

Cytokinesis in Higher Plants

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Annu. Rev. Plant Biol.
2005. 56:281–99

doi: 10.1146/
annurev.arplant.55.031903.141636

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First published online as a
Review in Advance on
February 15, 2005

1543-5008/05/0602-
0281\$20.00

Key Words

membrane traffic, membrane fusion, phragmoplast, cell plate, cytoskeleton, cell wall, cellularization, *Arabidopsis*

Abstract

Cytokinesis partitions the cytoplasm between two or more nuclei. In higher plants, cytokinesis is initiated by cytoskeleton-assisted targeted delivery of membrane vesicles to the plane of cell division, followed by local membrane fusion to generate tubulo-vesicular networks. This initial phase of cytokinesis is essentially the same in diverse modes of plant cytokinesis whereas the subsequent transformation of the tubulo-vesicular networks into the partitioning membrane may be different between systems. This review focuses on membrane and cytoskeleton dynamics in cell plate formation and expansion during somatic cytokinesis.

Contents

INTRODUCTION	282
MEMBRANE DYNAMICS.....	282
Vesicle Trafficking and Initiation of the Cell Plate.....	283
Membrane Fusion Machinery in Cytokinesis	284
Delivery of Cargo to the Cell Plate .	286
Membrane Dynamics During Cell Plate Expansion.....	287
Fusion with the Plasma Membrane .	288
CYTOSKELETON DYNAMICS	289
Roles of Microtubules and Actin Filaments in Phragmoplast- Assisted Cytokinesis.....	289
Formation and Dynamic Stability of the Phragmoplast Microtubules..	290
Lateral Translocation and Disassembly of the Phragmoplast Microtubules	292
CONCLUDING REMARKS.....	293

Higher plants display several cell type-specific modes of cytokinesis (61). The most common mode occurs in somatic cells (**Figure 1**). A plant-specific cytoskeletal array—the phragmoplast—delivers membrane vesicles to the center of the division plane. Vesicle fusion generates a novel transient compartment—the cell plate, which then grows out to fuse with the plasma membrane at the cell periphery. Endosperm cellularization is a closely related variant of somatic cytokinesis, involving “mini-phragmoplasts” and “mini-cell plates” and also sharing several genetically defined components (62, 63, 79). Female and male meiotic cells as well as microspores each undergo their own modes of cytokinesis, which are also distinct from embryo sac cellularization (61). However, cytokinesis of male meiotic cells in *Arabidopsis* was recently shown to resemble somatic cytokinesis in one important aspect—delivery of membrane vesicles to the plane of division and formation of membrane networks generated by vesicle fusion across the division plane, which contrasts with the previous notion of ingrowth from the cell wall (64). It is thus likely that all cell type-specific modes of cytokinesis share conserved features, which may reflect common underlying plant-specific mechanisms (51).

This review focuses on molecular mechanisms of cytokinesis, which have been mainly identified in somatic cells. Where appropriate, findings from other cell types are discussed. There is emphasis on membrane and cytoskeleton dynamics. For other aspects of plant cytokinesis, the reader is referred to excellent recent reviews (2, 9, 11, 15, 22, 51, 56, 61, 77, 88).

MEMBRANE DYNAMICS

Somatic cytokinesis starts with the accumulation and fusion of membrane vesicles in the center of the division plane from late anaphase on. The membrane fusion processes are spatially constrained such that a disk-shaped aggregate of fusion intermediates is produced. The intermediates are gradually transformed into a transient membrane compartment—the cell plate, which eventually gives rise to the plasma

INTRODUCTION

Cytokinesis partitions the cytoplasm of a dividing eukaryotic cell by laying down a stretch of plasma membrane between the forming daughter nuclei. Although common to all eukaryotes, cytokinesis is less conserved between higher plants and nonplant organisms than are other aspects of the cell cycle. Animal and fungal cells initiate cytokinesis at the periphery of the division plane, with the plasma membrane pulled in toward the center by a contractile actomyosin ring (5). Additional membrane material is delivered by vesicle trafficking to a site behind the tip of the ingrowing furrow. Ingrowth of the plasma membrane stops at the midbody, leaving a gap in the center. This gap is closed by vesicle trafficking and fusion, which completes the separation of the daughter cells (47). Although midbody closure resembles the initial stage of cytokinesis in higher plants, the overall process of cytokinesis is very different between higher plants and nonplant organisms.

Cell plate: a transient membrane compartment that is formed by the fusion of cytokinetic vesicles and eventually matures into the plasma membranes and cross-wall between daughter cells

Cellularization: simultaneous partitioning of a cell with more than two nuclei

Phragmoplast: a cytokinesis-specific array of organized microtubules, vesicles, and actin filaments that supports the formation and expansion of the cell plate

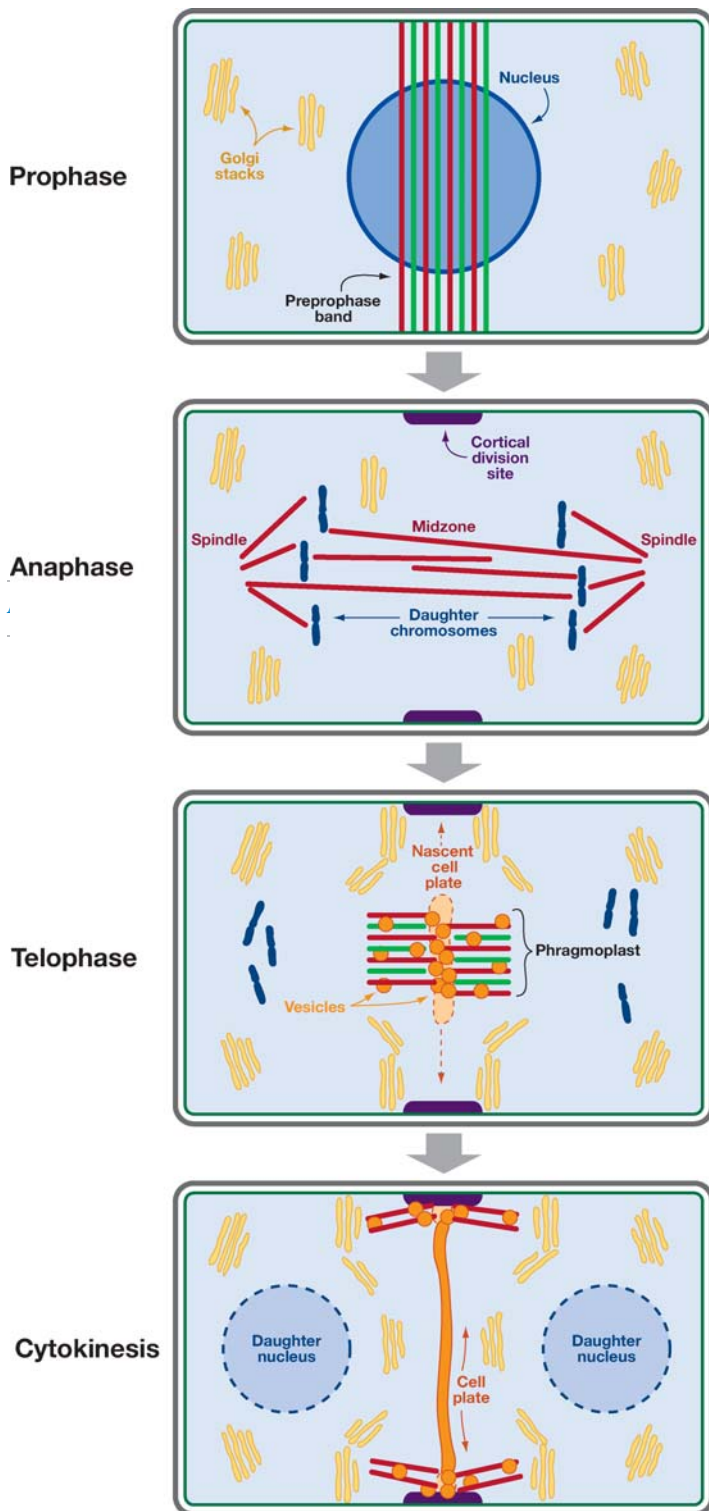
membranes that underlie the cross-wall between the daughter cells. The cell plate expands centrifugally by the fusion of later-arriving vesicles with its margin. Simultaneously, the cell plate undergoes a complex process of reorganization, which involves secretion of cell wall material into its lumen and removal of excess membrane material, resulting in a planar structure. Finally, the margin of the cell plate fuses with the parental plasma membrane at cortical division sites marked earlier by the transient preprophase band, physically separating the two daughter cells from one another (Figure 1).

Vesicle Trafficking and Initiation of the Cell Plate

There is evidence for transport vesicles being involved in cell plate formation in studies of both wild-type and mutant *Arabidopsis*, following the in-depth electron microscopy analysis of high-pressure frozen synchronized tobacco BY-2 cells (72). Noncoated vesicles were detected in the division planes of all three different cell types analyzed: meristematic cells, cellularizing endosperm, and male meiotic cells (63, 64, 74). In addition, embryo cells of cytokinesis-defective mutants impaired in vesicle fusion accumulate 60–80-nm vesicles in the plane of cell division, and these vesicles persist into

Figure 1

Cytokinesis of somatic cells. Prophase: Coaligned bundles of microtubules (*red*) and actin filaments (*green*) of the transient cortical preprophase band determine the future plane of division, marking the cortical division site. Anaphase: Spindle remnants facilitate initiation of the phragmoplast microtubules in the midzone between the two sets of daughter chromosomes. Telophase: Two antiparallel bundles, each of microtubules (*red*) and actin filaments (*green*), form the phragmoplast, their plus ends facing the nascent cell plate. Golgi stacks accumulate near the plane of division, forming a “Golgi belt.” Cytokinesis: Coordinated lateral expansion of the cell plate and lateral translocation of microtubules (*red*) terminate in the fusion of the cell plate with the plasma membrane at the cortical division site.



AP: adaptor protein

SNARE:

membrane-anchored protein involved in membrane fusion. A v-SNARE on the vesicle membrane (also called VAMP or R-SNARE because of a conserved arginine residue in its SNARE domain) interacts with two or three t-SNAREs on the target membrane, which are either a syntaxin (also called Qa-SNARE because of a conserved glutamine residue in its SNARE domain) and a SNAP-25 protein (also called Qb+Qc-SNARE) or a syntaxin and two t-SNARE light chains (also called Qb-SNARE and Qc-SNARE).

interphase (41, 92). Electron tomography has revealed two types of vesicles near forming cell plates: smaller dark vesicles approx. 51 nm in diameter and larger light vesicles approx. 66 nm in diameter (74). The smaller dark vesicles predominate initially, whereas the larger light vesicles appear more numerous at a later stage of cell plate formation. Both size calculations and the occurrence of hourglass-shaped putative vesicle fusion intermediates suggest that pairwise fusion of smaller dark vesicles results in larger light vesicles. Consistent with this, only smaller dark vesicles have been detected near Golgi stacks, which are thought to generate cytokinetic vesicles (74).

Golgi stacks and other organelles aggregate around the phragmoplast during telophase (**Figure 1**) (57). This spatial arrangement suggests direct delivery of Golgi-derived vesicles to the division plane via the phragmoplast. Alternatively, an intermediate endosomal compartment may be involved (see discussion in 9). A recent kinetic study of the endomembrane distribution of the endocytic tracer FM4-64 makes the latter possibility less likely as both Golgi stacks and cell plate are labeled only 30–60 min after uptake of the tracer (10). The machinery involved in the formation of cytokinetic vesicles has not been identified. However, the following components may be required, by analogy with vesicle budding in other post-Golgi trafficking pathways: an ADP-ribosylation factor (ARF)-type small GTPase, its guanine-nucleotide exchange factor (ARF-GEF) and GTPase-activating protein (ARF-GAP), AP complex coat proteins with or without clathrin, and dynamin GTPase for vesicle scission (32, 38). Cell plate formation is sensitive to the membrane-trafficking inhibitor brefeldin A (BFA) (97, 98), which blocks the activation of ARF-type GTPases by BFA-sensitive ARF-GEFs (69). Synchronized BY-2 cells treated with BFA from the onset of mitosis fail to form the cell plate, resulting in binucleate cells (98). BY-2 cells treated with BFA display characteristic Golgi abnormalities (70). In summary, several lines of evidence support the notion that the cell

plate originates from Golgi-derived transport vesicles.

Membrane Fusion Machinery in Cytokinesis

Membrane vesicles arriving at the plane of cell division initially fuse with one another and later with the tubulo-vesicular network derived from earlier fusion events (**Figure 2**). By analogy with other eukaryotic fusion events, cytokinetic membrane fusion should require Rab GTPases and their effectors for vesicle tethering to target membranes as well as soluble N-ethylmaleimide-sensitive factor adaptor protein receptor (SNARE) complexes mediating membrane fusion (30). SNARE complexes consist of membrane-anchored v-SNAREs on the vesicle membrane and t-SNAREs on the target membrane, which form 4-helical bundles by association of their coiled-coil domains. In cell plate formation, neither Rab GTPases nor Rab effectors have been identified, although electron tomographic evidence suggests the occurrence of vesicle linkers in the shape of exocyst complexes (74). Exocyst complexes tether vesicles to the plasma membrane in yeast and mammalian cells (30). In contrast to Rab GTPases, SNARE proteins and some of their interactors are among the best-studied molecular components of cytokinetic vesicle fusion. The syntaxin (Qa-SNARE) KNOLLE (also known as SYP111; for nomenclature see 73) was originally identified by cytokinesis-defective mutants that accumulate unfused vesicles in the plane of cell division (41, 48). KNOLLE protein is expressed only during M phase, localizing to Golgi stacks and the cell plate (41, 90). A KNOLLE-interacting t-SNARE (Qb + Qc-SNARE), the SNAP25 homolog SNAP33, localizes to the cell plate during cytokinesis and also to the plasma membrane in interphase or postmitotic cells (23). Inactivation of SNAP33 has only a minor effect on cytokinesis, possibly because of functional overlap with two other closely related KNOLLE-interacting SNAP25 homologs, SNAP29 and SNAP30, and causes lethality for other reasons

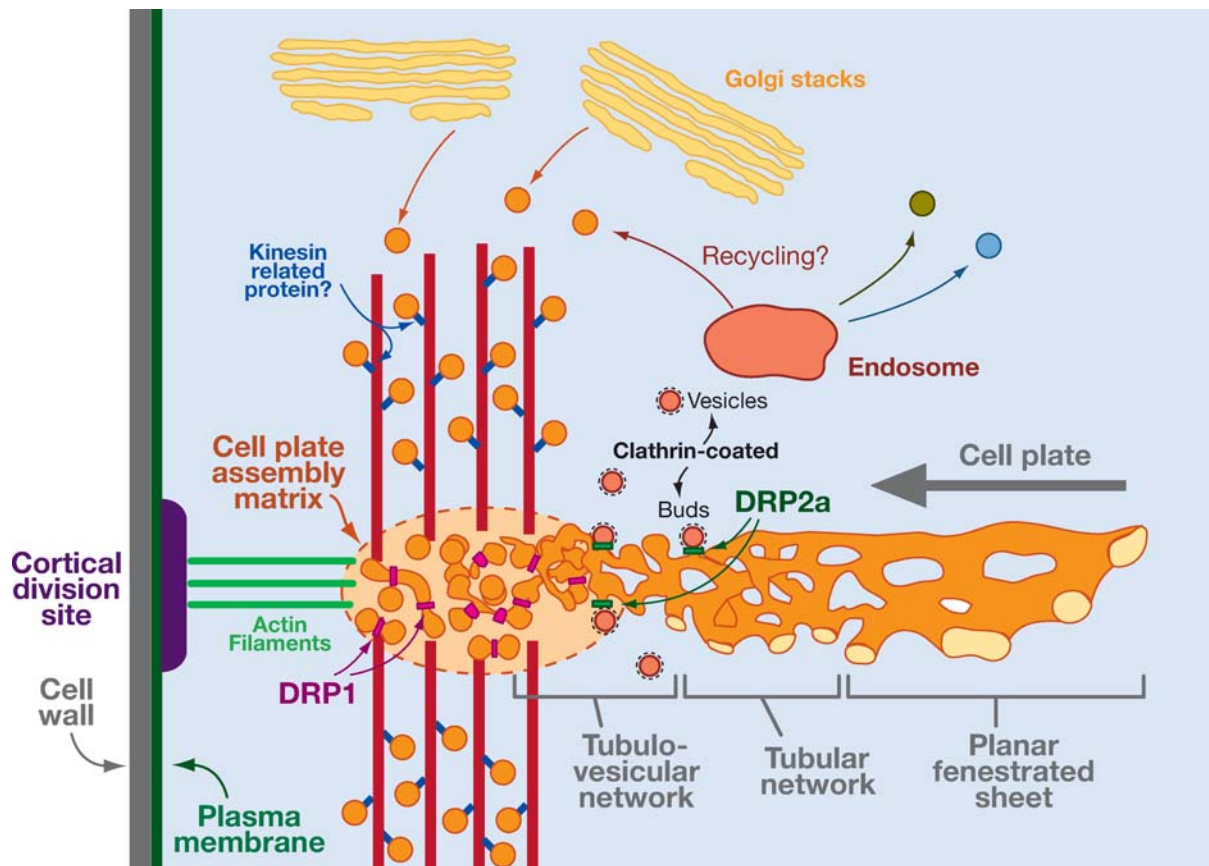


Figure 2

Membrane dynamics during cell plate development. Golgi-derived vesicles (*orange*) are delivered along phragmoplast microtubules (*red*), by a putative kinesin-related protein (*blue*), to the cell plate assembly matrix. Vesicle fusion generates fusion tubes and tubulo-vesicular networks as a result of the constricting activity of class I dynamin-related proteins (DRP1) (*magenta*). The tubulo-vesicular network is successively transformed into a tubular network and a planar fenestrated sheet. Lateral expansion of the cell plate (*large arrow*) toward the cortical division site is guided by actin filaments. Endocytosis from the tubulo-vesicular network and tubular network removes excess membrane, which is delivered to endosomes via clathrin-coated buds and vesicles. Dynamin-related protein 2a (DRP2a; *green*) is involved in the formation of clathrin-coated vesicles. The endosome sorts proteins for trafficking to various destinations (*blue, green, orange*), possibly including recycling to the margin of the cell plate.

(23). At the plasma membrane of yeast and animal cells, SNARE complexes form 4-helix bundles by interaction of the t-SNAREs syntaxin and SNAP25 with a specific v-SNARE, synaptobrevin/VAMP (R-SNARE) (30). By analogy, KNOLLE and SNAP33 might form a cytokinetic SNARE complex with an R-SNARE, which has not been identified. R-SNAREs encoded in the *Arabidopsis* genome do

not resemble R-SNAREs involved in plasma membrane trafficking in nonplant eukaryotes (73). However, an unusually large R-SNARE family is related to the mammalian VAMP7 involved in post-Golgi endomembrane trafficking (73). Recently, 5 of the 14 VAMP7 family members were localized to both the plasma membrane and endosomes in *Arabidopsis* suspension cells and thus, are potential candidates

for an R-SNARE involved in cytokinesis (86). Alternatively, the missing component of the cytokinetic SNARE complex might be a plant-specific SNARE such as NPSN11, which has been localized to the cell plate and interacts with KNOLLE (101). However, NPSN11 has the signature of a Qb-SNARE, which would imply the formation of an unusual Q-SNARE only complex in cytokinesis. It is also conceivable that KNOLLE and NPSN11 are part of another SNARE complex that still lacks both a Qc-SNARE and an R-SNARE. In contrast to KNOLLE, NPSN11 persists in the newly formed plasma membrane after the disassembly of the phragmoplast MTs (101). Inactivation of NPSN11 causes no obvious phenotype, which has been attributed to its presumed functional redundancy with two closely related homologs, NPSN12 and NPSN13 (101). Thus, the composition of the cytokinetic SNARE complex(es) remains unresolved.

Another syntaxin that accumulates at the plane of cell division is SYP31, which is most closely related to the cis-Golgi syntaxin Sed5/syntaxin5 of nonplant eukaryotes (67). SYP31 does not interact with KNOLLE and also accumulates in endomembranes such as Golgi during interphase (67). SYP31 interacts with the AAA-type ATPase CDC48, which has been localized to the division plane (17), whereas KNOLLE interacts with the AAA-type ATPase NSF (67). Whether these results suggest two separate pathways for cell plate formation or simply reflect dynamics of ER fusion and/or ER-Golgi trafficking near the cell plate remains to be determined (see 74 for ER dynamics in cell plate formation).

Sec1/Munc18 related (SM) proteins interact with syntaxins or assembled SNARE complexes, which is thought to increase the specificity of SNARE action (30). The SM protein KEULE was originally identified by mutants defective in cytokinetic vesicle fusion (4, 92) and interacts with KNOLLE both genetically and biochemically, whereas KNOLLE does not interact with AtSEC1a, a close homolog of KEULE (3, 92). However, KEULE is also expressed in nonproliferating tissue and

in contrast to *knolle* mutants, *keule* mutant seedlings fail to form long root hairs, suggesting another role of KEULE beyond cytokinesis (3, 78). Nonetheless, the very similar cytokinesis defects of *knolle* and *keule* mutants are consistent with the notion that KEULE may activate KNOLLE during cytokinetic vesicle fusion.

KNOLLE (SYP111) is not only a cytokinesis-specific SNARE protein but also a plant-specific member of the *Arabidopsis* SYP1 family of putative plasma-membrane syntaxins (73, 86). To determine what distinguishes KNOLLE from other syntaxins in regard to cytokinesis, several syntaxins were expressed under the cis-regulatory control of the *KNOLLE* gene and analyzed for both subcellular protein localization and their ability to functionally complement a *knolle* null mutant (53). The prevacuolar syntaxin PEP12 (SYP21) did not localize to the cell plate, whereas two members of the SYP1 family, SYP112 and SYP121 (SYR1/PEN1), accumulated at the plane of cell division. However, only SYP112 rescued the *knolle* mutant, although a *syp112* mutant had no obvious phenotype on its own nor did it enhance the *knolle* mutant phenotype. Thus, syntaxin specificity of cytokinesis is brought about by cell cycle-regulated gene expression, protein targeting, and protein activity at the cell plate. It is not known what features of KNOLLE syntaxin determine its activity during cytokinetic membrane fusion.

Delivery of Cargo to the Cell Plate

The cell plate may be viewed as an immature plasma membrane whose lumen is to be filled with cell wall material such as pectic polysaccharides and xyloglucans, which are synthesized in Golgi stacks and have to be delivered as vesicle cargo to the cell plate (100). Another major component—callose later to be replaced by cellulose—is synthesized by callose synthase at the cell plate (25). Thus, callose synthase and cell wall-modifying enzymes such as membrane-bound endo-1,4-beta-glucanase KORRIGAN (KOR) or secreted

endoxyloglucan transferase (EXGT) are putative cargo proteins of cytokinetic vesicles (100, 102).

In addition to cell plate-specific cargo, a number of proteins that accumulate at the plasma membrane of, or are secreted from, the interphase cell have been localized to the cell plate (reviewed in 33). For example, the plasma membrane-localized syntaxin PEN1 (SYP121/SYR1) accumulated at the cell plate when expressed during M phase (13, 53). Conversely, when ectopically expressed in post-mitotic cells, KNOLLE was also targeted to the plasma membrane (90). These observations suggest that the cell plate and the plasma membrane are interchangeable target membranes in exocytic vesicle trafficking. Exocytosis is the default pathway of membrane trafficking, which is taken in the absence of sorting signals, as supported by the following lines of evidence. GFP with a signal peptide for uptake into the ER (sp-GFP) is secreted from the cell during interphase (8) and accumulates in the cell plate of dividing tobacco BY-2 cells (100). Furthermore, the peptide ligand CLV3 fused to GFP is secreted from the cell, whereas an engineered variant carrying a C-terminal vacuolar sorting sequence accumulates in the vacuole (71). Similarly, the prevacuolar syntaxin PEP12 does not accumulate in the cell plate when expressed during M phase (53). These observations suggest that proteins trafficking to the plasma membrane or the cell plate lack sorting signals that would target them to endomembrane compartments. The proper localization of the membrane-bound endo-1,4-beta-glucanase KORRIGAN to the cell plate is proposed to involve two putative sorting motifs, a tyrosine-based motif and an acidic dileucine motif (102). However, both motifs are absent from PEN1 (SYP121/SYR1) syntaxin, which also accumulates at the cell plate when expressed during M phase (53). Thus, the significance of these sorting motifs in cytokinetic vesicle trafficking remains to be determined. In conclusion, the available evidence indicates that trafficking to the cell plate is an M phase-specific default pathway of proteins that lack sorting signals.

Membrane Dynamics During Cell Plate Expansion

The developing cell plate undergoes extensive reorganization from the initial fusion of vesicles via the formation of a lattice of membrane tubules, which is accompanied by the removal of excess membrane material to yield a planar membrane compartment (**Figure 2**). While this process is underway in the center of the division plane, newly arriving vesicles are targeted to the lateral margin of the cell plate, resulting in its expansion outward to the parental plasma membrane.

The formation of a lattice of tubules rather than a balloon-shaped membrane compartment has been attributed to the activity of dynamin-related proteins (DRPs) [formerly called phragmoplastins or *Arabidopsis* dynamin-like proteins (ADLs)]. Dynamin GTPases are eukaryotic mechano-enzymes that tubulate membranes and also mediate scission of clathrin-coated vesicles (66). Members of subgroup 1 of the *Arabidopsis* DRP family are associated with the developing cell plate, and the *drp1a drp1e* double mutant is embryo-lethal, displaying defects in cytokinesis (27, 28, 34–36). The tubular membranes are locally constricted by DRP1a (ADL1A), which also interacts with callose synthase and may facilitate callose deposition into the lumen of the incipient cell plate (25, 26, 63).

Removing membrane material from the maturing cell plate is estimated to reduce both surface area and volume by approximately 70% in the case of endosperm cellularization (63). Clathrin-coated buds on the cell plate and nearby clathrin-coated vesicles suggest that membrane material is removed by an endocytosis-related process (**Figure 2**) (63, 72, 74). Consistent with this notion, DRP2a (ADL6), which is related to animal classical dynamin involved in membrane scission, localizes to the cell plate, in addition to its association with the plasma membrane and Golgi stacks in interphase cells (28). Because DRP2a is required for trafficking of clathrin-coated vesicles from the trans-Golgi network to the lytic

Endocytosis:

internalization of membrane material from the plasma membrane, often via clathrin-coated vesicles

vacuole (31, 40), it may play a comparable role in the removal of excess membrane from the maturing cell plate.

Endocytosis during cell plate expansion may merely shape the cell plate. Alternatively, membrane removed from the center may be recycled, via endosomes, to the growing margin of the cell plate, which would speed up cytokinesis. At present, there is only limited evidence that recycling may occur. BFA treatment, which blocks cell plate expansion in BY-2 cells presumably by disrupting Golgi stacks (98, see above), appears to have a different effect in *Arabidopsis* root cells. BFA causes the formation of endosomal "BFA compartments," which accumulate the plasma-membrane protein PIN1, the endosomal ARF-GEF GNOM but not Golgi or trans-Golgi markers (18, 19). During cytokinesis, BFA compartments accumulate the cytokinesis-specific syntaxin KNOLLE, in addition to PIN1. In BFA-resistant GNOM transgenic seedlings, BFA compartments still accumulate KNOLLE but neither PIN1 nor GNOM, suggesting that KNOLLE trafficking from endosomes involves a different BFA-sensitive ARF-GEF (19). Nonetheless, recycling KNOLLE to the growing margin of the cell plate has not been conclusively demonstrated, in contrast to recycling PIN1 to the plasma membrane. In maize root cells, an ARF1-type GTPase has been localized to the cell plate, Golgi stacks, and the plasma membrane (14). Upon BFA treatment, ARF1 also colocalized with a Golgi marker in aggregates at the growing margin of the cell plate. Similar subcellular distributions were observed for several COPI coat proteins in both BFA-treated and -untreated cells (14). These results are difficult to interpret but may suggest the possibility of retrograde transport from Golgi stacks that accumulate near the leading edge of the expanding cell plate (57). The seemingly conflicting data on BFA effects in different plant species caution against a simplified view of membrane recycling from the expanding cell plate. Although local recycling of cell plate membrane via endosomes would provide an efficient mechanism for rapid

completion of cytokinesis, additional studies are required to determine whether this is true.

Fusion with the Plasma Membrane

To seal off the two daughter cells, the cell plate has to fuse with the parental plasma membrane. This fusion event triggers further maturation of the cell plate, including breakdown of callose and its replacement by cellulose (72). According to the classic model, the cell plate expands symmetrically from the center to the periphery before fusing with the parental plasma membrane (81). However, asymmetric expansion of the cell plate ("polar cytokinesis") has been described in vacuolate shoot cells of *Arabidopsis* (16). The cell plate appears to contact, and possibly fuse with, the plasma membrane on one side early during cytokinesis and then expand along the plasma membrane to the opposite side of the cell. In BY-2 cells, polar cytokinesis has been detected with the lipophilic dye FM4-64, which stained the developing cell plate together with the continuous plasma membrane, whereas symmetrically expanding cell plates were only stained after 30–60 min of incubation (10). The asymmetric expansion of the cell plate would easily account for the cell wall stubs observed upon genetic or experimental interference with cytokinesis (16). However, cell wall stubs also occur in cytokinesis-defective mutants that accumulate unfused vesicles across the entire plane of cell division (41, 92). This suggests that in the absence of cell plate formation, cell wall stubs may originate by local membrane fusion initiated from the cortical division site of the parental plasma membrane. A comparable process occurs normally in *Arabidopsis* male meiotic cytokinesis during which no cell plate is formed: Networks of tubular membranes formed by fusion of Golgi-derived vesicles are lined up across the division plane and are successively fused with the plasma membrane from the periphery to the center (64). It is thus conceivable that in somatic cells, the fusion of the cell plate margin with the plasma membrane is affected by different machinery than are the vesicle

fusion events that generate and expand the cell plate.

CYTOSKELETON DYNAMICS

Cytokinesis of somatic cells is assisted by two plant-specific cytoskeletal arrays, the preprophase band and the phragmoplast (**Figure 1**). The preprophase band forms transiently from late G2 phase to prometaphase and somehow marks the cortical division site at which the expanding cell plate fuses with the parental plasma membrane during cytokinesis. The phragmoplast originates in the interzone between the two sets of daughter chromosomes during late anaphase and undergoes lateral translocation during the expansion of the cell plate. These two cytoskeletal arrays consist of both MTs and AFs. Membrane dynamics during cytokinesis depends on MTs and AFs of the phragmoplast.

Roles of Microtubules and Actin Filaments in Phragmoplast-Assisted Cytokinesis

The phragmoplast contains MTs and AFs, which are aligned parallel to each other. Although both MTs and AFs are organized in two bundles, with their plus ends facing the plane of division, only the MTs interdigitate (81). During lateral expansion of the cell plate, MTs are translocated to its margin, whereas AFs remain present throughout the forming cell plate. A novel 190-kDa protein from tobacco BY-2 cells bundles both MTs and AFs *in vitro* and has been localized to the phragmoplast, suggesting a possible role in cross-linking MTs with AFs (**Figure 3A**) (29).

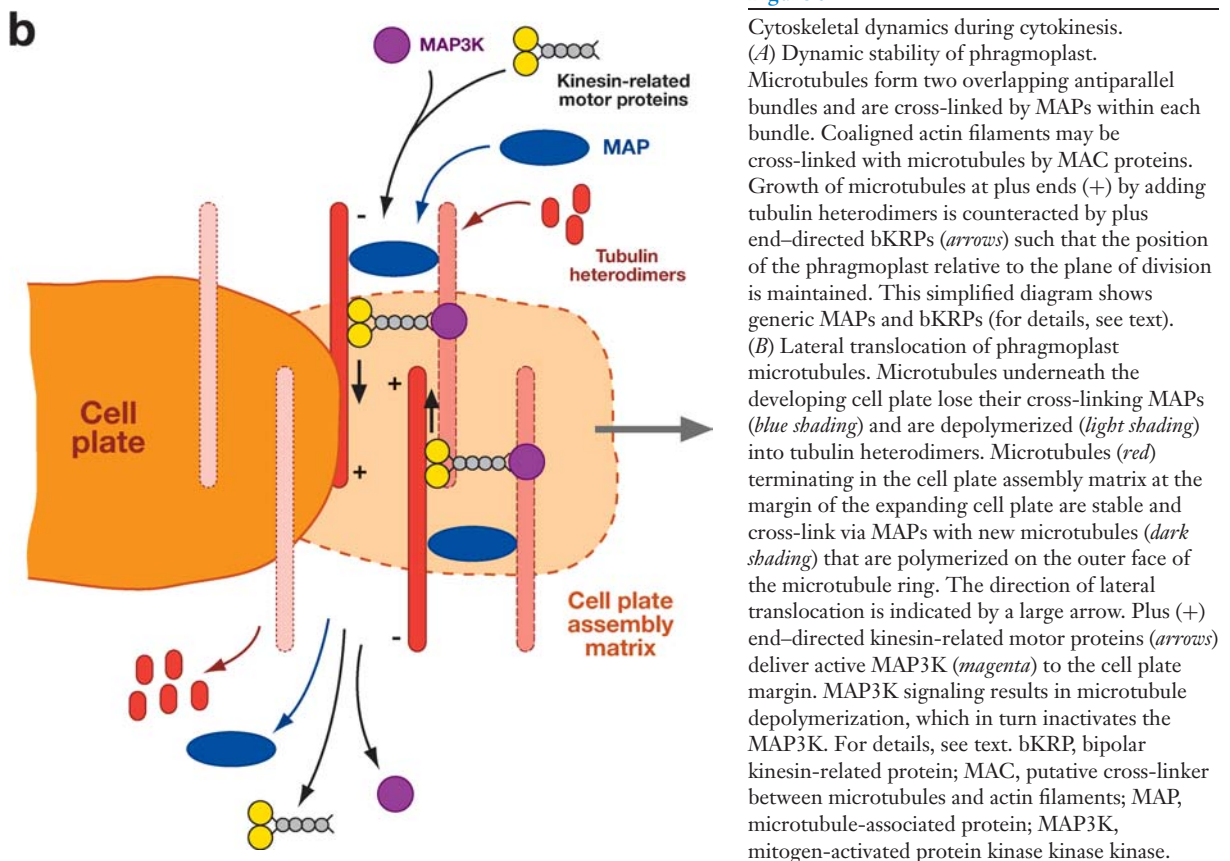
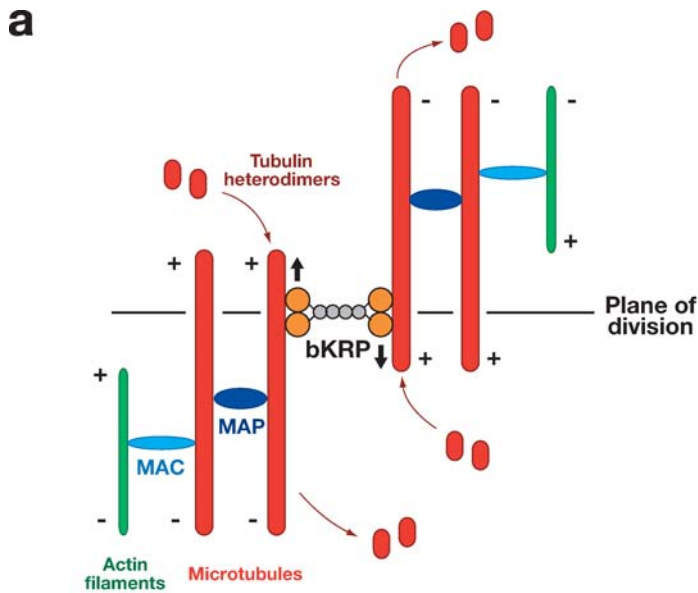
The phragmoplast plays two major roles: targeted delivery of Golgi-derived membrane vesicles to the plane of cell division and lateral expansion of the cell plate toward the cortical division site. Which of these roles can be attributed to MTs and/or AFs? In interphase cells, AFs but not MTs are involved in membrane trafficking to the plasma membrane, as evidenced by both drug studies and mu-

tant analysis (18, 82). Conversely, MTs but not AFs appear to mediate trafficking to the division plane because oryzaline inhibits delivery of KNOLLE (18). In addition, KNOLLE accumulation is dispersed rather than localized in mitotic cells of MT-deficient mutants (50, 82). Furthermore, noncoated vesicles appear to be linked, by structures resembling kinesins, to mini-phragmoplast MTs during endosperm cellularization (63). Thus, the available evidence favors a role for MTs in the targeted delivery of Golgi-derived membrane vesicles to the plane of cell division. However, no vesicle-carrying kinesin motor protein has been identified unambiguously. A possible candidate is PAKRP2, which colocalizes with KNOLLE at the cell plate and also shows a broader punctate distribution in the phragmoplast (43). However, there is no conclusive evidence that this protein is involved in transporting Golgi-derived vesicles along phragmoplast MTs.

Expanding the cell plate toward the cortical division site is a complex process involving both MTs and AFs (24). The lateral translocation of phragmoplast MTs is required for the targeted delivery of the vesicles to the growing margin of the cell plate (see below). The role of AFs has been probed by drug treatment and by injection of profilin into dividing stamen hair cells of *Tradescantia* (52, 87). Depending on the treatment, cell plate growth is impaired or the cell plate disintegrates, suggesting that AFs play a stabilizing role. In addition, AFs link the phragmoplast with the cortical division site during cell plate expansion and may thus direct the growing margin to the division site (24). So far, a role for AFs has not been supported by genetic evidence. A dominant-negative mutation in the *ACT2* gene, which disturbs actin polymerization, affects root hair initiation and root cell elongation but does not alter cell division (60). Also, an *act2 act7* double mutant is severely stunted but develops to the adult stage (20). These results are consistent with the stunted growth phenotype of seedlings germinated on the actin-depolymerizing drug latrunculin B (6). In summary, whatever role AFs play

AF: actin filament

Kinesin: a motor protein that travels along microtubules mostly to the plus end, sometimes to the minus end. Kinesins often carry cargo such as vesicles or microtubules but may perform other functions.



in cytokinesis, their contribution is less pronounced than that of the phragmoplast MT.

Formation and Dynamic Stability of the Phragmoplast Microtubules

The dynamics of phragmoplast MTs has been visualized with GFP-MAP4 or GFP-TUA6 fusion proteins in live BY-2 cells (21, 85). These MTs originate alongside remaining kinetochore MTs in the midzone during late anaphase, and they become more numerous and consolidate into two short bundles with their plus ends overlapping (Figure 1) (81). How the transition from anaphase MTs to phragmoplast MTs occurs is not known. Mitotic cyclin B1 must be degraded during metaphase-anaphase transition for cytokinesis to occur.

Figure 3

Cytoskeletal dynamics during cytokinesis. (A) Dynamic stability of phragmoplast. Microtubules form two overlapping antiparallel bundles and are cross-linked by MAPs within each bundle. Coaligned actin filaments may be cross-linked with microtubules by MAC proteins. Growth of microtubules at plus ends (+) by adding tubulin heterodimers is counteracted by plus end-directed bKRPs (arrows) such that the position of the phragmoplast relative to the plane of division is maintained. This simplified diagram shows generic MAPs and bKRPs (for details, see text). (B) Lateral translocation of phragmoplast microtubules. Microtubules underneath the developing cell plate lose their cross-linking MAPs (blue shading) and are depolymerized (light shading) into tubulin heterodimers. Microtubules (red) terminating in the cell plate assembly matrix at the margin of the expanding cell plate are stable and cross-link via MAPs with new microtubules (dark shading) that are polymerized on the outer face of the microtubule ring. The direction of lateral translocation is indicated by a large arrow. Plus (+) end-directed kinesin-related motor proteins (arrows) deliver active MAP3K (magenta) to the cell plate margin. MAP3K signaling results in microtubule depolymerization, which in turn inactivates the MAP3K. For details, see text. bKRP, bipolar kinesin-related protein; MAC, putative cross-linker between microtubules and actin filaments; MAP, microtubule-associated protein; MAP3K, mitogen-activated protein kinase kinase kinase.

Nondegradable cyclin B1 interferes with anaphase spindle disassembly and phragmoplast formation, although KNOLLE still accumulates at the midzone (93). Once phragmoplast MTs have formed their organization is dynamically maintained by MT-associated proteins (MAPs) and kinesin-related motor proteins (Figure 3A) (46). For example, MAP65-1 cross-bridges MTs and is normally associated with interzone anaphase MTs and plus ends of phragmoplast MTs (75, 76). However, if cyclin B1 is not degraded, MAP65-1 does not accumulate at the midline, which may account for the failure to form a stable phragmoplast (93).

MAPs have MT-bundling activity in vitro and colocalize with MT arrays in vivo (46). MAP65, the most abundant group of MAPs, is encoded by a family of nine genes in *Arabidopsis* (75, 76). MAP65-1 associates with all MT arrays during the cell cycle, and its dimerization is necessary to form MT cross-bridges (76). Another member of the MAP65 family, PLEIADE (PLE/MAP65-3), specifically localizes to interzone spindles in anaphase and to the overlapping plus ends of phragmoplast MTs but not to other MT arrays (55). In addition, *ple* mutants display cytokinesis defects, presumably due to compromised organization of phragmoplast MTs (54, 55). In the absence of PLE activity, the width of the phragmoplast increases, suggesting that PLE is essential for the integrity of the overlapped MTs. Another MAP with an essential role in cytokinesis is GEM1/MOR1, evidenced by the aberrant division of the microspore in *gem1* mutants (84). However, GEM1/MOR1 also stabilizes interphase cortical MT arrays, as indicated by the mutant phenotype of the temperature-sensitive allele *mor1* (94). GEM1/MOR1 localizes to all MT arrays and accumulates toward the plus ends of phragmoplast MTs (84). Its tobacco homolog has been isolated from telophase cells and cross-bridges MTs (99). Thus, GEM1/MOR1 may stabilize the growing plus ends of phragmoplast MTs (Figure 3A). In summary, several MAPs stabilize phragmoplast MTs but it is not known at present to what extent they are functionally distinct.

The *Arabidopsis* genome encodes some 60 kinesin-related motor proteins (KRPs) (68). Several KRPs localize to the phragmoplast and are implicated in the organization of phragmoplast MTs (Figure 3A) (reviewed in 44, 46). Bipolar KRPs such as TKRP125 are plus end-directed motors that slide overlapping MTs against each other and may thus compensate for MT growth at their overlapping plus ends (1). Whereas TKRP25 localizes along the MTs, related DcKRP120-2 mainly accumulates in the zone of overlap (7). PAKRP1 and its close homolog PAKRP1L represent a different group of phragmoplast-associated KRPs (42, 65). These proteins specifically localize to the interzonal MTs at anaphase and to the overlapping plus ends throughout phragmoplast development. Both may form dimers and thus maintain the organization of overlapping antiparallel bundles. However, their precise roles need to be clarified. A third group of KRPs, represented by ATK1/KatA, has C-terminal motor domains and may counteract the action of bipolar KRPs. ATK1/KatA is a minus end-directed KRP that specifically localizes to the midzone of the mitotic apparatus and the phragmoplast (45, 49). Disrupting the *ATK1* gene alters the spindle organization of male meiotic cells but does not affect mitotic spindle or phragmoplast. This finding has been attributed to functional overlap between members of the *ATK* family (12). Another minus-end-directed motor protein is the kinesin calmodulin binding protein (KCBP). Its C-terminal motor domain and an adjacent Ca calmodulin-binding domain bundle MTs in vitro in a calcium-dependent manner (37). Structural analysis of KCBP also predicts that Ca-calmodulin binding blocks motor motility and may initiate dissociation of KCBP from MTs (89). Injecting anti-KCBP antibody into anaphase cells activates KCBP and disrupts the phragmoplast, which suggests that KCBP is normally downregulated (91). This regulation may involve the calmodulin-binding domain of KCBP because limiting the rise of calcium concentration that normally occurs during cytokinesis has a similar disruptive effect (24).

MAP: a microtubule-associated protein. Some of these form cross-bridges between microtubules and thereby stabilize microtubule arrays.

In summary, both structural MAPs and KRPs play important roles in organizing the phragmoplast MTs at the plane of cell division. Their activity results in dynamic stability of the MT array perpendicular to the division plane during both the formation and the lateral translocation of the phragmoplast.

Lateral Translocation and Disassembly of the Phragmoplast Microtubules

As the cell plate develops in the center of the division plane, the associated phragmoplast MTs start to depolymerize, except those at the margin of the MT bundles. New MTs polymerize adjacent to the remaining ones, delivering Golgi-derived vesicles to the margin of the developing cell plate. Inner MTs then depolymerize and new MTs are added to the outer face of the ring-shaped array (**Figure 3B**). This cycle of phragmoplast translocation and cell plate expansion is repeated until the cell plate reaches the cortical division site marked earlier by the preprophase band (21, 81). In polar cytokinesis, the lateral translocation of the phragmoplast MTs occurs asymmetrically as does the expansion of the cell plate (85). What is the driving force behind the lateral progression of cytokinesis?

Lateral translocation of the phragmoplast is inhibited by the MT-stabilizing drug taxol, suggesting that polymerization of MTs at the margin requires depolymerization of MTs in the center (96). Taxol also inhibits cell plate expansion, presumably because no vesicles are delivered to the CPAM near its margin (**Figure 3B**). More recently, two orthologous plant-specific kinesin-related proteins, HINKEL (HIK) in *Arabidopsis* and NACK1 in BY-2 cells, were shown to play a role in phragmoplast dynamics during cell plate expansion (59, 83). The *HIK* gene is expressed in a cell cycle-regulated manner, and *bik* mutant embryos display characteristic cytokinesis defects. Phragmoplast MTs are stabilized beneath the expanding cell plate, as reported for taxol-treated cells, and the lateral translocation appears to continue (83). NACK1 colocalizes with the plus ends of phragmoplast

MTs during their lateral translocation (59). A dominant-negative form of NACK1 stabilizes the MTs beneath the cell plate but blocks lateral translocation of phragmoplast MTs and expansion of the cell plate, thus resembling the effect of taxol treatment (59). Regardless of the differences between the two systems, both studies indicate a role for HIK/NACK1 in the depolymerization of MTs beneath the cell plate. Further analysis shows that NACK1 activates and targets the MAP kinase kinase (MAP3K) NPK1 to the plus ends of phragmoplast MTs, which is required for cytokinesis (59). Overexpression of a kinase-negative variant of NPK1 causes essentially the same defects as the dominant-negative form of NACK1 (58). It is interesting to note that both NPK1 and its target MAP2K NQK1 are inactivated by MT depolymerization (80). It is thus conceivable that NPK1 signaling is controlled by a negative feedback loop, leading to MT depolymerization, which in turn results in NPK1 inactivation. NPK1-mediated MT depolymerization does not occur in the CPAM but only underneath the cell plate and may thus be linked to membrane fusion processes in the developing cell plate. This would ensure that membrane vesicles are only delivered to the growing margin of the cell plate and that the phragmoplast is disassembled when the fully expanded cell plate fuses with the plasma membrane.

The closest homolog of HIK/NACK1 is TETRASPORE (TES)/NACK2, which is required for male meiotic cytokinesis in *Arabidopsis* (95). In *tes* mutants, the radial MT arrays that mediate the formation of the partitioning membrane are disorganized, suggesting that the two homologous kinesin-related proteins perform comparable roles in two different types of cytokinesis. There is also indirect evidence that NPK1 signaling plays a role in male meiotic cytokinesis as well (39, 80). How TES/NACK2 and NPK1 signaling might affect disassembly of the radial MT arrays remains to be determined. Note that these arrays form simultaneously across the plane of division and the membrane fusion process starts at the plasma membrane and progresses to the center (64).

In summary, the directional progression of cytokinesis can in part be explained by the interaction of the NACK1-NPK1 signaling pathway with the fusion processes occurring in the adjacent cell plate, which accounts for the depolymerization of phragmoplast MTs as well as for the disassembly of the phragmoplast at the end of cytokinesis. However, it is not known at present how new MTs polymerize on the outer face of the ring-shaped MT array and thus deliver membrane vesicles required for the further expansion of the cell plate.

CONCLUDING REMARKS

Higher-plant cytokinesis is a highly orchestrated, cytoskeleton-assisted process of membrane targeting and fusion. The recent studies of diverse modes of cytokinesis, once considered mechanistically different, have revealed a remarkable similarity of the initial phase: in all three systems studied, transport vesicles accumulate in the division plane and fuse with

one another to give vesiculo-tubular networks. These results suggest common mechanisms in diverse modes of cytokinesis. However, our knowledge of the cytokinetic trafficking pathway and the fusion machinery is still fragmentary. Similarly, endocytosis from the expanding cell plate needs further study. We particularly need to determine whether there is a recycling pathway from endosomes to the growing margin of the cell plate, which would speed up the expansion of the cell plate. Regarding cytoskeleton dynamics in cytokinesis, the most important finding is the discovery of a kinesin-MAPkinase pathway that, in conjunction with fusion processes in the cell plate, mediates microtubule depolymerization. By contrast, it is not known how new microtubules polymerize at the outer face of the array, which delivers vesicles for cell plate expansion. Finally, the relative roles of microtubules and AFs have not been defined precisely. Addressing these and other cytokinesis-related problems will remain a challenge for the future.

SUMMARY POINTS

1. Higher plant cytokinesis involves a coordinated interplay of membrane and cytoskeleton dynamics.
2. Diverse modes of cytokinesis share common features during the early phase: targeted delivery of membrane vesicles, which fuse with one another to form vesiculo-tubular networks.
3. Membrane fusion during cell plate formation is mediated by a SNARE complex that contains a cytokinesis-specific syntaxin.
4. Vesiculo-tubular networks are locally constricted by dynamin-related proteins, which results in a lattice of membrane tubules.
5. Further flattening of the maturing cell plate is achieved by endocytosis of excess membrane material.
6. Cell plate expansion requires depolymerization of phragmoplast microtubules, which is mediated by a kinesin-MAPkinase pathway in conjunction with membrane fusion processes in the cell plate.

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This study demonstrates that syntaxin function in cytokinesis requires strong expression during mitosis, targeting to the plane of division and KNOLLE-like protein function.

This study provides evidence that a MAP kinase signaling pathway mediates depolymerization of phragmoplast microtubules during cell plate expansion.

This study demonstrates that the plus end-directed kinesin-related protein NACK1 activates the NPK1 kinase and that NACK1-mediated transport of NPK1 is required for microtubule depolymerization and cell plate expansion.

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This study demonstrates that cellularization of the endosperm is essentially a variant of phragmoplast-assisted cytokinesis of somatic cells.

This study demonstrates that in male meiotic cytokinesis, targeted delivery of vesicles and formation of membrane-tubule networks across the plane of division provide the material from which the partitioning membrane is made.

This study provides an in-depth analysis of cell plate formation in somatic-cell cytokinesis.

This study not only identifies a target for NPK1 signaling in cytokinesis but also demonstrates that the members of the MAPK cascade are inactivated by microtubule depolymerization.

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Contents

Fifty Good Years <i>Peter Starlinger</i>	1
Phytoremediation <i>Elizabeth Pilon-Smits</i>	15
Calcium Oxalate in Plants: Formation and Function <i>Vincent R. Franceschi and Paul A. Nakata</i>	41
Starch Degradation <i>Alison M. Smith, Samuel C. Zeeman, and Steven M. Smith</i>	73
CO ₂ Concentrating Mechanisms in Algae: Mechanisms, Environmental Modulation, and Evolution <i>Mario Giordano, John Beardall, and John A. Raven</i>	99
Solute Transporters of the Plastid Envelope Membrane <i>Andreas P.M. Weber, Rainer Schwacke, and Ulf-Ingo Flügge</i>	133
Abscisic Acid Biosynthesis and Catabolism <i>Eiji Nambara and Annie Marion-Poll</i>	165
Redox Regulation: A Broadening Horizon <i>Bob B. Buchanan and Yves Balmer</i>	187
Endocytotic Cycling of PM Proteins <i>Angus S. Murphy, Anindita Bandyopadhyay, Susanne E. Holstein, and Wendy A. Peer</i>	221
Molecular Physiology of Legume Seed Development <i>Hans Weber, Ljudmilla Borisjuk, and Ulrich Wobus</i>	253
Cytokinesis in Higher Plants <i>Gerd Jürgens</i>	281
Evolution of Flavors and Scents <i>David R. Gang</i>	301

Biology of Chromatin Dynamics <i>Tzung-Fu Hsieh and Robert L. Fischer</i>	327
Shoot Branching <i>Paula McSteen and Ottoline Leyser</i>	353
Protein Splicing Elements and Plants: From Transgene Containment to Protein Purification <i>Thomas C. Evans, Jr., Ming-Qun Xu, and Sribarsa Pradhan</i>	375
Molecular Genetic Analyses of Microsporogenesis and Microgametogenesis in Flowering Plants <i>Hong Ma</i>	393
Plant-Specific Calmodulin-Binding Proteins <i>Nicolas Bouché, Ayelet Yellin, Wayne A. Snedden, and Hillel Fromm</i>	435
Self-Incompatibility in Plants <i>Seiji Takayama and Akira Isogai</i>	467
Remembering Winter: Toward a Molecular Understanding of Vernalization <i>Sibum Sung and Richard M. Amasino</i>	491
New Insights to the Function of Phytopathogenic Bacterial Type III Effectors in Plants <i>Mary Beth Mudgett</i>	509

INDEXES

Subject Index	533
Cumulative Index of Contributing Authors, Volumes 46–56	557
Cumulative Index of Chapter Titles, Volumes 46–56	562

ERRATA

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