Vesicle Trafficking during Somatic Cytokinesis

Daniël Van Damme, Dirk Inzé, and Eugenia Russinova*

Department of Plant Systems Biology, Flanders Institute for Biotechnology, and Department of Molecular Genetics, Ghent University, B–9052 Ghent, Belgium

Plant cytokinesis, the final event of cell division, generates two daughter cells by partitioning the cytoplasm of a mother cell. This depends on targeted secretion to generate a new plasma membrane (PM; Whaley and Mollenhauer, 1963; Samuels et al., 1995; Jurgens, 2005a, 2005b). Plant cells control cytokinesis by constructing a plant-specific cytoskeletal configuration, the phragmoplast (Zhang et al., 1993; Granger and Cyr, 2000; Assaad, 2001). The phragmoplast consists of a double array of parallel-oriented microtubules, actin filaments, associated molecules, and a cell plate assembly matrix acting as a framework to transport cell plate-building vesicles to the cell equator (Lambert, 1993; Staehelin and Hepler, 1996; Otegui et al., 2001; Wasteneys, 2002; Segui-Simarro et al., 2004). Homotypic fusion of these vesicles, constricted by dynamins, gives rise to dumbbell-shaped tubular structures and the formation of a transient membrane compartment, the cell plate. The tubulovesicular network created by end-to-end fusion of vesicles evolves into a fenestrated sheet (Samuels et al., 1995; Otegui et al., 2001; Segui-Simarro et al., 2004). The cell plate grows outward to the cortex of the cell by peripheral vesicle fusion directed by the depolymerization of the central microtubules and polymerization of microtubules at the periphery (Strompen et al., 2002; Sasabe and Machida, 2006; Sasabe et al., 2006). When the cell plate reaches the cortex, it fuses with the parental PM, a process requiring heterotypic vesicle fusion. Fusion activates the maturation of the plate to a rigid cell wall, a process that includes closure of the plate fenestrae, removal of excess membrane, and replacement of callose by cellulose (Samuels et al., 1995; Segui-Simarro et al., 2004). This review provides an update on the membrane-trafficking events during plant somatic cytokinesis involved in initial plate formation, fusion of the cell plate with the parental PM, and trafficking events associated with cell plate maturation (Fig. 1).

ORIGIN OF CELL PLATE-BUILDING VESICLES

The textbook scheme of vesicle trafficking during plant somatic cytokinesis depicts the delivery of Golgi-derived vesicles to the forming cell plate along the parallel-oriented microtubules of the phragmoplast by means of plus-end-directed microtubule motor proteins. Indeed, ultrastructural analysis has revealed a close association of vesicles with the phragmoplast microtubules (Kakimoto and Shibaoka, 1987; Samuels et al., 1995), the integrity of which is essential for cell plate vesicle delivery (Steinborn et al., 2002). Proteins connecting vesicles to phragmoplast microtubules have been visualized during endosperm cellularization and pollen cytokinesis (Otegui et al., 2001; Otegui and Staehelin, 2004). The kinesin AtPAKRP2 is so far the best candidate motor protein to transport the cell plate-building vesicles to the division plane (Lee et al., 2001), although genetic data for this are lacking.

Recently, a lot of attention has been given to the origin of the cell plate-building vesicles. High-resolution electron tomography analysis reported the presence of two types of vesicles surrounding the forming cell plate: small, dark vesicles and larger, lighter stained vesicles. These vesicles are considered the initial building blocks of the cell plate, and the latter type is thought to occur through pairwise fusion of the darker vesicles (Segui-Simarro et al., 2004).

Vesicle fusion is accomplished through the action of SNARE proteins (for review, see Lipka et al., 2007), tethering factors (Rab GTPases; for review, see Zerial and McBride, 2001; Molendijk et al., 2004), and presumably aided by components of the exocyst complex (Segui-Simarro et al., 2004), although, to date, mutants defective in subunits of this complex have only been implicated in polar growth (Cole et al., 2005; Wen et al., 2005; Synek et al., 2006; Lavy et al., 2007). The dark-stained vesicles were also found in the vicinity of the Golgi apparatus, and it was concluded that they were Golgi derived. Prevacuolar compartment/multivesicular bodies (MVBs) or late endosomes were detected around the cell plate at its maturation phase, accompanied by an increased appearance of clathrin-coated vesicles (Otegui et al., 2001; Otegui and Staehelin, 2004; Segui-Simarro et al., 2004; Segui-Simarro and Staehelin, 2006). The observation that Arabidopsis (*Arabidopsis thaliana*) Golgi stacks double before mitosis (Segui-Simarro and Staehelin, 2006) supports the capacity for substantial vesicle secretion during plant cytokinesis. From mitosis on, Golgi stacks accumulate in a subcortical ring (the Golgi belt) surrounding the site of cell plate formation. Although the Golgi stacks do not show a preferential orientation toward the cell plate, the Golgi belt could facilitate directed delivery

* Corresponding author; e-mail eurus@psb.ugent.be.

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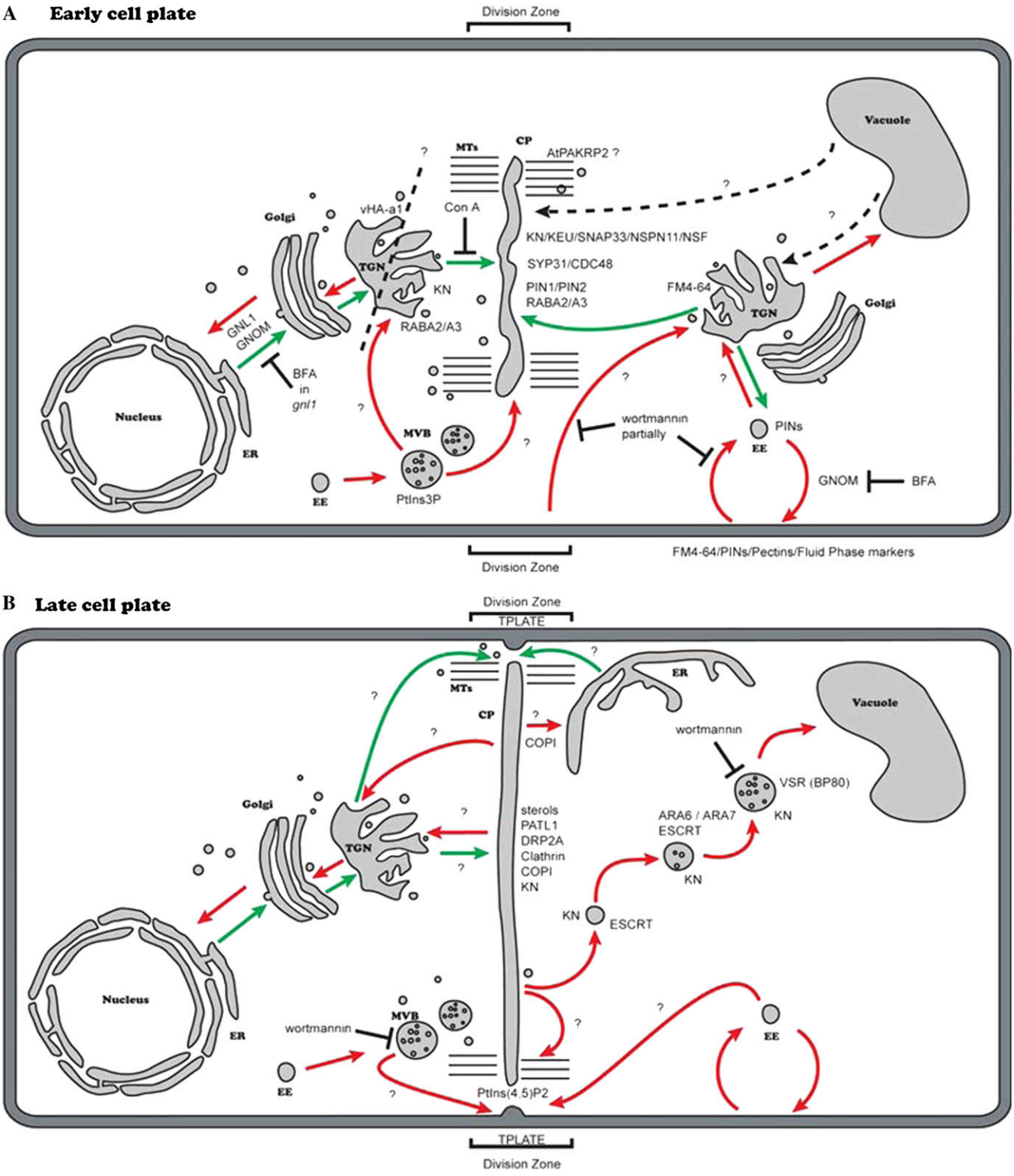

Figure 1. Membrane trafficking during early and late plant cell plate formation. A, During early cell plate formation, vesicle secretion becomes polarized to the cell equator. Two independent pathways, using either the KN/KEU/SNAP33/NSPN11/NSF or the SYP31/CDC48 machinery, operate in fusing the vesicles, resulting in the formation of a transient membrane compartment, the cell plate. Cell plate-building vesicles are likely transported by the AtPAKRP2 kinesin along the phragmoplast microtubules. Phragmoplast microtubules guide the cell plate as it grows outward to the correct insertion site at the center of the division zone. The PM at the division zone is at this stage marked by the absence of PM-associated KCA1 (data not shown), and this KCA1-depleted zone remains present throughout cytokinesis. Both secretory vesicles from the Golgi and endosome-derived vesicles

of Golgi-derived vesicles to the division plane. (Nebenfuhr et al., 2000; Segui-Simarro and Staehelin, 2006). Golgi-derived membrane and proteins, however, are not the only source of cell plate building blocks. When internalized by endocytosis in BY-2 cells and Arabidopsis seedlings, the styryl dye FM4-64 (Dettmer et al., 2006; Dhonukshe et al., 2006) and the fluid phase markers Alexa 633 and Lucifer Yellow (Dhonukshe et al., 2006) label the forming cell plate at a very early stage and within minutes after addition (Dettmer et al., 2006; Dhonukshe et al., 2006). The presence of cell wall-specific cross-linked rhamnogalacturonan and partially esterified homogalacturonan epitopes in the cell plate (Baluska et al., 2005; Dhonukshe et al., 2006) and the accumulation of YFP-2x-FYVE-positive endosomes in a belt accumulating at the periphery of the growing cell plate (Vermeer et al., 2006) strongly favor a contribution of the endocytic pathway to the construction of the cell plate.

Several drug studies have been carried out to unravel the contribution of the secretory and endocytic pathways in cell plate formation. To eliminate the contribution of the secretory pathway, the fungal toxin brefeldin A (BFA) is commonly used. BFA targets BFA-sensitive ARF-GEFs, causing a reversible inhibition of secretory vesicle trafficking (Renault et al., 2002; Geldner et al., 2003). BFA induces the loss of γ-COP-forming vesicles from the Golgi cisternae in BY-2 cells within minutes and subsequently leads to the formation of an endoplasmic reticulum (ER)-Golgi hybrid compartment (Ritzenthaler et al., 2002). BY-2 cells treated with BFA before chromosome condensation failed to construct a cell plate, while BFA addition at a later time point allowed initial cell plate formation (Yasuhara et al., 1995; Yasuhara and Shibaoka, 2000). This has been attributed to the formation of a pool of cell plate-forming vesicles before the onset of cytokinesis and was used to argue in favor of the contribution of endocytic vesicles to cell plate formation (Yasuhara et al., 1995; Dhonukshe et al., 2006). BFA sensitivity in BY-2 cells implicates ARF-dependent membrane trafficking, but not exclusively Golgi-derived vesicle trafficking, as the target of BFA in this system is not known (Schrick et al., 2004; Men et al., 2008). The nature and presence of the cell plate-forming vesicles remain to be addressed in BY-2 cells, but in Arabidopsis the RAB-A2/A3 compartment has been proposed to fulfill this function. The RAB-A2/A3 compartment functions in the secretory route, and YFP:RAB-A2 colocalizes with a newly synthesized cytokinesis-specific syntaxin, KNOLLE (KN; Lauber et al., 1997), at punctae, distinct from Golgi stacks, during metaphase, anaphase, and later at the cell plate, in contrast to VHA-a1 (Chow et al., 2008).

In Arabidopsis and maize (*Zea mays*) root cells, BFA treatment does not impair cell plate formation (Boutte et al., 2006; Dhonukshe et al., 2006). In Arabidopsis, this is likely due to the presence of a BFA-resistant ARF-GEF (GNOM-LIKE1 [GNL1]) mediating ER-to-Golgi trafficking in concert with the primary target of BFA, the ARF-GEF GNOM (Richter et al., 2007; Teh and Moore, 2007). Indeed, *gnl1* mutant seedlings treated with BFA (mimicking the *gn/gnl1* double mutant) show impaired cytokinesis and locked KN in the ER. This phenotype was used to argue that secretion of de novo synthesized proteins is essential for cytokinesis (Reichardt et al., 2007), in contrast to what was previously shown by Dhonukshe et al. (2006). In their experiments, cycloheximide treatment did not impair KN localization to the forming cell plate (Dhonukshe et al., 2006). As the authors do not report cell plate expansion after cycloheximide treatment, it cannot be excluded that these plates were pre-existing. Life cell imaging during cytokinesis of cycloheximide-treated cells will be required to clarify this point.

Reichardt and coworkers used another drug, wortmannin, which interferes with endocytosis by inhibiting phosphatidylinositol 3-kinase and 4-kinase (Matsuoka et al., 1995) and causes swelling of MVBs (Tse et al., 2004). In the presence of wortmannin, internalization of the styryl dye FM4-64 in Arabidopsis root cells was impaired without inhibiting cell plate formation (Reichardt et al., 2007). However, it has been reported that wortmannin only reduces the FM uptake in both BY-2 cells and Arabidopsis seedlings (Emans et al., 2002; Leshem et al., 2007). The latter experiments suggest that wortmannin

Figure 1. (*Continued.*)
from the PM and/or other endosomal organelles contribute to the formation of the cell plate. Secretory and endocytic pathways likely converge at the TGN, which explains the co-occurrence of KN, RAB A2/A3, PINs, FM4-64, pectins, and fluid phase markers at an early stage during cell plate formation. The dashed line over the TGN indicates a possible compartmentalization based on the localization of VHA-a1 and RAB A2/A3. MVBs, labeled with YFP-2x-FYVE, which recognizes PtIns3P, accumulate in a belt around the leading edge of the cell plate, but their contribution to the cell plate remains to be proven. B, During the late phases of cell plate formation, membrane addition occurs predominantly at the leading edge of the torus-like phragmoplast, next to removal and/or recycling of membrane at the maturing center of the plate. PtIns(4,5)P_2 accumulates at the leading edge of the cell plate, before fusion of the plate with the parental PM. The origin of the vesicles and the machinery required for fusion of the cell plate with the PM remain to be identified. TPLATE accumulates in the PM at the division zone during plate insertion and plays a role in anchoring of the cell plate. A close contact between the ER and the cell plate during the late phases of cell plate formation could reflect direct membrane transfer between the ER and the cell plate. The sites of action of commonly used drugs that interfere with membrane transport are indicated. CP, Cell plate; EE, early endosome; MTs, phragmoplast microtubules. Question marks indicate possible trafficking pathways for which no conclusive evidence has been presented so far. Green arrows represent anterograde transport, red arrows indicate retrograde transport and recycling, and black dashed arrows indicate the possible contribution of tubulated vacuolar membrane to the cell plate.

treatment does not totally abolish endocytosis and therefore cannot rule out a contribution of endocytic vesicles during early and/or late stages of cell plate formation.

Genetic interference of the endocytic pathway during cytokinesis, using a dominant negative (GDP-locked) form of the Rab5 homolog ARA7, or overexpression of the C-terminal part of clathrin heavy chain inhibited FM4-64 internalization and caused cytokinesis defects in Arabidopsis and BY-2, respectively (van der Bliek, 2005; Dhonukshe et al., 2006, 2007; Tahara et al., 2007). While these genetic approaches appear more robust than drug treatments, they cannot be used as conclusive proof for the endocytic requirement to build the cell plate, as a DN-ARA7 is likely to interfere with membrane removal from the cell plate besides endocytosis at the PM, and overexpression of a dominant negative clathrin also affects spindle and phragmoplast formation (Tahara et al., 2007).

A close contact between the trans-Golgi network (TGN) and the Golgi could explain the number of discussions and controversies regarding the origin of the cell plate-building vesicles. The TGN in Arabidopsis shoot meristem cells appears tightly associated with the trans-side of the Golgi apparatus, and no free-floating TGNs could be observed using high-pressure freezing and freeze substitution (Segui-Simarro and Staehelin, 2006). However, it was reported that the Golgi marker YFP-SYP31 and the TGN marker GFP-SYP41 did not show 100% co-occurrence when transiently expressed in Arabidopsis protoplasts (Uemura et al., 2004). Detailed ultrastructural analysis will be required to clarify whether the YFP-SYP41 punctae that do not appear in the vicinity of the Golgi represent true TGN compartments and whether they are involved in vesicle delivery during cytokinesis. It would be of interest to determine whether YFP-SYP41 punctae colocalize with the RAB-A2/A3 proteins, as the latter label endosomal compartments that contribute to the cell plate but show only a partial overlap with the VHA-a1-labeled TGN (Chow et al., 2008).

The TGN was recently reported to function as an early endosome compartment (Robinson et al., 2008). The TGN as grand central at the intersection of the secretory and endocytic pathways and the polarization of vesicle transport during cytokinesis could easily explain the presence of both secretory and endocytosed PM proteins at the forming cell plate. Concanamycin A (ConA), a specific V-ATPase inhibitor, blocks trafficking at the TGN, does not interfere with endocytosis at the PM, but causes cytokinesis defects and depletion of both KN and FM dyes from the cell plate (Robinson et al., 2004; Dettmer et al., 2005, 2006; Reichardt et al., 2007). Whether the secretory and the endocytic pathways that merge at the TGN mark distinct subdomains of this compartment remains to be proven, although the recently reported localization of RAB-A2/A3 hints in this direction (Chow et al., 2008). Because FM dyes, as free-flowing aspecific membrane markers, might not be sorted in a compartmentalized TGN, photoconvertible forms of GFP could be used to discriminate between recycling and newly synthesized membrane proteins. Converging endocytic and secretory pathways at the TGN, underlined by the localization of KN and FM dyes during cytokinesis (Reichardt et al., 2007), imply that the path of cell plate-building vesicles depends on an intact TGN. But is trafficking through the TGN the only way for cell plate building blocks?

The AtRAB4-mediated secretion of cell wall components during root hair expansion, which occurs between the Golgi and an endosomal compartment different from the TGN, is indicative of an alternative secretory route (Preuss et al., 2004), although it needs to be shown whether this occurs during cytokinesis. In the ConA-treated binucleated Arabidopsis root tip cells, fragmented cell plate-like structures and unfused membrane vesicles, resembling the vesicles that accumulate in the *kn* mutant, could be seen at the cell equator using electron microscopic resolution (Reichardt et al., 2007). What is the origin of these structures? And if the structures are derived from vesicle fusion, what mechanism is involved?

During the progression from mitosis to cytokinesis, the vacuole volume decreases by 80% and the surface membrane area halves, a process that may involve budding of vesicles or small tubules. An attractive hypothesis might be that the vesicles and fragmented cell plates are of vacuolar origin, as vacuoles tubulate and concentrate around the cell plate during early telophase (Segui-Simarro and Staehelin, 2006).Vacuolar membranes, however, are unlikely to contribute substantially to the cell plate, as the *vacuoleless* mutant of Arabidopsis does not show cytokinesis defects (Rojo et al., 2001). The use of large collections of membrane markers will be required to elucidate the nature of these vesicles and fragmented plates.

In conclusion, evidence has been presented that both secretory and endocytosed vesicles contribute to cell plate formation. Due to the complexity and cross talk of the plant endomembrane system and the diverse actions of membrane-trafficking drugs, it remains a challenge to distinguish between the contributions of secretory and retrograde transport pathways in the process of cell plate formation (Fig. 1A).

TRAFFICKING DURING CELL PLATE FUSION TO THE PARENTAL PM

While the early steps of cytokinesis have been elaborately studied, both genetically (Nacry et al., 2000; Sollner et al., 2002; Jurgens, 2005a, 2005b; Konopka et al., 2006; Sasabe and Machida, 2006) and structurally (Samuels et al., 1995; Otegui and Staehelin, 2004; Segui-Simarro et al., 2004; Austin et al., 2005; Segui-Simarro and Staehelin, 2006), few data have been generated regarding the final steps of cytokinesis (Fig. 1B). Some insight came from freeze fractionation of BY-2 cells, by which it was shown that the fusion between the transient cell plate and the parental PM occurs simulta-

neously at hundreds of sites by thin finger-like fusion tubes emanating from the forming cell plate (Samuels et al., 1995). Nevertheless, this closure of the physical barrier between the two daughter cells remains to date very poorly understood.

The observation that cytokinesis does not always proceed symmetrically within the volume of the cell (Cutler and Ehrhardt, 2002) led to the hypothesis that the fusion of the plate with the PM starts at a distinct position at the division zone (closest to the phragmoplast initiation point) and further expansion of the plate results in fusion along the cortex. The asymmetric initiation of cell plate formation is also used to explain the occurrence of short cell wall protrusions (stubs) and incomplete cell walls that are a hallmark of defective cytokinesis. However, this type of cytokinesis cannot account for the presence of stubs at each side of the cell in a midsection. Stubs at either side of the cell can be caused by defective maturation and tearing of the plate in mutants impeded in cell wall formation (Nickle and Meinke, 1998; Fagard et al., 2000; Schrick et al., 2000, 2004; Zuo et al., 2000; Lane et al., 2001; Lukowitz et al., 2001; Pagant et al., 2002). However, this does not explain the presence of stubs in mutants affected in early cell plate vesicle-trafficking events, like KN and KEULE (Lauber et al., 1997; Waizenegger et al., 2000), *stomatal cytokinesis defective1* (Falbel et al., 2003), the dynamin *drp1A drp1E* double mutant (Kang et al., 2003a), the double *gn/gnl1* mutant mimicked by BFA treatment of *gnl1* (Reichardt et al., 2007), and dominant inhibitory mutants of RAB-A2 (Chow et al., 2008). As the inhibition of both GN and GNL1 impairs ER-to-Golgi vesicle trafficking and traps KN in the ER, the cell wall stubs have to be generated by a KN-independent pathway (Lauber et al., 1997; Jurgens, 2005a). Based on this, one might speculate that vesicle fusion during cell plate insertion does not require de novo protein synthesis and Golgi trafficking. The latter would be in agreement with the effects of ConA treatment of Arabidopsis root cells, in which stubs were also formed (Reichardt et al., 2007). What then could be the origin of the vesicles, and which proteins are implicated in mediating their fusion? One hypothesis that certainly deserves testing is whether these vesicles are of endosomal origin.

It was recently reported that a PM-associated kinesin (KCA1) is selectively excluded from the PM at the division zone in BY-2 cells from prophase to the end of telophase. The PM band devoid of KCA1 was termed KCA1-depleted zone, by analogy to the actin-depleted zone (Vanstraelen et al., 2006), and it was hypothesized that this could be caused by an alteration of the membrane composition following preprophase band formation through localized endocytosis (Dhonukshe et al., 2005; Van Damme et al., 2007). As in animals, one way plant cells could specify their division zone PM is by accumulating phospholipids like phosphatidylinositol 4,5-bisphosphate [PtIns(4,5)P_2] (Emoto et al., 2005). Until now, accumulation of PtIns(4,5)P_2 at the division zone and in vesicles has not been reported in plants (Vermeer et al., 2006). However, PtIns(4,5)P_2 accumulated at the margin of the cell plate during its fusion with the parental PM in BY-2 cells (van Leeuwen et al., 2007), suggesting a role for these phosphoinositides in the final steps of cell plate formation.

During the anchoring of the cell plate with the parental PM, a cell plate-associated protein called TPLATE accumulates in a 5-μm region surrounding the cell plate insertion site (Van Damme et al., 2004, 2006). TPLATE is a plant-specific protein with similarity to coat and adaptin proteins, suggesting a function in vesicle trafficking. A T-DNA insertion mutant in *TPLATE* results in male sterility due to an altered vesicular composition and excessive callose accumulation in mature pollen grains, causing the pollen to collapse (Van Damme et al., 2006). This phenotype is highly similar to the *drp1c* mutant, which is proposed to function in PM dynamics (Kang et al., 2003b). Live cell imaging of BY-2 cells expressing a *TPLATE* RNA interference construct showed that although the initial steps of vesicle fusion and cell plate formation were not affected, cells failed to fuse or anchor their cell plates with the mother wall (Van Damme et al., 2006). The above experiments suggest that TPLATE likely functions in the vesicle fusion events occurring during the heterotypic fusion of the cell plate with the parental PM. The identification of TPLATE-interacting proteins will no doubt shed more light on the vesicle fusion events associated with cell plate anchoring.

VESICLE TRAFFICKING INVOLVED IN CELL PLATE MATURATION

Initial cell plate formation and cell plate anchoring are followed by a maturation phase in which, next to additional fusion events to close the fenestrae, endocytosis of excess membrane takes place (Samuels et al., 1995; Segui-Simarro et al., 2004). A question that remains unanswered is whether the closing of the fenestrae is performed by the same machinery as the initial fusion events. At least two nonredundant SNARE-mediated vesicle fusion pathways are involved in cell plate formation, utilizing either KN-KEULE-SNAP33-NSPN11-NSF or SYP31-CDC48 (Feiler et al., 1995; Lauber et al., 1997; Waizenegger et al., 2000; Heese et al., 2001; Rancour et al., 2002; Zheng et al., 2002). The first group is clearly essential for initial vesicle fusion events, as *kn* mutants fail to develop a plate (Lauber et al., 1997). SYP31 and CDC48 localize to the cell plate (Feiler et al., 1995; Rancour et al., 2002), but there is no information on whether they accumulate more in the center or at the leading edge. The finding that NSPN11 remained associated with the PM of recently completed cross walls (Zheng et al., 2002) suggests a function for the KN machinery during late vesicle fusion events.

It is estimated that about 70% of cell plate membrane is removed during the maturation process of the cell plate to a cell wall (Otegui et al., 2001). Membrane removal by endocytosis is necessary to establish the

identity of the cell plate membrane as PM and to re-establish polarity within the cell. This is embodied by the initial accumulation of the PIN2 protein at either side of the cell plate during the early phases of cell plate formation and endocytosis of PIN2 from the basal side of the cell once the cell plate is constructed. Side-specific removal of PIN2 from the basal PM is controlled by the sterol composition of the membrane, as the sterol biosynthesis mutant *cyclopropylsterol isomerase1-1* (*cpi1-1*) fails to remove PIN2 from the basal side of the fused cell plate following cytokinesis (Boutte et al., 2006; Men et al., 2008). Next to failure in establishing PIN2 polarity, *cpi1-1* shows cytokinesis defects, which are also reported for several other sterol biosynthesis mutants like *fackel* (*fk*), *hydra1* (*hyd1*), and *sterol methyltransferase1/cephalopod* (*smt1/cph*; Schrick et al., 2004; Men et al., 2008). For *fk*, *hyd1*, and *smt1/cph*, these defects are likely caused by defective cell plate maturation, as these mutants contain reduced levels of cellulose, together with ectopic callose and lignin deposits (Schrick et al., 2004). It would be of interest to investigate whether cellulose levels in the *cpi1-1* mutant are also reduced and whether the cytokinesis defects seen in these sterol mutants are linked with sterol-dependent membrane trafficking during cell plate maturation.

Endocytosed membranes from the center of the cell plate may also be recycled for peripheral membrane fusion to speed up cell plate formation, although no evidence for this has been reported so far. Interestingly, in BY-2 cells, clathrin as well as several DRP1 family proteins accumulate at the periphery of the cell plate, where vesicle fusion takes place (Hong et al., 2003; Kang et al., 2003a, 2003b; Tahara et al., 2007), which could reflect membrane recycling.

Independent of recycling, clathrin-mediated endocytosis removes excess membrane from the plate. Clathrin-coated vesicles and MVBs were observed at the newly formed cell plate using tomography (Samuels et al., 1995; Otegui et al., 2001; Segui-Simarro et al., 2004; Segui-Simarro and Staehelin, 2006). The volume and number of these MVBs increase during the late stages of cytokinesis, coinciding with enhanced clathrin-mediated endocytosis at the cell plate.

Similar to animal cells, clathrin-mediated endocytosis in plants is likely to be linked to phosphoinositide signaling (Simonsen et al., 2001; Lee et al., 2007). In agreement with this, phosphoinositides have been implicated in cell plate maturation. PATELLIN1 (PATL1), a SEC14-like protein that binds phosphoinositides, localizes to the cell plate and persists for some time after cytokinesis. PATL1 is likely to be involved in plate maturation, as it predominantly associates with the central region of the cell plate (Peterman et al., 2004).

Clathrin-mediated endocytosis of cell plate membranes would also require the function of dynamins in pinching off vesicles. The cell plate-localized dynamin DRP2A is a likely candidate to perform this function during cell plate maturation, as it localizes to the cell plate and is involved in trafficking from the TGN to the vacuole (Hong et al., 2003). Several members of the plant-specific dynamin family (DRP1) also remain present at the maturing region of the cell plate (Hong et al., 2003). Although DRP1A and DRP1C colocalize with clathrin at the cell cortex (Konopka and Bednarek, 2008), suggesting a function in endocytosis, their exact role during cytokinesis remains to be determined (for review, see Konopka et al., 2006). Endocytosis also removes KN from the cell plate, as KN accumulates in the MVB/prevacuolar compartment (Tse et al., 2004) in late mitotic cells (Reichardt et al., 2007), in agreement with the colocalization of KN in wortmannin-sensitive ARA7-positive endosomes (Dhonukshe et al., 2006).

Endocytosis from the plate to ARA7- and ARA6-positive MVBs (Haas et al., 2007; Jaillais et al., 2008) was recently suggested as an alternative degradation pathway to remove monoubiquitinated proteins by the ESCRT machinery (Winter and Hauser, 2006). In favor of this hypothesis, an Arabidopsis mutant in one of the ESCRT-I components (*elch*) was reported that produces multinucleated cells in several cell types. The protein colocalizes with ARA6- and ARA7-positive compartments and is thought to target a microtubule-associated protein for degradation (Spitzer et al., 2006). The animal homolog of *elch* has been reported to function in MVB formation and in midbody abscission, the final step of animal cytokinesis, suggesting a conserved role for the ESCRT machinery in vesicle formation at the MVB and cytokinesis in plants and animals (Hurley and Emr, 2006; Carlton and Martin-Serrano, 2007; Morita et al., 2007).

The presence of coatomer I (COPI) epitopes at the cell plate in maize and BY-2 cells suggests the removal of membrane by non-clathrin-coated vesicles (Couchy et al., 2003) and COPI machinery working not only at the retrograde transport between the Golgi and the ER but also at the cell plate. ER membranes gradually accumulate in the proximity of the cell plate during the late phases of cell plate formation (Segui-Simarro et al., 2004). Therefore, the presence of COPI proteins at the cell plate may point to trafficking events between the cell plate and the ER during the consolidation phase.

FUTURE PERSPECTIVES

Recently, it has become apparent that membrane trafficking during cytokinesis is not simply polarized secretion toward the cell equator (Fig. 1). Much progress has been made using high-resolution techniques like electron microscopic tomography and by analyzing marker proteins that label specific compartments. However, the necessity for additional markers to fine-tune and unambiguously mark various membrane compartments remains a challenge for the coming years. Many proteins, such as ROPs and RABs (for review, see this issue), that have key functions in membrane trafficking events in plants have already been identified. New insights into the process of vesicle

trafficking during cytokinesis will certainly emerge by analyzing the function of these proteins and their close homologues during cytokinesis. The recent developments in chemical genetics and high-throughput screening systems will no doubt provide scientists with new chemical compounds that specifically interfere with endocytic and exocytic trafficking that can be used as new tools to unravel the contribution of both pathways during the process of cell plate formation.

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