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Precision targeting by phosphoinositides: how PIs direct endomembrane trafficking in plants

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Each phosphoinositide (PI, also known as phosphatidylinositol phosphate, polyphosphoinositide, PtdInsP or PIP) species is partitioned in the endomembrane system and thereby contributes to the identity of membrane compartments. However, membranes are in constant flux within this system, which raises the questions of how the spatiotemporal pattern of phosphoinositides is established and maintained within the cell. Here, we review the general mechanisms by which phosphoinositides and membrane trafficking feedbacks on each other to regulate cellular patterning. We then use the specific examples of polarized trafficking, endosomal sorting and vacuolar biogenesis to illustrate these general concepts.

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Introduction

Membrane identities are acquired by the combined presence of specific lipids and proteins on each membrane. For example, small GTPases (Rab, Rho and Arf) are important contributors of organelle identity [1]. On the lipid side, major determinants of membrane identities are phosphoinositides, which are anionic phospholipids with an inositol head group that can be phosphorylated at various positions on their polar head (Figure 1a) [2]. Both groups of molecules also coordinate trafficking between different membranes and specific mechanisms are in place to ensure their correct spatiotemporal distribution. Here, we review the mechanisms by which phosphoinositides contribute to both membrane trafficking and organelle identity. Since the general mechanisms linking anionic phospholipids and trafficking are shared between all eukaryotes, we first describe these generic concepts, including plant examples when available. We then describe the subcellular localization of phosphoinositides in plants. Finally, to illustrate the aforementioned concepts, we use the examples of vacuolar sorting and polarized trafficking, notably in the context of tip growth.

General concepts linking phosphoinositides and membrane trafficking in eukaryotes

Importance of phosphoinositide cyclical and cascade regulation

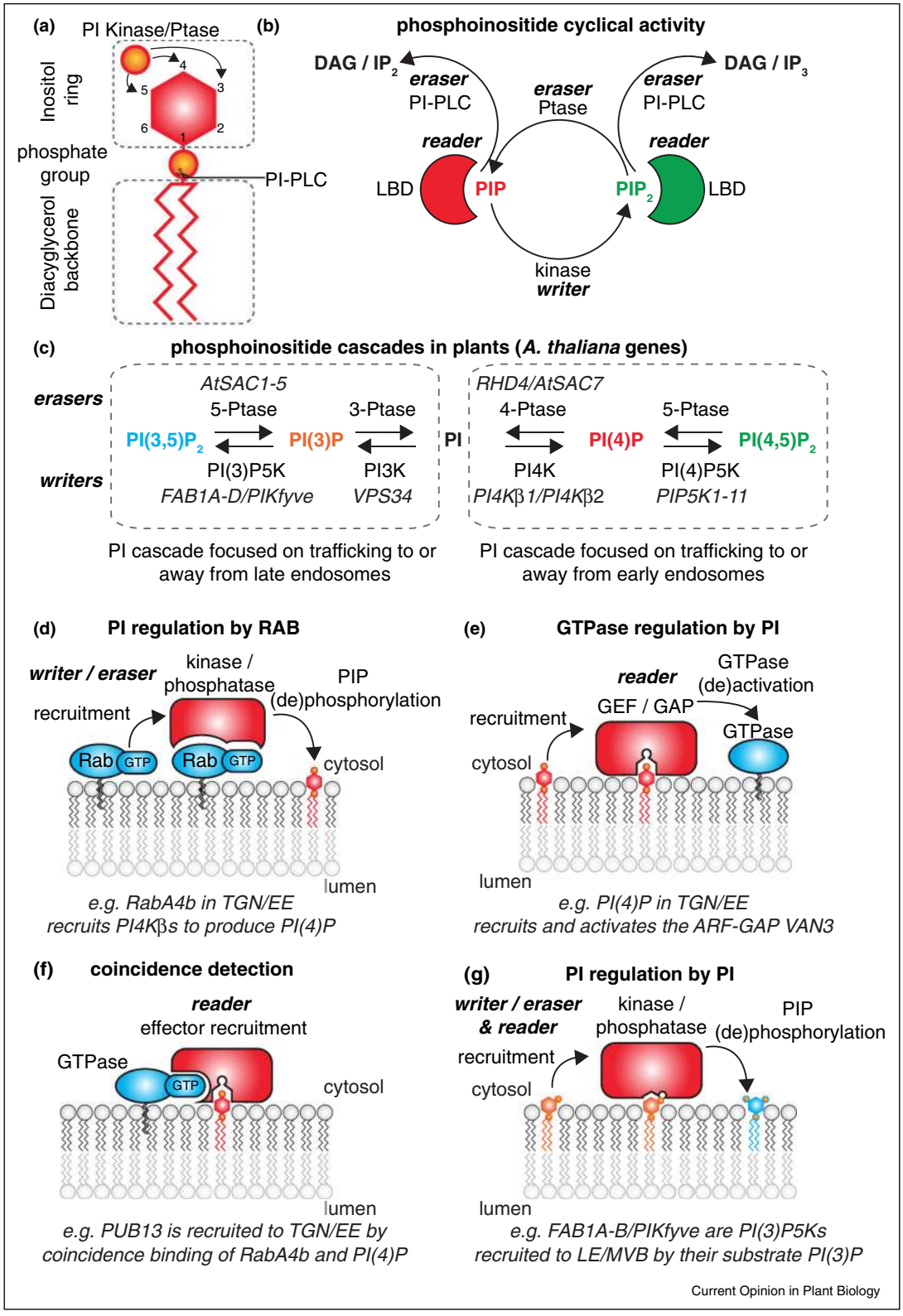
Phosphatidylcholine and phosphatidylethanolamine constitute more than 60% of total phospholipids, followed in abundance by phosphatidylinositol, phosphatidylglycerol, phosphatidylserine, and phosphatidic acid. Phosphatidylinositol is mainly found in the luminal side of the Endoplasmic Reticulum but a minor amount is exposed on organelle cytosolic leaflets, where it is phosphorylated into various phosphatidylinositol phosphate species (so called phosphoinositides or PIs). In all eukaryotes, phosphoinositides are quantitatively minor lipids, accounting for less than one percent of total phospholipids. Yet, they are set apart from other lipids because they are rapidly interconverted into one another by the action of lipid kinases and phosphatases, allowing their spatiotemporal control by cyclical regulation (Figure 1b) [1]. The combined action of kinases and phosphatases produces up to five phosphoinositides in plants, four of which have been involved in membrane trafficking (PI(3)P, PI(4)P, PI(4,5)P_2 and PI(3,5)P_2) (Figure 1c) [2,3]. In plants, PI(4)P constitutes about 80% of phosphoinositides, followed in abundance by PI(4,5)P_2, PI(3)P and PI(3,5)P_2.

Phosphoinositides are not only cycling between two states, but can cascade from one species to another and then another. In animals, a classical example of phosphoinositide cascade happens along the endocytic pathways [4]. This PI cascade starts from PI(4,5)P_2 at the plasma membrane (PM), utilizes transient accumulation of PI(4)P and PI(3,4)P_2 during endocytosis, and leads to accumulation of PI(3)P in early endosomes and then PI(3,5)P_2 in late endosomes [4]. By contrast, two shorter, largely independent phosphoinositide cascades exist in plants that are centered on early (trans-Golgi Network/Early Endosomes; TGN/EE) and late (Late Endosomes/Multivesicular Bodies; LE/MVB) endosomes (Figure 1c). Such cascades are a central theme in membrane trafficking as they allow membrane identity to evolve dynamically during the course of trafficking [1].

Phosphoinositides act as biochemical landmarks to recruit trafficking regulators

By analogy to the histone code, phosphoinositides generate a 'lipid code' that specifies membrane identity. The

Figure 1



The phosphoinositide code and interdependent relationship with small GTPases. **(a)** Schematic representation of phosphoinositides. Possible phosphorylation on the third, forth and/or fifth position on the inositol ring are indicated by arrows. Note that PI kinases or phosphatases act on these phosphorylation, while PI-PLC cut PIs below the phosphate group to release DAG and soluble inositol. Phosphorylations are represented by orange

three corner stones of the histone code hypothesis are the presence of proteins that can *create*, *remove* and *read* marks on histones (so called writer, eraser and reader modules). Lipid kinases and phosphatases represent writer and eraser molecules in such lipid code hypothesis. Phosphoinositide-specific phospholipases C (PI-PLCs) constitute another eraser module that terminates the PI cycle by metabolizing PI(4)P and PI(4,5)P_2 into Diacylglycerol (DAG) and soluble inositols (Figure 1b, note that the function of soluble inositols in endomembrane trafficking are not reviewed in this paper). The reader module is embodied by the presence of stereospecific lipid binding domains in many proteins (Figure 1b) [5]. Most trafficking regulators possess themselves such domains, which couple membrane recruitment and trafficking regulation. For instance, the three trafficking regulators AtSORTING-NEXINs (AtSNXs), PLEKSTRIN-HOMOLOGY1 (AtPH1) and FYVE-DOMAIN-PROTEIN1/FYVE-DOMAIN-PROTEIN-REQUIRED-FOR-ENDOSOMAL-SORTING1 (FYVE1/FREE1) are targeted to PI(3)P-containing endosomes via a PHOX (PX), PLECKRTSIN-HOMOLY (PH) or Fab1-YOTB-Vac1-EEA1 (FYVE) domain, respectively [6–9,10••].

Phosphoinositides cooperate with small GTPases to drive membrane traffic

Phosphoinositides are intimately linked with small GTPases (*e.g.* Rab, Arf and Rho), which are themselves master regulators of membrane trafficking [1]. Many phosphoinositide writer/eraser modules are effectors of activated GTPases, notably Rab proteins (Figure 1d). GTPase activities are also regulated by phosphoinositides and numerous GTPase-activating-proteins (GAPs) and GTPase-exchange-factors (GEFs) contain a lipid binding domain that drives their membrane association (Figure 1e). In addition, there are many examples of proteins that are recruited to a specific membrane via the coincidence binding of a phosphoinositide and a small GTPase (Figure 1f). For instance, RabA4b recruits PI4Kβs to control PI(4)P production in secretory vesicles emerging from the TGN/EE [11]. In turn, coincident detection of PI(4)P and RabA4b in the TGN/EE targets the PLANT-U-BOX13, which regulates the trafficking of the pattern-recognition receptor FLAGELIN-INSENSITIVE2 [12••].

Phosphoinositides influence membrane deformation and surface charges

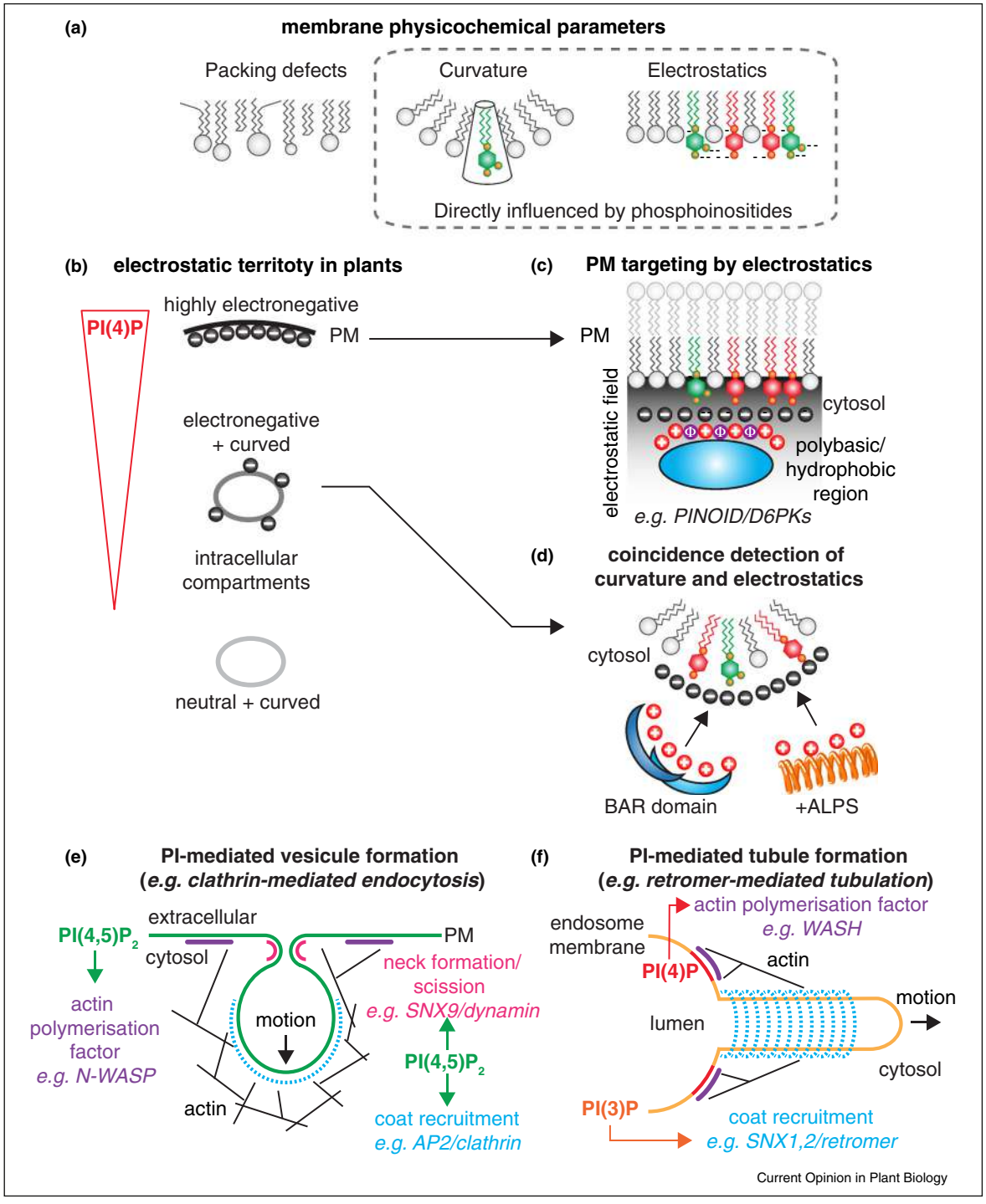
Phosphoinositides have a large head group that favor membrane with a positive curvature, for example in the bud of growing vesicles (Figure 2a) [13]. Anionic phospholipids also control membrane electrostatics by modulating surface charges in the cytosolic leaflet of membranes (Figure 2a, b) [14]. The plant PM has a specific electrostatic signature that is driven by PI(4)P and recruits proteins with polybasic sequence(s) (Figure 2b, c) [14,15•]. Interestingly, certain trafficking regulators are recruited to membrane through the coincident detection of curvature and electrostatics (Figure 2d) [5,16]. In turn, some of these proteins with a Bin-Amphyphisin-Rvs (BAR) domain, further induce membrane deformation; linking electrostatic and curvature regulation during trafficking [5,17]. Phosphoinositides also recruit coat proteins, such as the Adaptor Protein (AP) complexes involved in Clathrin-coated vesicle formation (Figure 2e) [4], or the retromer, involved in endosome tubulation (Figure 2f) [17,18•]. In addition, phosphoinositides recruit and activate the actin polymerization machinery, which generates a motile force required for membrane deformation and vesicle motion (Figure 2e, f) [18•,19].

The localization of phosphoinositides in plants

Studying lipid localization is complex. Endogenous lipids are not easily tagged by fluorescent moieties, lipid fixations are difficult to achieve, and phosphoinositides may diffuse or traffic away from their site of synthesis [2]. Yet, the localization of phosphoinositides in plants is getting clearer (Figure 3a), thanks to the combinatorial use of: (i) genetically encoded biosensors (*i.e.* fluorescent protein fused with stereospecific lipid binding domain), (ii) mutants impaired in phosphoinositide homeostasis, and (iii) localization studies of plant writer/eraser/reader proteins (see Figure 3b for a summary of writer/eraser known localization) [2,3]. Some notable differences can be noted between plants and animals. A prime example is the massive accumulation of PI(4)P at the plant PM (Figure 3a); whereas in animals, PI(4)P prominently resides in the Golgi/TGN compartments and to a lesser

(Figure 1 Legend Continued) circles. **(b)** Representation of the lipid code hypothesis embodied by the presence of writer, eraser and reader modules and the phosphoinositide cycle. **(c)** The two phosphoinositide cascades in plants. Generic enzyme names are written in capital letter, example of Arabidopsis proteins with proven enzymatic activities and roles in membrane trafficking are indicated in italic. **(d)** Schematic representation of Rab-regulated phosphoinositide phosphorylation or dephosphorylation. **(e)** Schematic representation of phosphoinositide-regulated small GTPase activation or deactivation. **(f)** Schematic representation of effector recruitment by coincidence binding of an activated small GTPase and a phosphoinositide. **(g)** Schematic representation of phosphoinositide-regulated phosphoinositide phosphorylation or dephosphorylation. (d–g) Plant examples are indicated in italics, and have been described in the following papers: PI4Kb [11], VAN3 [75], PUB13 [12••], FAB1A-B/PIKfyve [34]. DAG, diacylglycerol; IP2, inositol-1-4-biphosphate; IP3, inositol-1-4-5-triphosphate; PI-PLC, phosphoinositide-dependent phospholipase C; Ptase, phosphatase; PIP, phosphatidylinositol monophosphate; PIP2, phosphatidylinositol bisphosphate; LBD, lipid binding domain; SAC, SUPPRESSOR OF ACTIN; RHD4, ROOT HAIR DEFECTIVE4; PI(3)P, phosphatidylinositol-(3)-phosphate; PI(4)P, phosphatidylinositol-(4)-phosphate, PI(3,5)P_2, phosphatidylinositol-(3-5)-bisphosphate; PI(4,5)P_2, phosphatidylinositol-(4-5)-bisphosphate; PI(3)P5K, phosphatidylinositol-(3)-phosphate 5-kinase; PI(4)P5K, phosphatidylinositol-(4)-phosphate 5-kinase; PI4K, phosphatidylinositol 4-kinase; PI, phosphoinositides; TGN, *trans*-Golgi Network; EE, Early Endosomes; LE, Late endosomes; MVB, multivesicular bodies; GAP, GTPase Activating Protein; GEF, GTPase Exchange Factor; VAN3, VASCULAR NETWORK3; PUB13, PLANT U-BOX13; PIKfyve, PHOSPHOINOSITIDE KINASE FYVE FINGER CONTAINING.

Figure 2



Influence of phosphoinositides on physicochemical membrane parameters. **(a)** Membrane physicochemical properties may be conceptualized in three main categories including lipid packing defects, curvature and electrostatics. The latter two are directly influenced by phosphoinositides. **(b)** Schematic representation of the plant electrostatic territory, which includes membranes with varying degree of negative surface charges, the highest being at the PM. The red triangle indicates the concentration gradient of PI(4)P between the PM and TGN/EE and that controls the high PM electrostatic field. **(c)** Specific recruitment of proteins with polybasic/hydrophobic regions to the PM largely through PI(4)P-driven electrostatics. Note that PA and PI(4,5)P_2 are also involved in D6PK membrane association [76]. **(d)** Schematic representation of protein recruitment by coincidence detection of both curvature and electrostatics (*e.g.* proteins containing BAR domains or specialized amphipathic helices from the +ALPS class [5,16,17]). **(e)** Schematic representation of the importance of phosphoinositides in vesicle formation through the coordination of actin polymerization, and recruitment/activation of membrane deformation components, including coat proteins and the scission

extent at the PM [15•]. In addition, PI(3)P labels late endosomes in plants and early endosomes in animals [20,21]. By contrast, PI(4,5)P_2 localization at the PM and PI(3,5)P_2 in LE/MVB are conserved in eukaryotes [21,22,23•].

Phosphoinositides in endosomal sorting and vacuole biogenesis

The central vacuole is a plant specific organelle that can act both as a lytic and/or storage organelle [24]. Phosphoinositides, in particular the PI → PI(3)P → PI(3,5)P_2 cascade (Figure 1c), are involved in endosomal sorting events leading to membrane protein degradation or retrieval, vacuolar morphogenesis and autophagy.

PI(3)P and PI(3,5)P_2 sort PM proteins toward degradation or recycling

After endocytosis, membrane proteins can recycle back to the PM or be targeted to the vacuole for degradation. LE/MVBs mature from TGN/EE, which gradually loses PI(4)P on its membrane and acquires PI(3)P together with the RabF protein ARA7/RabF2b (Figure 3c) [25,26]. PI(3)P is synthetized by the phosphatidylinositol 3-kinase (PI3K) VACUOLAR PROTEIN SORTING34 (VPS34), while the concomitant PI(4)P hydrolysis might be regulated by ROOT-HAIR-DEFECTIVE4 (RHD4)/AtSUPRESSOR-OF-ACTIN7, a PI(4)P phosphatase that localizes in the TGN/EE (Figure 3b) [27].

Protein sorting during the maturation of TGN/EE into LE/MVB is orchestrated by the antagonistic activities of the ENDOSOMAL-SORTING-COMPLEX-REQUIRED-FOR-TRANSPORT (ESCRT) and the retromer complexes. ESCRT complexes mediate the internalization of ubiquitinated proteins into intraluminal vesicles of MVB/LE before their fusion with the lytic vacuole, where proteins are degraded. The retromer complex is involved in the retrieval of membrane proteins before their internalization in intraluminal vesicles, thereby allowing their subsequent recycling. In both cases, the function of these antagonistic complexes is intimately linked with PI(3)P (Figure 3c). Indeed, the plant specific ESCRT component FYVE1/FREE1 localizes in LE/MVB by binding concomitantly to PI(3)P, ubiquitinated cargos and ESCRT-I proteins [8,9,28,29•]. The seedling lethal *fyve1/free1* mutant shows MVB and vacuole defects and fails to target ubiquitinated proteins to the vacuole for degradation, which instead mislocalize to the tonoplast [9,29•]. In addition, the retromer components AtSNXs are also targeted to LE/MVB by PI(3)P [6,7]. In *snx1* and *retromer* mutants, the PIN auxin efflux carriers are not recycled back to the PM efficiently and are instead targeted to the vacuole for degradation [30–32]. The AtSNX1-interacting protein CYTOPLASMIC-LINKER-ASSOCIATED-PROTEIN (CLASP) links AtSNX1-containing endosomes with cortical microtubules to regulate PIN2 trafficking [33]. AtSNX1 also interacts with phosphatidylinositol 3-phosphate 5-kinases (PI(3)P5Ks) from the PHOSPHOINOSITIDE-KINASE-FYVE-FINGER-CONTAING (PIKfyve)/FAB1 family (Figure 1c, g), which regulates AtSNX1, CLASP and PIN2 localization [34•].

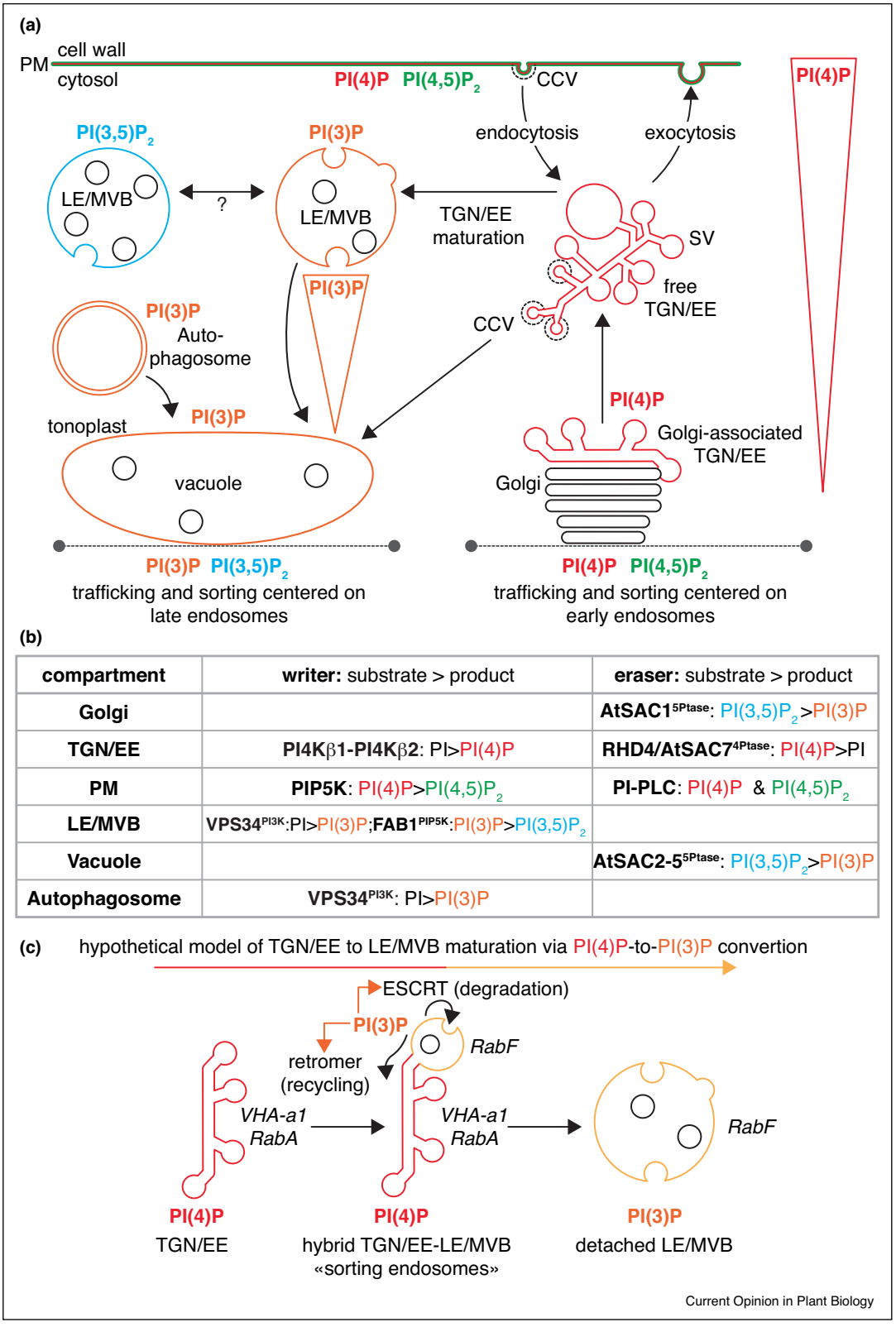
Phosphoinositides and the regulation of vacuole morphology

Phosphoinositides control the biogenesis and morphology of the central vacuole. The SOLUBLE-NSF-ATTACHMENT-RECEPTOR (SNARE) protein VTI11 is involved in the fusion of LE/MVB with the vacuole [24]. *vti11* mutants harbors small unfused vacuoles, a phenotype that is rescued by inhibition of PI3K activity [35]. *fyve1/free1* and retromer mutants also accumulates small fragmented vacuoles, supporting a role for PI(3)P in this process [9,29•,31,32,36•]. It is likely that PI(3)P-dependent endosomal sorting via the ESCRT and retromer complexes is important for the correct trafficking and localization of SNARE proteins involved in vacuole fusion.

PI(4)P also connects SNARE trafficking and vacuole morphology [37••,38•], although the mechanisms are still unclear and could be indirect. However, actin is important to shape vacuole morphology [35,38•]. Transient PI(4)P production has recently been described as an important regulator of actin synthesis on endosomal membranes in animal cells [18•]. It is therefore possible that a similar transient pool of PI(4)P might locally regulates vacuolar dynamics and/or sorting function at the LE/MVB, possibly by controlling actin dynamics. The notion that phosphoinositides might regulate actin dynamics in the late secretory pathway is supported by the vacuolar morphology defects displayed by loss- and gain-of-function

(Figure 2 Legend Continued) machinery. Note that the example shown in panel (e) relates to clathrin-mediated endocytosis, but similar concepts may be applied for the formation of other clathrin-coated vesicles, for example at the TGN. **(f)** Schematic representation of the importance of phosphoinositides in tubule formation through the coordination of actin polymerization, and the membrane tubulation machinery. Note that the examples shown in (d)–(e) have not been formally demonstrated in plants even tough proteins containing similar domains are found in plant genomes and the general concepts are likely applicable to plant endomembrane trafficking. LBD, Lipid Binding Domain; SNX, SORTING NEXIN; BAR, Bin Amphiphysinn Rvs; +ALPS, +amphipathic lipid-packing sensor; PX, PHOX Homology; LE, Late Endosomes; MVB, MultiVesicular Bodies; PM, plasma membrane; PI, phosphoinositides; N-WASP, NEURAL WISKOTT-ALDRICH SYNDROME PROTEIN; WASH, WASP AND SCAR HOMOLOGUE; AP2, ADAPTOR-COMPLEX2; D6PK, D6 PROTEIN KINASE. Negative charges carried by anionic phospholipids are represented by black circles; cationic residues by red circles; aromatic/hydrophobic residues by purple circles; phosphorylation by orange circles. PI(4)P distribution is shown in red, PI(4,5)P_2 in green, PI(3)P in orange, actin polymerization factors in purple, membrane coat in blue and scission machinery in pink. All lipid localizations shown are for the cytosolic membrane leaflet only.

Figure 3



compartment	**writer:** substrate > product	**eraser:** substrate > product
Golgi		**AtSAC1**^{5Ptase}: PI(3,5)P_2>PI(3)P
TGN/EE	**PI4Kβ1-PI4Kβ2**: PI>PI(4)P	**RHD4/AtSAC7**^{4Ptase}: PI(4)P>PI
PM	**PIP5K**: PI(4)P>PI(4,5)P_2	**PI-PLC**: PI(4)P & PI(4,5)P_2
LE/MVB	**VPS34**^{PI3K}:PI>PI(3)P;**FAB1**^{PIP5K}:PI(3)P>PI(3,5)P_2	
Vacuole		**AtSAC2-5**^{5Ptase}: PI(3,5)P_2>PI(3)P
Autophagosome	**VPS34**^{PI3K}: PI>PI(3)P	

Subcellular map of phosphoinositide localization in Arabidopsis root epidermis. **(a)** Schematic representation of endomembrane trafficking pathways. CCVs emanating from the TGN, and likely involving the AP3 clathrin adaptor, define a vacuolar trafficking pathway independent on canonical RabF-containing LE/MVB [77]. **(b)** Writer and eraser modules with known localization and likely contributing to phosphoinositide

mutants in *AtSUPPRESSOR-OF-ACTIN* genes (*AtSAC2* to *AtSAC5*) [39]. SAC proteins are phosphoinositide phosphatases (Figures 1c and 3b). AtSAC2-5 are homologous to the Golgi-localized AtSAC1 protein, which has a 5-phosphatase activity toward PI(3,5)P_2 (Figure 3b) [40]. AtSAC2-5 localize on the tonoplast and might control vacuolar morphology by promoting PI(3,5)P_2 hydrolysis and PI(3)P production in this compartment (Figure 3a, b). However, it remains to be determined whether AtSAC2-5 function actually links actin dynamics with phosphoinositide homeostasis to regulate vacuole morphogenesis.

PI(3)P orchestrates autophagy

Autophagy is a cellular degradation pathway that is initiated by the encapsulation of cytoplasmic cargos in a double membrane organelle called autophagosome and which culminates in the fusion of this compartment with the lytic vacuole. PI(3)P synthesis by the PI3K VPS34, which localizes to autophogosome in plant cells [41], is a key regulator of autophagosome formation across eukaryotes (Figure 3a and b). Furthermore, both ESCRT and retromer mutants are defective in the autophagy process [29•,36•,42–44]. SNAREs are involved in the fusion of autophagosomes with the vacuole; it is therefore likely that some of the autophagy-related phenotypes in ESCRT and retromer mutants arise from mis-sorting of SNARE complexes [43]. In addition, FYVE1/FREE1 interacts with the PI(3)P-binding SRC-HOMOLOGY3-CONTAINING-PROTEINs SH3P2 and SH3P3, which localize in preautophagosomal structures in a VPS34-dependent manner [29•,36•]. SH3P2 binds AUTOPHAGY-RELATED-PROTEIN8 (ATG8) and the PI3K complex and is actively involved in the deformation of this membrane [41]. SH3P2/SH3P3 contain a BAR domain, which could facilitate membrane bending during autophagosome formation.

To conclude, PI(3)P and PI(3,5)P_2 control protein sorting, including SNAREs, toward the vacuole or the TGN/EE, and may connect cortical microtubules with endosomal recycling and actin cytoskeleton with vacuole morphology.

Phosphoinositides and polarized trafficking

Phosphoinositides, in particular through the PI → PI(4)P → PI(4,5)P_2 cascade (Figure 1c), regulate polarized exo- and endocytosis. In the following section, we will focus on their role in tip growing cells, which are highly polarized, but we will also use additional examples in other cell types when opportune.

PI(4,5)P_2 targets endocytosis in pollen tube

In tip growing cells (root hairs and pollen tubes) growth occurs via a massive polarized exocytosis of cell wall components at the growing tip and is counterbalanced by endocytosis occurring in subapical regions of the tube, forming a reversed-fountain flow of membranes (Figure 4a) [45]. Loss-of function mutants of phosphatidylinositol 4-phosphate 5-kinases (*PIP5Ks)* show low pollen germination rate, slow tube elongation, endocytic defects and reduced transmission through the male gametophyte [46,47]. In growing pollen tube, PI(4,5)P_2 localizes at the flank of apex, in a region with both endocytic and exocytic activities (Figure 4a, b). This localization is maintained by a combination of local production of PI(4,5)P_2 by PIP5Ks, which localizes at the flank of apex [46–49] and degradation into DAG by PI-PLC, which localizes in the shank/subapical region (Figure 4b, c) [50,51].

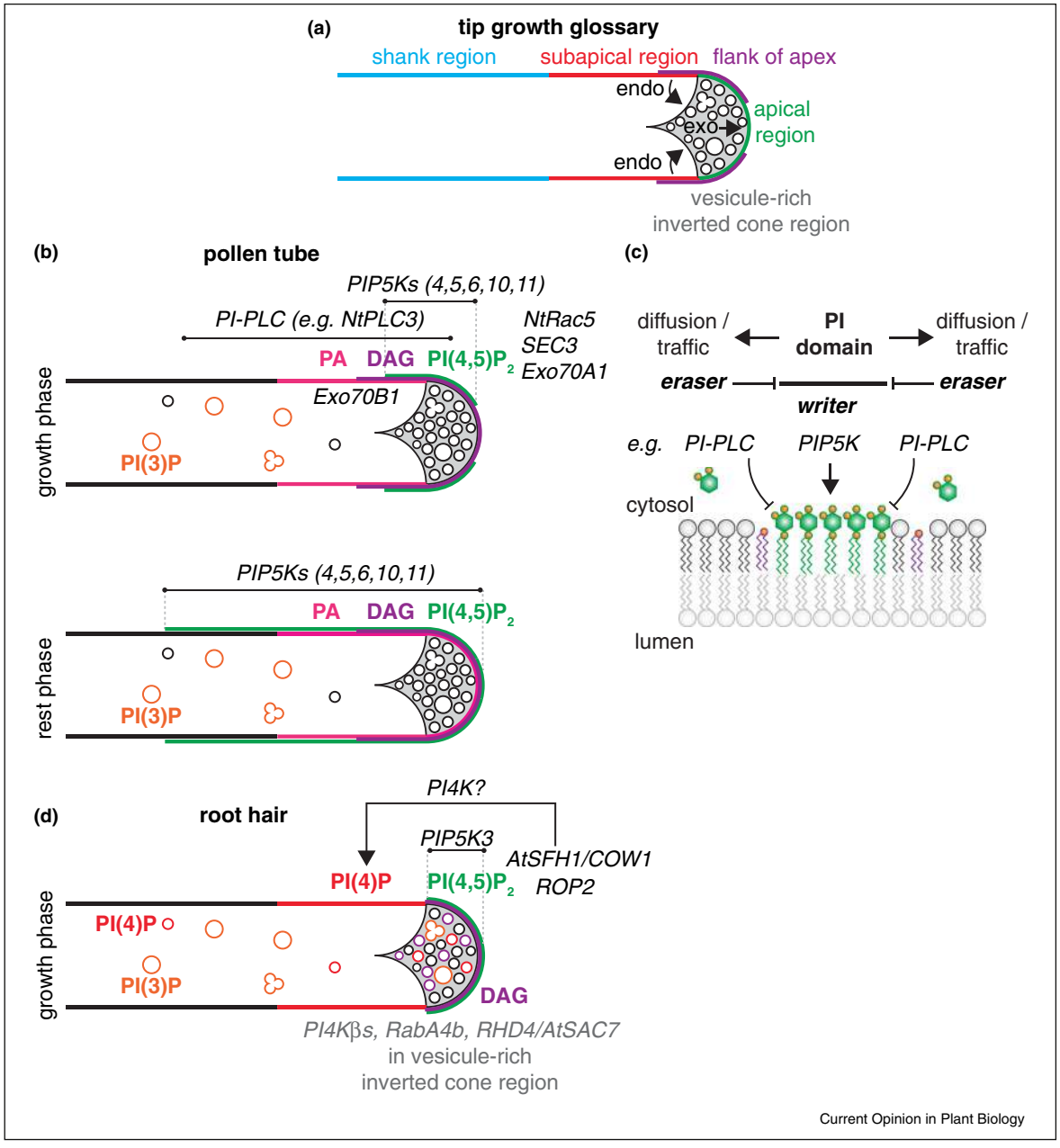
PIP5K6-OE enhances endocytosis, which results in PM invagination and the formation of several tip branches [48]. The expression of a clathrin dominant negative can rescue the *PIP5K6-OE* phenotype indicating that PI(4,5)P_2 synthesis regulates clathrin-mediated endocytosis [48]. A role of PI(4,5)P_2 in endocytosis in plants is further supported by the study of PIP5K1 and PIP5K2 in sporophytic tissues. *PIP5K1* and *PIP5K2* are auxin-induced genes that regulate clathrin membrane recruitment, endocytic trafficking and PIN polarity [3,52–54].

The exocyst is an anionic phospholipid effector involved in polarized secretion

The exocyst complex tethers secretory vesicles to the PM, a prerequisite for subsequent membrane fusion [55]. The exocyst binds the PM through the SEC3 and EXO70 subunits, which both have lipid binding activities. In plants, SEC3 has a PH domain that binds PI(4,5)P_2 *in vitro* and colocalizes with PI(4,5)P_2 *in vivo* [56••]. *sec3a* loss-of-functions show reduced pollen tube growth and fail to be transmitted through the male gametophyte

(Figure 3 Legend Continued) patterning. **(c)** Hypothetical model for ESCRT and retromer-mediated sorting during TGN/EE to LE/MVB maturation. Canonical markers of the TGN/EE (VHA-a1 and RabAs) and LE/MVB (RabFs, *e.g.* ARA7) are indicated in italics. Note that maturating TGN/EE displays both markers, suggesting the existence of transient hybrid organelles, where protein sorting might occur. The existence of such maturating TGN/EE, that contains RabF/PI(3)P as well as VHA-a1 markers was reported in [26]. PI(3)P distribution is shown in orange; PI(4)P in red; PI(4,5)P_2 in green and PI(3,5)P_2 in blue. Arrows represent trafficking pathways between compartments. PM, Plasma Membrane; TGN, *trans*-Golgi Network; EE, Early Endosomes; LE, Late Endosomes; MVB, MultiVesicular Bodies; CCV, Clathrin Coated Vesicle; SV, Secretory vesicle. Triangles indicate concentration gradient of PI(4)P between the PM and TGN/EE and PI(3)P between LE/MVB and tonoplast. PI(3)P, phosphatidylinositol-(3)-phosphate; PI(4)P, phosphatidylinositol-(4)-phosphate, PI(3,5)P_2, phosphatidylinositol-(3-5)-bisphosphate; PI(4,5)P_2, phosphatidylinositol-(4-5)-bisphosphate. SAC, SUPPRESSOR OF ACTIN; PI4K, phosphatidylinositol 4-kinase; PIP5K, phosphatidylinositol-(4)-phosphate 5-kinase; PI-PLC, phosphoinositide-dependent phospholipase C; AP3, ADAPTOR PROTEIN 3; VPS34, VACUOLAR PROTEIN SORTING34, ESCRT, ENDOSOMAL SORTING COMPLEX REQUIRED FOR TRANSPORT. All lipid localizations shown are for the cytosolic membrane leaflet only.

Figure 4



Subcellular maps of phosphoinositide localization in tip growing cells. **(a)** Schematic representation of the organization of tip growing cells. **(b)** and **(d)** are an attempt to draw maps of lipid localization in pollen tubes and root hairs, respectively. It is important to be cautious with such maps, since there is no consensus across different studies for the localization of PIP metabolic enzymes and PIP sensors in these systems. This is likely because anionic lipids localizations are highly dynamic in tip growing cells and vary with the mode of growth (*i.e.* oscillatory or steady growth, see for example Ref. [56••]). As such, snapshot images are unlikely to represent a full account of lipid/protein localizations. The localizations presented in Figure 4 are personal interpretations, which should be further studied using quantitative time-lapse imaging. **(c)** Conceptual model for the acquisition and maintenance of patterned phosphoinositide membrane domains/organelles. Polarized phosphoinositide kinases locally produce a given PI species. Phospholipases or phosphatases with complementary localization to the writer modules allow the establishment of sharp phosphoinositide boundaries by removing incoming PIs that escaped the polar domains either by lateral diffusion or vesicular trafficking. PI(3)P distribution is shown in orange (localization in root hair studied in [78]); PI(4)P in red; PI(4,5)P_2 in green, PI(3,5)P_2 in blue, PA in pink and DAG in purple (localization in pollen tube studied in [50] and in root hair in [79]). Chevron-shaped arrows represent trafficking pathways between compartments; triangle-shaped arrows indicate activation. Exo, exocytosis; endo, endocytosis; PM, Plasma Membrane; TGN, *trans*-Golgi Network; EE, Early Endosomes; LE, Late Endosomes; MVB, MultiVesicular Bodies; CCV, Clathrin Coated Vesicle; SV, Secretory vesicle. Name of enzymes/proteins involved in phosphoinositides metabolism and/or trafficking are in italic next to the compartment in which they reside. PI(3)P, phosphatidylinositol-(3)-phosphate; PI(4)P, phosphatidylinositol-(4)-phosphate, PI(3,5)P_2, phosphatidylinositol-(3-5)-bisphosphate; PI(4,5)P_2, phosphatidylinositol-(4-5)-bisphosphate; RHD4, ROOT HAIR DEFCTIVE4; SAC, SUPPRESSOR OF ACTIN; COW, CAN OF WORMS; ROP, RHO OF

[56••]. The localization of SEC3 determines the direction of the growth and the site of pollen germination [56••]. Furthermore, another anionic lipid, phosphatidic acid (PA), localizes in subapical PM regions of the pollen tube, which overlap only partially with tip-localized PI (4,5)P_2 [57]. In growing tobacco pollen tubes, two EXO70 isoforms (EXO70A1 and EXO70B1) overlap with PI(4,5)P_2 and PA markers, respectively (Figure 4b) [58••]. It is possible that the exocyst complex targets specific PM sites through coincidence binding with several anionic phospholipids depending of the lipid binding properties of the EXO70 isoforms. The exocyst is involved in cell polarity establishment in many cell types, not just pollen tube. Additional examples of exocyst-based polarity include PINs and PEN3 polar localization [59–61], the establishment of an Ortmannian ring in trichromes [62], the patterning of xylem secondary cell wall [63], or the definition of the casparian strip domain [64••]. In the later example, the localization of EXO70A1 in the casparian strip domain of the root endodermis nicely correlates with a local accumulation of PI(4,5)P_2, which is itself dependent on EXO70A1 activity [64••].

A precise phosphoinositide patterning coordinates membrane trafficking during root hair morphogenesis

Similar to pollen tubes, PI(4,5)P_2 is crucial to coordinate membrane trafficking during root hair growth. PIP5K3 and PI(4,5)P_2 localize at the tip of growing root hairs (Figure 4d) [65,66]. PIP5K3 is required for the polar localization of RHO-OF-PLANT2 (ROP2) and ROP6 during root hair initiation, which themselves coordinate endocytosis, exocytosis and cytoskeleton dynamics [67••]. *pip5k3* mutants have short root hairs, whereas *PIP5K3* overexpression induces root hair deformation [65,66]. The localization of PI(4,5)P_2 at the tip of growing root hair is maintained by the action of AtSFH1/CAN OF WORMS (COW1), a SEC14 domain-containing protein that is localized at the tip region via direct interaction with PI(4,5)P_2 [68,69•]. Yeast SEC14 and AtSFH1/COW1 stimulate PI4K activity [70], suggesting that AtSFH1/COW1 might promote local PI(4)P synthesis at the root hair tip, which could be subsequently channeled to PIP5K3 for local PI(4,5)P_2 synthesis (Figure 4d) [69•]. *AtSfh1* mutants have aberrant root hair morphology and show disperse secretory vesicles at their tip and disorganized actin and microtubule networks [68], highlighting the importance of precise phosphoinositide patterning for the coordination of membrane trafficking and cell polarity establishment.

PI(4)P localizes in root hair subapical regions [71], as well as some intracellular compartments, which are likely TGN/EE (Figure 4c). PI4Kβ1-β2 and RHD4/AtSAC7, a PI(4)P phosphatase (Figure 3b), localize in RabA4b-positive TGN/EE at the tip of growing root hairs (Figure 4d) [11,27]. *pi4kβ1/pi4kbβ2* double and *rhd4/sac7* single mutants have short bulged root hairs, with swollen TGN/EE, suggesting a crucial role for PI(4)P in secretion [11,27,72]. In *rhd4/sac7* mutants, PI(4)P accumulates in aberrant intracellular compartments that are likely TGN/EE [27,73]. It is possible that RHD4/AtSAC7 in TGN/EE erases incoming PI(4)P from the PM in order to maintain the PI(4)P gradient between these membranes [15•]. However, it is still unclear how PI(4)P kinases and phosphatases cohabitate in the same compartment and/or whether they are localized in complementary subdomains of the TGN/EE (Figure 3b).

The overall consensus is that patterned phosphoinositides are needed to mark specific domains at the PM for localized endocytosis and exocytosis. In addition PI(4)P sorting activity at the TGN/EE is also involved in polarized trafficking toward the PM.

Conclusions and future perspectives

While most concepts linking phosphoinositides with membrane trafficking are conserved throughout eukaryotes, there are a number of emerging plant specific innovations. First, phosphoinositide subcellular localizations are different in plants and animals and one of the future challenges will be to decipher the mechanisms enabling phosphoinositide subcellular localization and maintenance in plants. A key point will be to further understand the tight coupling between the writer and eraser modules that generates phosphoinositide spatiotemporal patterns despite their constant dispersal by lateral diffusion and vesicular trafficking. Furthermore, compartment interactions at membrane contact sites are emerging as key regulators of lipid homeostasis and their importance in phosphoinositide synthesis has not yet been studied in plants. Second, it remains to be fully explored whether a *bona fide* lipid code exists for polarized trafficking in plants. One of the keys behind such code might be the presence of multiple EXO70 exocyst subunits that could define several PM domains, at least in part through specific interactions with various anionic lipids or combinations thereof. Finally, a plant specific pathway heavily relying on polarized membrane trafficking and not treated in this review is the establishment of the cell plate during cytokinesis. Several mutants defective in phosphoinositide metabolism harbor aberrant cell division phenotypes [72,74] and PIPs have distinctive localizations during cell division [15•,20,22,73]. However, the exact roles of phosphoinositides in coordinating

(Figure 4 Legend Continued) PLANT; PI4K, phosphatidylinositol 4-kinase; PIP5K, phosphatidylinositol-(4)-phosphate 5-kinase; PI-PLC, phosphoinositide-dependent phospholipase C; DAG, Diacylglycerol; PA, phosphatidic acid. All lipid localizations shown are for the cytosolic membrane leaflet only.

membrane traffic during cytokinesis are still poorly understood.

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