the new wall and plasma membrane to the old, and/or in maturing the new wall. We now cite a number of relevant phenomena from the literature on plant cell division in support of this proposal.

First, there is direct evidence for a maturation phase in the development of new cell walls soon after attachment. Selective extraction combined with electron cytochemistry has shown changes in the pectic components, especially at the point of insertion into the parental walls (Matar and Catesson, 1988). Immunocytological localizations have revealed incorporation of the main pectic polysaccharide rhamnogalacturonan I into the new wall. It is absent from cell plates but appears after attachment, again especially at the sites of insertion (Moore and Staehelin, 1988). Conversely, callose is abundant in cell plates but is removed soon after their attachment, except from around plasmodesmata (Northcote *et al.* 1989).

Second, there is much evidence that the division site has wall-forming capacities that may contribute to the new wall in addition to the growth of the cell plate. It was reported over 50 years ago from observations of experimentally treated stamen hair cells that although cell plates grow centrifugally, their conversion to a 'hard new cell wall' proceeds centripetally from the site of attachment (Wada, 1939). Most of the additional evidence is based on the centripetal development of 'wall stubs' at division sites. They become conspicuous only when cell plate formation is disrupted by caffeine or anti-micro-

tubule drugs. Pickett-Heaps (1969) suggested that they are vestiges of a primitive cleavage mechanism of cytokinesis, and Hardham and McCully (1982) reviewed numerous observations of these ingrowing walls and suggested that their position and directionality are determined by the site occupied before mitosis by the microtubules of the preprophase band. Limited centripetal growth of wall flanges to meet extending cell plates under normal, drug-free conditions is also on record (Cronshaw and Esau, 1968; Pickett-Heaps, 1969; Mahlberg *et al.* 1975; Hepler, 1985).

Third, the portion of cell wall at the division site has been seen to become specialized in a number of situations. Dating from the work of Martens (1937) and discussed in detail in relation to his own observations on cell plate maturation by Ball (1969), there has been a very long-standing idea that for a new wall to become structurally linked to a pre-existing one, the latter must be modified in some way at the site of insertion. Ball (1969) illustrates minute 'receptive areas', visible using phase-contrast optics, formed in the parental wall in advance of the arrival of the edge of the cell plate, and interprets them as sites of localized hydrolysis to provide for insertion of the new wall. More recently, the wall underlying preprophase band microtubules has been seen to be differentially thickened in a variety of cell types and tissues (Packard and Stack, 1976; Galatis and Mitrakos, 1979; Galatis *et al.* 1982), so local deposition during preprophase of material that will react with the cell plate and aid its integration into the pre-existing wall framework is a real possibility. Rather more general evidence of a contribution from the parental walls is seen in the breakdown of cell plates if the parental wall is removed (Meyer and Abel, 1975; Meyer and Herth, 1978).

Lastly, and perhaps most relevant, it has been pointed out repeatedly that preprophase bands do not occur in classes of division in higher plants where new walls do not become inserted into parental walls. These are divisions in microspores and embryo sacs, meiotic divisions, and divisions that are not followed by cytokinesis, as in the free-nuclear phase of liquid endosperm development. The converse generalization is that there is a good correlation between insertion of new walls into the parental wall framework on the one hand and prior presence of preprophase bands on the other (reviewed by Gunning, 1982).

The threads of these observations can be drawn together with our own in a general proposal concerning the nature and mode of operation of the preprophase band, and its *raison d'être*. We retain the widely accepted view that the primary role of the division site is to generate histogenetic cell patterns through spatial regulation of cytokinesis. For the site to function, it has to be established in a narrow zone around the cell surface. We do not consider here the problem of how the position of the site is determined but propose that the actual process of establishing it involves: (1) localized deposition of insertion and maturation factors in a latent form; and (2) provision of a means that, later on, will guide the leading edge of the centrifugally extending phragmoplast to the site. Once the new wall has attached, the factors are activated and utilized to insert, anchor and integrate the new wall and contribute centripetally to its development.

If our proposal is correct, the guidance mechanism for phragmoplast expansion is essential because it brings the new wall to the only zone where it can be inserted and matured correctly. Maturation is usually incomplete if it inserts elsewhere. The correct site and plane of division are thus predetermined by localized deposition of insertion and maturation factors for the new wall. This localisation is the means of meeting histogenetic and morphogenetic requirements. It follows that if such factors are indeed required, the more they are restricted to a limited site, the more efficient the system is in terms of resource allocation in the dividing cell.

Localised deposition of the factors demands that there is a very precise deposition mechanism. It is an obvious suggestion that the necessary spatial guidance could be provided by the microtubules of the preprophase band, and that this is one operational role of the preprophase band. Note that the role of the microtubules would have to be accomplished before spindle and phragmoplast development exert an all-consuming requirement for the cell's tubulin, i.e. in preprophase–prophase at the latest. A detailed investigation of preprophase band development by double immunolabelling of microtubules and incorporated bromodeoxyuridine has shown that the band is initiated at the end of the S-phase and gradually narrows throughout G_2 until it becomes a sharply circumscribed girdle that exists only briefly at prophase (Gunning and Sammut, 1990). One possibility is that the insertion and maturation factors are produced just before mitosis in a form that can interact with cortical microtubules; the preprophase band by then being the only target for them because all other microtubules have been withdrawn from the cell cortex. Another possibility is that the prolonged narrowing phase of preprophase band development is functionally active in 'sweeping' membrane-bound factors into the final, accurately placed division site.

One other phenomenon that has been puzzling now falls into place. In at least some cells, tissues and organs it is known that cell plates are placed with sub-micrometre accuracy at or very close to the mid-line of the zone where the preprophase band microtubules formerly lay (reviewed by Gunning, 1982). Does histogenesis really require such remarkable precision? Our proposal provides the rationale that the immediate requirement is to ensure accurate juxtaposition of the edge of the new wall to the position of the insertion and maturation factors, which were stored very locally where the preprophase band used to be. The histogenetic role is, of course, intrinsic. We do not discuss phragmoplast guidance in detail here, but concur with others in the belief that a 'fine-tuning' mechanism produces the final alignment once the leading edge of the cell plate is within a few micrometres of the division site (Galatis *et al.* 1984; Gunning and Wick, 1985; Palevitz, 1986; Apostolakos and Galatis, 1987). In small cells where the nucleus occupies much of the cell diameter this local capture zone may suffice (McCurdy and Gunning, 1990), but in larger, vacuolated cells there is also a first-stage mechanism (Gunning and Wick, 1985), which is very probably based on actin (reviewed by Lloyd, 1988).

Although the argument that the preprophase band functions in localizing wall maturation factors is in accord with its own precise localization in the dividing cell, it must be reiterated that division sites and centripetally ingrowing wall stubs do also occur in lower plants (e.g. algae) where there are no preprophase bands (see Gunning, 1982). It could be that the higher plants elaborated the pre-existing mechanism for defining division sites by evolving the preprophase band system to give more accurate localization of the factors. Additionally, or alternatively, the preprophase band may have been added to the pre-existing mechanism to confer new

functions such as orienting the spindle at its inception and ensuring that it is bipolar (Mineyuki *et al.* 1988*a*; Cho and Wick, 1989).

We recognize that elements of this proposal have been published before, especially the concept of a receptive site for new wall insertion (Ota, 1961; Ball, 1969) and, independently, the suggestion that the preprophase band helps to prepare such a site (Palevitz and Hepler, 1974*a*); also the recognition that wall stubs may be a manifestation of wall-forming activity at that site (Palevitz and Hepler, 1974*a*; Hardham and McCully, 1982; Hepler, 1985). Our observations on cell plate maturation have allowed us to draw these and other elements together into a cohesive general proposal on the nature and functions of the division site and its component parts in higher plant cells. There are several testable sections of the proposal, and we hope that these will soon be subjected to experimental appraisal.

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