Cell Plate Restricted Association of DRP1A and PIN Proteins Is Required for Cell Polarity Establishment in Arabidopsis

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Summary

The polarized transport of the phytohormone auxin [1], which is crucial for the regulation of different stages of plant development [2,3], depends on the asymmetric plasma membrane distribution of the PIN-FORMED (PIN) auxin efflux carriers [4,5]. The PIN polar localization results from clathrin-mediated endocytosis (CME) from the plasma membrane and subsequent polar recycling [6]. The Arabidopsis genome encodes two groups of dynamin-related proteins (DRPs) that show homology to mammalian dynamin—a protein required for fission of endocytic vesicles during CME [7,8]. Here we show by coinmunoprecipitation (coIP), bimolecular fluorescence complementation (BiFC), and Förster resonance energy transfer (FRET) that members of the DRP1 group closely associate with PIN proteins at the cell plate. Localization and phenotypic analysis of novel drp1 mutants revealed a requirement for DRP1 function in correct PIN distribution and in auxin-mediated development. We propose that rapid and specific internalization of PIN proteins mediated by the DRP1 proteins and the associated CME machinery from the cell plate membranes during cytokinesis is an important mechanism for proper polar PIN positioning in interphase cells.

Results and Discussion

To identify proteins associated with PIN-FORMED (PIN) proteins that may be required for their function and trafficking, we initially utilized coimmunoprecipitation (coIP) of green fluorescent protein (GFP)-tagged PIN1 (the most prominent member of the PIN family) from seedlings expressing pPIN1::PIN1-GFP, followed by trypsin digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. Among the proteins identified, we found the dynamin-like protein DRP1A with 14 peptide hits (Table 1). Label-free relative quantification revealed that DRP1A was one of the most enriched proteins when compared to the wild-type control. Interestingly, the DRP1A protein as well as some other significant hits from the coIPs (such as HSP70) had previously been found in the complexes binding the auxin transport inhibitor naphthylphthalamic acid (NPA) [9]. In addition, the presence of the AVP1 regulator of auxin transport [10] in the fraction further indicates the specificity of the results and the plausibility of the association between PIN and DRP1 proteins (see Table S1 available online; Figure S1).

The dynamin superfamily is formed of structurally related, but functionally diverse, high-molecular weight GTPases generally functioning in membrane scission and tubulation [11,12]. In plants, two distinct families of dynamin-related proteins (DRPs), DRP1 and DRP2, have been implicated in clathrin-mediated endocytosis (CME). Members of the DRP2 family are most similar in domain structure to the mammalian dynamin. In contrast, the DRP1 members are plant specific and lack any recognized lipid or protein interaction domains (pleckstrin homology and proline rich) found in dynamin and DRP2 that are required for the interaction with the clathrin-coat accessory proteins and lipids, respectively [8]. The Arabidopsis DRP1 family consists of five members (isoforms A–E), and recent evidence suggests that they play a role in the membrane dynamics during CME, but their precise function in this process is not well understood [13,14]. drp1 mutants display defects in cell plate biogenesis during cytokinesis, polarized cell expansion, altered cell wall composition, and decreased endocytic uptake of the lipophilic sterol dye FM4-64 [15–17]. DRP1A and DRP1C predominantly localize to the cell plate as well as the plasma membrane of expanding and fully expanded interphase cells [13,15,16,18].

To determine where in the cell the PIN and DRP1 proteins co-localize in planta, we generated an Arabidopsis line expressing DRP1A-mRFP1 under the control of the strong 35Spro::CaMV constitutive promoter. The 3SSpro::DRP1A-mRFP1 construct rescued the developmental phenotypes of drp1a mutant plants (Figure S2A) and its subcellular distribution (Figures S2B–S2E) was similar to that observed for the native DRP1A and the DRP1A-GFP fusion protein (Figures S2H and S2I) [15], demonstrating that the DRP1A-mRFP1 fusion protein is functional. Time-lapse imaging of Arabidopsis roots showed that DRP1A-mRFP1 predominantly accumulates at the growing cell plate and largely disappears after cell plate fusion with the plasma membrane (Figure 1A; Figure S2), resembling the dynamic distribution of the syntaxin KNOLEE [19]. Faint plasma membrane staining was also observed in interphase cells. However, when 3SSpro::DRP1A-mRFP1 was introgressed into pPIN1::PIN1-GFP and pPIN2::PIN2-GFP lines, the most prominent colocalization of PIN-GFP and DRP1A-mRFP1 was observed at the growing cell plates (Figure 1A; Figures S2C–S2F).
and S2I). As reported, no PIN polarization was observed before the completion of the cell plate [20]. Within the maturing regions of the cell plate, DRP1A also colocalized with clathrin light chain-GFP (CLC-GFP) (Figure 1 B), suggesting that clathrin and DRP1A function in the retrieval of PIN1 from the cell plate.

Next we tested the association of PIN1 and DRP1 proteins by in vivo methods. We used bimolecular fluorescence complementation (BiFC) in Arabidopsis protoplasts or in seedlings coexpressing DRP1A and the PIN1 hydrophilic loop, fused with the C- or N-terminal parts of the yellow fluorescent protein (YFP), respectively [21]. We observed both oligomerization of DRP1 proteins and interaction with PIN1 that were confined predominantly to the growing cell plate, although a weak staining was also detected at the plasma membrane (Figure 2A; Figure S3A). To independently test the association of DRP1 and PIN1 at the cell plate, we used the Förster resonance energy transfer (FRET), which is so far one of the most reliable methods for studying protein-protein interaction in vivo [22]. Because of the size and continuous growth, Arabidopsis root cells appeared to be unsuitable for reliable FRET measurements. Therefore, we used stably transformed tobacco BY-2 cell lines coexpressing PIN1-GFP and DRP1A-mRFP1 fusion proteins. After excitation with a 477 nm laser used typically for the stimulation of GFP fluorescence, an additional signal with maximum at 610 nm was detected at the cell plate with spectral imaging (Figure 2B; Figures S3C–S3L), implying FRET between PIN1-GFP and DRP1A-mRFP1 molecules. We measured the average fluorescence intensity over the emission spectrum in the three specified regions of interest: middle region of the cell plate, growing ends of the cell plate, and transversal plasma membrane (Figure 2C). The highest FRET was detected on the growing ends of the cell plate in contrast to mature plasma membranes, which is additional in vivo proof for the cell plate specificity of the association between PIN and DRP1. However, in a yeast two-hybrid experiment, the PIN1 hydrophilic loop showed no interaction with the DRP1A full-length protein (Figure S3B), which might indicate that the close proximity of PIN1 and DRP1 molecules is facilitated by other cofactors.

The DRP1 and PIN association at the cell plate might be important for the PIN trafficking or dynamics in dividing cells. To test this hypothesis, we studied the PIN1 and PIN2 distribution in drp1a null mutant by immunolocalization as well as by pPIN2::PIN2-GFP construct. The drp1a mutation causes the pleiotropic cellular defects, especially misshapen and bifurcated cell plates and cell wall stubs [15, 17]. In the wild-type, PIN1 and PIN2 localize on the basal or apical site of the given

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**Table 1. PIN1-GFP CoIP Experiments Identify DRP1A**

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<tr>
<th>Protein</th>
<th>Peptides</th>
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<td></td>
<td>K.ISVPQNSNOQYVER.E</td>
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<td>R.FLAADSLOK.V</td>
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The PIN1- and DRP1A-derived peptides identified by coimmunoprecipitation (coIP) and mass spectrometry (MS). Protein extracts from a plant line expressing PIN1-GFP were incubated with GFP-antibody coupled Miltenyi beads. Precipitated proteins were eluted, digested by trypsin, and measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Peptide identification was performed with Bioworks 3.3.1 with the following filter settings: ΔCn > 0.08, Xcorr > 1.5 for charge state 2+, Xcorr > 3.3 for charge state 3+, and Xcorr > 4 for charge state 4+. A list of the proteins identified is presented in Table S1. A complete list of all proteins identified including the fold enrichment over background and their relative abundance as derived from MaxQuant analysis is given in Figure S1.

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**Figure 1. Colocalization of PIN, DRP1A, and CLC Proteins**

(A) Time-lapse scanning during cytokinesis (20 min interval) in DRP1A-mRFP1/PIN2-GFP double-transgenic lines. Proteins largely colocalize on the forming cell plate (arrowheads).

(B) Indirect immunofluorescence localization of CLC-GFP and DRP1A proteins showing partial colocalization on the cell plate (arrowheads). α-DRP1A and α-GFP antibodies were used for the immunolocalization.

See also Figure S2.
Strong accumulation and persistence of the PIN signal on the endomembrane cell plate structures of drp1a cells compared to the wild-type cells (Figures 3I and 3J; Figures S4I and S4J) therefore suggests that the drp1a mutation inhibits selective PIN retrieval from the cell plate during cytokinesis.

To explore whether the loss of DRP1 function and its effect on PIN trafficking has some consequence on the auxin-mediated development, we analyzed phenotypes of drp1 mutants. For our analyses, we used novel insertional mutants in the DRP1A and DRP1C genes (Figure SSA) and generated lines carrying estradiol-inducible artificial microRNA against DRP1A, DRP1C, and DRP1E—the most abundant isoforms—artificial microRNA (amiRNA) drp1a/c/e (Figure S5B). The mutant drp1a2 is a null mutant and shows the already described phenotypes [15, 17], including seedling growth retardation (Figure 4A). Because the full knockout drp1c1 allele is gameto-phytically lethal [16], we isolated the drp1c2 allele that is leaky (Figure SSA) and viable. Both mutants were defective in root growth and gravitropic response as measured by the vertical growth index (Figure 4B). After crossing drp1a2 and drp1c2 mutants, no double homozygous seedlings were recovered, but a combination of homozygous drp1a2 with heterozygous drp1c2 resulted in minute lethal seedlings (Figure 4C) that resemble some of the mutants in auxin-mediated patterning [4]. Importantly, we were able to recapitulate the seedling phenotypes in the line conditionally expressing the drp1a/c/e amiRNA. Seedlings germinated on medium supplemented with estradiol were extremely dwarfed and pale with strongly agravitropic roots (Figures S5C and S5D). Agravitropism could also be induced in seedlings transferred 3 days after germination to plates with estradiol (Figure 4D). Next, we examined the embryo development of the drp1a2+/−/drp1c2−/− plants and observed the typical apical-basal patterning phenotypes, including extra cell divisions in the root pole or doubling of the proembryo (Figures 4E and 4F) that are specific for mutants defective in auxin response and PIN-dependent auxin transport [24]. Adult plants of both drp1a2 and drp1c2 mutants showed relatively normal growth but were semisterile (Figure 4G) and showed defects in flower development. drp1a2 mutant flowers occasionally formed five petals (in 19% cases), and drp1c2 flower organs were unusually elongated (Figure 4H). Notably, the activity of the auxin response reporter DR5rev::GFP [24] was strongly reduced in drp1a2 mutant root tips (Figure 4l; Figure S5E), consistent with defects in auxin distribution. In summary, the phenotypic analysis revealed that although the gross of the morphological phenotypes might be caused by defective cell divisions [15, 16], some typical phenotype features, including agravitropism, apical-basal embryo, or organ positioning defects, suggest that the loss of synergistically and/or redundantly acting DRP1A and DRP1C proteins indeed affects transport and distribution of auxin.

Figure 2. Analyses of the DRP1A-PIN Association
(A) In planta analysis of PIN1-DRP1A association by bimolecular fluorescence complementation (BiFC). Top: control line coexpressing DRP1A fused with N- and C-terminal parts of the YFP (SYN or SYC) confirms dimerization and oligomerization of DRP1A proteins at the cell plate. Bottom: line coexpressing DRP1A-SYN and PIN1 hydrophilic loop fused with SYC. The signal is again visible at the cell plate.
(B and C) Förster resonance energy transfer (FRET) analysis of PIN1-GFP/DRP1-mRFP1 in the stable double-transformed BY-2 cells. (B) Unmixed emission of GFP donor at 500–520 nm (green) and mRFP1 acceptor at 600–620 nm (red) from the emission spectrum detected after excitation with a 477 nm laser. (C) The fluorescence emission spectrum measured in the selected regions of interest (ROI, inset) numbered from 1 to 5 (1, middle of the cell plate; 2 and 3, growing ends of the cell plate; 4 and 5, transversal and longitudinal plasma membranes). Emission peak at 600–620 nm implies highest FRET at growing edges of the cell plate, lower in the middle of the cell plate, and no FRET detected at the plasma membrane.
See also Figure S3.

In drp1a2 mutants, the overall PIN1 and PIN2 localization as well as the sensitivity to an inhibitor of vesicle trafficking, brefeldin A (BFA), did not change severely (Figure 3B; Figures S4A–S4D). However, we found in approximately 28% of all cells (n = 367), especially those visibly affected by the drp1a mutation, several types of defects in the subcellular PIN2 distribution (Figure 3B–3H; Figures S4F–S4H). The changes ranged from complete loss of the PIN polarity (Figure 3D), high accumulation of the PIN signal on the newly formed septum (Figure 3E), aberrant cell division planes (Figure 3F), and cell wall stubs (Figure 3G) to accumulation of PIN proteins in intracellular round compartments (Figure 3H). In the wild-type, PIN accumulation on the cell plate is transient and increases only after the new septum is formed; this is in contrast to the observed accumulation of some plasma membrane and extracellular markers at the cell plate [23].

In interphase cells, respectively (Figure 3A; Figures S4A and S4E). In drp1a2 mutant, the overall PIN1 and PIN2 localization as well as the sensitivity to an inhibitor of vesicle trafficking, brefeldin A (BFA), did not change severely (Figure 3B; Figures S4A–S4D). However, we found in approximately 28% of all cells (n = 367), especially those visibly affected by the drp1a mutation, several types of defects in the subcellular PIN2 distribution (Figure 3B–3H; Figures S4F–S4H). The changes ranged from complete loss of the PIN polarity (Figure 3D), high accumulation of the PIN signal on the newly formed septum (Figure 3E), aberrant cell division planes (Figure 3F), and cell wall stubs (Figure 3G) to accumulation of PIN proteins in intracellular round compartments (Figure 3H). In the wild-type, PIN accumulation on the cell plate is transient and increases only after the new septum is formed; this is in contrast to the observed accumulation of some plasma membrane and extracellular markers at the cell plate [23].
PIN distribution must always be established de novo on the newly formed septum between daughter cells. This presumably involves sterol-dependent endocytosis specific for mitotic cells as shown for the PIN2 polarity acquisition in interphase cells [20]. However, the dynamics and trafficking of PIN proteins on the site of the division plane are unknown. Function of DRP1 proteins is strongly required for the formation of the cell plate, most likely by tubulation and constriction of the fusion membranes and vesicles [26, 27]. The role of DRPs in regulating dynamics of clathrin-mediated endocytosis at the plasma membrane was also proposed, but their precise mechanistic role in this process is unknown [13, 28]. PIN proteins are also cargos of clathrin-mediated endocytosis at the plasma membrane [29].

Our results reveal that PIN and DRP1 proteins form transient complexes during cell plate formation. This association is presumably important for the clathrin-mediated retrieval of PIN proteins from the cell plate, as suggested by more intensive PIN accumulation at newly formed intracellular plasma membranes in drp1 mutant root cells. Animal dynamin interacts with various SH3 domain-containing proteins, such as Src kinases, amphiphysin, Grb2, and other factors whose function is usually to modify or recruit dynamin to the site of its action [30], but no direct interaction with cargo proteins, such as receptors or transporters, has been described. Also in this case, we assume that the interaction between PIN and DRP1 is not direct and can be better described as a close association. It is feasible that DRP1 is preferentially recruited to the sites of the PIN internalization from the cell plate where it assists its retrieval. Detailed analyses of the molecular action of DRP1 proteins at the cell plate would be therefore necessary to understand the process of dynamic PIN trafficking during cytokinesis and the reestablishment of cell polarity after cell division.

Supplemental information
Supplemental Information includes five figures, one table, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.05.018.

Acknowledgments
We thank Caterina Brancato and Vassilena Gaykova for technical assistance, European Arabidopsis Stock Centre (NASC) for providing SALK insertion lines, Martine De Cock for help in preparing the manuscript, and Herman Höfte for enabling J.M. to finalize the work in his laboratory. This work was supported by the Volkswagenstiftung (J.F. and J.M.), the Odysseus Programme of The Research Foundation—Flanders (FWO) (J.F., J.M., J.Z. and T.N.), the project of The Ministry of Education, Youth and Sports of the Czech Republic LC06034 (J.P., M.P. and E.Z.), the European Fund for Regional Development, the Operational Programme Prague—Competitiveness, project number: CZ.1.07/2.3.00/20.0070, the Centre for Biosystems Genomics part of the Netherlands Genomics Initiative/Netherlands Organization for Scientific Research (N.L.) and ERA-Plant Genomics (R.K.), and the Agrotechnology and Food Sciences Group of Wageningen University (S.d.V.). J.M. is indebted to the Federation of the Societies of Biochemistry and Molecular Biology (FEBS) for a long-term fellowship.

Received: March 9, 2011
Revised: April 27, 2011
Accepted: May 9, 2011
Published online: June 9, 2011

References


