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Phosphatidylinositol 4-phosphate: a key determinant of plasma membrane identity and function in plants

Vedrana Marković¹ and Yvon Jaillais^{1*}

Vedrana Marković <https://orcid.org/0000-0002-0310-5102>

Yvon Jaillais <https://orcid.org/0000-0003-4923-883X>

¹Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, CNRS, INRAE, F-69342, Lyon, France

* Correspondance : yvon.jaillais@ens-lyon.fr

Summary: Phosphatidylinositol 4-phosphate (PI4P) is an anionic phospholipid, which has been described as a master regulator of the Golgi apparatus in eukaryotic cells. However, recent evidence suggests that PI4P mainly accumulates at the plasma membrane in all plant cells analyzed so far. Besides, many functions that are typically attributed to phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] in animal and yeast cells are also supported by PI4P in plants. For example, PI4P is the key anionic lipid that powers the strong electrostatic properties of the plasma membrane. PI4P is also required for the establishment of stable membrane contacts between the endoplasmic reticulum (ER) and the plasma membrane, for exocytosis and to support signaling pathways. Thus, we propose that PI4P has a prominent role in specifying the identity of the plasma membrane and in supporting some of its key functions and should be considered a hallmark lipid of this compartment.

Key words: plant cell biology, membrane lipid, PtdIns4P, phosphoinositides, trafficking, endocytosis, nanodomains, membrane contact sites.

Introduction.

Within the endomembrane system of eukaryotic cells, anionic lipids are low abundant lipids but they are key landmarks that contribute to the identity of membrane compartments (Noack & Jaillais, 2017, 2020). Anionic phospholipids have a negatively charged head group that each can recruit a suite of effector proteins. Phosphatidylinositol 4-phosphate (PI4P) is a derivative of phosphatidylinositol, which is phosphorylated on the fourth position of its inositol head group. Early studies in animal and yeast cells established PI4P as a master regulator of the Golgi apparatus (De Matteis *et al.*, 2005). Indeed, many PI4P binding proteins localize to the Golgi. Such as, for example, the oxysterol-binding protein (OSBP), four-phosphate-adaptor protein 1 (FAPP1), ceramide transfer (CERT), Golgi-localized gamma-ear-containing ADP-ribosylation factor-binding proteins (GGAs) or Golgi-phosphoprotein3 (GOLPH3) (Santiago-Tirado & Bretscher, 2011). In addition, PI4P is a key trafficking regulator of the Golgi and *trans*-Golgi Network (TGN) thanks to its interplay with small GTPases from the Rab and Arf families (Noack & Jaillais, 2017).

Although PI4P mainly accumulates in the Golgi in animal cells, its function extends beyond the Golgi (De Matteis *et al.*, 2013). New generation of PI4P biosensors together with PI4P immunolocalization established that PI4P also accumulates at the plasma membrane, in late endosomes and in autophagosomes (De Matteis *et al.*, 2013). In plants, the situation is even more extreme since PI4P accumulates as a major pool at the plasma membrane and is present

as a minor pool in the TGN (Noack & Jaillais, 2020). In this review, we present how PI4P localization was studied in plants and what are the evidences for a massive accumulation of PI4P at the cell surface by comparison to its intracellular localization. We describe the known mechanisms that regulate this PI4P balance between the plasma membrane and the TGN. Finally, we summarize some of the key functions of PI4P at the plasma membrane, including many cellular pathways that are traditionally associated with PI(4,5)P₂ function in animal cells.

An inverted balance of PI4P between the TGN and the plasma membrane in plants.

The plekstrin homology (PH) domain of FAPP1 and OSBP interacts with PI4P and when fused to a fluorescent protein, they almost exclusively localize to the Golgi in both animal and yeast cells (Levine & Munro, 1998, 2002; Simon *et al.*, 2014). By contrast, expression of the same domains in plant cells, including tobacco BY-2 cells and all plant cell types analyzed in transgenic *Arabidopsis thaliana*, leads to a drastically different localization (Vermeer *et al.*, 2009; Simon *et al.*, 2014). Indeed, PH^{FAPP1} is targeted to the TGN but also to a greater extent to the plasma membrane (Simon *et al.*, 2014). PI4P sensor with higher avidity, consisting of two (2xPH) or three (3xPH) repeats of the FAPP1 PH domain, are even more associated with the plasma membrane than 1xPH^{FAPP1}, which hints that the plasma membrane accumulates more PI4P than the TGN membrane (Simon *et al.*, 2014, 2016). This hypothesis is supported by additional observations. The PH domain of FAPP1 does not only bind to PI4P but also to the small GTPase ARF1, which localizes at the TGN (Levine & Munro, 2002; He *et al.*, 2011). However, the two binding sites are distinct, so it is possible to mutate the ARF1 binding site while leaving the PI4P interacting domain fully functional (He *et al.*, 2011). Such mutant strictly localizes to the plasma membrane in plants (Simon *et al.*, 2016; Ito *et al.*, 2021). By contrast, mutants still able to interact to ARF1 but not PI4P localize to the TGN and not the plasma membrane (Simon *et al.*, 2016). Thus, PI4P binding is dispensable for PH^{FAPP1} to associate with the TGN, while it is necessary and sufficient for plasma membrane targeting. Moreover, the P4M domain from the SiDM protein of *Legionella pneumophila*, which specifically binds to PI4P but not ARF1 (Hammond *et al.*, 2014), is strictly localized at the plasma membrane in all cell types analyzed (i.e., various root tissues, hypocotyl, leaf epidermis, shoot apical meristem) in *Arabidopsis* and in *Nicotiana benthamiana* leaves (Simon *et al.*, 2016).

So far, only two endogenous *Arabidopsis* protein, PLANT U-BOX13 (PUB13) and VASCULAR NETWORK DEFECTIVE3/ FORKED 1 (VAN3/FKD1) have been shown to bind PI4P and to localize at the TGN (**Table 1**) (Koizumi *et al.*, 2005; Naramoto *et al.*, 2009; Antignani *et al.*, 2015). Of note, both proteins also require interaction with TGN-associated proteins for localization, confirming that PI4P binding alone may not be sufficient for TGN targeting (Table 1). In addition, recent reports found these proteins to also localize at the plasma membrane (Zhou *et al.*, 2018; Adamowski & Friml, 2021), again suggesting that PI4P binding favors plasma membrane targeting in plants.

Regulation of the PI4P balance between the plasma membrane and the TGN

PI4K α 1 localize at the plasma membrane and is essential for both gametophyte and sporophyte development, which is consistent with a prominent functional role of PI4P at the cell surface (**Figure 1a**) (Noack *et al.*, 2022). PI4K β 1 and PI4K β 2 localize to the TGN (Preuss *et al.*, 2006). These two kinases are redundant, with each single mutant presenting no phenotype, while the double mutant shows root hair and cytokinesis defects, as well as innate immunity-related phenotypes (Preuss *et al.*, 2006; Šašek *et al.*, 2014; Antignani *et al.*, 2015; Lin *et al.*, 2019). In addition, electron tomography showed that the TGN of the *pi4k β 1 β 2* double mutant has an aberrant morphology (Kang *et al.*, 2011). Together, the presence of these kinases at the TGN

and the phenotype of the double mutant argue in favor of PI4P being synthesized and present in this compartment. Note that PI4K β 1 also localizes at the cell plate in dividing cells (Lin *et al.*, 2019), and that this compartment strongly accumulates PI4P sensors (Simon *et al.*, 2016).

The existence of a minor pool of PI4P at the TGN was also revealed thanks to the use of a specific tool designed to deplete PI4P at the plasma membrane. This tool, coined MAP-SAC1, consists of a plasma membrane targeting sequence (myristoylation and palmitoylation sequence, MAP), fused with a fluorescent protein and the isolated catalytic domain of the yeast PI4P phosphatase SAC1 (Simon *et al.*, 2016; Platre *et al.*, 2018; Doumane *et al.*, 2022). MAP-SAC1 is specifically decreasing the amount of PI4P at the plasma membrane, but is not impacting the pool of PI4P at the TGN. In this condition, PH^{FAPP1} and P4M^{SidM} relocate from the plasma membrane to the TGNs, demonstrating that the two PI4P pools are in competition to attract PI4P-binding proteins (Simon *et al.*, 2016; Platre *et al.*, 2018). Thus, it is likely that two pools of PI4P exist in plant cells, a major one at the cell surface and a minor one in the TGN.

This hypothesis is also supported by the fact that the PI4P balance between the TGN and the plasma membrane is actively regulated by at least two pathways (Figure 1). The first one was described in root hairs. In this cell type, the PI4P phosphatase SAC7, also called ROOT HAIR DEFECTIVE4 (RHD4), localizes to the TGN (Thole *et al.*, 2008). In the *sac7* mutant, PI4P sensors accumulate in intracellular compartments, likely corresponding to TGNs. This phenotype is even stronger in *sac6sac7* and *sac7sac8* double mutants, SAC6 and SAC8 being closely related to the SAC7 protein (Dubois & Jaillais, 2020; Song *et al.*, 2021). This balance is also maintained by the activity of a phosphoinositide-specific phospholipase C (PI-PLC) at the TGN (Ito *et al.*, 2021). Chemical inhibition of PI-PLCs increases the amount of PI4P sensors in the TGN. In addition, inhibition of very long chain fatty acid synthesis, which mainly targets sphingolipids, decreases the amount of PI-PLC2/7 at the TGN and thereby increases the amount of PI4P in this compartment (Ito *et al.*, 2021). These results were confirmed by direct lipid measurements using mass spectrometry, thereby confirming data obtained with translocation PI4P sensors. Altogether, it appears that PI4P phosphatases and PI4P phospholipases cooperate to actively maintain a low concentration of PI4P at the TGN membrane (Boutté & Jaillais, 2020).

PI4P is essential for many cellular functions at the cell surface

One possible explanation for the continuous and active removal of PI4P at the TGN in plants, is to target PI4P-binding proteins to the plasma membrane. Indeed, PI4P not only accumulates at the cell surface, but it is absolutely crucial for many cellular processes that are known to rely on PI(4,5)P₂ in other eukaryotic systems.

Plasma membrane electrostatics. The plasma membrane is highly electronegative in yeast, animal and plant cells, but these organisms use different anionic lipids to power the electrostatic field of this compartment (Platre & Jaillais, 2017). In animals, PI(4,5)P₂ has a central role but PI4P and PI(3,4,5)P₃ are redundantly involved in the regulation of membrane surface charges. In yeast, phosphatidylserine is the main lipid that drives plasma membrane electrostatics. In plants, the MAP-SAC1 assay, as well as pharmacological inhibition, suggest that PI4P is the main driver of plasma membrane electrostatic (Simon *et al.*, 2016). Phosphatidic acid (PA), phosphatidylserine and PI(4,5)P₂ contribute to the surface charges of the plant plasma membrane but to a lesser extent (Barbosa *et al.*, 2016; Platre *et al.*, 2018; Synek *et al.*, 2021).

ER/PM contact sites. PI4P is also required for the localization of tethering proteins involved in the establishment of membrane contact sites (Noack & Jaillais, 2020; Kang *et al.*, 2022) (**Table 1**). SYNAPTOTAGMIN1 (SYT1) is a tethering protein that is embedded in the ER membrane via transmembrane domains and which contacts the plasma membrane through lipid-binding C2 domains (Pérez-Sancho *et al.*, 2015) (**Figure 2a**). SYT1 is homologous to mammalian extended-synaptotagmin (E-SYT), which require PI(4,5)P₂ for association with the plasma membrane (Giordano *et al.*, 2013). By contrast, SYT1 localize in the bulk ER when coexpressed with MAP-SAC1, suggesting that PI4P is critical for SYT1 plasma membrane association (Ruiz-Lopez *et al.*, 2021). PI4P is also required for the localization of MULTIPLE C2 DOMAIN AND TRANSMEMBRANE PROTEINs (MCTP) (Brault *et al.*, 2019). MCTPs are tethering the ER and the plasma membrane within plasmodesmata, which are membrane-lined pores that connect plant cells (Kang *et al.*, 2022). Upon pharmacological inhibition of PI4P, MCTP4 does not accumulate in plasmodesmata anymore (Brault *et al.*, 2019).

Exocytosis and endocytosis. The exocyst is an octameric protein complex that tethers transport vesicles to the target membrane and represents one of the fundamental regulators of exocytosis in eukaryotes (Žárský *et al.*, 2013). In yeast and animal cells, the exocyst recruitment to the plasma membrane depends on the direct interaction of Exo70 subunit with PI(4,5)P₂ (Liu *et al.*, 2007). In *Arabidopsis*, the exocyst is targeted to the plasma membrane via the EXO70A1 subunit (Synek *et al.*, 2021). The plasma membrane localization of EXO70A1 was completely lost upon the pharmacological depletion of PI4P, and diminished upon inhibition of PA synthesis, while mutants with low levels of PI(4,5)P₂ had a minor impact on exocyst membrane association (**Table 1**). Thus, unlike in yeasts and animals, PI4P seem to be more important than PI(4,5)P₂ in exocyst membrane targeting in *Arabidopsis* root cells (**Figure 2b**).

A similar situation may also exist for clathrin-mediated endocytosis. Indeed, PI(4,5)P₂ is absolutely required for endocytosis in mammalian cells and depletion of this lipid from the plasma membrane completely and acutely inhibits endocytosis (He *et al.*, 2017). In plants, PI(4,5)P₂ is also involved in endocytosis but depletion of PI(4,5)P₂ from the cell surface does not fully block endocytosis (Doumane *et al.*, 2021). It is thus possible that other anionic lipids may replace or compensate, at least partially, PI(4,5)P₂ during endocytosis. For example, the μ -homology domain (μ HD) of the TML protein interacts with PI4P in vitro, clusters PI4P molecules in silico and depends on PI4P for plasma membrane localization (Yperman *et al.*, 2021) (**Table 1**). TML is part of the T-PLATE complex, which is essential for clathrin-mediated endocytosis in plants (Gadeyne *et al.*, 2014). Several plant dynamin-related proteins have also been shown to interact with PI4P in vitro (Kim *et al.*, 2001; Lam *et al.*, 2002; Lee *et al.*, 2002; Bednarek & Backues, 2010; Minami *et al.*, 2015), however the exact roles of PI4P in dynamin function at the plasma membrane remain to be fully evaluated in planta (**Table 1**).

Signaling. A number of proteins involved in cell signaling also rely on PI4P for plasma membrane association (**Table 1**). This is the case for example of AGC kinases such a PINOID or D6PKs and the Rho-GTPase of Plant6 (ROP6) (Barbosa *et al.*, 2016; Simon *et al.*, 2016; Platré *et al.*, 2019). In addition, NON-PHOTOTROPIC HYPCOTYL3 (NPH3), a substrate of the AGC kinase and blue photoreceptor Phot1, also localizes to the plasma membrane in a PI4P-dependent manner (Reuter *et al.*, 2021) (**Figure 2c**). NPH3 association with the plasma membrane is dynamically regulated by lights and is dependent on NPH3 phosphorylation and 14-3-3 association (Reuter *et al.*, 2021). BRI1 KINASE INHIBITOR1 (BKI1) and MEMBRANE ASSOCIATED KINASE REGULATOR2 (MAKR2), which are receptor kinase inhibitors, also localize to the plasma membrane in a PI4P-dependent manner (Jaillais *et al.*, 2011; Simon *et al.*, 2016). Like for NPH3, the association between BKI1/MAKR2 and the cell

surface is actively regulated by phosphorylation (Jaillais *et al.*, 2011; Marquès-Bueno *et al.*, 2021) (**Figure 2c**).

Moreover, using MAP-SAC1, PI4P was shown to be required for the plasma membrane localization and function of ACTIVATED DISEASE RESISTANCE1 (ADR1) and RESISTANCE TO *P. SYRINGAE* PV MACULICOLA 1 (RPM1). ADR1 and RPM1 are immune receptors (Saile *et al.*, 2021). Interestingly, the effect is specific for PI4P, since depletion of PI(4,5)P₂ using MAP-OCRL, a synthetic plasma membrane-targeted 5-phosphatase, did not impact the localization of ADR1 or RPM1 (Doumane *et al.*, 2021; Saile *et al.*, 2021; Doumane *et al.*, 2022). Thus, PI4P is not only essential for development but also for signaling events critical to immune responses.

Nanodomain formation. The plasma membrane is not uniform and instead is made of a myriad of domains that have specific lipid and protein compositions (Jaillais & Ott, 2020). REMORINs (REM) are well characterized proteins that localize in nanodomains in a sterol dependent manner. Their C-terminal anchor (REM-CA) binds to PI4P *in vitro* (Gronnier *et al.*, 2017) (Table 1). In addition, the targeting of StREM1.3 is dependent on PI4P at the plasma membrane, as demonstrated by the MAP-SAC1 assay. Thus, PI4P is not only required for the localization and function of many proteins at the plasma membrane, but might also be important for their targeting at the nanoscale (Jaillais & Ott, 2020) (**Figure 2d**). In addition, PI4K α 1 and its protein partners are not uniformly localized at the plasma membrane, but are also present in nanodomains (Noack *et al.*, 2022). However, how PI4K α 1-containing nanodomains relate to the function of PI4P in organizing plasma membrane nanodomains remains to be fully established.

Conclusion

PI4P has an unusual accumulation pattern in plants by comparison with animal and yeast cells. It is seldomly found in the TGN, thanks to an active and local removal by phosphatases and phospholipases. By contrast, most PI4P concentrate at the plasma membrane, where it serves essential cellular functions, in term of structural organization, intracellular trafficking and signaling. However, these functions are just beginning to emerge and it is likely that many more PI4P-dependent phenomena will be discovered in the near future. Another challenge will be to decipher whether the balance of PI4P between the TGN and the plasma membrane is dynamically regulated by signaling pathways or during development. The exact function of PI4P in the Golgi/TGN also remains poorly understood. Given the specificities of the plant TGN (Kang *et al.*, 2022), it is likely that PI4P may have unique functions in this compartment.

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Protein	Localization	Mode of binding	Lipid binding method	Function of the protein	References
PUB13	TGN, plasma membrane#	<i>PI4P binding via ARM domain and co-incident binding to RabA4B</i>	<i>In vitro</i> : lipid overlay assay, liposomes	signaling	Antignani <i>et al.</i> , 2015 Zhou <i>et al.</i> , 2018
VAN3/FKD1	TGN or plasma membrane*	<i>PI4P binding via PH domain and co-incident binding to CVP2 and VAB</i>	<i>In vitro</i> : lipid overlay assay, stimulation of enzymatic activity	membrane trafficking	Koizumi <i>et al.</i> , 2005 Naramoto <i>et al.</i> , 2009
DRP2A/ADL6	TGN, plasma membrane	<i>Anionic lipids binding via PH domain</i>	<i>In vitro</i> : lipid overlay assay, liposomes	membrane trafficking	Lam <i>et al.</i> , 2002
AGD1	Plasma membrane	<i>PI4P and other monophosphorylated phosphoinositides binding via PH domain</i>	<i>In vitro</i> : lipid overlay assay	membrane trafficking	Yoo <i>et al.</i> , 2012
SYT1	Plasma membrane	<i>Anionic lipids binding via C2 domain</i>	<i>In vivo</i> : MAP-SAC1, PAO <i>In vitro</i> : lipid overlay assay, liposome	membrane contact site	Ruiz-Lopez <i>et al.</i> , 2021 Perez-Sancho <i>et al.</i> , 2015
MCTP	Plasma membrane	<i>PI4P binding via C2 domain</i>	<i>In vivo</i> : PAO <i>In silico</i> : molecular dynamics	membrane contact site	Brault <i>et al.</i> , 2019
D6PK	Plasma membrane	<i>Anionic lipids binding via polybasic stretch</i>	<i>In vitro</i> : lipid overlay assay, liposomes <i>In vivo</i> : PAO	signaling	Barbosa <i>et al.</i> , 2016
EXO70A1	Plasma membrane	<i>Anionic lipids via polybasic surface + co-incident binding?</i>	<i>In vitro</i> : lipid overlay assay, liposomes <i>In vivo</i> : PAO <i>In silico</i> : molecular dynamics	membrane trafficking	Synek <i>et al.</i> , 2021
PID	Plasma membrane	<i>Anionic lipids via polybasic stretch in kinase insertion domain</i>	<i>In vivo</i> : PAO <i>In vitro</i> : lipid overlay assay	signaling	Simon <i>et al.</i> , 2016
ROP6	Plasma membrane	<i>Anionic lipids via polybasic C-terminal tail + Geranylgeranyl lipid anchor</i>	<i>In vitro</i> : lipid overlay assay <i>In vivo</i> : PAO	signaling	Platre <i>et al.</i> , 2019
NPH3	Plasma membrane	<i>Anionic lipids binding via a C-terminal amphipathic helix with a polybasic face</i>	<i>In vivo</i> : MAP-SAC1 <i>In vitro</i> : lipid overlay assay, liposomes	signaling	Reuter <i>et al.</i> , 2021
BK11	Plasma membrane	<i>Anionic lipids binding via polybasic stretch</i>	<i>In vitro</i> : lipid overlay assay <i>In vivo</i> : BK11 localization in WT and cho1Δ yeast	signaling	Jaillais <i>et al.</i> , 2011 Simon <i>et al.</i> , 2016
MAKR2	Plasma membrane	<i>Anionic lipids binding via polybasic stretch</i>	<i>In vitro</i> : lipid overlay assay <i>In vivo</i> : PAO; MAKR2 localization in WT and cho1Δ yeast	signaling	Simon <i>et al.</i> , 2016 Marquès-Bueno <i>et al.</i> , 2021
MAKR1, MAKR3, MAKR4	Plasma membrane	<i>Anionic lipids binding via polybasic stretch</i>	<i>In vitro</i> : lipid overlay assay	signaling	Simon <i>et al.</i> , 2016
ADR1	Plasma membrane	<i>Anionic lipids binding via CC_R domain</i>	<i>In vivo</i> : MAP-SAC1 <i>In vitro</i> : lipid overlay assay	signaling	Saile <i>et al.</i> , 2021
RPM1	Plasma membrane	<i>Anionic lipids binding via CC domain</i>	<i>In vivo</i> : MAP-SAC1 <i>In vitro</i> : lipid overlay assay	signaling	Saile <i>et al.</i> , 2021
REM1.3	Plasma membrane	<i>Phosphoinositide binding via REM-C anchor</i>	<i>In vivo</i> : MAP-SAC1 <i>In vitro</i> : liposomes <i>In silico</i> : molecular dynamics	nanodomain formation	Gronnier <i>et al.</i> , 2017
TML	Plasma membrane	<i>PI4P binding and clustering via μHD domain</i>	<i>In vivo</i> : PAO <i>In vitro</i> : lipid overlay assay <i>In silico</i> : molecular dynamics	membrane trafficking	Yperman <i>et al.</i> , 2021
POL/PLL	Plasma membrane	<i>PI4P and PS and targeting to the plasma membrane via myristoylation anchoring</i>	<i>In vitro</i> : lipid overlay assay, regulation of enzymatic activity	signaling	Gagne <i>et al.</i> , 2010

PATL2	Plasma membrane	Anionic lipid binding via SEC14 domain	In vitro: lipid overlay assay	membrane trafficking	Suzuki <i>et al.</i> , 2016
PATL3	Plasma membrane	co-incident binding to EXO70A1	In vitro: lipid overlay assay	membrane trafficking	Wu <i>et al.</i> , 2017
DRP1A	Plasma membrane	unknown	In vitro: liposomes	membrane trafficking	Backues and Bednarek, 2010
DRP1E	Plasma membrane	unknown	In vitro: lipid overlay assay	membrane trafficking	Minami <i>et al.</i> , 2015

Table 1. Summary of Arabidopsis proteins known to rely on PI4P for localization or to interact with PI4P in vitro. PLANT U-BOX13 (PUB13), VASCULAR NETWORK DEFECTIVE3/ FORKED 1 (VAN3/FKD1), DYNAMIN-RELATED PROTEIN 2A/ ARABIDOPSIS DYNAMIN-LIKE PROTEIN 6 (DRP2A/ADL6), ARF-GAP DOMAIN 1 (AGD1), SYNAPTOTAGMIN 1 (SYT1), MULTIPLE C2 DOMAIN AND TRANSMEMBRANE REGION PROTEIN (MCTP), D6 PROTEIN KINASE (D6PK), EXOCYST SUBUNIT EXO70 FAMILY PROTEIN A1 (EXO70A1), PINOID (PID), RHO-RELATED PROTEIN FROM PLANTS 6 (ROP6), NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3), BRI1 KINASE INHIBITOR 1 (BKI1), MEMBRANE-ASSOCIATED KINASE REGULATOR 1-4 (MAKR1-4), ADR1 ACTIVATED DISEASE RESISTANCE 1 (ADR1), RESISTANCE TO PSEUDOMONAS SYRINGAE PV MACULICOLA 1 (RPM1), REMORIN 1.3 (REM1.3), TPLATE COMPLEX MUNISCIN-LIKE (TML), POLTERGEIST/POLTERGEIST LIKE 1 (POL/PLL1), PATELLIN 1-3 (PATL1-3), DYNAMIN-RELATED PROTEIN 1A (DRP1A), DYNAMIN-RELATED PROTEIN 1E (DRP1E), ARMADILLO DOMAIN (ARM), PLECKSTRIN HOMOLOGY DOMAIN (PH), SECRETION 14 DOMAIN (SEC14), COILED-COIL DOMAIN (CC), HELO/RPW8-LIKE COILED-COIL DOMAIN (CC_R), REMORIN C-TERMINAL ANCHOR (REM-CA), M-HOMOLOGY DOMAIN (μHD).

PUB13 was found at the TGN in Antignani *et al.*, 2015 but Zhou *et al.*, (2018) also reported a plasma membrane localization.

* VAN3 was shown to localize in the TGN by Naramoto *et al.*, 2009 but was found at the plasma membrane in a recent preprint (Adamowski and Friml, 2021).

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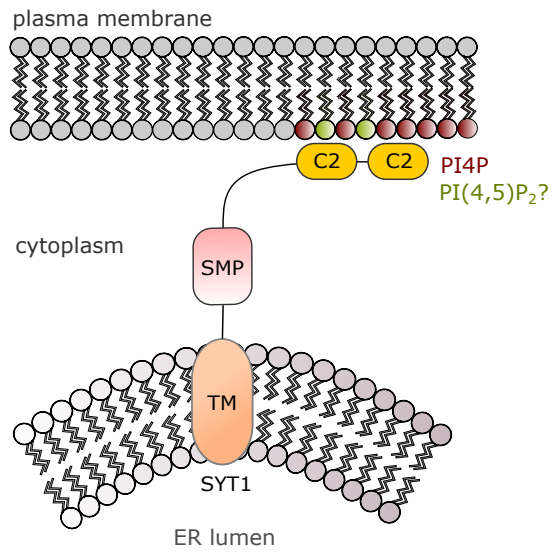
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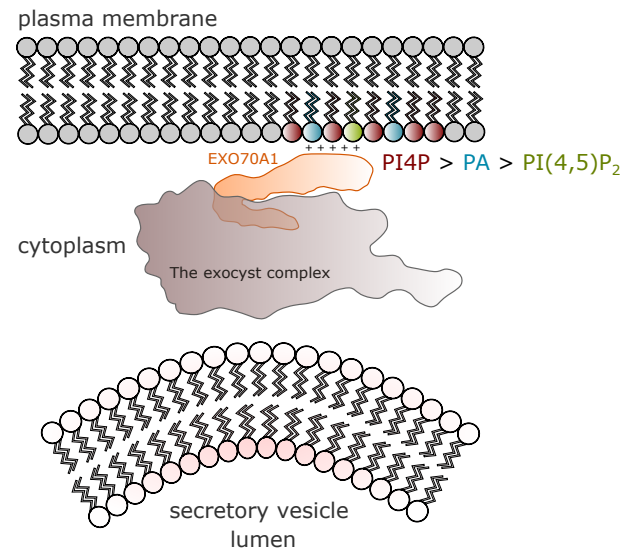
Figure 1. Enzymes involved in balancing PI4P between the plasma membrane and the TGN. In *Arabidopsis thaliana*, PI4P is produced at the plasma membrane by the PI4K α 1 complex (shown with a higher magnification in a) and in the TGN by the redundant PI4K β 1 and PI4K β 2 (shown with a higher magnification in b). The concentration of PI4P in the TGN is maintained at a low level thanks to the continuous action of PI-PLC2/7 and the 4-phosphatases SAC7. PI, phosphatidylinositol; PI4P, phosphatidylinositol 4-phosphate; DAG, diacylglycerol; IP₂, inositol (1,4)-biphosphate; PI-PLC, phosphoinositide-specific phospholipase C; SAC7, SAC-DOMAIN CONTAINING7; TGN, trans-Golgi Network; EE, early endosome; ALPS motif, amphipathic lipid packing sensor motif; HYC, HYCCIN-CONTAINING; EFOP, EFR3-OF-PLANTS; NPG, NO POLLEN GERMINATION; NPGR, NPG-RELATED; PI4K, phosphatidylinositol 4-kinase.

Figure 2: Examples of key PI4P function at the plasma membrane. Examples illustrating some of the key functions of PI4P at the plant plasma membrane: in membrane contact site establishment (a), exocytosis (b), signaling (c) and the formation of nanodomains (d). Note that most of these cellular functions are mainly driven by PI(4,5)P₂ in animals, while they are mediated, at least in part, by PI4P in plants. The examples shown in this figure were investigated in *Arabidopsis thaliana* (a-c) and *Nicotiana benthamiana* leaf (a and d). PI4P, phosphatidylinositol 4-phosphate; PI(4,5)P₂, phosphatidylinositol 4,5-bisphosphate; PA, phosphatidic acid; ER, endoplasmic reticulum; SYT, SYNAPTOTAGMIN; TM, transmembrane region; SMP, Synaptotagmin-like mitochondrial-lipid binding domain; C2, C2 domain; StREM1.3, *Solanum tuberosum* REMORIN1.3; MAKR2, MEMBRANE ASSOCIATED KINASE REGULATOR2; TMK1, RECEPTOR-LIKE TRANSMEMBRANE KINASE1; NPH3, NON-PHOTOTROPIC HYPOCOTYL3.

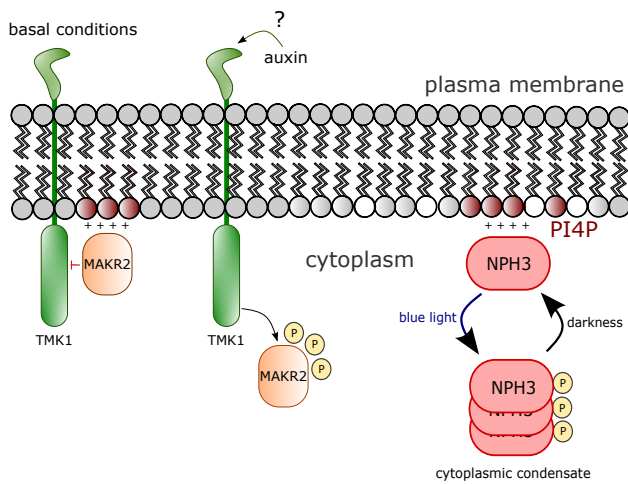
a) Membrane contact site



b) Exocytosis



c) Plasma membrane/cytoplasm switch signaling



d) Nanodomain formation

