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Phragmoplasts in the Absence of Nuclear Division

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Abstract

All land plants (embryophytes) use a phragmoplast for cytokinesis. Phragmoplasts are distinctive cytoskeletal structures that are instrumental in the deposition of new walls in both vegetative and reproductive phases of the life cycle. In meristems, the phragmoplast is initiated among remaining nonkinetochore spindle fibers between sister nuclei and expands to join parental walls at the site previously marked by the preprophase band of microtubules (PPB). The microtubule cycle and cell cycle are closely coordinated: the hoop-like cortical microtubules of interphase are replaced by the PPB just prior to prophase, the PPB disappears as the spindle forms, and the phragmoplast mediates cell plate deposition after nuclear division. In the reproductive phase, however, cortical microtubules and PPBs are absent and cytokinesis may be uncoupled from the cell cycle resulting in multinucleate cells (syncytia). Minisyncytia of 4 nuclei occur in microsporocytes and several (typically 8) nuclei occur in the developing megagametophyte. Macrosyncytia with thousands of nuclei may occur in the nuclear type endosperm. Cellularization of syncytia involves formation of adventitious phragmoplasts at boundaries of nuclear-cytoplasmic domains (NCDs) defined by radial microtubule systems (RMSs) emanating from non-sister nuclei. Once initiated in the region of microtubule overlap at interfaces of opposing RMSs, the adventitious phragmoplasts appear structurally identical to interzonal phragmoplasts. Phragmoplasts are constructed of multiple opposing arrays similar to what have been termed microtubule converging centers. The individual phragmoplast units are distinctive fusiform bundles of anti-parallel microtubules bisected by a dark mid-zone where vesicles accumulate and fuse into a cell plate.

Key words: Cytokinesis; Endosperm; Microsporogenesis; Nuclear-cytoplasmic domain; Phragmoplast; Radial microtubule system; Seed; Syncytium

Introduction

Control of the placement and subsequent expansion of walls, basic features of plant growth at the cellular level, are functions of the cytoskeleton. Although the various roles of microtubules and actin filaments remain imperfectly understood, considerable evidence of their essential role in cell division and morphogenesis has accumulated from observation of patterns of organization during development in normal and perturbed systems. This paper deals principally with the organization and inferred role of microtubules in controlling the placement of walls when cytokinesis is uncoupled from nuclear division.

Vegetative Growth

In vegetative tissues, cytokinesis is so typically tied to mitosis that it is often considered part of the M phase of the cell cycle. The process of meristematic

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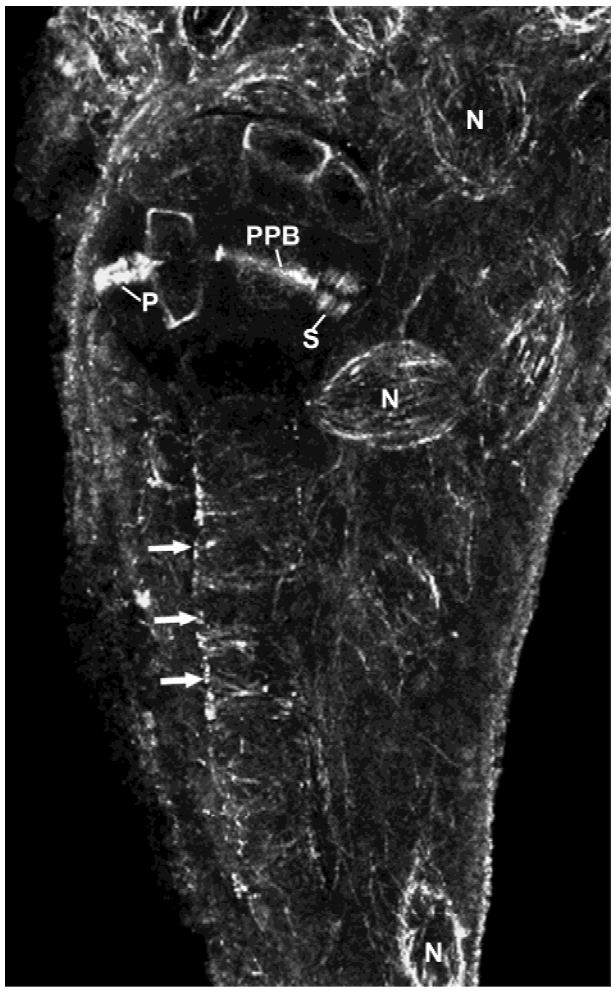

Figure 1. Syncytial development of the endosperm and cellular development of the embryo proceed side by side in the micropylar chamber of mustard seeds (*Coronopus didymus* illustrated here). The embryo exhibits the 4 cycling microtubule arrays of meristems: cortical (arrows), PPB, spindle (S), and phragmoplast (P); at this early stage of development in the syncytium, microtubules sheath the fusiform nuclei (N) and form a reticulum in the cytoplasm.

cell division is the quintessential example of elegant coordination of events that together insure that the two new cells will be strategically placed within the existing framework of walls. Wall formation is mediated by a phragmoplast that begins between anaphase/telophase nuclei and expands to junction with the parental cell wall at the site predicted before mitosis by a band of cortical microtubules. Thus, two microtubule systems are involved in cytokinesis of meristematic cells, the preprophase band of microtubules (PPB), and the phragmoplast (Gunning 1982), each of which is distinct as to location, function, and time of appearance in the cell cycle (Figure 1). The PPB is part of the cortical (wall) microtubule system of interphase, whereas the phragmoplast is endoplasmic; the PPB predicts the division site and disappears before metaphase, whereas the phragmoplast mediates deposition of the cell plate that completes cytokinesis after mitosis.

The phragmoplast is a complex organelle of cytoskeletal and other proteins that redirects the secretory mechanism to deposit the new cell plate in plant cytokinesis. First described as a fibrous spindle-like structure, the phragmoplast was soon discovered to be a distinct birefringent structure like the spindle (Inoué and Bajer 1961). The cytoskeletal system consists of highly aligned microtubules and F-actin. Numerous additional proteins have putative functions in cytoskeletal organization, vesicle trafficking, and wall synthesis (Sylvester 2000). Microtubules and actin filaments of the phragmoplast are organized into two opposing sets of dense brush-like arrays on either side of a mid-zone where vesicles are concentrated in the plane of cytokinesis (Schopfer and Hepler 1991; Staehelin and Hepler 1996; Sylvester 2000). The microtubules are initially arranged with tips overlapping in the equatorial region whereas the actin filaments occur as two non-overlapping sets on either side of the equatorial region (Zhang and others 1993; Staehelin and Hepler 1996). Both microtubules (Euteneuer and others 1982) and F-actin (Kakimoto and Shibaoka 1988) are oriented with plus (rapidly assembling ends) toward the division plane. Arguments have been put forth for each in vesicle transport (Kakimoto and Shibaoka 1988; Staehelin and Hepler 1996; Sylvester 2000). Typically, the phragmoplast is initiated in the interzonal array of microtubules that proliferates between the sister groups of chromosomes (Figure 2A) in anaphase/telophase (Bajer 1968; Gunning 1982; Staehelin and Hepler 1996). Quite unlike the animal cell midbody which elongates as sister nuclei move apart, the phragmoplast shortens and spreads as a ring at the leading edge of the forming discoid cell plate (Figure 2B). The somewhat autonomous nature of the expanding phragmoplast suggests the incorporation of microtubule organizing centers (MTOCs) within the phragmoplast itself (Gunning 1982).

The processes that control phragmoplast expansion are little understood. One hypothesis (Gunning 1982; Mineyuki 1999) is that a division site, which is somehow prepared by the PPB, controls growth of the cell plate once it approaches within a critical distance. It has been suggested that the actin component has a role in preserving the memory of the

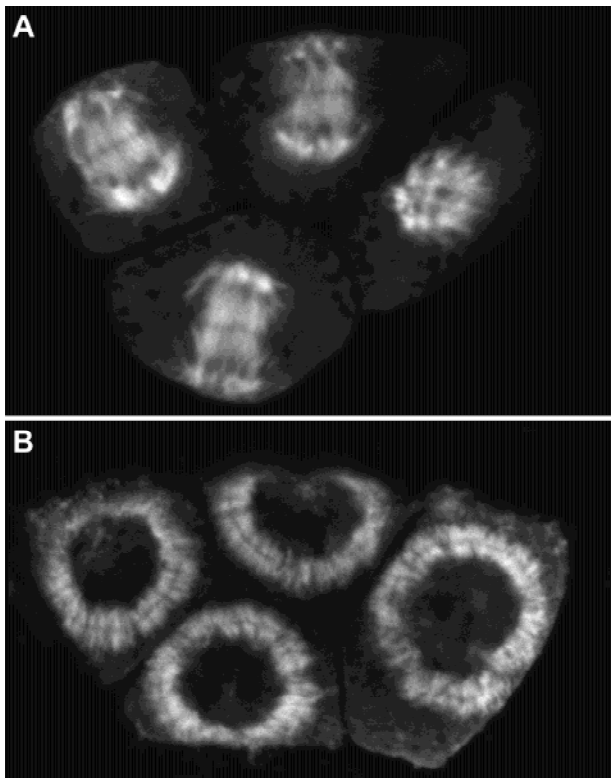

Figure 2. Microtubules in development of interzonal phragmoplasts in the permanent tetrad of an orchid. (**A**) Phragmoplasts are initiated in the interzone between sister nuclei and immediately appear as brushlike arrays bisected by a dark zone. (**B**) Phragmoplasts narrow and expand as a ring at the edge of the forming discoid cell plate.

division site and in guiding the leading edge of the expanding cell plate (Lloyd and Traas 1988; Valster and Hepler 1997). The division site F-actin may be derived evolutionarily from cleavage furrow F-actin, as suggested by studies of *Spirogyra* (McIntosh and others 1995). In some densely cytoplasmic cells, actin is noticeably absent from the late division site, the so-called actin-depleted zone (Liu and Palevitz 1992; Cleary and others 1992; Cleary 1995). Instead, F-actin is distributed throughout the two incipient daughter cytoplasts. It may be that interaction of the leading edge of the expanding phragmoplast with inner surfaces of actin in the daughter cytoplasts is instrumental in guiding the cell plate to junction with the parental wall at the prescribed division site (Pickett-Heaps and others 1999). In this sense, distribution of the actin cytoskeleton may reflect cytoplasmic determination of the daughter cell domains before the mitotic apparatus delivers the nuclei, a view consistent with the cytoplasmic domain or cytoplast concept (Pickett-Heaps and others 1999).

Reproductive Phase

The life cycle of a plant includes not only vegetative growth, but a reproductive phase as well. Cells entering the reproductive lineage are no longer part of a multicellular tissue and often pass through a multinucleate stage. In angiosperms, cytokinesis may be uncoupled from nuclear division at various points in the reproductive phase. Pollen development often includes a brief syncytial stage during which time four haploid nuclei resulting from meiosis reside in the common cytoplasm of the microsporocyte before simultaneous cytokinesis. Likewise, the megasporocyte may undergo simultaneous cytokinesis, as is the case in *Arabidopsis* (Battaglia 1991; Webb and Gunning 1990). Megagametophyte (embryo sac) development following megasporogenesis always includes a syncytial stage before simultaneous cytokinesis occurs and cells of both the egg apparatus and antipodals are walled off from the central cell (for review see Battaglia 1991; Russell 1993). The most common type of endosperm development (nuclear) includes a syncytial stage of sometimes thousands of nuclei in a common cytoplasm before cytokinesis results in its cellularization. In all of these examples, the microtubule cycle comprises 3 arrays—spindle, radial, and phragmoplast. Both cortical microtubules and PPBs are absent and the radial microtubules determine wall placement.

Microtubule Cycle in Plant Syncytia

Radial microtubules are important in the cellularization of plant syncytia. The radial microtubule system (RMS) is nucleated in the perinuclear area and serves to organize the cytoplasm into nuclear-cytoplasmic domains (NCDs) at the borders of which walls will be deposited in association with adventitious phragmoplasts. As far as we know, this model accounts for all examples of cell wall placement in plant syncytia. The concept of the cytoplasmic domain (Brown and Lemmon 1992a) in the control of wall placement in syncytia stems from studies of sporogenesis in lower plants where the spore domains are defined by RMSs before meiosis (Brown and Lemmon 1997). In these cases, the RMSs do not emanate from the undivided nucleus, but rather from either a single plastid or from a polar organizer positioned in each of the future spore domains which are delimited in advance of nuclear division (Brown and Lemmon 1997). In syncytia of higher plants, domains of cytoplasm to be walled off are defined by nuclear-based RMSs. Our current concept holds that the RMS is a fundamental component of the control of wall placement in plants. Dur-

ing evolution, there has been a temporal shift from before nuclear division to after nuclear division and a corresponding change in site of organization (Brown and Lemmon 1993). To include all cases from sporocytes to endosperm, the NCD definition has been broadened to "a portion of cytoplasm either nucleate or destined to contain a nucleus with wall development at its periphery" (Brown and Lemmon 2001). Not only is the establishment of NCDs via nuclear-based RMSs the mechanism of cytoplasm apportionment in syncytial systems but it appears to function as a default mechanism in meristems as well. For example, in *Arabidopsis* mutants that lack PPBs such as *fass* and *ton* (Torres-Ruiz and Jürgens 1994; Traas and others 1995), it appears that radial microtubules may control wall placement (McClinton and Sung 1997). Even though the *fass* mutant plants are misshapen (dumpy), they are capable of some complex developmental sequences such as those leading to reproductive tissues, stomates, and trichomes. That plants carrying these mutations would not likely compete successfully in nature reinforces the concept that the PPB has a role in the precise control of division plane in vegetative growth.

The process of cellularization in syncytia affords extraordinary opportunity to elucidate the fundamental nature of plant cytokinesis because PPBs are absent and phragmoplasts are uncoupled from nuclear division. Large numbers of phragmoplasts, and therefore subtle differences in their development, can be observed in a single preparation. The two principal examples of plant syncytia are microsporocytes and nuclear endosperm. Sporocytes can develop into minisyncytia with 4 nuclei and the macrosyncytium of nuclear endosperm may contain thousands of nuclei.

In cellularization of syncytia, phragmoplasts are typically the instruments of cytokinesis and form among all nuclei, non-sister as well as sister. The phragmoplasts that form between non-sister nuclei in microsporogenesis have been termed "secondary spindles" (Heslop-Harrison 1971) to recognize their unusual point of origin other than in the interzone between sister nuclei. In the early literature, phragmoplasts were often referred to as spindles because of their structural similarity to the mitotic apparatus. The numerous phragmoplasts responsible for wall deposition in the large syncytium of nuclear endosperm have been termed cytoplasmic (Brown and others 1994), or adventitious (Olsen and others 1995) in keeping with the conventional designation for plant structures of unusual origin. These phragmoplasts are not initiated on an existing scaffold of non-kinetochore spindle fibers in anaphase/telophase, as is generally thought to be the origin of interzonal phragmoplasts in vegetative cell division. Instead, phragmoplasts that are uncoupled from nuclear division are initiated at the interfaces of opposing RMSs emanating from nuclei in the common cytoplasm.

Microsyncytia

Meiotic cytokinesis can occur successively after each round of nuclear division, or simultanously after the completion of meiosis. When walls fail to form after first meiosis, a band of organelles, which may include plastids, mitochondria, and lipid droplets, frequently forms in the equatorial region between the dyad domains (Rodkiewicz and Duda 1988; Brown and Lemmon 1991a). Simultaneous cytokinesis that accomplishes the apportionment of a 4 nucleate microsyncytium into a tetrad of free spores (Figure 3) results from merger of two interzonal phragmoplasts between telophase nuclei of the second meiosis and adventitious phragmoplasts formed among the non-sister nuclei. Although the widespread occurrence of simultaneous cytokinesis in sporogenesis of lower land plants may indicate a primitive condition, most plant groups exhibit both simultaneous and successive cytokinesis. Meiotic cytokinesis in bryophytes (Brown and Lemmon 1988a) and ferns (Verma and Khullar 1976; Sheffield and Bell 1987) is predominately simultaneous, whereas both types occur in lycopsids (Brown and Lemmon 1991b). Among flowering plants, cytokinesis in microsporocytes of dicots is generally considered to be simultaneous and monocots successive. However, certain large groups of monocots (for example, Iridales and Orchidales) exhibit simultaneous cytokinesis (Brown and Lemmon 1991c; Furness and Rudall 1999). Multiple modes of meiotic cytokinesis are reported for dicots (Sampson 1969; Brown and Lemmon 1991a; Furness and Rudall 1999) with *Magnolia* representing an intermediate condition between successive and simultaneous.

Sporocytes of the lower land plants typically prepare for simultaneous cytokinesis before karyokinesis. In monoplastidic meiosis of bryophytes, plastid division and placement of a single plastid at each of the future tetrad poles is followed by development of an elaborate quadripolar microtubule system (QMS) (Brown and Lemmon 1997). The QMS, which subsequently merges into a functionally bipolar meiotic spindle, predicts the future planes of cytokinesis prior to nuclear division, as does the PPB in meristems. Unlike the PPB, however, microtubules of the QMS are at right angles to the plane of cytokinesis and, in this respect, are more like phrag-

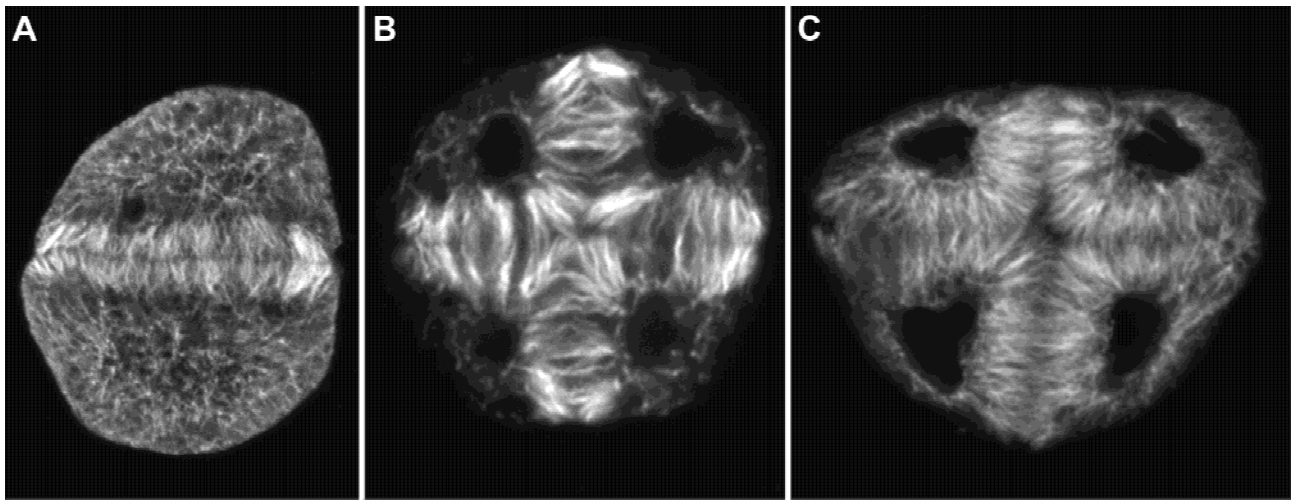

Figure 3. Microtubules in simultaneous cytokinesis of microsporocytes. (**A**) A typical phragmoplast may develop and expand without the deposition of a cell plate after first meiosis. (**B**) Following second meiosis, a complex of identical phragmoplasts guides wall deposition to produce the tetrad. Only two of these phragmoplasts were formed in the interzone between sister nuclei; all others were formed adventitiously among non-sister nuclei. (**C**) The newly separated microspores of the tetrad with their prominent radial microtubule systems.

moplasts and the midbodies of animal cells. By establishing the four future spore domains and determining orientation of the first and second meiotic divisions, as well as predicting location of the complex of phragmoplasts after meiosis, the QMS functions to spatially coordinate cytoplasmic and nuclear division and insures that a plastid as well as nucleus is inherited by each spore of the tetrad (Brown and Lemmon 1997).

In microsporogenesis of seed plants, the organization of cytoplasm into spore domains is typically delayed until after meiosis, with the position of nuclei determining the placement of intersporal walls via nuclear-based RMSs. This is especially obvious in sporocytes with no set arrangement of tetrads, for example, polliniate orchids (Brown and Lemmon 1991c). Following first meiosis in the moth orchid *Phalaenopsis,* a phragmoplast develops in the interzone but a wall may or may not be deposited. When no wall is present, a conspicuous organelle band in the equatorial region defines the dyad domains. Following second meiosis, two interzonal phragmoplasts form between pairs of sister nuclei and radial microtubules emanating from all nuclei interact to trigger secondary phragmoplasts among non-sister nuclei. The primary and secondary phragmoplasts soon become indistinguishable and the expanding complex of merged phragmoplasts mediates wall deposition that neatly separates four spores of approximately equal volume in a pattern that reflects the arrangement of nuclei at the end of meiosis. Interestingly, the mechanism for the cellularization of irregular spore tetrads in the polyplastidic marchantialian liverwort *Conocephalum conicum* (Brown and Lemmon 1988b) is identical to that in *Phalaenopsis.*

Further proof that spore domains in simultaneous cytokinesis of microsporocytes of angiosperms are determined by nuclear position rather than being prepatterned comes from examples of the cleavage of supernumerary spores following faulty meiosis in normal and perturbed systems. Experimental treatment of microsporocytes of *Magnolia* with griseofulvin, a drug that affects microtubule organization, results in multipolar spindles and faulty distribution of chromosomes, leading to micronuclei and/or unusual arrangements of tetrad nuclei that never occur normally (Brown and Lemmon 1992b). The interaction of RMSs from atypical arrangement or numbers of nuclei defines domains of cytoplasm (NCDs) that are subsequently walled off. In untreated microsporocytes of triploid daylilys and certain complex orchid hybrids, meiotic mishaps result in stranded chromosomes and supernumerary micronuclei (Brown and Lemmon 1992a). At cytokinesis, RMSs emanating from all nuclei claim proportionate amounts of cytoplasm. Phragmoplasts formed at borders of the NCDs mediate wall deposition resulting in spores of various sizes and location.

The inclusion of a syncytial stage in development provides an opportunity for free nuclei to become variably placed in response to the establishment of polarity before cellularization locks them into position. Megagametophyte development in angiosperms is known to include a brief syncytial stage

before simultaneous cytokinesis occurs to produce the highly polar megagametophyte or embryo sac (see for example, Battaglia 1991). The unusual patterns of simultaneous wall deposition in embryo sac development have long puzzled embryologists. Russell (1993) suggested that cellularization of the megagametophyte can be explained by the NCD model. Cell plates deposited at the boundaries of NCDs are guided by phragmoplasts formed at the interfaces of the nuclear-based RMSs and thus reflect the position of the nuclei that have become unequally placed in the common cytoplasm. The control of this complex polarity and the mechanisms of nuclear migration are unknown, but it is clear that the phenomenon requires the flexibility of a syncytium with wall placement reflecting nuclear position.

In the eight nucleate endosporic megagametophyte, which is present in 70% of flowering plants (Reiser and Fischer 1993), the two nuclei resulting from mitosis of the functional megaspore move to opposite poles of the cell and undergo two more rounds of karyokinesis without cytokinesis to produce two quartets of nuclei. Three nuclei from the chalazal quartet are walled off to become antipodals and three nuclei of the micropylar quartet are at least partially walled off to become egg and flanking synergids. The remaining two polar nuclei (one from each of the opposing quartets) are not walled off from each other but reside in the bulk of the cytoplasm comprising the central cell. These nuclei fuse to become the secondary endosperm nucleus which is fertilized by the second sperm in double fertilization to initiate endosperm development.

Macrosyncytia

In the nuclear type of endosperm development, the primary endosperm nucleus resulting from fusion of sperm and polar nuclei undergoes nuclear divisions uncoupled from cytokinesis. Mitotic waves, which appear to originate in the vicinity of the embryo, are responsible for populating the enlarging mass of cytoplasm with nuclei. Although phragmoplasts are initiated in the interzonal region between telophase nuclei, they fail to develop and no walls are deposited (Brown and others 1994). The mechanism of wall placement and growth in nuclear endosperm remained enigmatic until the advent of appropriate techniques for three-dimensional imaging of *in situ* developing systems. Of special importance is immunolocalization of the cytoskeleton to provide global views of the participating arrays (van Lammeren 1988; Brown and others 1994; Nguyen and others 2001). More recently, endosperm fixed by high pressure freezing and viewed by transmission electron microscopy (TEM) has provided high resolution tomographic data on redirection of the secretory apparatus at boundaries of the NCDs (Otegui and Staehelin 2000).

The dramatic difference in microtubule systems involved in syncytial development compared with embryogenesis can be seen in the micropylar chamber where the cellular embryo is surrounded by the syncytial endosperm (Figure 1). Unusual fusiform endosperm nuclei are sheathed with parallel microtubules that are connected to a reticulate network of microtubules in the syncytium (Nguyen and others 2001) whereas the interphase cells of the embryo have hoop-like cortical microtubules. With the cessation of proliferative nuclear division in the syncytium, there is a reorganization of the cytoskeleton from a reticulate pattern throughout the syncytium into nuclear-based RMSs (Nguyen and others 2001). The macrosyncytium is prepared for cellularization by RMSs which serve to define NCDs and position them into a regular hexagonal pattern prior to the formation of adventitious phragmoplasts at their boundaries (Figure 4A).

Development of adventitious phragmoplasts is initiated when subsets of the RMS of two adjacent nuclei interact to become directly opposed. At the site of interaction, a region of microtubule overlap is presumably established and microtubules proliferate into phragmoplast fibers (Figure 4A). This establishes the dark zone that remains unstained in immunofluorescence preparations of microtubules. Increased organization of the microtubules results in the distinctive units of phragmoplasts, that is, fusiform bundles bisected by the dark zone. These subsets of microtubules appear similar to microtubule-converging centers (MTCCs) described during mitosis in extruded *Haemanthus* endosperm (Smirnova and Bajer 1994). Phragmoplasts initiated between adjacent NCDs merge to form a continuous system of phragmoplasts in which cell plates are deposited (Figure 4B). In the narrow micropylar chamber of mustards, where the syncytium is not forced to the periphery by a large central vacuole, the adventitious phragmoplasts may completely surround the NCDs (Nguyen and others 2001).

Recent state of the art TEM using high pressure freezing fixation has provided high resolution images of the sequence of events in wall formation in the micropylar chamber (Otegui and Staehelin 2000). Cell plates are initiated in individual phragmoplast units of 4–12 microtubules ("mini-phragmoplasts") at the perimeter of NCDs. The cell plates initiated in this way are unlike those in somatic cells in that they lack 20 nm wide membrane

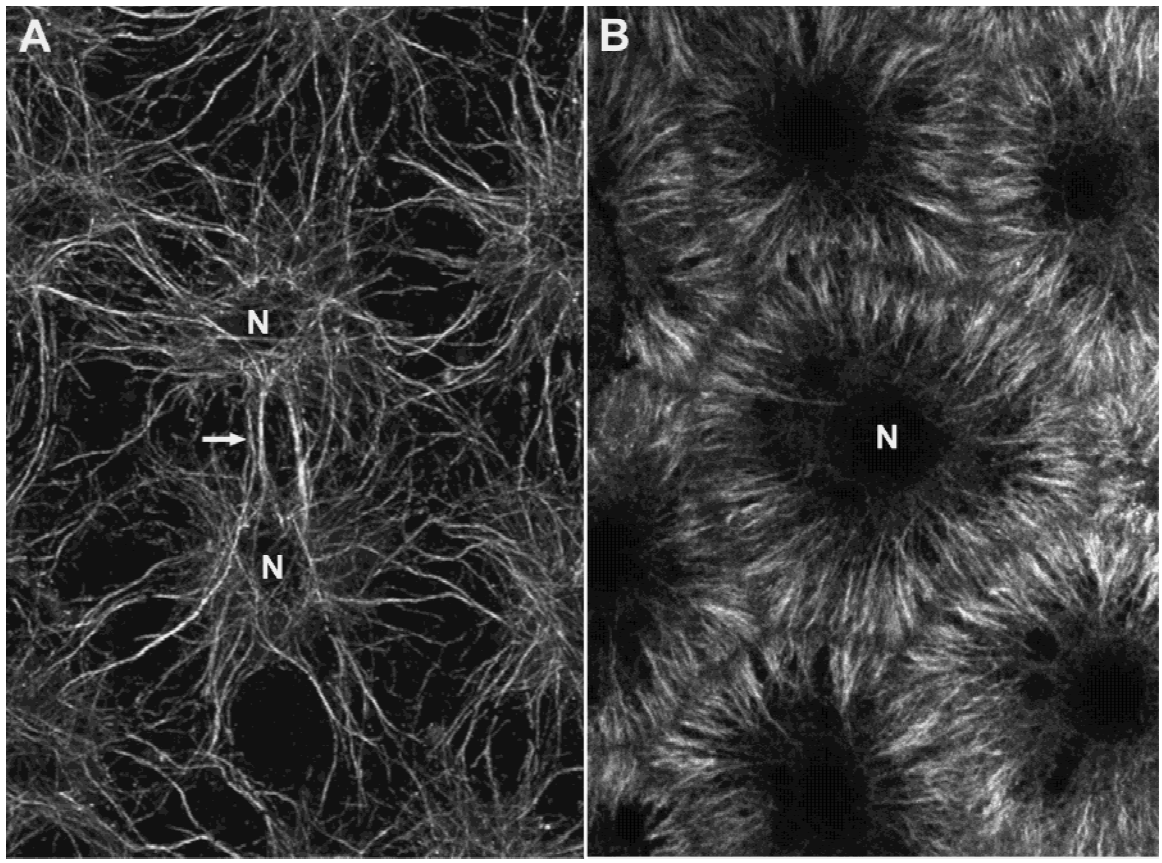

Figure 4. Microtubules in adventitious phragmoplast development in endosperm syncytium shown in face view. (**A**) NCDs defined by RMSs are packed in an orderly hexagonal pattern. At the interface of RMSs, initial phragmoplast fibers (arrow) are established by microtubule alignment on either side of a dark mid-zone. (**B**) Adventitious phragmoplasts in the canopy of cytoplasm adjacent to central vacuole. Fusiform bundles comprised of opposite sets of microtubules bisected by a dark zone (site of fusing cell plate vesicles) make up the complex multifaceted alveolar phragmoplast.

fusion tubes; Golgi-derived vesicles fuse directly into hour-glass intermediates. The numerous cell plates initiated in phragmoplast units at the periphery of NCDs fuse with each other to form continuous cell plates. Cell plate maturation after fusion with the central cell wall is similar to final cell plate assembly in somatic cells described by Samuels and others (1995). However, the endospermic cell plates remain callosic for a prolonged period (Brown and others 1997; Otegui and Staehelin 2000).

The same basic process of wall placement via the NCD mechanism also occurs in the large central chamber, but with variations that result in a unique pattern of cellularization termed alveolation. Alveolation begins with the delimitation of NCDs by nuclear-based RMSs in the thin layer of peripheral syncytium between the central cell wall and the large central vacuole. The NCDs become polarized in axes perpendicular to the central cell wall and anticlinal wall formation results in open-ended compartments termed alveoli. Numerous works (see for example, Olsen and others 1995; XuHan 1995; Brown and others 1999; Nguyen and others 2001) have demonstrated that alveolation is the typical mechanism for cellularization of macrosyncytia peripheral to a large central vacuole. In some developing seeds such as the elongate cereal grains, this is a very large region and the process of alveolation dominates endosperm development. It is even more prominent in the large megagametophytes of gymnosperms where in some cases the entire process of initial cellularization is attributable to a single peripheral layer of alveoli that elongate centripetally until they meet in the center to effect closure (Singh 1978).

In both cereals (Olsen and others 1995) and mustards (Brown and others 1999; Nguyen and others 2001), there is a short period during which phragmoplasts are not prominent but walls are nevertheless initiated at the boundaries of NCDs. The term "free-growing" to indicate this enigmatic type of wall deposition has led to much confusion particu-

larly since some workers (for example, Morrison and O'Brien 1976) interpreted the origin of these walls as ingrowths of the central cell wall. The explanation for anticlinal walls as ingrowths was discounted in favor of the NCD model in a series of endosperm studies in which no evidence could be found that the first walls begin as ingrowths (Brown and others 1994, 1999; Olsen and others 1995). The NCD model explains the control of wall placement in endosperm as a function of nuclear-based RMSs. Further, it states that formation of adventitious phragmoplasts at the boundaries of opposing RMSs is responsible for wall deposition. A summary diagram (Figure 2A in Olsen and others 1995) illustrated the "free growing anticlinal wall" starting at sites along shared boundaries of NCDs and not yet fused with each other or with the central cell wall. The mini-phragmoplasts at common boundaries of NCDs, as demonstrated by Otegui and Staehelin (2000), provide the most plausible explanation for the unusual phenomenon of cell plate initiation in the absence of prominent phragmoplasts. These initial anticlinal walls fuse with the central cell wall and merge with each other laterally to establish the continuous network of open hexagonal alveolar compartments. All subsequent anticlinal wall formation is a function of alveolar phragmoplasts.

A model proposed by Fineran and others (1982) for anticlinal wall formation in association with interzonal phragmoplasts has likewise been discounted by recent studies (van Lammeren 1988; Brown and others 1994; Olsen and others 1995; Otegui and Staehelin 2000; Nguyen and others 2001). However, it is possible that a fast moving wave of anticlinal wall formation can overtake the final wave of mitosis in the chalazal region of the central cell and both types of phragmoplasts (interzonal and adventitious) could contribute to alveolar wall formation. This has been reported to occur in *Ranunculus* (Chitraleka and Bhandari 1993). In this case, a six-sided alveolus would have one wall formed in association with an interzonal phragmoplast between sister nuclei and the other five walls formed between non-sister nuclei. As previously mentioned, it is the normal situation in meiotic simultaneous cytokinesis where two phragmoplasts are interzonals between sister nuclei and all others are adventitious phragmoplasts between non-sister nuclei.

Two phenomena contribute to alveolation. Each NCD becomes polarized in an axis perpendicular to the central cell wall and a ring of vacuoles accumulates around the nucleate central part of the cytoplasm (Brown and others 1996a). The vacuoles serve to isolate a thin layer of shared cytoplasm between adjacent NCDs in which the anticlinal walls are formed. Little is known of either process but it seems likely that vacuolation is important in driving the elongation of NCDs. Certainly vacuolation is conspicuous as anticlinal walls continue to grow.

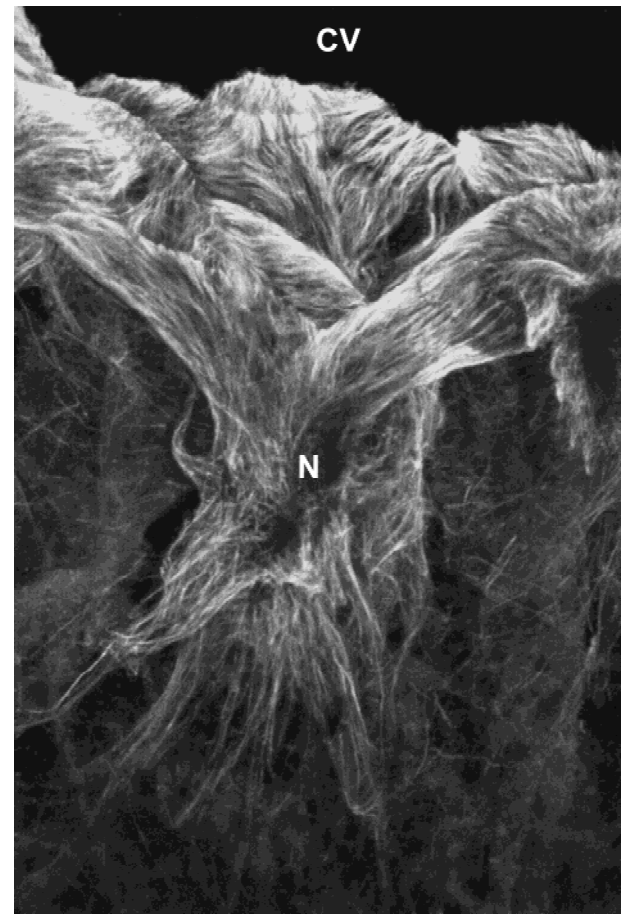

Figure 5. Polarized microtubule system in a single alveolar NCD shown in side view. Adventitious phragmoplasts, which form at interfaces of microtubules emanating from nuclei in adjacent NCDs, fill the canopy of syncytial cytoplasm adjacent to the central vacuole (CV). Microtubules extend from the nucleate column of cytoplasm toward the central cell wall. Delicate callosic anticlinal walls, usually six around each NCD, are deposited in dark mid-zones of the phragmoplasts.

Concomitant with elongation of alveoli is a dramatic reorganization of the nuclear-based microtubules into highly polar systems (Figure 5). As seen from the side, the microtubule system in an elongating alveolus resembles a tree with axially aligned microtubules in the column of cytoplasm containing the nucleus, root-like processes at the peripheral wall, and a canopy extending into the syncytial cytoplasm adjacent to the central vacuole and overtopping the anticlinal walls. Adventitious phragmo-

plasts form at the interfaces of opposing microtubule systems emanating from tips of elongated nuclei in adjacent NCDs (Figure 5). These adventitious phragmoplasts mediate continued unidirectional growth of the anticlinal walls; growth of walls is centrifugal relative to their point of origin and centripetal relative to the central cell. In face view, as seen from the central vacuole, the alveoli are arranged in a honeycomb-like pattern (Figure 4). Hereafter, leading edges of the anticlinal walls continue to grow unidirectionally in association with these phragmoplasts which remain tethered to adjacent nuclei. The complex of merged alveolar phragmoplasts themselves and the syncytial front of cytoplasm in which they are formed is continuously elevated as the six walls surrounding each alveolar NCD grow into the center of the central cell.

Each alveolus, starting with only one wall (the central cell wall) and growing six merged anticlinal walls, remains open-ended until it is divided periclinally by mitosis followed by cytokinesis. Prior to the wave of periclinal divisions, the alveolar phragmoplasts are disassembled and nuclei become more centrally located in the alveoli. The factors involved in positioning nuclei are not known. Alveoli are of nearly uniform length and the prophase nuclei are suspended in rafts of cytoplasm (phragmosomes) nearly equidistant from the central cell wall. Phragmosomes predict the plane of the future division, as is typical of vacuolate cells (Sinnott and Bloch 1941; Lloyd and Traas 1988; Lloyd and others 1992) but no PPBs are formed in alveoli (Brown and others 1994, 1999). Interzonal phragmoplasts/cell plates expand to junction with the anticlinal walls of the alveoli. In this manner, the peripheral portion of each alveolus receives its final wall and becomes a cell while the inner portion remains an alveolus. Following the wave of periclinal divisions, microtubules again emanate from the tips of interphase nuclei in the alveolar layer (all of which are non-sisters), interact at their interfaces, and organize phragmoplasts that direct renewed growth of the anticlinal walls.

The restarting of wall growth at the outer edge of a cell plate after an interruption is well documented in microsporogenesis. For example, forming cell plates after first meiosis may be abandoned before they join with the microsporocyte wall and remain as floating discs in the cytoplasm (see for example, Brown and Lemmon 1991c). After second meiosis, when interzonal phragmoplasts are depositing walls between sister nuclei, RMSs emanating from nuclei trigger phragmoplast formation to restart at the edges of the disc and complete the interrupted process of cytokinesis.

The second period of alveolar growth in endosperm is identical to the first and again is followed by a periclinal division. This repeated cycle of anticlinal wall formation between non-sister nuclei followed by periclinal wall formation between sister nuclei completes cellularization of the endosperm. Following the initial cellularization, cell divisions occur in both the starchy endosperm and peripheral aleurone. These later divisions depart from the strictly RMS-driven developmental pathway of initial cellularization. Cells of the starchy endosperm develop cortical microtubules but no PPBs (Brown and others 1994; Clore and others 1996). Cells of the multilayered peripheral aleurone of barley develop both hoop-like cortical microtubules and PPBs (Brown and others 1994). It is significant that the switch to the PPB microtubule cycle typical of histogenesis occurs only in later stages of endosperm development when cells are added in an orderly fashion to the growing aleurone. Whether or not PPBs develop in the single-layered aleurone (endosperm epidermis) that occurs in many seed types is not known.

Conclusions

A comparison of the specialized alveolar phragmoplast to the phragmoplast in meristematic cells is informative. They appear identical in structure, being a complex of individual units consisting of fusiform bundles bisected by a dark zone in which vesicles accumulate and fuse. Expansion of both types of phragmoplasts (and cell plates) is centrifugal from the point of origin between nuclei. However, phragmoplasts in meristematic cells are symmetrical and follow a path previously marked by a PPB, whereas the alveolar phragmoplasts become asymmetrical and are guided solely by microtubules emanating from the tips of adjacent nuclei. Whereas an interzonal phragmoplast forms a single wall, several alveolar phragmoplasts (usually six) surround each NCD and merge to function as a single unit during the initial stages of anticlinal wall formation. Finally, the fact that nuclei move from their original position at the start of phragmoplast formation, ride the syncytial front of advancing cytoplasm, and continue to guide the centrifugally expanding phragmoplasts/cell plates is unique. The entire complex of hexagonal walls grows centripetally into the center of the central cell.

In conclusion, it appears that the phragmoplast is a structure of uniform organization that can be triggered to assemble at the interface of microtubules of opposite polarity. This inherent ability of plant cy-

toplasm to organize phragmoplasts is responsible for the appearance of phragmoplasts at places and times other than in the interzone between sister nuclei after nuclear division. The concept of the phragmoplast and its role in cytokinesis is constantly being revised and restated from its original description as a spindle associated with the cell plate. Dissection of the process of cytokinesis can be reduced to control points, the most basic of which is probably the interaction of microtubules of opposite polarity and development of a dark zone defining the site of vesicle accumulation and fusion. Once initiated, phragmoplasts merge easily, for instance, the multiple mini-phragmoplasts initiated at boundaries of NCDs, and even those of different origin (one interzonal, the other adventitious) which fuse in simultaneous cytokinesis of microsporocytes. Phragmoplast/wall deposition can be interrupted and restarted at a later time, the newly formed phragmoplast joining with the abandoned cell plate. This occurs in alveolar walls and in microsporocytes when the dyad wall remains a floating disc. A second control point is the coalescence of vesicles to initiate a cell plate. Not all phragmoplasts function in deposition of a cell plate; this occurs in formation of syncytia and in the phragmoplast-like structures adjacent to the meiotic prophase nucleus in lower land plants where vesicles accumulate but do not fuse (Brown and Lemmon 1991b, 1997). Another control point establishes the self-generating nature of the phragmoplast that drives its centrifugal expansion. Not all phragmoplasts expand; for example, proliferative divisions in nuclear endosperm and in certain sporocytes.

Recognition of the common structural features of interzonal phragmoplasts and phragmoplasts in syncytial systems between non-sister nuclei (and even in isolated bits of cytoplasm), should contribute to a model of the minimal requirement for construction of the functional phragmoplast component of the cytokinetic apparatus.

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