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Plant dynamin-related protein families DRP1 and DRP2 in plant development

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Abstract

Two separate families of *Arabidopsis* dynamin-related proteins, DRP1 and DRP2, have been implicated in clathrin-mediated endocytosis and cell plate maturation during cytokinesis. The present review summarizes the current genetic, biochemical and cell biological knowledge about these two protein families, and suggests key directions for more fully understanding their roles and untangling their function in membrane trafficking. We focus particularly on comparing and contrasting these two protein families, which have very distinct domain structures and are independently essential for *Arabidopsis* development, yet which have been implicated in very similar cellular processes during cytokinesis and cell expansion.

Introduction

Plant morphogenesis depends largely on three key processes: the timing of cell division, cell expansion and the establishment of the division plane, which involves the construction of the cell plate. Members of the DRP (dynamin-related protein) 1 and DRP2 families [1] have been implicated in the initiation and maturation [2–8] of the cell plate and in cell expansion [5–7] (Figure 1). In the present review, we summarize our current knowledge of the requirements for DRP1 and DRP2 proteins in plant development, with an emphasis on their role in membrane trafficking during plant cell cytokinesis and cell expansion.

Plant cytokinesis is achieved via *de novo* formation of the cell plate, which expands across the division plane to separate cytosol, organelles and nuclei of the daughter cells. Assembly of this unique organelle, which will mature into new PM (plasma membrane) and extracellular matrix (i.e. the cell wall), is initiated through the action of a cytoskeleton-based scaffold, the phragmoplast. During cytokinesis, Golgi-derived exocytic [9] and potentially endocytic [10,11] vesicles carrying membrane and cell wall components are trafficked to the phragmoplast mid-zone, where they fuse. Subsequent expansion and maturation of the cell plate occurs via the incorporation of additional endomembrane vesicles at the margins of the growing cell plate and retrieval of excess lipids and proteins via endocytic-like events. Ultimately, the cell plate fuses with the parental PM and cell wall.

Following cytokinesis, the two daughter cells rapidly expand. Plant cell expansion, which controls cell shape and ultimately plant morphology, is accomplished by the polarized targeting and localized release of secretory-pathway-derived membrane and cell wall material at specific sites on the PM. Directional cell expansion in plants occurs through two distinct mechanisms: diffuse polar and tip growth [12], which differ in terms of their polarized exocytosis and cytoskeletal requirements. Diffuse polar growth occurs in the majority of plant cells, and is characterized by the uniform incorporation of new PM and cell wall material across the expanding cell surface. In contrast, exocytic vesicles are targeted to a small area of the PM during asymmetric tip growth, resulting in a unidirectional expansion of the cell surface. The two best-characterized plant cell types that employ tip growth are pollen tubes and root hairs.

Polarized delivery of exocytic endomembrane-derived vesicles during cell plate formation and cell expansion must be balanced by selective retrieval of membrane and proteins via endocytosis [13]. Morphometric studies have estimated that $\geqslant$75% of the total membrane incorporated into the PM of an expanding cell or cell plate during cytokinesis is recycled [14–16], most likely via CME (clathrin-mediated endocytosis) [17–19]. In mammalian cells, CME involves a co-ordinated interplay of accessory and regulatory proteins that is initiated by binding of the cargo adaptor complex AP-2 (adaptor protein 2). Subsequently, the polymerization of clathrin triskelia, which are composed of CHC (clathrin heavy chain) and CLC (clathrin light chain) subunits, and the membrane remodelling activities of accessory proteins, including dynamin, are necessary for the invagination and release of the clathrin-coated bud [20].

Clathrin-coated structures at the PM of plant cells are evident by EM (electron microscopy) [21–25], and homologues of CHC, CLC, AP-2 subunits and several other mammalian accessory proteins are present in the *Arabidopsis* genome [26,27]. Expression of a DN (dominant-negative) CHC inhibits uptake of the lipophilic tracer dye FM4-64

Key words: cell plate, clathrin, cytokinesis, dynamin-related protein, endocytosis, lipid.

Abbreviations used: AP, adaptor protein; ARF, ADP-ribosylation factor; *At*, *Arabidopsis thaliana*; CHC, clathrin heavy chain; CLC, clathrin light chain; CME, clathrin-mediated endocytosis; DN, dominant-negative; DRP, dynamin-related protein; EM, electron microscopy; FFP, fluorescent fusion protein; GED, GTPase effector domain; GFP, green fluorescent portin; *Gm*, *Glycine max*; MT, microtubule; PATL1, patellin1; PH, pleckstrin homology; PM, plasma membrane; PRD, proline-rich domain; PS, phosphatidylserine; SH3, Src homology 3; TGN, *trans*-Golgi network; TyrA23, tyrphostin A23; VAEM, variable-angle epifluorescence microscopy.

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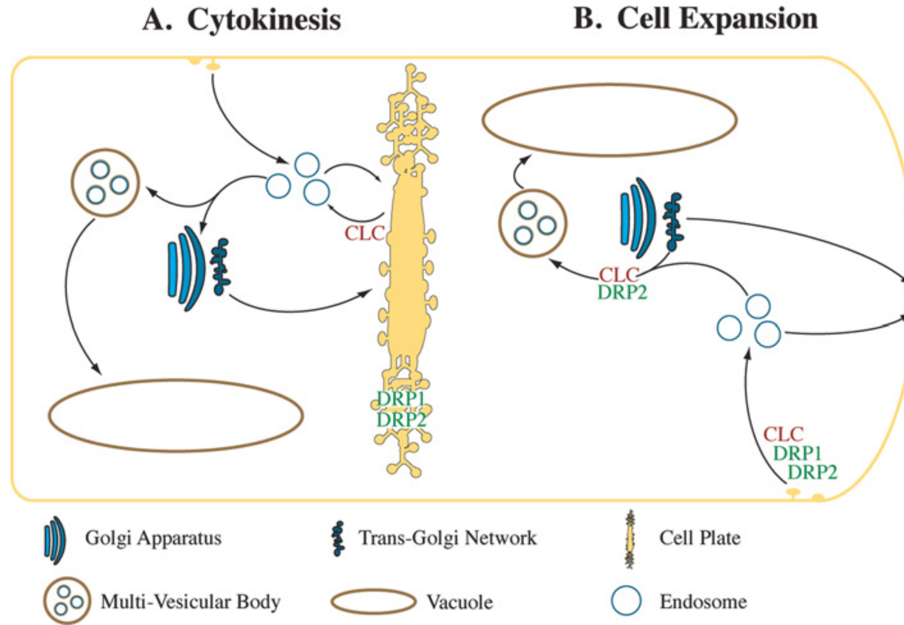
Figure 1 | DRP1 and DRP2 in cytokinesis and cell expansion

(**A**) During cytokinesis, Golgi- and early-endosome-derived vesicles fuse at the division plane to form the cell plate. Consolidation and maturation of the cell plate requires DRP1 and DRP2, which may act through clathrin-dependent and -independent processes. (**B**) In tip-growing or diffuse-growing plant cells, proteins, lipids and cell wall material are likely to be delivered via multiple exocytic pathways toward the PM. Membrane is recycled via DRP1, DRP2 and clathrin-dependent and possibly -independent mechanisms from the PM and delivered to an endocytic compartment.



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and several PM proteins [25,28]. In addition, use of the mammalian AP-2 adaptor complex inhibitor TyrA23 (tyrphostin A23), causes a reduction in endocytosis [25]. Although the existence of CME in plants is now widely accepted, our understanding of the molecular machinery responsible for the regulated uptake of membrane and endocytic cargo [29–31] is rudimentary. Among the proteins critical for plant cell CME are members of the dynamin superfamily, which are the focus of this review.

Dynamin and dynamin-related proteins

Dynamin and dynamin-related proteins are a superfamily of structurally related, but functionally diverse, high-molecular-mass GTP-binding proteins [32,33]. All members contain a conserved N-terminal GTP-binding (GTPase) domain and a C-terminal assembly domain/GED (GTPase effector domain). In addition, animal dynamin, the founding and most well-characterized member of the superfamily, contains a PH (pleckstrin homology) domain and a C-terminal PRD (proline-rich domain), which have been demonstrated to bind phosphoinositides and to interact with clathrin coat accessory proteins via SH3 (Src homology 3) domains respectively [34]. Dynamin subunits are recruited early in the process of CME, eventually forming oligomeric rings and spirals around the necks of the invaginating clathrin-coated buds [35]. GTP-hydrolysis-mediated conformational changes in the dynamin oligomer [36] and the activity of additional membrane modifying proteins promotes the release the clathrin-coated vesicle from the PM.

Plants have six distinct dynamin-related protein families (DRP1–DRP6) on the basis of their predicted domain structure and amino acid conservation [1]. Members of the DRP3–DRP6 families function in mitochondrial, chloroplast [1] and peroxisome [37] biogenesis/maintenance and/or have no known function. An exception to this is DRP5A, which has recently implicated in cytokinesis [38]. DRP5A is expressed in a cell-cycle-dependent manner and was observed to initially appear during prophase as bright spots around the nucleus, which subsequently become localized to the division plane during cytokinesis. Consistent with a role in cytokinesis, *drp5A* null mutants display defects in cell plate maturation in the root, but only at elevated temperatures [38]. The mechanistic details of the function of DRP5A in cytokinesis remain to be determined. For the remainder of this review we will focus only on the DRP1 and DRP2 families.

In vivo analysis of DRP1 and DRP2

Of the *Arabidopsis* dynamin-related proteins, DRP2A/DRP2B are the most similar in domain structure and organization to mammalian dynamin 1 (Figure 2), and are thus are hypothesized to represent the *bona fide* dynamins in plants, even though they have only approx. 20% amino

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Figure 2 | Modular domain structure of DRP2 and DRP1

Dynamin and dynamin-related proteins contain an N-terminal GTPase domain followed by a conserved middle region and a C-terminal GED. The region between the middle and GED domains has low homology within the plant dynamin superfamily. DRP1A–DRP1E isoforms can be distinguished from one another by a ∼15-amino-acid hypervariable hydrophilic region preceding the GED. Identical and conserved amino acid residues are outlined in black and grey respectively.

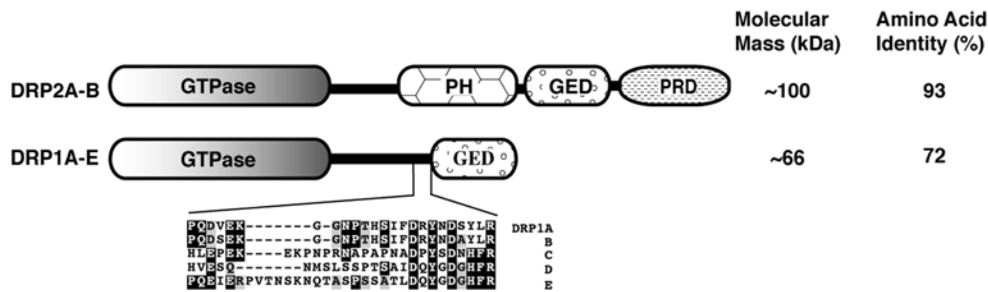
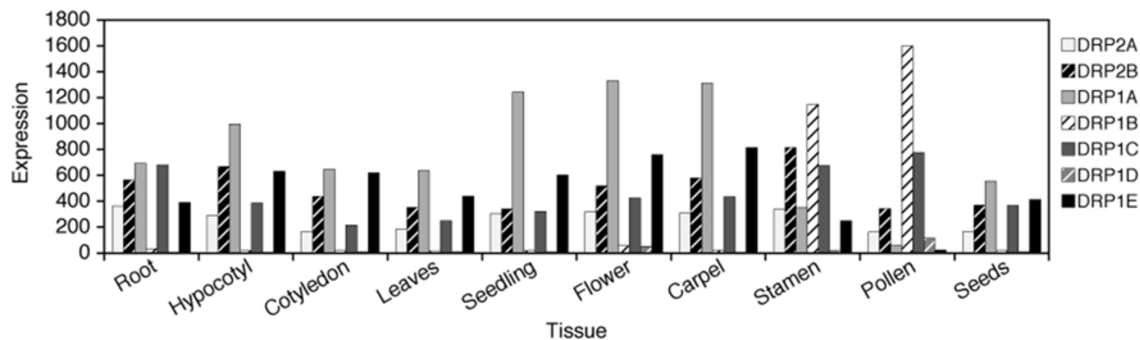


Figure 3 | *Arabidopsis DRP1* and *DRP2* expression profiles

Expression profiles of genomic *DRP1* and *DRP2* at selected stages of development in wild-type *Arabidopsis* were analysed using the AtGenExpress Visualization Tool (AVT; http://jsp.weigelworld.org/expviz/expviz.jsp) [88]. Tissue, stage/age: root, 7 days; hypocotyl, 7 days; cotyledon, 7 days; leaves, first and second/7 days; flowers, stage 9; carpels, stage 12 flowers; stamens, stage 12 flowers; pollen, mature/6 weeks; seeds, siliques/8 weeks.



acid identity with mammalian dynamin 1. Both *DRP2* homologues appear to be expressed throughout development in *Arabidopsis* (Figure 3) and, on the basis of their high degree of amino acid sequence identity (93%), are likely to be partially or completely functionally redundant. Transient overexpression analysis of a DN form of DRP2A in protoplasts has suggested a function for this dynamin-related protein in vesicular trafficking from the Golgi to the vacuole [39]. Interestingly, individual *drp2A* and *drp2B* null mutants show no gross defects in *Arabidopsis* morphogenesis, whereas *drp2A*/*drp2B* double mutants display developmental arrest defects in both female and male gametogenesis (S.K. Backues and S.Y. Bednarek, unpublished results). Similar to mammalian dynamin 1, GFP (green fluorescent protein)-tagged forms of DRP2A and DRP2B localize to the TGN (*trans*-Golgi network) [39,40], PM and cell plate [8,40], suggesting that these protein may be involved in multiple membrane trafficking steps at the TGN/endosomal compartment and PM.

In contrast with the DRP2s, the DRP1 subfamily appears to be plant-specific [1]. In *Arabidopsis*, this family comprises five highly-related members, DRP1A–DRP1E [4], which share approx. 65–84% amino acid sequence identity with the soya bean dynamin-like protein, phragmoplastin [*Gm*DRP1 (*Glycine max* DRP1)] [2]. Unlike *DRP2A* and *DRP2B*, *Arabidopsis DRP1* homologues show highly variable and tissue-type expression profiles (Figure 3) and *drp1A*, *drp1C* and *drp1E* null mutants display distinct phenotypes. Consistent with a role of DRP1 proteins in cytokinesis, *drp1A*^{rsw9} (Columbia ecotype; Col) and *drp1A;drp1E* (*drp1A;E*) double mutants (Wassilewskijia ecotype; WS) display defects in cell plate assembly in roots and arrested embryos respectively [5,7]. Interestingly, *drp1A*^{WS} null mutants do not show the root swelling or cytokinetic defects observed in *drp1A*^{rsw9Col} plants; instead, an early seedling arrest (perhaps due to defects in cell expansion) is apparent under some growth conditions. These differences may point to ecotype differences in expression level of the DRP1 family members or other differences in the processes of endocytosis and cell wall deposition between ecotypes.

In addition to differences in expression levels, it is likely that the *Arabidopsis* DRP1 homologues may have distinct

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functions and/or be only partially redundant. In developing pollen grains, multiple *DRP1* homologues are expressed, with the highest expression being shown by *DRP1B* and *DRP1C*. Yet, *drp1C* mutant pollen grains show PM defects and collapse during desiccation [6], whereas single *drp1A*^{rsw9Col} [7] and *drp1B*^{WS} [6] or the double *drp1A;E*^{WS} [5] null mutants display no defects in male gametogenesis. Furthermore, exogenous expression of DRP1C under control of the *DRP1A* native promoter can only partially compensate for the lack of DRP1A in *drp1A* mutant plants, and DRP1C and DRP1A proteins displays subtle differences in their dynamics at the PM [41]. Therefore differences in expression patterns, as well as functional differences at a protein level between the DRP1 isoforms, probably explain the distinct phenotypes observed in the single *drp1* null mutants. A key to these differences may be found in the hypervariable region preceding the GED of DRP1A–DRP1E (Figure 2), which may be a site of differential regulation or protein–protein interactions.

Despite these isoform- and ecotype-specific differences, a unifying theme observed in *drp1* null mutants is the requirement for DRP1 function in cytokinesis and/or cell expansion. The defects displayed in cytokinesis-defective *drp1A;E*^{WS} embryonic cells closely match those in the embryonic lethal cytokinesis-defective mutant, *cyt1*, and in embryos treated with an inhibitor of cellulose biosynthesis [42,43], suggesting that loss of DRP1A and DRP1E proteins affects normal cell wall biosynthesis and/or PM biogenesis. Indeed, *drp1A;E*^{WS} double and *drp1A*^{WS} and *drp1C*^{WS} single mutants display defects in polarized cell expansion, with mutant cells showing abnormal cell walls and PM proliferation. On the basis of these mutant phenotypes, we have hypothesized previously that the defects in cell wall formation and PM morphology observed in *drp1* mutants results from defects in the endocytic trafficking of PM lipids and proteins, including enzymes involved in cell wall biosynthesis [5,6]. Subsequent isolation and analysis of the *drp1A*^{rsw9Col} mutant have strengthened this idea, as *drp1A*^{rsw9Col} mutant cell walls have reduced cellulose content, decreased endocytic uptake of the lipophilic sterol dye FM4-64 relative to wild-type [7] and defects in the restriction of the cell-plate-associated syntaxin KNOLLE to the cell plate during late cytokinesis [44].

Interestingly, the *edr3* mutant, which confers resistance to powdery mildew in a salicylic-acid-dependent manner, was recently shown to be the result of an amino acid replacement (P77L) in the GTPase domain of DRP1E [45]. This pathogen-resistant phenotype was not observed in a *drp1E* T-DNA (transferred DNA) line and is therefore related to the synthesis of a GTP-hydrolysis-defective form (i.e. DN) of DRP1E, even though the *edr3* mutation is recessive in nature. Further research will be required to understand this interesting phenotype and whether it has any connection to known roles of the DRP1s in cell expansion and cell division or reflects a separate cellular function.

In addition to the roles for DRP1 discussed above, DRP1s have also been suggested to be required for thylakoid morphogenesis [46], mitochondrial biogenesis and signalling [45,47], trafficking of secretory vesicles along phragmoplast MTs (microtubules) [8] and vesicle budding from the TGN [48]. The proposed role for DRP1 in thylakoid morphogenesis is puzzling as all five *Arabidopsis* DRP1 homologues are nuclear-encoded and lack any recognizable chloroplast-transit sequences. Furthermore, the suggested roles for DRP1 isoforms at the mitochondria and phragmoplast MTs were based on experiments using DN DRP1s and/or the subcellular localization analysis of heterologously overexpressed FFP (fluorescent fusion protein)-tagged DRP1s and have not been supported by other studies using immunolocalization, subcellular fractionation and/or native-promoter-driven FFP constructs [4,8,41,49]. Caution with the interpretation of the subcellular distribution of overexpressed FFP-tagged DRPs is suggested, and should be confirmed through additional complementary approaches. An additional concern with the use of DN *drp1* and *drp2* mutants is the possibility that they may interact with, and thereby inhibit, other dynamin superfamily members, thus complicating the analysis of the resulting phenotypes (see below).

DRP1 and DRP2 localization and dynamics

Immunolocalization studies using DRP1 antibodies and native promoter-driven functional DRP1A–FFP and DRP1C–FFP expressed in *drp1A* and *-1C* null mutants show localization of DRP1A, -1C and -1E to the forming cell plates, consistent with the requirement for DRP1s in cytokinesis [2,4–8,50,51] (Figure 4). Both DRP1A and DRP1C are both very early and late markers of cell plate initiation and maturation. During cell plate initiation and expansion DRP1A and DRP1C localize prominently to the leading edge of the organelle, and DRP1A has been detected by immuno-EM at ring-like structures that encircle constricted regions of syncytial-type cell plates during endosperm cellularization [18]. Slightly reduced levels of DRP1A and DRP1C are observed within the central region of the cell plate, where clathrin-coated vesicles have been observed [17–19], which are presumably involved in the recycling of excess cell plate material. DRP1A and DRP1C also linger at the newly formed cross walls shortly after fusion of the cell plate with the parental cell PM.

Throughout the cell cycle, DRP1A– and DRPC–FFPs also show both a partial cytoplasmic localization, as well as association with the cell cortex. In the case of DRP1C, this localization to the cell cortex is particularly intense in areas of the cell that are undergoing rapid expansion, such as the tips of growing pollen tubes and root hairs (Figures 4B–4D) [51]. Time-course studies of expanding root hairs demonstrated that DRP1C–GFP localizes predominantly at the subapical flanks of the growing tip (Figure 4) [51] where clathrin-coated structures are abundant [23], compared with the apical dome where membrane addition is occurring.

Visualization of the cell-cortex-associated pool of DRP1A– and DRPC–FFPs with high-temporal and -spatial

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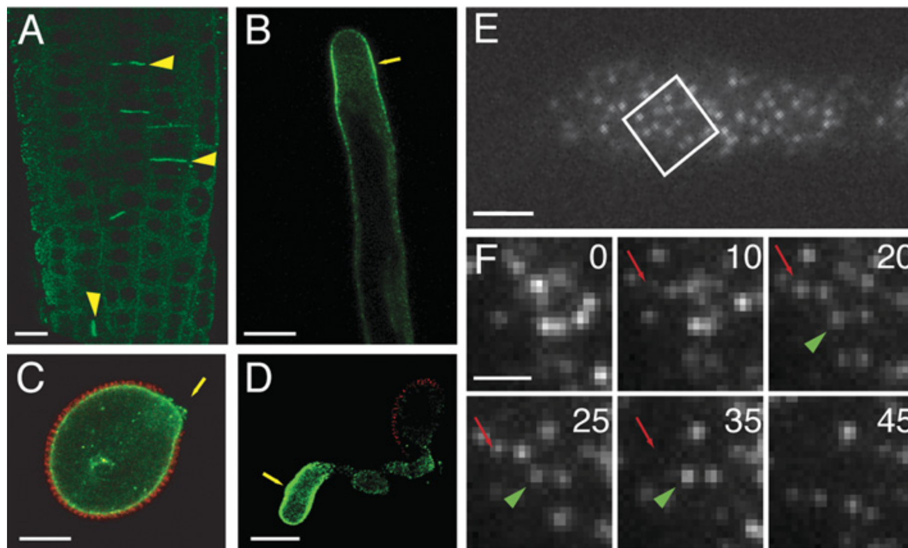
Figure 4 | **DRP1–FFPs localize to regions of active membrane trafficking**

(**A** and **B**) Laser-scanning confocal microscopy image of DRP1C–GFP (green) in cell plate of dividing cortical root cells (yellow arrowheads) and at the subapical PM (yellow arrow) of an expanding root hair. DRP1A, DRP2A and DRP2B show a similar localization at the cell plate in dividing *Arabidopsis* root cells. (**C** and **D**) Pollen from a *drp1C-1/DRP1C–GFP* plant imaged with laser-scanning confocal microscopy. DRP1C–GFP (green) and autofluorescence of pollen coat (red) are evident. DRP1C–GFP is present at the PM at the aperture of the germinating pollen grain (**C**) and at the distal end of the pollen tube (**D**). (**E** and **F**) DRP1C–GFP expressing root hair PM imaged with VAEM showing protein in discreet dynamic foci. Time series of boxed area in with two foci indicated with arrow (red) and arrowhead (green) that appear gradually and then vanish over time course (**E**). Numbers indicate elapsed time from start of imaging in s. Scale bars, 10 μm (**A–D**); 2 μm (**E**); 1 μm (**F**). Images reprinted from [51] (http://www.plantcell.org) with permission. Copyright American Society of Plant Biologists.



resolution by VAEM (variable-angle epifluorescence microscopy) [41,51] revealed that these protein were not uniformly distributed within the plane of the PM, but instead form mobile foci. These foci have dynamics similar to clathrin-associated mammalian dynamin [52], and partially co-localize with CLC–FFPs [41,51]. The mobility of these foci is inhibited by treatment with TyrA23, which has been demonstrated to block plant endocytosis [25]. These analyses support the hypothesis that DRP1A and DRP1C are components of the CME machinery in plants. Interestingly, TyrA23 treatment did not affect the gross localization of DRP1 to the cell plate, suggesting that DRP1 cell plate localization maybe independent of cell-plate-associated clathrin and/or its accessory adapter proteins [51].

Unlike in animal and yeast cells where there is little evidence of MT involvement in CME, MT dynamics affect DRP1C and CLC foci formation [51]. Upon treatment with a MT depolymerization agent, the DRP1C–GFP and CLC–FFP foci were more stable and longer lived. This was surprising because neither DRP1C–GFP nor CLC–GFP foci are organized in filamentous-like arrays or move in a linear fashion [41,51] that is typical of other MT-associated proteins [53,54]. However, clathrin-coated structures clustered around cortical MTs have been observed in EM micrographs [24] and purified *Gm*DRP1 has been reported to bind MTs *in vitro* [8]. In contrast with MT disruption, disruption of actin dynamics with latrunculin B only showed an effect on foci dynamics at concentrations that stop cytoplasmic streaming. Likewise, treatment with 2,3-butanedione monoxime, a myosin inhibitor that also interferes with cytoplasmic streaming, halted foci dynamics. We hypothesize that cytoplasmic streaming may promote endocytosis in plant cells, whereas cortical MTs act as a trellis for stabilization of the endocytic protein network or as a diffusion barrier similar to the cortical actin network in mammalian cells [55]. If this hypothesis is correct, then the mechanism of DRP1-mediated endocytosis would differ significantly from that observed in yeast and mammalian cells, where actin polymerization helps drive CME and MTs are not thought to be involved.

In contrast, a common feature of endocytosis in yeast, mammals and plants is a requirement for PM sterols [56]. Studies of yeast sterol biosynthetic mutants [57] and mammalian cells treated with the sterol-depleting drug methyl-β-cyclodextrin [58] show defects in endocytosis of specific cargo. Similarly, endocytosis defects are seen in an *Arabidopsis* sterol biosynthesis mutant, *cpi1-1* [59], which shows synthetic lethality with the *drp1A*^{rsw9Col} mutant [44], and treatment of plants with the sterol synthesis inhibitor fenpropimorph inhibits the dynamics of DRP1A, DRP1C and CLC at the cell cortex [41,51].

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DRP2A– and DRP2B–FFPs expressed under viral [61] or native (S.K. Backues and S.Y. Bednarek, unpublished work) promoters show patterns of localization nearly identical with those of DRP1 in *Arabidopsis* roots, including a precise temporal co-localization with DRP1A at the growing edges and maturing regions of forming cell plates [61]. In addition, DRP2B–GFP expressed in suspension-cultured tobacco cells [40] or under the native promoter in *Arabidopsis* roots (S.K. Backues and S.Y. Bednarek, unpublished results) forms foci at the cell cortex, with an appearance similar to those formed by DRP1A and DRP1C. The dynamics and drug sensitivities of these foci have not been studied, nor is it known whether these foci co-localize with either clathrin or DRP1A or DRP1C; however, their appearance is suggestive of a similar role for DRP2 in endocytosis. An important caveat of all DRP2–FFP localization studies to date is that none of the constructs used have been shown to be functional (i.e. through complementation analysis of *drp2* mutants). Without this direct evidence questions remain whether the observed localization and dynamics of DRP2–FFPs accurately reflect the behaviour of the endogenous proteins.

Dynamin-related protein interaction networks

Animal dynamin participates in a large network of protein–lipid and protein–protein interactions at sites of endocytosis [62]. The large number of low-affinity interactions not only serves to recruit each component specifically to endocytic sites, but also contributes to the regulation of the entire complex, allowing productive bud formation only if every piece is in place [62,63].

To date, too few DRP1 or DRP2 interaction factors have been described to assemble a tenable model of the role of these dynamin-related proteins in regulating membrane dynamics involved in cell plate biogenesis and endomembrane trafficking. In the next section, we summarize our current knowledge of the interactions of DRP1 and DRP2 with lipids and proteins.

DRP1– and DRP2–lipid interactions

Animal dynamin-1 binds via its PH domain to PtdIns(4,5)P_2 [64,65], which is found in relatively high concentrations throughout the inner leaflet of the PM [66]. PtdIns(4,5)P_2 functions in the recruitment of dynamin to the PM, and also has a positive role in clathrin bud maturation. Specifically, the recruitment of phosphoinositol phosphatases to the maturing bud acts as part of a bud maturation checkpoint by destabilizing non-productive complex interactions and thereby promoting the abortion of non-productive vesicle buds [67]. Dynamin I activity is probably also a critical determinant in this regulatory decision [68].

DRP2A, similar to dynamin-1, has a PH domain (Figure 2) that binds PtdIns(4,5)P_2, as well as PtdIns3*P* and PtdIns4*P* [69,70]. In contrast, full-length DRP2A or the C-terminal half of DRP2A shows a preference for PtdIns3*P*, with less binding to PtdIns4*P* and no binding to PtdIns(4,5)P_2 in lipid-overlay assays. The binding to PtdIns3*P* and PtdIns4*P* was verified by liposome sedimentation [70]. PtdIns3*P* is reported to localize to the endosomes [71], whereas PtdIns4*P* is found at the Golgi, the PM and the newly formed cell plate [72]. Consistent with the subcellular localization of PtdIns3*P* and PtdIns4*P*, DRP2A [39,69] and DRP2B [61] have been reported to be associated with Golgi/endosomes, PM and cell plate, suggesting that protein–lipid interactions play a role in targeting of DRP2; whether these interactions also play a regulatory role in DRP2 action at the target membranes remains to be determined.

In contrast with DRP2, DRP1 isoforms do not contain PH or other identifiable lipid-binding domains that may function in their targeting and association with the PM and cell plate. However, in preliminary liposome-binding studies, purified DRP1A interacted with PtdIns3*P*, PtdIns4*P*, PtdIns5*P* and PS (phosphatidylserine) [72a]. Given the high levels of PS (approx. 10 mol%) in the inner leaflet of the plant PM relative to other negatively charge phospholipids [73,74], binding of DRP1A to PS is probably the dominant interaction involved in recruiting DRP1A to the PM (i.e. docking). Conversely, this interaction is unlikely to serve a regulatory role (at specific sites of action), because of the constitutive presence of PS within the PM.

DRP1– and DRP2–protein interactions

Although many of the proteins involved in clathrin-mediated endocytosis are conserved in *Arabidopsis*, direct homologues of many of the dynamin-1-associated proteins (e.g. amphiphysin, intersectin and cortactin) have not been identified in the *Arabidopsis* genome [75]. However, proteins with similar domains, such as ENTH, BAR and SH3 domains, have been identified [27,76,77]. SH3 domains, in particular, are known to serve as dynamin-binding motifs, interacting with the C-terminal PRD of dynamin. Three closely related proteins with C-terminal SH3 domains are found in *Arabidopsis*, and one of these, *At*SH3P3 (*Arabidopsis thaliana* SH3P3), binds the C-terminal PRD of DRP2A [69]. These SH3 proteins lack the N-terminal membrane-bending BAR domain found in amphiphysin, but in its place have an unidentified α-helical domain that may also interact with membranes, perhaps allowing SH3P3 to play an analogous role [77]. Consistent with a role for DRP2 in trafficking between the TGN and vacuole, DRP2A also binds γ-adaptin, a subunit of the AP-1 complex involved in clathrin-mediated trafficking at the Golgi in animals, through another PXXP motif found in the GED of DRP2A [69]. Plant dynamins may of course also have additional interactions that have not been described in animal endocytosis; for example, *At*Seh1 binds to the C-terminus of DRP2A, thereby blocking its interaction with PtdIns3*P* and potentially regulating its membrane association *in vivo* [78]. Further studies of these DRP2-binding partners and the identification of additional interactors will be necessary to define the

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interaction network of clathrin-mediated trafficking in plants.

Recently, a rice dynamin that is homologous with the *Arabidopsis* DRP2 family was reported to interact with rice GIGANTEA, a component of the circadian clock [79], raising the possibility that this interaction is also conserved in *Arabidopsis*. However, it is unclear if and how this would relate to membrane trafficking processes.

Three DRP1-binding proteins have been identified to date: VAN3 and two enzymes involved in callose synthesis at the cell plate. Callose, a β-1,3-glucan polymer, is a major polysaccharide component of the developing cell plate whose deposition precedes the synthesis of cellulose [17]. It has been suggested that callose deposition within the developing cell plate provides a spreading force that widens the tubules and converts the network into a fenestrated sheet [9,17]. Two callose synthases GSL6 and GSL8 [80,81] were found to be associated with the cell plate and defects in *GSL8* results in cytokinetic abnormalities [81]. The soya bean DRP1 homologue, *Gm*DRP1, interacts with GSL6 and with a cell-plate-specific UDP-glucose transferase [80,82]. Thus DRP1A may help to localize enzymes required for callose biosynthesis at the cell plate through their direct interaction with membrane-associated DRP1. Alternatively, DRP1 may function in the recycling of these enzymes via endocytosis.

VAN3, an ARF (ADP-ribosylation factor) GAP (GTPase-activating protein) that probably regulates membrane trafficking at the TGN, was found to interact with DRP1A via yeast two-hybrid and co-immunoprecipitation assays [48,76]. Both *van3* [76] and *drp1A*^{WS} [48] loss-of-function mutants display defects in vascularization. The *van3*/*drp1A*^{WS} double mutants fail to germinate or show enhanced defects in vascular structure relative to the single mutants, indicating genetic interaction between *DRP1A* and *VAN3* [48]. Consistent with a role of DRP1A in ARF-dependent trafficking, *DRP1A* was also shown to genetically interact with *GNOM* [48], which encodes an ARF GEF (guanine-nucleotide-exchange factor) involved in membrane recycling between the PM and endosomes [83]. Sawa et al. [48] postulated, on the basis of upon transient expression analysis of DRP1A– and VAN3–FFPs in protoplasts, that these proteins function co-ordinately in vesicle trafficking at the TGN. However, stable expression of DRP1A–FFPs under their native promoter in *Arabidopsis* roots does not show significant TGN localization, leaving it unclear whether the interaction of DRP1A with VAN3 at the TGN is only transient and possibly stabilized under the assay conditions utilized in the analysis of VAN3 and DRP1A co-localization [48].

Lastly, DRP1A, β-adaptin and PATL1 (patellin1) were found to co-purify with several multidrug-resistance/P-glycoproteins by affinity chromatography of a detergent-resistant microsomal membrane fraction on immobilized naphthylphthalamic acid [84]. PATL1 is a SEC14-like phosphoinositol lipid transfer protein that is recruited to the cell plate during late cytokinesis and, like DRP1A and DRP1C, persists for a time in the newly formed cross wall post-telophase [85]. However, unlike DRP1, PATL1 is not associated with the leading edge of the cell plate, but instead exclusively with the region undergoing maturation and membrane recycling. In plant cells, β-adaptin is presumed to be a subunit of both the PM AP-2 and Golgi-associated AP-1 clathrin-coated vesicle cargo–adaptor complexes [86]. The association of DRP1A with VAN3, PATL1 and β-adaptin further support a role for the DRP1 family in clathrin-mediated vesicular transport between the PM and TGN/endosomes, as well as in clathrin-mediated membrane retrieval at the cell plate.

All known dynamin superfamily members have been shown to self-interact to form large homopolymeric spirals, and this ability is shared by members of the DRP1 and DRP2 families [8,70,87]. Given the sequence homology between the polymerization domains of the DRP1 and DRP2 family members, as well as the fact that all of these seem to show generally similar subcellular localizations at the PM and cell plate [41,61], a major question is whether these dynamin-related protein may also form heteropolymers and whether or not these mixed subunit protein complexes have any distinct functional roles. Indeed, yeast two-hybrid interactions between *Gm*DRP1A and both *Arabidopsis* DRP1 and DRP2 family members have been reported [8]. However, an *in vivo* demonstration of DRP1 and DRP2 intra- or inter-family heteropolymerization through co-immunopurification, Förster resonance energy transfer and/or bimolecular fluorescence complementation will be essential. Such studies would also give insight into the physical properties and cellular roles of these putative heteropolymers, which have not been reported for dynamin superfamily members in any other organism, and might be unique to plant cells.

Perspectives

The DRP1 family of plant-specific dynamins play essential roles in cell division and polarized cell expansion, and participate in clathrin-mediated endocytosis at the cell cortex. Similarly, the DRP2 family of classical dynamins are essential for plant development, localize to the forming cell plate, and appear to participate in clathrin-mediated trafficking at the Golgi apparatus and possibly cell cortex. However, the DRP1 and DRP2 families do not function redundantly, and many questions about their function remain to be untangled. Key among these questions are: (i) What are the DRP1s (and DRP2s) doing at the cell plate during cytokinesis, and is it related or unrelated to their endocytic function(s) at the cell cortex; and (ii) are there separate endocytic pathways utilizing different dynamin family members (DRP1A compared with DRP1C compared with DRP2s), and, if so, do these pathways carry distinct cargo or function in distinct types of transport or signalling or at different stages of plant development?

The role of DRP1 and DRP2 in clathrin-mediated membrane trafficking at the PM and/or Golgi suggests that they may also be involved in clathrin-mediated membrane recycling at the cell plate during cytokinesis. In addition, these dynamin-related proteins are associated with the leading

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edge of the cell plate where Golgi-derived exocytic vesicles fuse. Hypothetical functions for dynamin-related-protein-containing membrane encircling rings [18] at the leading edge of the cell plate include: (i) formation and or stabilization of cell plate membrane tubules; (ii) generation of regions of high membrane curvature that could promote membrane fusion [17]; and (iii) targeting or restricting the lateral diffusion of enzymes (e.g. callose synthases) and other proteins required for cell plate formation and maturation. More must be done to test these hypotheses and to unravel the individual and perhaps combined contributions of DRP1 and DRP2 isoforms both during cell plate formation, as well as during endocytosis. Co-localization of FFP-fused endocytic cargo, as well as other accessory proteins and the characterization of dynamin-related-protein interactions with other proteins and lipids, will be necessary to assemble a clear picture of dynamin-related-protein-dependent endocytic network(s). Finally, functional studies of different types of endocytosis, perhaps in single mutants or carefully targeted knockdowns, will provide the link between each protein and its actual developmental role, and begin to untangle the network of DRP1 and DRP2 functions in plant cells.

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