PCP
PLANT & CELL PHYSIOLOGY

Trafficking of Plant Plasma Membrane Aquaporins: Multiple Regulation Levels and Complex Sorting Signals

Adrien S. Chevalier and François Chaumont*

Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud 4, L7.07.14, B-1348 Louvain-la-Neuve, Belgium
*Corresponding author: E-mail, francois.chaumont@uclouvain.be; Fax, +32-10-47-38-72.
(Received November 21, 2014; Accepted December 4, 2014)

Aquaporins are small channel proteins which facilitate the diffusion of water and small neutral molecules across biological membranes. Compared with animals, plant genomes encode numerous aquaporins, which display a large variety of subcellular localization patterns. More specifically, plant aquaporins of the plasma membrane intrinsic protein (PIP) subfamily were first described as plasma membrane (PM)-resident proteins, but recent research has demonstrated that the trafficking and subcellular localization of these proteins are complex and highly regulated. In the past few years, PIPs emerged as new model proteins to study subcellular sorting and membrane dynamics in plant cells. At least two distinct sorting motifs (one cytosolic, the other buried in the membrane) are required to direct PIPs to the PM. Hetero-oligomerization and interaction with SNAREs (soluble *N*-ethylmaleimide-sensitive factor protein attachment protein receptors) also influence the subcellular trafficking of PIPs. In addition to these constitutive processes, both the progression of PIPs through the secretory pathway and their dynamics at the PM are responsive to changing environmental conditions.

Keywords: Aquaporin • ER export • PIP • SNARE • Sorting motif • Trafficking.

Abbreviations: A23, tyrphostin A23; At, *Arabidopsis thaliana*; BFA, brefeldin A; ER, endoplasmic reticulum; ERES, endoplasmic reticulum exit sites; FRAP, fluorescence recovery after photobleaching; FRET/FLIM, Förster resonance energy transfer/fluorescence lifetime imaging microscopy; GFP, green fluorescent protein; Lp_r, root water conductivity; MβCD, methyl-beta-cyclodextrine; NAA, naphthalene-1-acetic acid; NIP, nodulin26-like intrinsic protein; Nt, *Nicotiana tabacum*; P_f, membrane water permeability coefficient; PIP, plasma membrane intrinsic protein; PM, plasma membrane; PVC, pre-vacuolar compartment; ROS, reactive oxygen species; SA, salicylic acid; SNARE, soluble *N*-ethylmaleimide-sensitive factor protein attachment protein receptor; TGN, *trans*-Golgi network; TIP, tonoplast intrinsic protein; TM, transmembrane domain; Zm, *Zea mays*.

Introduction

Aquaporins are a family of channel proteins which facilitate the passive diffusion of water and/or small neutral solutes across biological membranes (Bienert and Chaumont 2011). In plants, aquaporins are crucial molecular players involved in numerous essential processes (Chaumont and Tyerman 2014, Li et al. 2014). These proteins assemble as tetramers, in which each subunit forms a functional channel (Törnroth-Horsefield et al. 2006). The monomers are constituted of six transmembrane domains (TM1–TM6), which are linked by five loops (A–E). The N- and C-termini and the B and D loops are cytosolic.

Membrane proteins are co-translationally inserted into the membrane of the endoplasmic reticulum (ER). From there, most cargo proteins follow the secretory pathway (Bassham et al. 2008). They are exported towards the Golgi apparatus in COPII vesicles, then to the *trans*-Golgi network (TGN). Proteins leaving the TGN are sorted to their final destinations, mostly the vacuole or the plasma membrane (PM; Gendre et al. 2015). The first plant aquaporins to be identified were clearly associated with specific membranes (i.e. the vacuolar membrane and the PM). However, with the increasing number of reported plant aquaporin genes, the family appeared to exhibit a large variety of subcellular localization patterns (Wudick et al. 2009, Hachez et al. 2013, Luu and Maurel 2013). Recent work on aquaporins belonging to the plasma membrane intrinsic protein (PIP) subfamily demonstrated that their trafficking along the secretory pathway to their destination membrane is dependent on complex sorting determinants, and that their dynamics in their target membrane are dependent upon environmental conditions.

Plants are not able to escape from unfavorable growth conditions, such as drought or salinity. Therefore, aquaporin regulation processes are essential for plants to adapt the density of active channels in their membranes in response to changing environmental conditions. In the past few years, the usefulness of PIP aquaporins as an experimental model in subcellular trafficking studies has been demonstrated, both to identify new sorting signals and to characterize dynamic processes in response to various environmental conditions (Luu and Maurel 2013, Nebenfuhr 2014, Zelazny and Vert 2014). This review aims to bring this information together to show that the journey of aquaporins from their site of synthesis towards the PM depends on multiple molecular factors, and that their transport and dynamics are highly regulated.

Plant Aquaporins Display an Unexpected Variety of Subcellular Localization Patterns

The first aquaporins discovered in plants were classified on the basis of their subcellular localization. NIPs (nodulin26-like

Review

Editor-in-Chief's Choice

Plant Cell Physiol. 56(5): 819–829 (2015) doi:10.1093/pcp/pcu203, Advance Access publication on 16 December 2014, available FREE online at www.pcp.oxfordjournals.org
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intrinsic proteins) were identified in the peribacteroid membrane of soybean (*Glycine max*) nodules (Fortin et al. 1987), TIPs (tonoplast intrinsic proteins) were found in the bean vacuolar membrane (tonoplast) (Johnson et al. 1989), and PIPs were detected in the PM of *Arabidopsis thaliana* cells (Daniels et al. 1994, Kammerloher et al. 1994). However, as a whole, the subcellular localization of proteins belonging to the plant aquaporin family is complex and highly regulated. Proteins of the PIP subfamily have been shown in many cases indeed to be localized in the PM, but this is not a completely general feature. When expressed in tobacco, maize (*Zea mays*) ZmPIP1;2 displays a dual localization in the PM and in the ER of root elongating cells (Chaumont et al. 2000). It has been shown that ZmPIP1 isoforms are unable to reach the PM when they are expressed alone in maize mesophyll protoplasts (Zelazny et al. 2007). The tobacco (*Nicotiana tabacum*) PIP1 NtAQP1 is found both in the PM and in the chloroplast inner envelope, where it is thought to facilitate CO_2 transport (Uehlein et al. 2008). In salt stress conditions, Arabidopsis PIPs are relocated to intracellular vesicles (Boursiac et al. 2005, Boursiac et al. 2008). These observations underline the complex nature of aquaporin subcellular localization. Nevertheless, PIPs are often used as PM markers, notably, but not only, as controls in studies pertaining to auxin efflux transporters (PINs) (Paciorek et al. 2005, Takano et al. 2005, Dhonukshe et al. 2007, Jaillais et al. 2007, Dhonukshe et al. 2008, Men et al. 2008, Feraru et al. 2010, Zhang et al. 2011, Feraru et al. 2012). The use of plant PIPs as reference PM proteins in many studies has led to the accumulation of a considerable amount of experimental data which are of primary interest for the understanding of their endomembrane trafficking. However, many mechanisms which determine the routing of these proteins to their destination membranes remain to be uncovered (Hachez et al. 2013, Luu and Maurel 2013).

Heteromerization Regulates Maize PIP Routing to the PM

Early co-expression experiments in *Xenopus laevis* oocytes demonstrated a functional synergy between maize ZmPIP1s and ZmPIP2s, although the ZmPIP1s were inactive when expressed alone (Fetter et al. 2004). This could be attributed to the inability of ZmPIP1s to reach the PM and/or to their instability in the PM of the oocyte in the absence of ZmPIP2s. These initial experiments in oocytes were not completely sufficient to distinguish the activity and stability effects from trafficking aspects. The expression of ZmPIPs fused to fluorescent proteins in maize mesophyll protoplasts allowed for the trafficking properties of ZmPIP1s and ZmPIP2s to be specifically studied (Zelazny et al. 2007). When expressed alone, ZmPIP2s reach the PM, whereas ZmPIP1s are retained in the ER. However, when co-expressed, ZmPIP1s and ZmPIP2s co-localize in the PM. These localization patterns were conserved for all of the ZmPIP isoforms and all combinations tested. Therefore, the differential localization of ZmPIP1s and ZmPIP2s, as well as the co-localization of both in the PM upon co-expression, is likely to be a general feature for all ZmPIPs. The Förster resonance energy transfer/fluorescence lifetime imaging microscopy (FRET/FLIM) technique has been used to demonstrate that the relocalization of ZmPIP1s to the PM upon co-expression with ZmPIP2s is the consequence of a physical interaction between the ZmPIP1s and ZmPIP2s. The interaction has additionally been confirmed by co-immunopurification experiments in maize roots and suspension cells (Zelazny et al. 2007, Cavez et al. 2009). ZmPIP2s are specifically required for ZmPIP1s to leave the ER, since physical interaction between different ZmPIP1 isoforms in the ER does not result in the export of the proteins to the PM. The relocalization of ZmPIP1s to the PM by ZmPIP2s does not require the disulfide bridge in loop A (Bienert et al. 2012). These findings imply that, in a hetero-oligomer, the ER export capacity of ZmPIP2s overcomes the ER retention properties of ZmPIP1s. The reason why ZmPIP1s are retained in the ER could be the lack of ER export signals, or the presence of a specific retention signal. The association with ZmPIP2s may either provide an export signal or hide the retention motif, allowing the complex to reach the PM. ZmPIP transcripts are not very abundant in maize mesophyll protoplasts. This explains why ZmPIP1s are found in the ER when expressed alone, because the expression level of endogenous ZmPIP2s is not sufficient to relocalize them to the PM (Zelazny et al. 2007).

Functional interactions between PIP1s and PIP2s from many other plant species expressed in oocytes have been reported (reviewed in Chaumont and Tyerman 2014). However, a systematic analysis of the subcellular localization of the PIP1s and PIP2s in a plant system is often lacking. Confirmation that PIP1s need to interact with PIP2s to leave the ER in a plant species other than maize has been lacking, and this is due to the fact that it is difficult to analyze PIP1s in plant cells independently of endogenous PIP2s.

A Diacidic Motif is Responsible for the Export of Some PIP2s from the ER

Diacidic motifs (D/ExD/E sequences) have been shown to be required for the ER export of some proteins in yeast, animal and plant cells (Nishimura and Balch 1997, Votsmeier and Gallwitz 2001, Hanton et al. 2005, Dunkel et al. 2008, Yang et al. 2014). In ZmPIP2;4 and ZmPIP2;5, which are localized at the PM when expressed in mesophyll protoplasts, the DIE sequence in position 4–6 is necessary for both proteins to reach the PM (**Fig. 1**) (Zelazny et al. 2009). Co-localization with an ER marker revealed that this diacidic motif was necessary for the exit of ZmPIP2;5 out of the ER. Both ZmPIP2;4 and ZmPIP2;5 contain an additional diacidic sequence in their N-terminal tail (DAE; **Fig. 1**). However, since deactivation of the DIE motif is sufficient to prevent ZmPIP2;5 completely from reaching the ER, the DAE motif is apparently not sufficient to drive the exit of ZmPIP2;4 and ZmPIP2;5 from the ER. Indeed, it is known that the functionality of trafficking motifs in endomembrane sorting is largely dependent on the molecular context (Ohno et al. 1996, Nufer et al. 2002, Bonifacino and Traub 2003, Wolfenstetter et al. 2012, Gershlick et al. 2014). However, it cannot be excluded that this motif is necessary, in combination with the

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ZmPIP1;1 MEG-------KEEDVRLGANKFSERQPIGTAAQGT----DDKDYKEPPPAPLFEPGELK 48
ZmPIP1;2 MEG-------KEEDVRLGANKFSERQPIGTAAQGA---ADDKDYKEPPPAPLFEPGELK 49
ZmPIP1;3 MEG-------KEEDVRLGANKFSERQPIGTAAQGAGAGDDDKDYKEPPPAPLFEPGELK 52
ZmPIP1;4 MEG-------KEEDVRLGANKFSERQPIGTAAQGAGAGDDDKDYKEPPPAPLFEPGELK 52
ZmPIP1;5 MEG-------KEEDVRLGANRYSERQPIGTAAQGT----EEKDYKEPPPAPLFEAEELT 48
ZmPIP1;6 MAGGTLQDRSEEEDVRVGVDRFPERQPIGTAADDLG-----RDYSEPPAAPLFEASELS 54
ZmPIP2;1 MGKD------DVIESGAGGGEFAA-----------------KDYTDPPPAPLIDAAELG 36
ZmPIP2;2 MGKD------DVVQSGAGGGEFAA-----------------KDYTDPPPAPLVDAAELG 36
ZmPIP2;3 MAKQ------DIEASGPEAGEFSA-----------------KDYTDPPPAPLIDADELT 36
ZmPIP2;4 MAK-------DIEASGPEAGEFSA-----------------KDYTDPPPAPLIDAEELT 35
ZmPIP2;5 MAK-------DIEAAAAHEG---------------------KDYSDPPPAPLVDAEELT 31
ZmPIP2;6 MGK-------EVDVSTLEAGGVRD-----------------RDYADPPPAPLIDIDELG 35
ZmPIP2;7 MAK-------DVEQV-TEQGEYSA-----------------KDYHDPPPAPLIDPDELT 34
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Fig. 1 Alignment of the N-terminal sequences of ZmPIPs. Putative diacidic motifs are shown in bold. The motifs that are necessary for the ER export of ZmPIP2;4 and ZmPIP2;5 are highlighted in blue. The alignment was performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

DIE motif, for ER export. Surprisingly, the fusion of the N-terminal portion of ZmPIP2;5, which contains the diacidic motif, to ZmPIP1;2 is not sufficient to bring the latter to the PM, indicating that ZmPIP1;2 contains a strong ER retention motif that overcomes the ER export capacity of the DIE motif of ZmPIP2;5 (Zelazny et al. 2009). On the other hand, replacement of the N-terminal portion of ZmPIP2;5 with that of ZmPIP1;2 results in the retention of ZmPIP2;5 in the ER, probably due to the removal of the diacidic motif. This showed that the acidic sequences present in the N-terminal tail of ZmPIP1;2 are not functional for ER export. Finally, ZmPIP2;1 is localized in the PM (Zelazny et al. 2007) even though no putative diacidic motif can be found in its N-terminal sequence (**Fig. 1**).

The diacidic motif of ZmPIP2;4 and ZmPIP2;5 is conserved in AtPIP2;1 (DVE; in position 4–6). Similarly, as has been observed for ZmPIP2;4 and ZmPIP2;5, this motif is necessary for the protein to progress beyond the ER in the secretory pathway (Sorieul et al. 2011). Mutation of either D4 or E6 to alanine (AtPIP2;1D4A or AtPIP2;1E6A, respectively) is sufficient to induce a significant intracellular retention of AtPIP2;1. Modified versions of AtPIP2;1 with mutations in the diacidic ER export motif which preserve the acidic nature of the residues (AtPIP2;1D4E and AtPIP2;1E6D) were generated. Unexpectedly, those mutated proteins were retained in the ER, similar to the alanine mutants (Sorieul et al. 2011). These results point towards the requirement for a strict DxE sequence instead of a more accommodating 'diacidic' motif for the export of AtPIP2;1 out of the ER. Similar results were obtained for the vesicular stomatitis virus glycoprotein (VSV-G) (Nishimura and Balch 1997), and for the mammalian K^+ inward rectifying channels Kir1.1 and Kir1.2 (Ma et al. 2001). In transgenic Arabidopsis plants expressing ER-retained mutated versions of AtPIP2;1, the root water conductivity (Lp_r) was reduced by 36–45% compared with the controls (Sorieul et al. 2011). Upon co-expression with the ER-retained AtPIP2;1 variant, both wild-type AtPIP2;1 and AtPIP1;4 were blocked in the ER. Thus, the expression of the ER-retained AtPIP2;1 dramatically disturbs the trafficking of other PIPs to the PM, resulting in the decreased Lp_r. This effect is specific to the AtPIP subfamily of aquaporins, since AtTIP1;1 is correctly localized to the tonoplast in transgenic plants expressing the ER-retained mutant of AtPIP2;1.

These studies demonstrate the importance of diacidic motifs in the export of PIPs from the ER. However, a sequence alignment of the N-terminal region of maize PIPs indicates that these motifs are not sufficient to account for their differential localization. All of the ZmPIP1s which have been investigated to date are retained in the ER, even though all contain diacidic sequences in their N-terminus (**Fig. 1**). Reciprocally, ZmPIP2;1 does not contain a diacidic sequence in its N-terminal tail, but is able to reach the PM. ZmPIP2;6 contains an ExD sequence at a conserved position, similar to the DxE motif of ZmPIP2;4 and ZmPIP2;5. Analyzing its subcellular localization would help to determine whether strict DxE motifs are required for PIP routing to the PM, as suggested by Sorieul et al (2011).

Even though the ER export mechanism might not be identical for all PIPs, the requirement for DxE diacidic motifs in this essential trafficking step has been clearly established. It is likely that these sequences interact with the COPII coat component Sec24, as has been shown for the K^+ transporter KAT1 (Sieben et al. 2008). It would be of interest to investigate whether specific Sec24 isoforms interact with specific PIPs, and whether this specificity varies according to the environmental conditions.

Maize PIP2s Rely on Their Third Transmembrane Helix to Reach the PM

The diacidic ER export motif identified in some PIP2 isoforms (Zelazny et al. 2009, Sorieul et al. 2011) is not fully sufficient to account for the differential sorting of ER-retained ZmPIP1s and PM-localized ZmPIP2s (Zelazny et al. 2007). To obtain more insights into the distinct localization of these two groups of proteins and to identify new trafficking signals in ZmPIPs, a systematic domain-swapping approach has been taken (Chevalier et al. 2014). Localization experiments in maize protoplasts and leaf epidermal cells showed that, unexpectedly, the soluble regions (N-terminus, C-terminus and loops A–E) of ZmPIP1;2 and ZmPIP2;1 do not contain major trafficking determinants. Instead, TM3 emerged as a critical region influencing the progression of ZmPIPs towards the PM. Site-directed mutagenesis of diverging residues within the TM3 region of ZmPIP1;2 and ZmPIP2;5 showed that the Leu127 and Ala131

residues of ZmPIP2;5 are needed for its anterograde trafficking in the secretory pathway. Although the transport step in which this LxxxA motif is involved could not be unambiguously identified, it seems to be required for an efficient routing of ZmPIP2;5 towards the PM, as early as ER to Golgi transport. Truncated versions of ZmPIP2;5 and ZmPIP1;2 consisting of only the TM3 region fused to a fluorescent protein reflect the localization of the full-length proteins, proving the different trafficking properties of the TM3 of ZmPIP2;5 and ZmPIP1;2. The LxxxA motif is responsible for this differential localization, as its mutation results in the retention of the TM3 of ZmPIP2;5 inside the cell, and its insertion into TM3 of ZmPIP1;2 allows the protein partially to reach the PM (Chevalier and Chaumont, unpublished results). Nevertheless, the addition of the LxxxA motif to the full-length ZmPIP1;2 does not allow the protein to reach the PM, even in combination with the previously identified N-terminal diacidic motif of ZmPIP2;5 (Chevalier et al. 2014).

Despite its striking role in the anterograde routing of ZmPIP2;5 along the secretory pathway, how the TM3-based LxxxA motif is recognized by trafficking proteins remains unknown. The vast majority of trafficking motifs have been identified in the cytoplasmic regions of cargo proteins (Barlowe 2003). Proteins bearing trafficking motifs exposed to the cytosol can be recruited directly for incorporation into nascent vesicles. Cytoplasmic motifs are accessible to the trafficking machinery, and are able to interact physically with specific transport proteins, a process that is often required for cargo transport. In contrast, TM-based trafficking signals cannot directly interact with transport proteins. However, examples of subcellular routing regulated by TM-based sorting signals have been reported. Unlike soluble sorting determinants, TM-based trafficking signals do not consist of conserved ‘motifs’, but rather of physical properties of the TM segment (Cosson et al. 2013). The length of the TM influences the progression of single-TM membrane proteins along the secretory pathway in yeast, mammalian and plant cells (Brandizzi et al. 2002, Ronchi et al. 2008, Sharpe et al. 2010). Proteins with a short TM are retained at the level of the ER or the Golgi, while a longer TM allows the protein to reach the PM. However, TM-based protein sorting does not rely only on TM length. For example, some well-defined regions in the TM of the ORF7b protein from the severe acute respiratory syndrome coronavirus have been found to be responsible for sorting (Schaecher et al. 2008). Subcellular sorting of some polytopic plant membrane proteins relies on TM-based information. The bean tonoplast aquaporin α-TIP is driven to the vacuolar membrane by its final transmembrane helix (Höfte and Chrispeels 1992). The rice secretory carrier membrane protein 1 (SCAMP1) relies on its TM2 and TM3 for Golgi export, and on TM1 for TGN to PM routing (Cai et al. 2011).

The mechanisms regulating TM-based sorting are not very clear yet, but partitioning into specific membrane subdomains and/or interactions with receptor proteins are thought to mediate this process (Cosson et al. 2013). The recruitment of artificial C-tail-anchored membrane proteins to ER exit sites (ERES) requires a long TM segment (Ronchi et al. 2008). Otherwise, the protein remains in export-incompetent domains of the ER membrane. In yeast and mammalian cells, many proteins need to be recruited into lipid rafts to be properly routed to their final PM destination (Surma et al. 2012). Localization of the model transmembrane protein Linker for Activation of T-cells (LAT) in membrane rafts is necessary and sufficient for PM localization (Diaz-Rohrer et al. 2014). Palmitoylation and transmembrane domain length are determinants for the association of LAT with membrane rafts. In yeast and animal cells, transmembrane receptor proteins recognize TM segments of secretory proteins for sorting at the ER–Golgi interface (Dancourt and Barlowe 2010). In the anterograde direction, Erv14p binds cargo proteins to load them into COPII vesicles. Erv14p has recently been shown to interact with the transmembrane segments of cargo proteins (Herzig et al. 2012). In the Golgi to ER direction, Rer1p binds TM-based signals to retrieve proteins towards the ER. Erv14p and Rer1p homologs exist in plants. Their role in cargo transport has, to our knowledge, not been demonstrated, but Arabidopsis Rer1 homologs complement the yeast *rer1* mutant (Sato et al. 1999). These findings show that subcellular sorting of transmembrane proteins depends on TM-based trafficking determinants in addition to well-characterized soluble motifs. How TM-based sorting information integrates into well-characterized endomembrane processes has only started to be uncovered (Cosson et al. 2013). In this respect, the discovery of the TM-based Leu127 Ala131 PM targeting motif in ZmPIP2;5 (Chevalier et al. 2014) opens up exciting new perspectives in plant protein trafficking research (Nebenfuhr 2014).

Phosphorylation- and Ubiquitylation-Dependent Trafficking of Arabidopsis PIP2;1

Phosphorylation affects the gating of plant aquaporins (Törnroth-Horsefield et al. 2006). Multiple adjacent phosphorylation sites (up to three) have been detected in the C-terminus of several AtPIPs by mass spectrometry (Prak et al. 2008). Most interestingly, AtPIP2;1 contains two phosphorylation sites in that region (Ser280 and Ser283). Mutations which abolished phosphorylation (serine to alanine), or mimicked constitutive phosphorylation (serine to aspartate) demonstrated that phosphorylation on Ser283 is necessary for the trafficking of AtPIP2;1 from the ER to the PM. On the other hand, mutation of Ser280 has no effect on AtPIP2;1 localization under normal growth conditions. **Fig. 2** summarizes the molecular determinants governing the export of PIPs out of the ER.

A salt stress treatment (100 mM NaCl; 2 or 4 h) modifies both the localization and phosphorylation status of AtPIP2;1 (Prak et al. 2008). AtPIP2;1 is relocalized to ‘fuzzy structures’ and to a lesser extent to intracellular ‘spherical bodies’ tentatively identified as late endosomal compartments (pre-vacuolar compartment, PVC). The non-phosphorylated forms of AtPIP2;1 are primarily relocalized to fuzzy structures, while the relocalization to spherical bodies requires the phosphorylated form of Ser283. Because salt stress has been shown to reduce Lp_r (100 mM NaCl; 45 min) (Boursiac et al. 2005), it is tempting to assume that, upon salt treatment, the cell reduces the amount of water

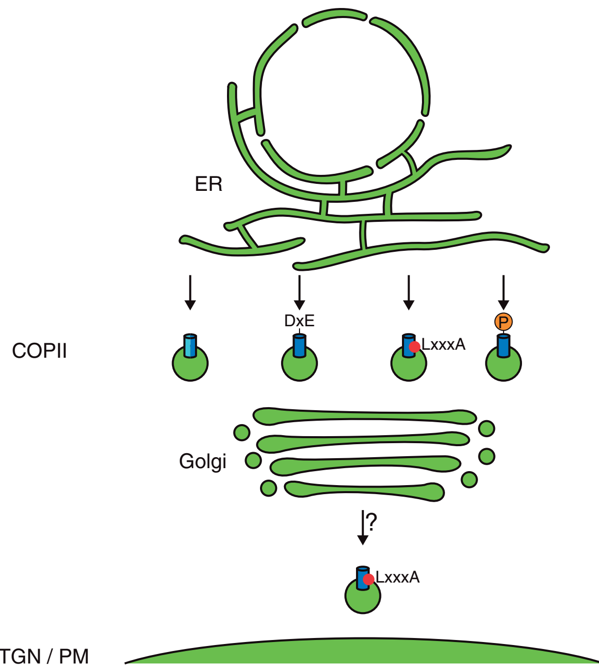

Fig. 2 The exit of PIPs from the ER depends on multiple factors. In maize, ZmPIP1s need to form a hetero-oligomer (light and dark blue cylinder) with ZmPIP2 to leave the ER. Diacidic motifs (DxE) required for ER export have been identified in PIP2 proteins from maize and Arabidopsis. In addition, a TM-based sorting signal (LxxxA) is required for the anterograde sorting of ZmPIP2;5. Although it is required as early as ER to Golgi transport, a role for this motif in Golgi export and/or post-Golgi transport is not excluded. Finally, phosphorylation of AtPIP2;1 in its C-terminal tail promotes ER export.

channels in the PM by (i) preventing neosynthesized PIPs from leaving the ER and (ii) internalizing PM-localized PIPs (AtPIP2;1S283D) to endosomal compartments for degradation (**Fig. 3**). This is supported by the observation that salt induces a reduction in the abundance of the phosphorylated Ser283 form, which is able to reach the PM. These data underline the major regulatory role of phosphorylation in PIP localization and dynamics (Verdoucq et al. 2014).

In Arabidopsis, AtPIP2;1 is retained in the ER by the overexpression of an E3 ubiquitin ligase (Rma1), which is induced by abiotic stresses including dehydration (Lee et al. 2009). Rma1 is localized in the ER membrane and confers resistance to drought stress when overexpressed in transgenic Arabidopsis plants. AtPIP2;1 is ubiquitinated by Rma1 and is consequently degraded in the proteasome. These observations suggest a mechanism by which the density of AtPIP2;1 channels in the PM is reduced under drought stress conditions by the E3 ubiquitin ligase Rma1 to ensure plant survival. This model correlates with the observed down-regulation of AtPIP2;1 (and most other PIPs) under drought stress conditions (Alexandersson et al. 2005).

PIPs Interact with SNAREs to Reach the PM

SNAREs (soluble *N*-ethylmaleimide-sensitive factor protein attachment protein receptors) are a family of proteins that mediate vesicle fusion events in the endomembrane system. SNAREs associated with a vesicle and with its target membrane, respectively, interact to form a coiled-coiled structure to overcome hydrophobic forces and achieve membrane fusion between the two compartments (Grefen and Blatt 2008). In addition to their role in vesicle trafficking, SNAREs can specifically interact with cargos to regulate their activity. For example, the regulatory K^+ channel AtKC1 interacts with AtSYP121 to promote the gating of the inward-rectifying K^+ channel AtAKT1 through the formation of a tripartite complex (Honsbein et al. 2009).

Trafficking of ZmPIP2;5 to the PM requires the SNARE ZmSYP121 (Besserer et al. 2012) (**Fig. 3**). The localization of ZmPIP2;5 at the PM is partially inhibited by a dominant-negative fragment of SYP121 (SYP121-sp2). This effect is specific to SYP121, as inhibitory fragments of both ZmSYP121 and AtSYP121 but not of the PM-localized AtSYP122 and AtSYP71 nor of the TGN SYP21 decrease the amount of ZmPIP2;5 in the PM. A direct physical interaction between ZmPIP2;5 and ZmSYP121 has been demonstrated. Protoplast swelling assays showed that the membrane water permeability coefficient (P_f) is reduced in cells co-expressing SYP121-sp2 and ZmPIP2;5 compared with cells expressing ZmPIP2;5 alone. Thus, the transport of water across the membrane is directly affected by the defect in ZmPIP2;5 trafficking to the PM. Together with previous findings regarding K^+ channels, these data point to a general role for SYP121 in the regulation of the cell osmotic homeostasis. The involvement of SYP121 in PIP2 delivery to the PM has been confirmed in Arabidopsis where an interaction between AtPIP2;7 and AtSYP121 is also observed (Hachez et al. 2014). SYP121-mediated PIP2 trafficking to the PM seems therefore to be a conserved mechanism. Whether all PIPs, and in particular PIP1 isoforms, interact with SYP121 has not been investigated. However, not all PM proteins depend on SYP121 to reach the PM, since the localization of the H^+-ATPase PMA2 was not affected by SYP121-sp2 (Besserer et al. 2012).

AtPIP2;7 interacts with a second SNARE, AtSYP61 (Hachez et al. 2014). The localization of AtPIP2;5 in the PM requires AtSYP61 to be functional. When overexpressed in an AtSYP61 T-DNA insertion line (*osm1*), AtPIP2;7 specifically accumulates in abnormal intracellular structures which were identified as a modified ER. This aberrant localization was not observed for the auxin transporter AtPIN2, and could be rescued by the expression of an AtSYP61-encoding construct. Both SNAREs AtSYP121 and AtSYP61 physically interact putatively to form a SNARE complex (Hachez et al. 2014). However, AtSYP121 appears to be mainly localized at the PM, while AtSYP61 is more endosomal even if it accumulates in the PM upon treatment with the endocytosis inhibitor tyrphostin A23 (A23). From these data, it has been suggested that PIPs might reach the PM from the TGN by two non-exclusive pathways: one mediated by SYP121 alone, and the other mediated by the SYP121/SYP61 SNARE complex. On the other hand, internalization from the PM might be driven either by SYP61 alone or by the SYP121/SYP61 complex (Hachez et al. 2014) (**Fig. 3**). The involvement of SNARES in both endomembrane transport and

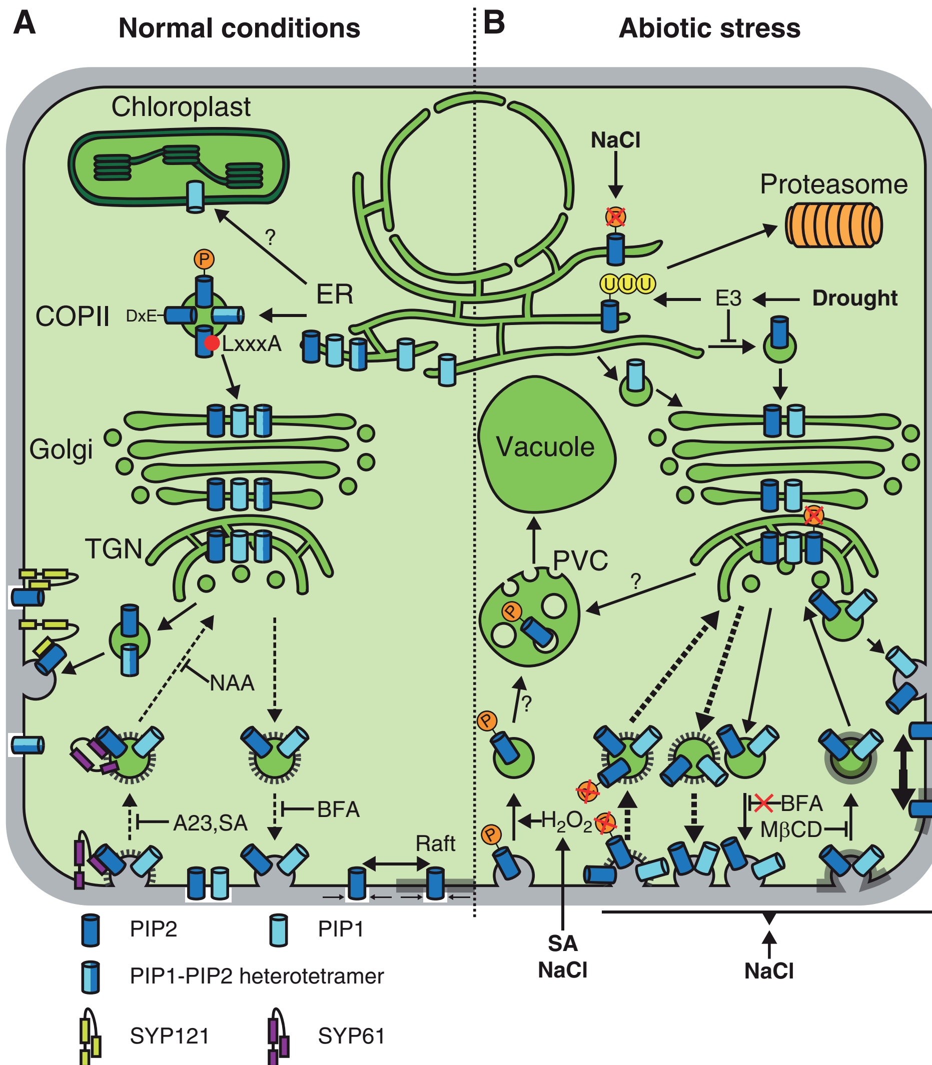

Fig. 3 Intracellular trafficking of PIPs. (A) PIP transport through the secretory pathway under normal conditions. The PIPs (blue cylinders) rely on various mechanisms to reach the Golgi. Some PIP1s are targeted to the chloroplast inner membrane by an unknown mechanism. Post-TGN transport of PIPs depends on SNARE proteins, and PIPs undergo constitutive cycling (dashed arrows). The cell wall restricts PIP lateral movement within the PM. A part of the PM pool of PIP is associated with rafts. (B) Modifications of PIP subcellular transport under abiotic stress conditions. Salt stress reduces ER export, increases cycling and diffusion, and causes intracellular accumulation of PIPs. Under drought stress, PIP2s are degraded via the proteasome as a result of polyubiquitination. See text for more details and references.

K^+ channel gating led to the hypothesis that SNAREs might act as molecular governors, co-ordinating the cell osmotic homeostasis (via water and solute uptake and membrane transporter traffic) together with membrane expansion in growing cells (Grefen and Blatt 2008, Honsbein et al. 2009, Honsbein et al. 2011, Besserer et al. 2012, Hachez et al. 2013, Hachez et al. 2014).

As suggested in Hachez et al. (2013), it is tempting to assume that different members of the multigenic SNARE family regulate different steps of the subcellular routing of PIPs from their site of synthesis to their final destination. An example supporting this idea is the mislocalization of AtPIP2;1 in the Arabidopsis *vamp721 vamp722* double mutant. Although the specificity of such a mechanism to PIPs is unlikely, the correct localization of AtTIP1;1 in the tonoplast in this mutant suggests a specific role for the VAMP721 and VAMP722 SNAREs in TGN to PM trafficking (Zhang et al. 2011, Hachez et al. 2013).

Some Plant Aquaporins Exhibit a Polarized Localization in the PM

The majority of PIP aquaporins show a uniform distribution at the cell periphery (Chaumont et al. 2000, Boursiac et al. 2005, Boursiac et al. 2008, Prak et al. 2008, Sorieul et al. 2011). However, several PIP members are directionally distributed in the PM. ZmPIP2;5 and ZmPIP2;1/ZmPIP2;2 display a polar localization in root and leaf epidermis, respectively. ZmPIP2;5 strongly labels the exofacial PM of root epidermal cells (Hachez et al. 2006), while ZmPIP2;1/ZmPIP2;2 labels the internal and anticlinal membranes in leaf epidermis more intensively (Hachez et al. 2008). In rice, OsPIP2;1 and OsPIP2;5 are specifically localized in the internal PM of root endodermal cells (Sakurai-Ishikawa et al. 2011). Interestingly, many NIPs that have been shown to be localized in the PM are asymmetrically

distributed (Ma et al. 2006, Mitani et al. 2009, Takano et al. 2010, Mitani et al. 2011). It is likely that other isoforms with a polar distribution face these aquaporins in the opposite membrane (Luu and Maurel 2013). The mechanism generating and maintaining PIP and NIP polarization is not known, but a role for the cell wall in limiting PIP lateral mobility in the PM has been shown (Martinière et al. 2012). PIPs (AtPIP1;1 and AtPIP2;1) have been used as control non-polarized PM proteins in PIN polarization studies (Dhonukshe et al. 2008, Kleine-Vehn et al. 2011). Polar endocytic recycling and restricted lateral mobility were crucial for the maintenance of PIN polarization, but had no influence on PIP distribution within the PM. Furthermore, because PINs are polarized following the longitudinal axis whereas NIP and PIP polarization follows the radial axis, the mechanisms establishing the asymmetrical distribution of PINs, NIPs and PIPs must differ at some point.

Mobility of PIPs in the PM

When observed by standard confocal microscopy, the fluorescence signal associated with PIPs appears to be continuous in the membrane (Dhonukshe et al. 2007, Zelazny et al. 2007, Boursiac et al. 2008, Kleine-Vehn et al. 2011, Besserer et al. 2012). However, PIPs have been identified in detergent-insoluble membranes in a number of studies (Borner et al. 2005, Morel et al. 2006, Lefebvre et al. 2007, Minami et al. 2009, Belugin et al. 2011, Takahashi et al. 2013) (**Fig. 3**). In accordance with these findings, PIPs display a relatively low lateral mobility in the membrane under normal growth conditions (Kleine-Vehn et al. 2011, Li et al. 2011, Sorieul et al. 2011, Besserer et al. 2012, Luu et al. 2012, Martinière et al. 2012). The lateral mobility of ER-retained AtPIP2;1 mutants is much higher than that of PM-localized wild-type AtPIP2;1, suggesting the existence of a PM-specific immobilization mechanism (Sorieul et al. 2011). The use of high-end microscopy technologies recently demonstrated the localization of AtPIP2;1 in microdomains (Li et al. 2011). This partitioning is dynamic, since AtPIP2;1 is able to move in and out of these microdomains. Surprisingly, the low lateral mobility of AtPIP2;1 and other PM proteins of Arabidopsis is independent of the organization of the membrane into microdomains (Martinière et al. 2012). The low lateral diffusion does not rely on protein concentration, protein interactions or cytoskeletal elements either. Instead, the cell wall was shown to restrict the diffusion of those proteins. Cell wall-restricted diffusion is maximal for proteins with large extracytoplasmic domains. Consequently, the cell wall meshwork was suggested to constrain the diffusion of membrane proteins mechanically (Martinière et al. 2012).

In resting conditions, AtPIP2;1 continuously cycles to and from the PM (Paciorek et al. 2005, Luu et al. 2012) (**Fig. 3**). The constitutive endocytosis of AtPIP2;1 from the PM is clathrin mediated (Dhonukshe et al. 2007, Li et al. 2011), and its recycling to the PM from the TGN probably involves Bex5/RabA1b (Feraru et al. 2012). Fluorescence recovery after photobleaching (FRAP) and photoconverted fluorescence monitoring were used as new tools to dissect the mechanisms underlying the constitutive cycling of PIPs to and from the PM (Luu et al. 2012). FRAP experiments performed on root epidermal cells of Arabidopsis transgenic plants expressing green fluorescent protein (GFP)-tagged AtPIP2;1 and AtPIP1;2 confirmed the very low lateral mobility of PIPs in the PM. The FRAP response of AtPIPs is biphasic, with an early response occurring within 60 s, and a late phase that is achieved after 30 min. Because the contribution of lateral mobility to fluorescence recovery is negligible, other factors must explain the biphasic behavior of the recovery. Vesicles containing GFP–AtPIPs flowing with the fast cytoplasmic streaming beneath the PM were suggested to be responsible for the early response, while the recycling of GFP–AtPIPs from vesicles to the PM accounted for the late response. This model was validated by coupling FRAP with the use of inhibitors of exocytosis (brefeldin A; BFA) and clathrin endocytosis (A23). A23 inhibited the fast response, and thus the accumulation of PIPs in endosomal compartments, and BFA inhibited the late recovery response. The synthetic auxin analog NAA (naphthalene-1-acetic acid) inhibited a step downstream of endocytosis and upstream of the TGN. This showed that, in normal conditions, PIPs continuously cycle to and from the PM in a three-step sequence: endocytosis, transfer to the TGN and recycling to the PM. The clathrin-mediated endocytosis step of the cycle is inhibited by salicylic acid (SA) (Du et al. 2013). The reason why PIPs undergo constitutive cycling remains somewhat obscure (Hachez et al. 2013). Endocytic cycling of PINs, for example, is involved in the maintenance of their polar localization (Kitakura et al. 2011) and regulates the amount of channels in the PM (Paciorek et al. 2005, Robert et al. 2010). In contrast, one could imagine that PIP cycling maintains a uniform repartition in the PM despite their low intrinsic mobility.

Upon exposure to salt stress conditions, the cycling rate of AtPIP2;1 to and from the PM is increased (Luu et al. 2012) (**Fig. 3**). During this process, AtPIP2;1 clathrin-dependent endocytosis takes place, but a second, raft-associated, internalization pathway is activated (Li et al. 2011). Thus, one of the reasons for AtPIP2;1 to be incorporated into microdomains may be the need of the raft-associated internalization pathway in response to salt stress conditions. Segregation of PIN1 and PIN2 into membrane clusters contributes to the dramatically low lateral mobility of these proteins and to the maintenance of their polar PM localization. In contrast to PIN proteins, AtPIP2;1 is not detected in 'membrane clusters', and its recovery after photobleaching is insensitive to sterol disruption by filipin under normal conditions (Kleine-Vehn et al. 2011). Together, the studies of Kleine-Vehn et al. (2011), Li et al. (2011) and Luu et al. (2012) indicate that PINs and PIP2;1 are located in different microdomains which are functionally specialized to carry out different functions (PIN immobilization vs. PIP2;1 internalization upon exposure to salt stress conditions).

Abiotic Stress-Induced Aquaporin Reorganization

Salt has diverse effects on aquaporins in plants (Vera-Estrella et al. 2004, Boursiac et al. 2005, Boursiac et al. 2008, Prak et al.

2008, Li et al. 2011, Luu et al. 2012). A 100 mM NaCl treatment results in a 70% decrease in Lp_r (Boursiac et al. 2005). The half-time of this response is 45 min, and the reduced Lp_r is stable over at least 24 h. The transcript levels of all highly expressed *PIP* and *TIP* genes are dramatically reduced (60–70%) after 2–4 h of exposure to stress conditions. Surprisingly, PIP1 abundance is reduced by 40% after 30 min, whereas the PIP2 and TIP levels decrease by 20–40% only after 6 h. The use of GFP-tagged aquaporins showed that AtTIP1;1 is relocalized to intravacuolar invaginations after 45 min, while the localization of AtTIP2;1 and AtPIPs remains unchanged. Only after 2 h of exposure to salt stress conditions are the AtPIP1 and AtPIP2 proteins occasionally found in intracellular structures instead of the PM. Root treatment with an equivalent osmotic concentration of mannitol (200 mM) induces a similar reduction in Lp_r, showing that the effect of NaCl on Arabidopsis roots is perceived as an osmotic challenge, rather than as an ionic toxicity (Boursiac et al. 2005). Ice plant (*Mesembryanthemum cristallinum*) root treatment with 200 mM mannitol also induces a partial aquaporin relocalization (Vera-Estrella et al. 2004). The tonoplast-localized McTIP1;2 is specifically translocated to intracellular vesicles upon osmotic challenge in a glycosylation-dependent way. Together with the data obtained by Boursiac et al. (2005), this work points to a redistribution of some, but not all, TIP aquaporins under abiotic stress conditions, but the mechanisms responsible for this aquaporin reorganization are poorly characterized. The effect of salt on plant PM aquaporin localization seems to be dose dependent, since a 150 mM NaCl treatment has effects in nature identical to a 100 mM stress, but the relocalization of AtPIPs to intracellular structures is faster (45 min) and more pronounced (Boursiac et al. 2008). Both salt (150 mM; 45 min) and SA (0.5 mM; 1 h) treatments induce the accumulation of reactive oxygen species (ROS), decrease Lp_r and cause the accumulation of GFP-tagged AtPIPs to intracellular structures (**Fig. 3**). When exogenously applied, catalase (a ROS scavenger) counteracts the effects of SA on Lp_r and AtPIP localization, and H_2O_2 reduces Lp_r by 90% in <15 min. Due to the fact that H_2O_2 has no influence on the water channel activity of AtPIPs expressed in oocytes, it has been concluded that ROS act in the signaling cascade in response to NaCl. Accordingly, H_2O_2 enhances the accumulation of GFP–AtPIP in intracellular structures (vesicles and small vacuoles) in <15 min. These findings show that H_2O_2 mediates a signaling cascade in response to salt exposure, which redirects AtPIPs to intracellular compartments in order to reduce the permeability of the PM to water. However, these results have partially been contradicted in a recent study showing that SA inhibits clathrin-mediated endocytosis of AtPIP2;1 and other PM proteins from the cell surface (Du et al. 2013).

Single-particle tracking by variable-angle evanescent wave microscopy showed that, very shortly after exposure to salt stress conditions (100 mM NaCl; 10 min), the diffusion coefficient of AtPIP2;1 in the PM of root epidermal cells doubled (Li et al. 2011). In addition to this quantitative change, the proportion of GFP–AtPIP2;1 that exhibits a restricted diffusion mode increases by 60%. As shown by fluorescence correlation spectroscopy, the density of GFP–AtPIP2;1 in the PM is 30.3 ± 5.1 molecules μm^{-2} in control conditions, and this decreases by 46% under salt stress conditions. Finally, while the internalization of AtPIP2;1 under normal conditions is predominantly linked to clathrin-mediated endocytosis, a raft-associated endocytic pathway is specifically induced upon exposure to NaCl. The higher proportion of particles with a restricted diffusion mode might reflect a specific recruitment of AtPIP2;1 to raft microdomains under salt stress conditions. This process might be facilitated by the increased diffusion coefficient of AtPIP2;1 in NaCl-treated root cells. As a result, this facilitates the reduction of the density of water channels at the cell surface by raft-mediated endocytosis under osmotic stress conditions.

In salt stress conditions (100 mM), the rate of PIP constitutive cycling is increased, but the lateral mobility of AtPIPs in the PM remains negligible, and no net increase in endosomal labeling is observed (Luu et al. 2012). All three steps of the cycle are intensified: endocytosis, transfer to the TGN and exocytosis. In contrast to control conditions, A23 is not sufficient to inhibit the early response. This is explained by two non-exclusive mechanisms: an enhancement of clathrin-mediated endocytosis (which would then over-ride the effect of A23), and the specific activation upon salt stress conditions of an A23-insensitive (probably clathrin-independent) endocytic pathway. The latter hypothesis confirms previous observations according to which raft-associated internalization would be activated in salt-exposed cells (Li et al. 2011). Finally, in salt stress conditions, the exocytosis step of the cycle is no longer fully blocked by BFA, indicating the induction of an alternative recycling route to the PM (Luu et al. 2012). The exact role of the increased rate of AtPIP constitutive cycling in the salt stress response has yet to be determined. The link between enhanced cycling and decreased root hydraulic conductivity (Boursiac et al. 2005) following NaCl exposure is not straightforward. Luu et al. (2012) suggested that the increased cycling rate might be coupled to a modification of the gating status of PIPs in order to modulate the permeability of the cell membrane to water. Previous studies showed that GFP–AtPIPs are relocalized to intracellular structures, but that this effect largely depends on the NaCl concentration. While GFP–AtPIP internalization is only occasionally observed after 2 h of treatment with 100 mM NaCl (Boursiac et al. 2005), AtPIPs are observed in intracellular structures in 30% of the cells after only 45 min when the NaCl concentration is increased to 150 mM (Boursiac et al. 2008). In contrast, no net increase in intracellular PIPs after 30 min of exposure to 100 mM NaCl was observed in the study conducted by Luu et al. (2012). This might, at least in part, be due to the different spatiotemporal resolutions of the experimental systems used in all of these studies.

Conclusions and Future Challenges

Plant aquaporins are essential for many physiological processes in plants and have been found in the membranes of a variety of cell compartments. They were first extracted from tonoplast- and PM-enriched fractions, and have been used as reference organelle markers

in many studies. Recent research on the subcellular routing of PIPs from the ER to the PM and on their dynamics at the PM revealed unexpected levels of complexity.

Maize PIP co-expression experiments demonstrated that not all ZmPIPs are equally able to reach the PM, as ZmPIP1s need to interact with ZmPIP2s to leave the ER. Whether this mechanism applies to other plant species remains unclear, as it is difficult to study PIP1 trafficking alone. Studies carried out in maize and Arabidopsis showed that the targeting of some, but not all, PIP2s to the PM requires an N-terminal diacidic motif. It has been suggested that a strict DxE sequence, and not only a diacidic motif is necessary. This should be investigated by mutating the ExD sequence located, in ZmPIP2;6 for instance, at the same conserved position as the DxE ER export motif of ZmPIP2;4, ZmPIP2;5 and AtPIP2;1. Intriguingly, ER-retained ZmPIP1s contain diacidic sequences in their N-terminus. Whether these play a role in bringing the ZmPIP1–ZmPIP2 complex to the PM should be investigated. More recently, another anterograde trafficking signal (LxxxA), surprisingly located within a transmembrane helix, has been identified in ZmPIP2;5. However, it remains unclear whether this motif drives recruitment to ERES, export from the ER or Golgi exit. The mechanism responsible for the recognition and sorting of this motif remains to be identified. Lipid and protein interaction studies should be performed to investigate this point. Post-translational modifications have also been shown to influence PIP subcellular transport in response to environmental changes. While phosphorylation promotes ER export, ubiquitylation prevents PIPs from further progress in the secretory pathway. This shows that the journey of PIPs to the PM does not occur by default, but instead requires multiple signals to leave the ER. However, more motifs regulating PIP anterograde progression are likely to be uncovered in the future. The factor retaining ZmPIP1s in the ER has still not been identified. Additionally, no sorting determinants have been identified for post-ER steps.

In the course of their journey across the cell, PIPs probably interact with numerous transport proteins. These aspects of PIP trafficking were shown recently in two studies demonstrating a physical interaction between PIP2s and the SYP121 and SYP61 SNAREs. However, knowledge of the 'trafficking interactome' of PIPs remains fragmentary, as only post-TGN interactions have been demonstrated. Efforts have to be made to identify more transport proteins interacting with PIPs, in order to draw a global map of PIP interactions during their movement through the secretory pathway. Finally, significant progress has recently been made in deciphering regulatory processes under changing environmental conditions. Both the anterograde trafficking of PIPs from the ER and their dynamics at the PM are regulated by environmental conditions; however, the understanding of this regulation remains incomplete. Since PIPs are emerging as a new model for endomembrane studies, additional knowledge of the cellular and molecular mechanisms governing PIP density in the membrane under abiotic stress conditions would be of great interest, not only for aquaporin trafficking research, but more broadly for the understanding of plant responses to stress.

Funding

This work was supported by the Belgian National Fund for Scientific Research; the Interuniversity Attraction Poles Programme-Belgian Science Policy (IAP7/29); the Communauté française de Belgique-Actions de Recherches Concertées (11/16-036); the Francqui Foundation; the Bauchau Award; the Fonds de Formation à la Recherche dans l'Industrie et l'Agriculture [grant No. FC89796 to A.S.C.].

Acknowledgments

We apologize to colleagues whose work could not be cited due to space restrictions.

Disclosures

The authors have no conflicts of interest to declare.

References

Alexandersson, E., Fraysse, L., Sjovall-Larsen, S., Gustavsson, S., Fellert, M., Karlsson, M. et al. (2005) Whole gene family expression and drought stress regulation of aquaporins. *Plant Mol. Biol.* 59: 469–484.

Barlowe, C. (2003) Signals for COPII-dependent export from the ER: what's the ticket out?. *Trends Cell Biol* 13: 295–300.

Bassham, D.C., Brandizzi, F., Otegui, M.S. and Sanderfoot, A.A. (2008) The secretory system of Arabidopsis. *In* The Arabidopsis Book. pp. 1–29. The American Society of Plant Biologists, Rockville, MD.

Belugin, B.V., Zhestkova, I.M. and Trofimova, M.S. (2011) Affinity of PIP-aquaporins to sterol-enriched domains in plasma membrane of the cells of etiolated pea seedlings. *Biochemistry (Moscow) Suppl. Ser. A: Membr. Cell Biol* 5: 56–63.

Besserer, A., Burnotte, E., Bienert, G.P., Chevalier, A.S., Errachid, A., Grefen, C. et al. (2012) Selective regulation of maize plasma membrane aquaporin trafficking and activity by the SNARE SYP121. *Plant Cell* 24: 3463–3481.

Bienert, G.P., Cavez, D., Besserer, A., Berny, M.C., Gilis, D., Rooman, M. et al. (2012) A conserved cysteine residue is involved in disulfide bond formation between plant plasma membrane aquaporin monomers. *Biochem. J.* 445: 101–111.

Bienert, G.P. and Chaumont, F. (2011) Plant aquaporins: roles in water homeostasis, nutrition, and signaling processes. *In* Transporters and Pumps in Plant Signaling. Edited by Geisler, M. and Venema, K. pp. 3–36. Springer, Berlin.

Bonifacino, J.S. and Traub, L.M. (2003) Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu. Rev. Biochem.* 72: 395–447.

Borner, G.H., Sherrier, D.J., Weimar, T., Michaelson, L.V., Hawkins, N.D., Macaskill, A. et al. (2005) Analysis of detergent-resistant membranes in Arabidopsis. Evidence for plasma membrane lipid rafts. *Plant Physiol.* 137: 104–116.

Boursiac, Y., Boudet, J., Postaire, O., Luu, D.T., Tournaire-Roux, C. and Maurel, C. (2008) Stimulus-induced downregulation of root water transport involves reactive oxygen species-activated cell signalling and plasma membrane intrinsic protein internalization. *Plant J.* 56: 207–218.

Boursiac, Y., Chen, S., Luu, D.T., Sorieul, M., van den Dries, N. and Maurel, C. (2005) Early effects of salinity on water transport in Arabidopsis roots.

Molecular and cellular features of aquaporin expression. *Plant Physiol.* 139: 790–805.

Brandizzi, F., Frangne, N., Marc-Martin, S., Hawes, C., Neuhaus, J.M. and Paris, N. (2002) The destination for single-pass membrane proteins is influenced markedly by the length of the hydrophobic domain. *Plant Cell* 14: 1077–1092.

Cai, Y., Jia, T., Lam, S.K., Ding, Y., Gao, C., San, M.W. et al. (2011) Multiple cytosolic and transmembrane determinants are required for the trafficking of SCAMP1 via an ER–Golgi–TGN–PM pathway. *Plant J.* 65: 882–896.

Cavez, D., Hachez, C. and Chaumont, F. (2009) Maize black Mexican sweet suspension cultured cells are a convenient tool for studying aquaporin activity and regulation. *Plant Signal. Behav.* 9: 890–892.

Chaumont, F., Barrieu, F., Jung, R. and Chrispeels, M.J. (2000) Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity. *Plant Physiol.* 122: 1025–1034.

Chaumont, F. and Tyerman, S.D. (2014) Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiol.* 164: 1600–1618.

Chevalier, A.S., Bienert, G.P. and Chaumont, F. (2014) A new LxxxA motif in the transmembrane helix3 of maize aquaporins belonging to the plasma membrane intrinsic protein PIP2 group is required for their trafficking to the plasma membrane. *Plant Physiol.* 166: 125–138.

Cosson, P., Perrin, J. and Bonifacino, J.S. (2013) Anchors aweigh: protein localization and transport mediated by transmembrane domains. *Trends Cell Biol.* 23: 511–517.

Dancourt, J. and Barlowe, C. (2010) Protein sorting receptors in the early secretory pathway. *Annu. Rev. Biochem.* 79: 777–802.

Daniels, M.J., Mirkov, T.E. and Chrispeels, M.J. (1994) The plasma membrane of *Arabidopsis thaliana* contains a mercury-insensitive aquaporin that is a homolog of the tonoplast water channel protein TIP. *Plant Physiol.* 106: 1325–1333.

Dhonukshe, P., Aniento, F., Hwang, I., Robinson, D.G., Mravec, J., Stierhof, Y.D. et al. (2007) Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in Arabidopsis. *Curr. Biol.* 17: 520–527.

Dhonukshe, P., Tanaka, H., Goh, T., Ebine, K., Mähönen, A.P., Prasad, K. et al. (2008) Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions. *Nature* 456: 962–966.

Diaz-Rohrer, B.B., Levental, K.R., Simons, K. and Levental, I. (2014) Membrane raft association is a determinant of plasma membrane localization. *Proc. Natl Acad. Sci. USA* 111: 8500–8505.

Du, Y., Tejos, R., Beck, M., Himschoot, E., Li, H., Robatzek, S. et al. (2013) Salicylic acid interferes with clathrin-mediated endocytic protein trafficking. *Proc. Natl Acad. Sci. USA* 110: 7946–7951.

Dunkel, M., Latz, A., Schumacher, K., Müller, T., Becker, D. and Hedrich, R. (2008) Targeting of vacuolar membrane localized members of the TPK channel family. *Mol. Plant* 1: 938–949.

Feraru, E., Feraru, M.I., Asaoka, R., Paciorek, T., De Rycke, R., Tanaka, H. et al. (2012) BEX5/RabA1b regulates trans-Golgi network-to-plasma membrane protein trafficking in Arabidopsis. *Plant Cell* 24: 3074–3086.

Feraru, E., Paciorek, T., Feraru, M.I., Zwiewka, M., De Groodt, R., De Rycke, R. et al. (2010) The AP-3 β adaptin mediates the biogenesis and function of lytic vacuoles in Arabidopsis. *Plant Cell* 22: 2812–2824.

Fetter, K., Van Wilder, V., Moshelion, M. and Chaumont, F. (2004) Interactions between plasma membrane aquaporins modulate their water channel activity. *Plant Cell* 16: 215–228.

Fortin, M.G., Morrison, N.A. and Verma, D.P. (1987) Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment. *Nucleic Acids Res.* 15: 813–824.

Gendre, D., Jonsson, K., Boutté, Y. and Bhalerao, R.P. (2015) Journey to the cell surface—the central role of the trans-Golgi network in plants. *Protoplasma* 252: 385–398.

Gershlick, D.C., Lousa, C.e.M., Foresti, O., Lee, A.J., Pereira, E.A., daSilva, L.L. et al. (2014) Golgi-dependent transport of vacuolar sorting receptors is regulated by COPII, AP1, and AP4 protein complexes in tobacco. *Plant Cell* 26: 1308–1329.

Grefen, C. and Blatt, M.R. (2008) SNAREs—molecular governors in signalling and development. *Curr. Opin. Plant Biol.* 11: 600–609.

Hachez, C., Besserer, A., Chevalier, A.S. and Chaumont, F. (2013) Insights into plant plasma membrane aquaporin trafficking. *Trends Plant Sci.* 18: 344–352.

Hachez, C., Heinen, R.B., Draye, X. and Chaumont, F. (2008) The expression pattern of plasma membrane aquaporins in maize leaf highlights their role in hydraulic regulation. *Plant Mol. Biol.* 68: 337–353.

Hachez, C., Laloux, T., Reinhardt, H., Cavez, D., Degand, H., Grefen, C. et al. (2014) Arabidopsis SNAREs SYP61 and SYP121 coordinate the trafficking of plasma membrane aquaporin PIP2;7 to modulate the cell membrane water permeability. *Plant Cell* 26: 3132–3147.

Hachez, C., Moshelion, M., Zelazny, E., Cavez, D. and Chaumont, F. (2006) Localization and quantification of plasma membrane aquaporin expression in maize primary root: a clue to understanding their role as cellular plumbers. *Plant Mol. Biol.* 62: 305–323.

Hanton, S.L., Renna, L., Bortolotti, L.E., Chatre, L., Stefano, G. and Brandizzi, F. (2005) Diacidic motifs influence the export of transmembrane proteins from the endoplasmic reticulum in plant cells. *Plant Cell* 17: 3081–3093.

Herzig, Y., Sharpe, H.J., Elbaz, Y., Munro, S. and Schuldiner, M. (2012) A systematic approach to pair secretory cargo receptors with their cargo suggests a mechanism for cargo selection by Erv14. *PLoS Biol.* 10: e1001329.

Höfte, H. and Chrispeels, M.J. (1992) Protein sorting to the vacuolar membrane. *Plant Cell* 4: 995–1004.

Honsbein, A., Blatt, M.R. and Grefen, C. (2011) A molecular framework for coupling cellular volume and osmotic solute transport control. *J. Exp. Bot.* 62: 2363–2370.

Honsbein, A., Sokolovski, S., Grefen, C., Campanoni, P., Pratelli, R., Paneque, M. et al. (2009) A tripartite SNARE-K+ channel complex mediates in channel-dependent K+ nutrition in Arabidopsis. *Plant Cell* 21: 2859–2877.

Jaillais, Y., Santambrogio, M., Rozier, F., Fobis-Loisy, I., Miège, C. and Gaude, T. (2007) The retromer protein VPS29 links cell polarity and organ initiation in plants. *Cell* 130: 1057–1070.

Johnson, K.D., Herman, E.M. and Chrispeels, M.J. (1989) An abundant, highly conserved tonoplast protein in seeds. *Plant Physiol.* 91: 1006–1013.

Kammerloher, W., Fischer, U., Piechottka, G.P. and Schäffner, A.R. (1994) Water channels in the plant plasma membrane cloned by immunoselection from a mammalian expression system. *Plant J.* 6: 187–199.

Kitakura, S., Vanneste, S., Robert, S., Löfke, C., Teichmann, T., Tanaka, H. et al. (2011) Clathrin mediates endocytosis and polar distribution of PIN auxin transporters in Arabidopsis. *Plant Cell* 23: 1920–1931.

Kleine-Vehn, J., Wabnik, K., Martinière, A., Łangowski, Ł., Willig, K., Naramoto, S. et al. (2011) Recycling, clustering, and endocytosis jointly maintain PIN auxin carrier polarity at the plasma membrane. *Mol. Syst. Biol.* 7: 540.

Lee, H.K., Cho, S.K., Son, O., Xu, Z.Y., Hwang, I. and Kim, W.T. (2009) Drought stress-induced Rma1H1, a RING membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination in transgenic Arabidopsis plants. *Plant Cell* 21: 622–641.

Lefebvre, B., Furt, F., Hartmann, M.A., Michaelson, L.V., Carde, J.P., Sargueil-Boiron, F. et al. (2007) Characterization of lipid rafts from *Medicago truncatula* root plasma membranes: a proteomic study reveals the presence of a raft-associated redox system. *Plant Physiol.* 144: 402–418.

Li, G., Santoni, V. and Maurel, C. (2014) Plant aquaporins: roles in plant physiology. *Biochim. Biophys. Acta* 1840: 1574–1582.

Li, X., Wang, X., Yang, Y., Li, R., He, Q., Fang, X. et al. (2011) Single-molecule analysis of PIP2;1 dynamics and partitioning reveals multiple modes of Arabidopsis plasma membrane aquaporin regulation. *Plant Cell* 23: 3780–3797.

Luu, D.T., Martinière, A., Sorieul, M., Runions, J. and Maurel, C. (2012) Fluorescence recovery after photobleaching reveals high cycling dynamics of plasma membrane aquaporins in Arabidopsis roots under salt stress. *Plant J.* 69: 894–905.

Luu, D.T. and Maurel, C. (2013) Aquaporin trafficking in plant cells: an emerging membrane-protein model. *Traffic* 14: 629–635.

Ma, D., Zerangue, N., Lin, Y.F., Collins, A., Yu, M., Jan, Y.N. et al. (2001) Role of ER export signals in controlling surface potassium channel numbers. *Science* 291: 316–319.

Ma, J.F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M. et al. (2006) A silicon transporter in rice. *Nature* 440: 688–691.

Martinière, A., Lavagi, I., Nageswaran, G., Rolfe, D.J., Maneta-Peyret, L., Luu, D.T. et al. (2012) Cell wall constrains lateral diffusion of plant plasma-membrane proteins. *Proc. Natl Acad. Sci. USA* 109: 12805–12810.

Men, S., Boutté, Y., Ikeda, Y., Li, X., Palme, K., Stierhof, Y.D. et al. (2008) Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier polarity. *Nat. Cell. Biol.* 10: 237–244.

Minami, A., Fujiwara, M., Furuto, A., Fukao, Y., Yamashita, T., Kamo, M. et al. (2009) Alterations in detergent-resistant plasma membrane microdomains in *Arabidopsis thaliana* during cold acclimation. *Plant Cell Physiol.* 50: 341–359.

Mitani, N., Yamaji, N., Ago, Y., Iwasaki, K. and Ma, J.F. (2011) Isolation and functional characterization of an influx silicon transporter in two pumpkin cultivars contrasting in silicon accumulation. *Plant J.* 66: 231–240.

Mitani, N., Yamaji, N. and Ma, J.F. (2009) Identification of maize silicon influx transporters. *Plant Cell Physiol.* 50: 5–12.

Morel, J., Claverol, S., Mongrand, S., Furt, F., Fromentin, J., Bessoule, J.J. et al. (2006) Proteomics of plant detergent-resistant membranes. *Mol. Cell. Proteomics* 5: 1396–1411.

Nebenfuhr, A. (2014) Targeting of polytopic proteins to the plasma membrane. *Plant Physiol.* 166: 3–4.

Nishimura, N. and Balch, W.E. (1997) A di-acidic signal required for selective export from the endoplasmic reticulum. *Science* 277: 556–558.

Nufer, O., Guldbrandsen, S., Degen, M., Kappeler, F., Paccaud, J.P., Tani, K. et al. (2002) Role of cytoplasmic C-terminal amino acids of membrane proteins in ER export. *J. Cell Sci.* 115: 619–628.

Ohno, H., Fournier, M.C., Poy, G. and Bonifacino, J.S. (1996) Structural determinants of interaction of tyrosine-based sorting signals with the adaptor medium chains. *J. Biol. Chem.* 271: 29009–29015.

Paciorek, T., Zazímalová, E., Ruthardt, N., Petrásek, J., Stierhof, Y.D., Kleine-Vehn, J. et al. (2005) Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature* 435: 1251–1256.

Prak, S., Hem, S., Boudet, J., Viennois, G., Sommerer, N., Rossignol, M. et al. (2008) Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins. *Mol. Cell. Proteomics* 7: 1019–1030.

Robert, S., Kleine-Vehn, J., Barbez, E., Sauer, M., Paciorek, T., Baster, P. et al. (2010) ABP1 mediates auxin inhibition of clathrin-dependent endocytosis in Arabidopsis. *Cell* 143: 111–121.

Ronchi, P., Colombo, S., Francolini, M. and Borgese, N. (2008) Transmembrane domain-dependent partitioning of membrane proteins within the endoplasmic reticulum. *J. Cell Biol.* 181: 105–118.

Sakurai-Ishikawa, J., Murai-Hatano, M., Hayashi, H., Ahamed, A., Fukushi, K., Matsumoto, T. et al. (2011) Transpiration from shoots triggers diurnal changes in root aquaporin expression. *Plant Cell Environ.* 34: 1150–1163.

Sato, K., Ueda, T. and Nakano, A. (1999) The *Arabidopsis thaliana* RER1 gene family: its potential role in the endoplasmic reticulum localization of membrane proteins. *Plant Mol. Biol.* 41: 815–824.

Schaecher, S.R., Diamond, M.S. and Pekosz, A. (2008) The transmembrane domain of the severe acute respiratory syndrome coronavirus ORF7b protein is necessary and sufficient for its retention in the Golgi complex. *J. Virol.* 82: 9477–9491.

Sharpe, H.J., Stevens, T.J. and Munro, S. (2010) A comprehensive comparison of transmembrane domains reveals organelle-specific properties. *Cell* 142: 158–169.

Sieben, C., Mikosch, M., Brandizzi, F. and Homann, U. (2008) Interaction of the K+-channel KAT1 with the coat protein complex II coat component Sec24 depends on a di-acidic endoplasmic reticulum export motif. *Plant J.* 56: 997–1006.

Sorieul, M., Santoni, V., Maurel, C. and Luu, D.T. (2011) Mechanisms and effects of retention of over-expressed aquaporin AtPIP2;1 in the endoplasmic reticulum. *Traffic* 12: 473–482.

Surma, M.A., Klose, C. and Simons, K. (2012) Lipid-dependent protein sorting at the trans-Golgi network. *Biochim. Biophys. Acta* 1821: 1059–1067.

Takahashi, D., Kawamura, Y. and Uemura, M. (2013) Changes of detergent-resistant plasma membrane proteins in oat and rye during cold acclimation: association with differential freezing tolerance. *J. Proteome Res.* 12: 4998–5011.

Takano, J., Miwa, K., Yuan, L., von Wirén, N. and Fujiwara, T. (2005) Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. *Proc. Natl Acad. Sci. USA* 102: 12276–12281.

Takano, J., Tanaka, M., Toyoda, A., Miwa, K., Kasai, K., Fuji, K. et al. (2010) Polar localization and degradation of Arabidopsis boron transporters through distinct trafficking pathways. *Proc. Natl Acad. Sci. USA* 107: 5220–5225.

Törnroth-Horsefield, S., Wang, Y., Hedfalk, K., Johanson, U., Karlsson, M., Tajkhorshid, E. et al. (2006) Structural mechanism of plant aquaporin gating. *Nature* 439: 688–694.

Uehlein, N., Otto, B., Hanson, D.T., Fischer, M., McDowell, N. and Kaldenhoff, R. (2008) Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. *Plant Cell* 20: 648–657.

Vera-Estrella, R., Barkla, B.J., Bohnert, H.J. and Pantoja, O. (2004) Novel regulation of aquaporins during osmotic stress. *Plant Physiol.* 135: 2318–2329.

Verdoucq, L., Rodrigues, O., Martinière, A., Luu, D.T. and Maurel, C. (2014) Plant aquaporins on the move: reversible phosphorylation, lateral motion and cycling. *Curr. Opin. Plant Biol.* 22C: 101–107.

Votsmeier, C. and Gallwitz, D. (2001) An acidic sequence of a putative yeast Golgi membrane protein binds COPII and facilitates ER export. *EMBO J.* 20: 6742–6750.

Wolfenstetter, S., Wirsching, P., Dotzauer, D., Schneider, S. and Sauer, N. (2012) Routes to the tonoplast: the sorting of tonoplast transporters in Arabidopsis mesophyll protoplasts. *Plant Cell* 24: 215–232.

Wudick, M.M., Luu, D.T. and Maurel, C. (2009) A look inside: localization patterns and functions of intracellular plant aquaporins. *New Phytol.* 184: 289–302.

Yang, H., Krebs, M., Stierhof, Y.D. and Ludewig, U. (2014) Characterization of the putative amino acid transporter genes AtCAT2, 3 &4: the tonoplast localized AtCAT2 regulates soluble leaf amino acids. *J. Plant Physiol.* 171: 594–601.

Zelazny, E., Borst, J.W., Muylaert, M., Batoko, H., Hemminga, M.A. and Chaumont, F. (2007) FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. *Proc. Natl Acad. Sci. USA* 104: 12359–12364.

Zelazny, E., Miecielica, U., Borst, J.W., Hemminga, M.A. and Chaumont, F. (2009) An N-terminal diacidic motif is required for the trafficking of maize aquaporins ZmPIP2;4 and ZmPIP2;5 to the plasma membrane. *Plant J.* 57: 346–355.

Zelazny, E. and Vert, G. (2014) Plant nutrition: root transporters on the move. *Plant Physiol.* 166: 500–508.

Zhang, L., Zhang, H., Liu, P., Hao, H., Jin, J.B. and Lin, J. (2011) Arabidopsis R-SNARE proteins VAMP721 and VAMP722 are required for cell plate formation. *PLoS One* 6: e26129.