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Review

Membrane Trafficking During Plant Cytokinesis

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Plant morphogenesis is regulated by cell division and expansion. Cytokinesis, the final stage of cell division, culminates in the construction of the cell plate, a unique cytokinetic membranous organelle that is assembled across the inside of the dividing cell. Both during cell-plate formation and cell expansion, the secretory pathway is highly active and is polarized toward the plane of division or toward the plasma membrane, respectively. In this review, we discuss results from recent genetic and biochemical research directed toward understanding the molecular events occurring during cytokinesis and cell expansion, including data supporting the idea that during cytokinesis one or more exocytic pathways are polarized toward the division plane. We will also highlight recent evidence for the roles of secretory vesicle transport and cytoskeletal machinery in cell-plate membrane trafficking and fusion.

Key words: cell division, cell plate, cytoskeleton, dynamin, exocytosis, Rop, SNARE

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Cytokinesis, the grand finale of a cell division, involves the partitioning of cytosol and organelles and the completion of a membranous barrier between daughter cells. Formation of this membranous barrier in higher plant cells involves the *de novo* assembly of the unique cytokinetic organelle, the cell plate, a process that involves polarized trafficking of massive amounts of membrane and cell-wall material into the division plane. The extent of this flow of membrane is dramatically illustrated in dividing cambial initials during wood development where the cell plate is several millimeters long and requires approximately a day to complete (see Figure 1). Cell-plate assembly is orchestrated by the phragmoplast, a specialized cytoskeletal structure, composed of microtubules (MTs) and microfilaments (MFs) (1). During cytokinesis, secretory vesicles carrying membrane and cell-wall components are guided along the phragmoplast toward its center, where they fuse to form a membranous tubular-vesicular network (TVN) (2), within which cell-wall biosynthesis is initiated. As additional vesicles are added, the TVN gradually develops into a smoother, more plate-like structure that expands outward toward the margin of the cell. Ultimately the cell plate fuses with the parental plasma membrane at a site coincident with the previous location of the preprophase band, a transient ring of cortical MTs that forms briefly during the G2 phase of the cell cycle. Alignment of the expanding cell plate with the fusion site is regulated by an actomyosin system (3–5) which appears to push the cell plate outward. Conversely, in animal cells, a contractile actomyosin ring brings the plasma membrane inward. Recent studies in animal cells and fungi (6,7) now demonstrate the importance of targeted secretion of membranes for furrowing and resolution of the constricted plasma membrane [for review, see (8–10]. Therefore, although the mechanism for cytokinesis in plants and fungi and animals has long been considered wholly distinct, these systems in fact bear significant similarities.

In this review we will focus primarily on recent studies of membrane trafficking during plant cytokinesis, making comparisons with other systems where possible, as well as highlighting significant remaining questions about the molecular mechanisms involved in cell-plate membrane trafficking and fusion. For additional discussion of the various topics touched upon here, the reader is referred to several other recent reviews (11–15).

Organization of the Secretory Machinery During Cytokinesis

Differences exist in the organization of the secretory pathway between plant and animal cells. Most notably, plant cells contain multiple independent Golgi stacks (i.e. often hundreds) that do not vesiculate during mitosis as is the case in animal cells. This is important because the process of secretion in plants remains active throughout the cell cycle. Plant Golgi stacks are highly mobile, moving actively throughout the cytoplasm along actin filaments via an actomyosin system (16–18). During mitosis these stacks accumulate in a subcortical ring, the so-called 'Golgi Belt', surrounding the future site of cell-plate formation (17). This localization pattern does not depend upon cytoskeletal interactions, but is thought to be required for partitioning of the Golgi into the daughter cells as well as for delivery of secretory vesicles to the cell plate. During mitosis and cytokinesis, tubular components of the endoplasmic reticulum (ER) are also recruited to the division plane, as shown by electron microscopy (19,20) and three-dimensional confocal imaging (21) to form a reticular network surrounding the cell plate. Analogous to the cortical ER, which is ad-

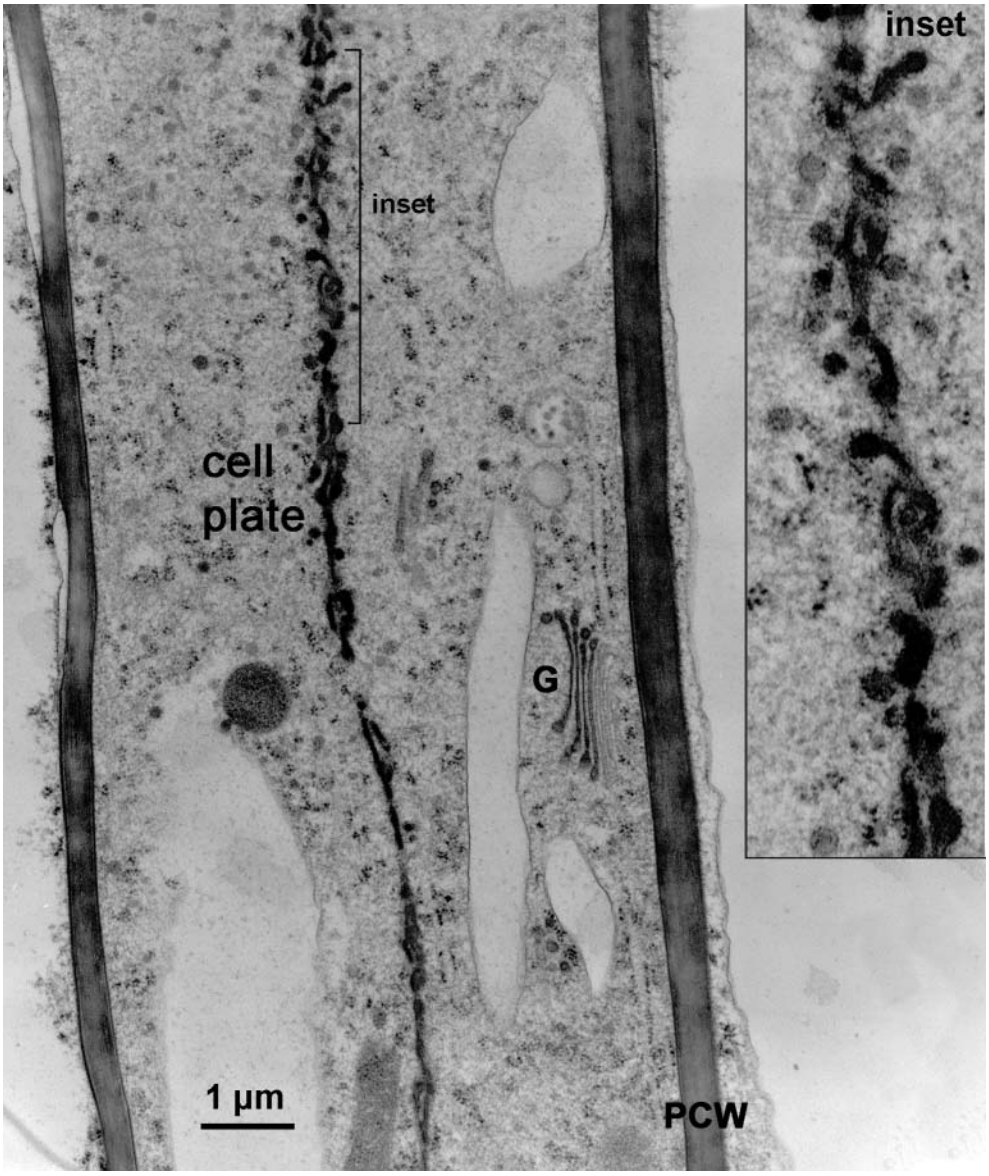

Figure 1: Ultrastructure of a dividing vascular cambial cell in developing wood tissue of Pine. The cell plates of these dividing cambial initials can be several millimeters long. An immature region of this cell plate is shown at the top part of the figure and in the inset. Secretory vesicles are seen starting to coalesce into a tubulo-vesicular network. The lower part of the figure shows an older region of the cell plate where the membrane has flattened into a more plate-like structure that will further mature and thicken as cellulose biosynthesis begins to form the new cell wall. PCW: parental cell wall. G: Golgi stack. Tissue samples were preserved by high-pressure freezing and freeze substitution (85). Image courtesy of Drs Lacey Samuels and Kim Rensing (University of British Columbia).

jacent to the plasma membrane, the cell-plate-associated ER may mediate direct lipid transfer to the cell plate and/or provide the appropriate ionic environment (e.g. supplying Ca^{2+} ions) necessary for cell-plate membrane fusion and cytoskeletal organization. Tubular elements of the cell-plate-associated ER network may also fuse and become entrapped, forming the desmotubules that traverse plasmodesmata. How these membranes are recruited to the division plane and assembled remains to be determined.

Multiple Secretory Routes May Lead to the Parental Plasma Membrane and Cell Plate

Plant cell expansion is mediated through the delivery and fusion of Golgi-derived exocytic vesicles carrying membranes and cell-wall components to specific sites on the plasma membrane. Likewise, the vesicles that fuse at the site of cell-plate formation are thought to bud from the Golgi apparatus. However, very little is known about the molecular machinery involved in their formation and targeting in plant cells. For

example, it remains to be determined whether cell-plate formation is mediated by a single homogeneous vesicle population or if multiple Golgi-derived vesicle types, carrying distinct sets of cargo, converge and fuse to form a new plasma membrane and cell wall *de novo*. Recent studies have demonstrated the existence of multiple exocytic pathways that transport distinct sets of cargo to the cell surface in yeast and mammalian cells (22–24). Similarly the presence of multiple exocytic pathways in plant cells (Figure 2A) has been suggested, based upon the polarized distribution of Golgi-synthesized polysaccharides in the cell walls of root tip and suspension-cultured cells (25,26). The recent localization and characterization of the polarized distribution of several cell-wall-associated and plasma membrane proteins, including COBRA (27) and the putative auxin transporters PIN1, PIN3 and AUX1 (28–30), lends further support to this model [for discussion, see the article by Jürgens and Geldner in this issue (31)].

During cell-plate formation, one or more of these pathways may become polarized toward the plane of division, as they appear to do during cell division in *Saccharomyces cerevisiae* (32,33). Alternatively, it has been suggested, based upon the localization of the cell-plate SNARE, KNOLLE, that a

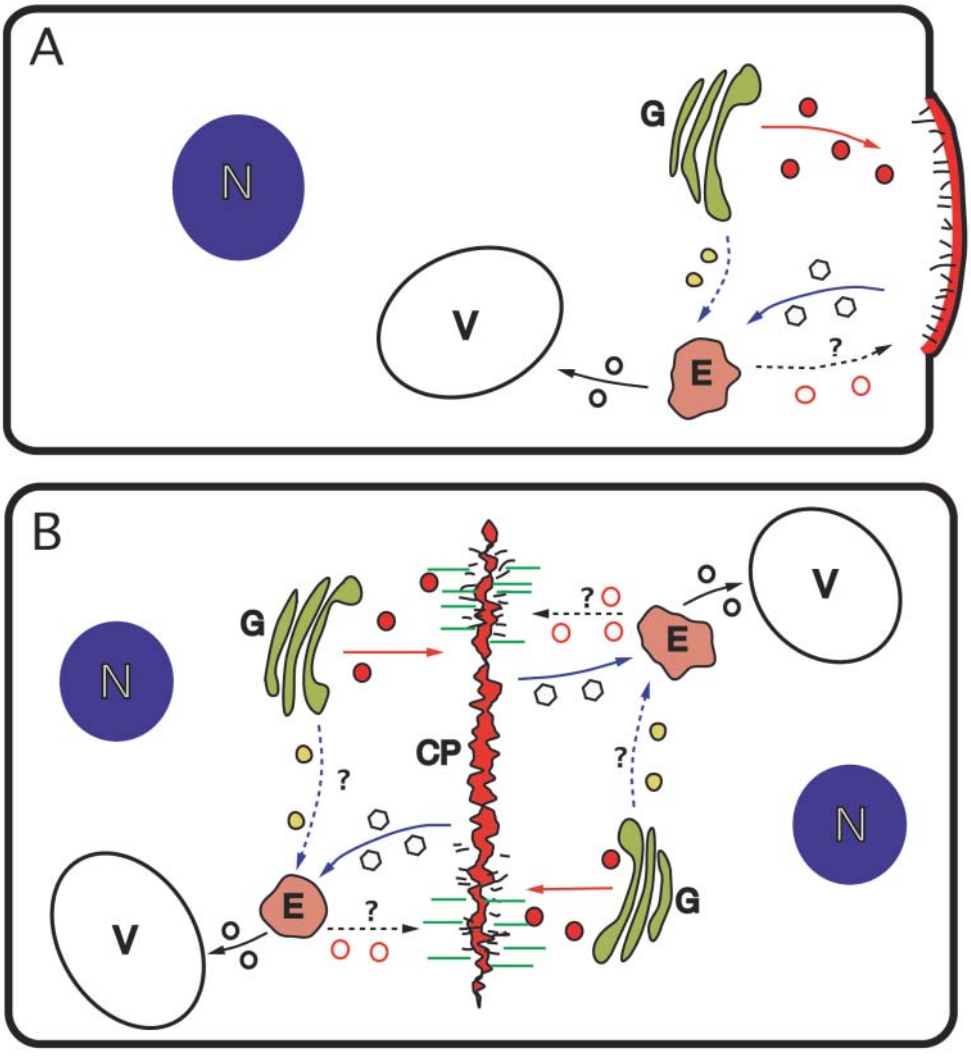

Figure 2: Speculative model for polarized vesicular trafficking during (A) polarized cell-surface expansion and (B) cell-plate formation. A: In polarized growth, vesicles are delivered by multiple exocytic pathways (open and closed red circles) toward sites of expansion on the plasma membrane (red). Targeting of these vesicles may be guided by short MFs (black). B: During cytokinesis, exocytic vesicles are directed toward the cell plate (red). Targeting of these vesicles is guided by phragmoplast MTs (green) and short MFs (black). Cell-plate transport vesicles may originate directly from the Golgi apparatus or from an intermediate compartment (e.g. an early and/or late endocytic/prevacuolar endocytic compartment). Membrane is recycled via clathrin-coated vesicles (white hexagons) from the plasma membrane and cell plate (blue arrows) and delivered to an endocytic compartment (pink). See text for additional details. CP: cell plate, E: endocytic compartment, G: Golgi, N: nuclei, V: vacuole. The ER is not shown.

unique class of cell-plate-specific transport vesicle(s) may be generated during cytokinesis (34). KNOLLE was found to be expressed exclusively in dividing cells and targeted specifically to the cell plate during cytokinesis. More recent studies show several constitutively synthesized proteins, including soluble endoxyloglucan transferase (35), and the plasma-membrane-associated proteins KORRIGAN (36), PIN1 (37) and plasma membrane PM-ATPase (Dickey and Bednarek, unpublished) are localized at the cell plate during cell division. These data argue in favor of a model in which the exocytic secretory pathway becomes polarized toward the division plane during cytokinesis, and suggests that it is the cell-cycle timing of secretory protein synthesis that dictates whether they are transported to the cell surface or the cell plate. In support of this proposal, constitutive expression of KNOLLE resulted in its localization to the plasma membrane in nondividing cells and to the cell plate in dividing cells (38). Several questions that remain to be addressed are whether the exocytosis of plasma membrane and extracellular proteins to the parental cell surface continues during cytokinesis, and whether one or more exocytic pathways are directed toward the cell plate. With regard to the latter, it will be interesting to determine if in addition to PIN1 other asymmetrically distributed plasma membrane proteins such as AUX1 (30) are also targeted to the cell plate.

Sorting signals

Polarized trafficking of KORRIGAN, an endo-1,4-beta-glucanase required for cell expansion and cytokinesis, has been shown to depend on two sorting signals within the cytosolic N-terminal domain of the protein (36). Mutation of either one of these signals, an acidic di-leucine (LL) motif [typically a pair of leucines located 4–5 amino acids C-terminal of an acidic residue (39)] or a YXXϕ [Y is tyrosine, X is any amino acid and ϕ a hydrophobic amino acid with a large side chain (40)] resulted in the persistent mislocalization of KORRIGAN at the plasma membrane. Both of these sorting motifs are also present in the cytosolic domains of PIN1, KNOLLE, and another cell-plate-associated SNARE NSPN11 (41), but their role in trafficking to the cell plate remains to be defined. Studies in yeast and mammalian cells have demonstrate that the YXXϕ and di-LL motifs are recognized by GGAs (Golgi-localized γ-ear-containing ARF binding proteins) and the adaptor protein complexes AP-1, AP-2, AP-3 [for review, see (42) and references therein] which are responsible for trafficking of cargo proteins via clathrin-coated vesicles between the TGN, plasma membrane and endocytic compartments. Similarly a YXXϕ motif in the C-terminus of the putative plant vacuolar cargo receptor that traffics between the TGN and a prevacuolar compartment has been shown to interact with the AP-1 complex (43). Interestingly, the asymmetric plasma membrane distribution of PIN1 involves the cycling of the protein between the plasma membrane and an endosomal compartment (38). KNOLLE and NSPN11 have also been observed in punctate subcellular organelles resembling endosomes in dividing and nondividing Arabidopsis cells (41). These observations suggest the intriguing possibility that the polarized trafficking of proteins containing the acidic-LL and YXXϕ sorting motifs to the cell plate may involve transport through the endocytic pathway (see Figure 2B). Consistent with this hypothesis, one of two exocytic pathways from the TGN to the plasma membrane in yeast has recently been shown to involve an intermediate endocytic compartment, the prevacuolar compartment (24). Whether a similar mechanism exists in plants and whether the acidic-LL and YXXϕ sorting signals mediate the general sorting of transmembrane proteins including KNOLLE, NSPN11, and PIN1 to the cell plate during cytokinesis remains to be determined.

Cell-Plate Secretory Trafficking and Fusion Machinery

The formation, targeting and fusion of transport vesicles within each branch of the plant exocytic and endocytic secretory pathway is assumed to be regulated by a wide variety of cytosolic and membrane-associated factors that are highly conserved among all eukaryotic organisms [for review, see (44)].

Dynamins

Formation of transport vesicles involves the assembly of distinct coat complexes that drive membrane budding and the selection of cargo proteins. This process is regulated by small GTPases such as ARF (ADP-Ribosylation Factor) that are required for the formation of TGN-derived clathrin coated vesicles. In mammalian cells, release of the clathrin-coated TGN-derived and endocytic vesicles from the plasma membrane involves the action of another GTPase, dynamin, the defining member of structurally related but functionally diverse family of large GTP-binding proteins (45).

Dynamin has been shown to form multimeric rings around the necks of invaginating clathrin-coated vesicles and other membrane tubules. In the presence of GTP these dynamin-coated membranes have been observed to fragment, suggesting that the dynamin rings function as a macromolecular garrote. An alternative but not mutually exclusive idea is that the dynamin rings serve to recruit various binding partners, such as the lipid modification enzyme endophilin and other vesicle budding components that function in concert with dynamin to promote membrane scission (46). In addition to their role in vesicular trafficking, dynamin and dynamin-related proteins have been linked to a number of diverse processes including actin dynamics (47).

The plant-specific, dynamin-related protein from soybean, phragmoplastin (48), and its Arabidopsis homologue, the Arabidopsis dynamin-like protein (ADL1) (34,40–51), have been localized to the cell plate. The ADL1 protein family has five members which are $>80\%$ similar in sequence. Like dynamin, phragmoplastin and ADL1A have been shown to form helical structures both *in vitro* (52) and *in vivo* on syncytial-type cell plates during the endosperm cellularization in developing Arabidopsis seeds (51). ADL1A rings were found at $\sim$20 nm constrictions throughout the initial wide tubular

membrane system of the developing syncytial-type cell plates. In contrast to dynamin, these rings do not appear to sever the membrane tubules, suggesting that they may be required to maintain the tubular nature of the initial syncytial cell plate prior to its consolidation (51). Similar to its proposed role in syncytial-type cell-plate development, ADL1A and phragmoplastin have been suggested to play a role in the formation/stabilization of ~20 nm diameter fusion tubules that have been observed to connect fusing cell-plate vesicles (2,15). However, the latter association of these dynamin-like proteins to these tubules remains to be confirmed by immuno-electron microscopy (EM).

Analysis of *adl1A* loss-of-function mutants has confirmed that ADL1A is required for various stages of plant growth and development; however, the mutants do not display defects in cytokinesis (50). One explanation for this is that functionally redundant ADL1 proteins may compensate for the loss of ADL1A during cell division. Indeed, other members of the ADL1 gene family display overlapping expression patterns throughout plant development, and moreover colocalize with ADL1A at the cell plate during cytokinesis (Kang and Bednarek, unpublished). More recent studies have suggested a role for ADL1A in polarized vesicular trafficking and endocytosis. ADL1A and other proteins involved in vesicular transport between the TGN, plasma membrane and endocytic compartments including β-adaptin, a subunit of the clathrin adaptor protein complex, and AtSEC14 cofractionated by affinity chromatography on immobilized-naphthylphthalmic acid (NPA) (53). NPA is an inhibitor of polar auxin transport that has been shown to block trafficking of PIN1, KNOLLE, and the PM-ATPase (37). Consistent with this, *adl1A* mutants display cell-type specific defects in the polarized growth and endocytosis (Kang and Bednarek, unpublished). These results suggest that during cytokinesis ADL1A may function in the formation of cell-plate transport vesicles and in the recycling of membranes and vesicles from the developing cell plate. Nearly 75% of the total membrane delivered to the cell plate has been estimated to be removed during cell-plate maturation (51) indicating that regulation of membrane recycling is likely to be critical for cytokinesis. Other additional plant dynamin-related proteins, including the mammalian dynamin homolog ADL6 (54), have been localized to the cell plate (15) and may be required for cell-plate formation.

In addition to their potential roles in cell-plate membrane trafficking, ADL1A and phragmoplastin may also regulate 1,3-β-glucan (callose) biosynthesis (15). Callose deposition within the cell-plate lumen precedes cellulose synthesis and is thought to help drive the spreading and stabilization of cell-plate membranes (1). The catalytic subunit of callose synthase has been localized to the cell plate (55) and shown to interact directly with phragmoplastin. Overexpression of phragmoplastin has also been found to stimulate the accumulation of callose (15), suggesting that it may regulate the activity or localization of the callose synthase complex. Interestingly, putative acidic LL and YXXϕ sorting motifs are present in the N-terminal cytosolic domain of callose synthase, which could facilitate its targeting to the cell plate and plasma membrane.

GNOM and cell-plate formation

With the possible exception of the above-mentioned dynamin-like proteins, no other factors involved in the biogenesis of transport vesicles that are directed to the cell plate have been identified to date. GNOM/EMB30 is an ADP-ribosylation factor-guanine nucleotide exchange factor (ARF-GEF) that is required for the polarized transport of PIN1 to the plasma membrane (56). Yet, surprisingly, *gnom/emb30* mutants show no obvious cytokinesis defects. One explanation for this is that disruption of GNOM/EMB30 may only affect one of several exocytic pathways that are directed to the cell plate (Figure 2) and that loss of a single vesicular transport pathway to the cell plate could be compensated for by the other cell-plate-directed exocytic pathways. Similarly, yeast mutants lacking one of the two exocytic branches are viable because the remaining functional pathway to the cell surface begins to transport the cargo proteins of the other pathway (24).

Rabs and tethering systems

Following secretory vesicle formation, another class of small GTPases, Rabs, function to coordinate the docking (also known as tethering) of transport vesicles to their appropriate acceptor membranes (57). Each branch of the secretory pathway is thought to contain a unique Rab that recruits a large fibrous protein or protein complex, a vesicle-tethering factor, to sites where transport vesicle and their target membrane fuse. The yeast genome encodes only 11 Rabs, whereas animals and plants have over 50 distinct Rabs (58). This significant difference may be related to specific secretory needs of complex multicellular organisms, including multiple polarized exocytic pathways. With the exception of Rab1 and Rab2, which function in ER-to-Golgi trafficking (18,59), little is known about the function of other plant Rabs. The roles of other plant Rabs have only been inferred based upon sequence homology and localization studies. Recently, Rab11 (which regulates trafficking between the TGN, endosomes and plasma membrane domains) was demonstrated to be required for the completion of cytokinesis in early *Caenorhabditis elegans* embryos (60). The importance of Rabs for cell-plate formation is supported by the recent identification of *SCD1* (*stomatal cytokinesis defective*), a gene that is required for cytokinesis in developing stomates and the expansion of various other cell types (Falbel et al., unpublished data). *SCD1* encodes a protein homologous to guanine nucleotide exchange factors for Rab GTPases, putatively representing the first regulatory link to Rab GTPases and tethering systems in the pathway of cell-plate formation.

Compared to plant Rabs, even less is known about the existence and function of plant secretory vesicle-tethering factors. The Arabidopsis genome appears to encode homologs of some subunits of the *S. cerevisiae* exocyst complex, an eight-subunit plasma membrane-associated tethering complex required for exocytosis (57). However, no functional

analysis has been performed yet for the putative Arabidopsis exocyst or other tethering complexes.

Cell-plate membrane fusion factors

Following tethering it has been proposed that pairing of cognate SNAREs, that reside on the opposing membranes (i.e. *v*esicle v-SNAREs pair with *t*arget membrane t-SNAREs) drives transport vesicle fusion. The post-fusion or cis-SNARE complex is comprised of a highly stable parallel-four helix bundle in which one of the helices is contributed from a v-SNARE, one from a syntaxin-type t-SNARE, and two from either a single SNAP-25-type SNARE or two other individual t-SNAREs [for review, see (44)]. The recent identification and characterization of several Arabidopsis membrane-fusion factors that localize to the division plane have provided some insight into the membrane-fusion mechanism(s) required for plant cytokinesis.

KNOLLE, the cell-plate-associated t-SNARE (34,61), has been shown to bind to SNAP33 (a SNAP25-type SNARE) (62), the plant-specific SNARE, NSPN11 (41) and the Sec1p homolog, KEULE (63). Sec1 family members exhibit specificity for particular syntaxins. In concert with Rabs and vesicle-tethering factors, they regulate syntaxin's ability to interact with other SNAREs (57). Consistent with this, cells of severely malformed *knolle* and *keule* mutant *Arabidopsis* seedlings display defects in cell-plate assembly and *knolle*/*keule* double mutants are synthetically lethal (63,64), suggesting that these two genes are part of the same fusion pathway. It has been proposed that the interaction of KEULE and KNOLLE on the target membrane could prime a cell-plate membrane domain for the addition of the next vesicle (13,63). In contrast to *knolle* and *keule*, *snap33* and *nspn11* mutants show either very mild or no obvious cytokinesis defects (41,62). However, *keule*/*snap33* and *knolle*/*snap33* double mutants are synthetically lethal, providing genetic evidence that SNAP33 functions along with KNOLLE and KEULE during cell-plate–membrane fusion. Analysis of *nspn11*/*keule* and *nspn11*/*knolle* double mutants remains to be done. One possible explanation for the phenotypes of the *snap33* and *nspn11* mutants is that SNAP33 and NSPN11 are members of multigene families (65) and thus mutations in either SNARE may be suppressed by expression of other family members. Indeed, KNOLLE interacts in a yeast two-hybrid assay with two other Arabidopsis SNAP25-type SNAREs, SNAP29 and SNAP30 (62); however, the expression and localization of these SNAREs remains to be determined. Unlike KNOLLE, which is expressed only in dividing cells, SNAP33, NSPN11 and KEULE are expressed in dividing as well as nondividing cells, suggesting that these proteins function not only in cell-plate formation but also in other exocytic vesicle fusion events. In support, SNAP33 has been demonstrated to interact with the plasma membrane syntaxin SYR1 (SYP121) (66).

SNARE complexes are enzymatically disassembled by the AAA (ATPases *a*ssociated with various cellular *a*ctivities) (67) chaperone NSF prior to or concomitant with tethering in order to prime the individual SNAREs for fusion (44). A second AAA ATPase, CDC48 (p97 in mammalian cells) has also been shown to interact with SNAREs during the homotypic fusion of ER-ER and transitional ER membranes (68,69). Interestingly, Cdc48p (p97) and NSF along with the SNARE, syntaxin 5 mediate the fusion of mitotic Golgi membranes in mammalian cells through two separate pathways. Likewise, some or all of the stages of ER and cell-plate membrane dynamics at the division plane, including cell-plate vesicle fusion and consolidation of the TVN into the smooth plate, may involve NSF- and/or Cdc48p-dependent mechanisms. Indeed, KNOLLE has been shown to interact with NSF *in vitro* (Rancour et al., unpublished data). Furthermore, the plant ortholog of CDC48, AtCDC48, is localized at the division plane in dividing Arabidopsis cells (70). *In vitro* binding studies, however, reveal that AtCDC48 does not interact with KNOLLE but rather with another division plane localized SNARE, SYP31, the plant ortholog of the ER-to-Golgi syntaxin 5 (Rancour et al., manuscript submitted). Although the significance of AtCDC48 and SYP31 localization at the division plane remains to be determined, these results suggest that there are at least two distinct membrane fusion pathways involving CDC48p/p97 and Sec18p/NSF that operate at the division plane to mediate plant cytokinesis.

Cytoskeletal Guidance of Cell-Plate Membrane Trafficking

The phragmoplast guides secretory vesicle trafficking to the division plane and the expansion of the developing cell plate outward toward the cell cortex. This cytoskeletal scaffold is made up of two antiparallel arrays of MTs and MFs, with the MT and MF plus ends directed toward the central equatorial region of the structure. In animal cells, there appear to be functional equivalents of the phragmoplast; the spindle midzone (60,71) and in some cell types the furrow microtubule array (10) direct the insertion of membrane into the cleavage furrow and are required for proper cytokinesis. Like the phragmoplast, each of these structures is composed of two arrays of MTs with plus ends directed toward the center. Cell-plate formation is blocked by MT depolymerization and in mutants that disrupt tubulin folding (72). Similarly, MT depolymerization in animal cells prevents the addition of new membrane in the furrow and results in furrow regression (10). Thus, the arrival of membrane via a MT scaffold appears to be a plant feature that is conserved in animals to accomplish cytokinesis.

Microtubule-associated motors

Plus end-directed MT motor proteins are thought to drive vesicles toward the equatorial plane of the phragmoplast during cell-plate formation. Ultrastructural analysis of developing cell plates has revealed that phragmoplast MT-associated transport vesicles are connected to the MT via rod-like 10–30 nm structures that resemble kinesin-type motor proteins (51). At-PAKRP2 (73) is a recently identified plus end-directed kinesin-like protein (KLP) that may function in vesicular transport

during cytokinesis. AtPAKRP2 is localized normally to phragmoplast MTs, but the ARF-GEF inhibitor, Brefeldin A, which blocks secretory vesicle trafficking, disrupts this localization in dividing cells (73). AtPAKRP2 does not, however, colocalize with KNOLLE, a marker of cell-plate transport vesicles. Therefore additional functional and immuno-EM studies are necessary in order to establish the role of AtPAKRP2 in cell-plate vesicle trafficking.

Several other plus end-directed KLPs, such as TKRP125, NACK1/Hinkel, NACK2 and KCBP have been found to be associated with the phragmoplast. However, they appear to play a role in the assembly and maintenance of the phragmoplast MT organization [for review, see (74,75)] and in phragmoplast-guided lateral expansion of the cell plate (76,77), but not in vesicle trafficking to the cell plate.

Role for the actin cytoskeleton in cell-plate vesicle targeting and fusion?

In contrast to the MT-dependent trafficking of vesicles to the cell plate during cytokinesis, vesicle trafficking to the cell surface continues in the absence of MTs (72). Conversely, studies with actin-disrupting drugs have suggested a role for MFs in vesicular trafficking to the plasma membrane but not to the cell plate (5,37).

Two types of MFs have been observed in tip-growing cells such as pollen tubes: large actin cables, which are thought to mediate long-distance transport of secretory vesicles; and short MFs, which are localized at the tip where the vesicles fuse (78). The role for the short MFs is not understood; however, they may serve as a landmark for targeting and fusion of the exocytic vesicles (Figure 2). Recent studies suggest that members of the plant-specific Rho GTPase subfamily, Rops (*R*ho related GTPase *o*f *p*lants), regulate polarized cell expansion by modulating the formation of short MFs at the cell cortex (78–80). Overexpression of constitutively active and dominant-negative Rops resulted in the disassembly or mislocalization of short cortical MFs, respectively, both causing defects in cell expansion (78). Consistent with their role in polarized membrane expansion, Rops have been localized to the apical plasma membrane of tip-growing cells and to sites on the surface of cells undergoing localized expansion.

During cytokinesis, Rops and short MFs are associated with the cell plate and may function in its assembly. Similar to the short cortical MFs involved in cell expansion, short MFs are associated with the leading edge of the cell plate (81), which is the predominant site where new membrane is incorporated into the expanding cell plate. A green fluorescent protein tagged-Rop, GFP-AtRop4, was shown to be localized to the cell plate in transgenic tobacco BY2 cells (80) potentially directing the assembly of the short MFs. In addition, the Rop1GTPase was shown to interact in its GTP-bound state with a UDP-glucose transferase, a subunit of the cell-plate-associated phragmoplastin/callose synthase complex (82), suggesting that it may regulate callose biosynthesis. Alternatively, the interaction between Rop and the callose synthase complex may serve to coordinate membrane deposition and localized callose biosynthesis during cell-plate assembly.

Recent studies in *S. cerevisiae* have demonstrated that Rho GTPases, MFs, and Rab GTPases are all required for the spatial regulation of the vesicle tethering complex, the exocyst, thereby regulating the site at which vesicles fuse with the plasma membrane (83,84). In the fission yeast, *Schizo-saccharomyces pombe*, exocyst proteins localize in an F-actin-dependent manner to regions of active exocytosis, including the growing ends of interphase cells and medial regions of cells undergoing cytokinesis (7). During cytokinesis in *S. pombe,* mutants lacking the exocyst subunit Sec8p accumulate unfused vesicles in the cytoplasm near the division septum, which morphologically resembles the build-up of unfused cell-plate vesicles in *knolle* mutant cells (34). Further genetic and biochemical experiments will help to establish whether Rops and short MFs regulate the polarized targeting and fusion of transport vesicles at the cell plate and facilitate the identification of other components of the cell-plate vesicle targeting machinery, including the necessary Rabs and tethering factors.

Final Perspective

It has only been a few short years since the first high-resolution images of the dynamic stages of cell-plate formation were obtained and the first cell-plate membrane fusion factor, *KNOLLE* was identified. Since then, our understanding of the 3D ultrastructure of cell-plate formation has been enhanced by the use of high-voltage EM tomography, and by the characterization of many more cell-plate-associated factors through the use of forward and reverse genetics, which are aided by the completion of the Arabidopsis genome sequence. The combination of cell biological tools now available in plants is likely to test and render many of the hypotheses and questions raised in this review obsolete within a very short time. Recent studies have highlighted some of the dramatic similarities and differences between animal, fungal, and plant cytokinesis. As more information unfolds, the network of interactions becomes more interconnected and complete through more genetic and biochemical analyses. The complexity, similarity, and uniqueness of each organism's and each cell type's approach to the separation of one cell into two will show us just how many ways there are to divide a cell.

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