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Opinion

Phosphorylation-regulated SEC14-GOLD PATELLIN lipid transfer proteins

Jannik Hornbergs [1,*] and Petra Bauer [1,2,*]

The SEC14-GOLD family of phosphatidylinositol (PI) transfer proteins, known as PATELLIN (PATL) proteins in plants, are key regulators of plasma membrane (PM)-related signaling processes. They function through multifaceted interactions involving a SEC14 lipid-binding domain, GOLD domain, and their N-terminal region. Protein phosphorylation is crucial for modulating protein and phospholipid interactions, but phosphorylation of SEC14 proteins remains understudied. Phosphoproteomics data from *Arabidopsis thaliana* indicates two major phosphorylation hubs within the N-terminal and the conserved SEC14-GOLD regions in the PATLs. These phosphorylation patterns vary in response to environmental and hormonal stress-related factors. Understanding how PATL proteins are phosphorylated can offer insights into PATL–membrane interactions and their functional roles in cell physiology, providing new strategies for plant adaptation and stress resilience in crops.

Highlights

The SEC14-GOLD family of phosphatidylinositol (PI) transfer proteins, known as PATELLIN (PATL) proteins in plants, play essential roles in regulating plasma membrane (PM)-related processes through their SEC14 lipid-binding and GOLD domains.

PATLs are implicated in membrane signaling processes, yet the phosphorylation of SEC14 proteins has not been thoroughly studied, despite its importance in modulating protein and phospholipid interactions during developmental and environmental signaling.

Phosphoproteomics data from *Arabidopsis thaliana* reveal phosphorylation regions of PATLs, influenced by environmental and hormonal stress factors. Investigating the phosphorylation patterns of PATLs can enhance our understanding of their interactions with membranes and their functions in plant physiology, potentially leading to new strategies for improving crop resilience to stress.

SEC14-GOLD PI transfer proteins act in cellular signaling at the PM

Cells rely on rapid and precise signaling at their **plasma membrane (PM)** (see Glossary) to perceive and respond to environmental cues. This process requires tailored cellular-level signaling mechanisms for proper perception and signal transmission. Central to this are **post-translational modifications (PTMs)**, such as protein phosphorylation, which modify transmembrane and peripheral membrane proteins. These modifications control how proteins interact with each other and with the membrane, playing vital roles for cell physiological processes such as secretion, endocytosis, and maintaining the complex organization of the PM.

A critical aspect of membrane signaling involves proteins that regulate the composition of **PI-phosphates (PIPs)**, a group of lipids essential for cellular communication [1–7]. Such proteins are phosphatidyl inositol transfer proteins (PITPs), including SEC14-like PITPs (Box 1). Their canonical **SEC14 domain** can associate with membranes while also constituting a **lipid-binding site (LBS)**, facilitating the transfer and regulation of lipids within the membrane. Notably, **PATELLINs (PATLs)** are a unique family of multi-domain PIPTs [8,9] (Figure 1A). PATLs play essential roles in cell plate formation during cell division [8,10–13]. Specific members like PATL1, PATL2 and, PATL4 are implicated in stress responses [14–17].

SEC14-GOLD PI transfer proteins are phosphorylated in plants

Protein phosphorylation within the SEC14 protein family remains largely unexplored. The **PhosPhAt** server provides hints on SEC14 protein phosphorylation [18,19] (Table S1 in the supplemental information online). An analysis of the PhosPhAt database uncovers previously unconsidered phosphorylation events of certain PATLs, some also in response to environment and hormone signals [16,20–24]. This opens up a fascinating possibility that PATLs can be controlled

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Box 1. Multidomain SEC14 proteins in land plants – diversification and functional specificities

SEC14 proteins can bind lipophilic ligands, such as PI or PIPs or other lipid substrates [37,66,67]. The SEC14 domain also binds and presents PI for phosphorylation, or transfers and exchanges PI against other lipids in the membrane or between membranes. Thereby, SEC14 proteins effectively can change the PIP landscape at a membrane [28,37,67–69].

The SEC14 domain has undergone substantial evolutionary diversification in land plants. While just six SEC14-like proteins are present in *Chlamydomonas reinhardtii*, there are over 35 such proteins in *Arabidopsis thaliana* or other angiosperms. Moreover, the *Chlamydomonas* SEC14 proteins are of a very simple type, consisting merely of the SEC14 domain itself. In contrast, the SEC14 protein family expansion in the green lineage is largely attributed to fusions with multiple functional domains, providing higher land plants with new functional contexts for SEC14 proteins, enabling them to engage in higher-order protein complexes and to perform specialized physiological roles at distinct membrane sites in cells [9]. Examples for multi-domain SEC14 proteins are *A. thaliana* SFH1 and PATELLINs (PATLs). SFH1 belongs to the SEC14-Nodulin domain family [9,28,65,67,70] and harbors a nodulin-like domain, which contributes to $PI(4,5)P_2$ targeting and regulates oligomerization of the protein (e.g., in pollen tube development) [67,70,71]. PATLs are distinguished by their jelly roll-like GOLD domain, which directs them to $PI(4,5)_2$-specific PM sites and enables interaction with proteins for vesicle trafficking [8–13]. The six PATL proteins share relatively conserved CTN-SEC14-GOLD domains but diversify in their N-terminal regions (here called N-regions) specifying each PATL protein form [8,9]. However, the PATLs act in a redundant manner as only sextuple *patl* knockout phenotypes were reported as embryo-lethal [12]. With the exception of PATL6, the N-regions are acidic, harboring glutamate repeats neighbored by lysins, providing a certain electrostatic character within intrinsically disordered regions (IDRs) [8,9,16].

through protein phosphorylation to link extracellular signals to changes in PM protein function and localization.

Very interestingly, **phosphopeptides** were identified in only seven out of 35 SEC14-like PITP proteins and notably in the SEC14-GOLD PATL family. A total of 933 phosphopeptides represented 54 experimentally proven phosphorylation sites. An additional 18 phosphorylation sites are there in the SEC14 family, but their exact positions cannot be deduced from the phosphopeptides (hence termed 'putative', Table S1 in the supplemental information online). Among these proteins, SFH1 stands out as an exception [25] (Box 1): it has a phosphorylation site in the linker region between the SEC14 domain and the C-terminal nodulin-like domain. Interestingly, this site's phosphorylation status can be influenced by treatment with sucrose and rapamycin, indicating a possible regulatory role under specific conditions [25].

The N-regions of PATL proteins (Box 1) serve as major hubs for phosphorylation. For example, PATL1 contains 12 confirmed phosphoserines (pS) and eight phosphothreonines (pT), along with two additional putative pS and four putative pT sites (Figure 1B and Table S1 in the supplemental information online). Most of these modifications are located in the N-region, with eight pS and seven pT confirmed there, as well as two putative pS and four putative pT (Figure 1B). Similarly, PATL2 has six confirmed pS, three pT, and one phosphotyrosine (pY), as well as four more putative pS, three putative pT, and another putative pY. All confirmed pT and pY as well as three confirmed pS are located in the N-region (Figure 1B). The N-region of PATL2 has phosphorylation at S77 and S79 under iron deficiency and during salt stress, in response to oligogalacturonides and brassinosteroid signaling [16,20–24]. Because of the involvement of the N-regions in binding salt and iron transporters, protein phosphorylation in the N-regions may indeed interfere with protein–protein interactions in response to environmental stress or hormones. Notably, for PATL3, PATL4, PATL5, and PATL6, there are much less phosphopeptides as compared with PATL1 and PATL2, indicating less regulation by protein phosphorylation (Figure S1 in the supplemental information online).

Phosphopeptides in the SEC14 and neighboring regions outside of the N-region were mainly detected for PATL1 and PATL2, especially in four different regions, namely at the very beginning of the SEC14 (S403 and S409 of PATL2), in the central portion of the SEC14 sequence

Glossary

Lipid/ligand-binding site (LBS): protein site favoring the binding of a ligand through a specific shape but also electrostatic, hydrophobic, Van der Waals interactions, or by formation of hydrogen bonds. Binding sites can reside on the protein surface, or be located in grooves or pockets. The SEC14 domain harbors a lipophilic ligand (lipid) binding site in a pocket form.
PATELLIN (PATL): SEC14-Golgi dynamics (GOLD) domain-containing protein, named after 'patella', due to localization to $PI(4,5)P_2$ patches on the cell plate during plant cell division in plants.
PhosPhAt: protein phosphorylation site database. Summarizes the outcome of studies on the phosphorylation of *A. thaliana* proteins.
Phosphatidylinositol-phosphates (PIPs): phospholipids derived from phosphorylation of phosphatidylinositol (PI); they are part of cell membranes. They play roles in dynamic signaling processes at membranes and recruit specific proteins. They can confer processes such as membrane curvature, vesicle trafficking, cytoskeletal rearrangement, and protein complex assembly.
Phosphopeptide: a peptide harboring one or more phosphorylated serine, threonine or tyrosine residues. Here, phosphopeptides are generated and identified in phosphoproteomics studies and detected by mass spectrometry techniques.
Plasma membrane (PM): cell membrane separating the inside cell from the extracellular environment, composed of membrane lipids of different kinds and transmembrane and peripheral membrane proteins.
Post-translational modifications (PTMs): protein modifications at specific amino acid residues, altering charges, conformation, function, and protein interaction platforms. A frequently found type is protein phosphorylation at serine, threonine, or tyrosine residues.
SEC14 domain: globular domain as in human CRAL/TRIO and yeast SEC14p. The SEC14 domain of PATLs is preceded by CRAL/TRIO-N (CTN), a small domain portion of three alpha helices governing the entrance to the lipid-binding pocket of the large SEC14 domain.

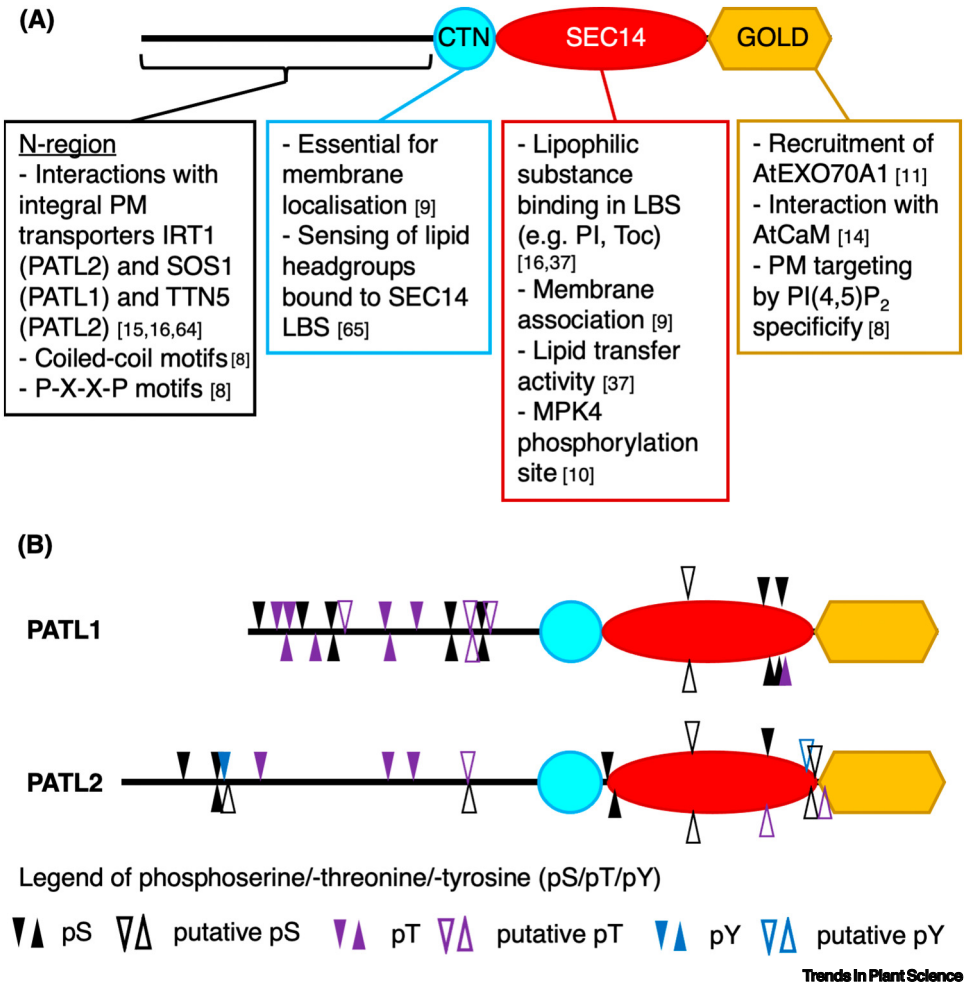

Figure 1. SEC14 PATELLIN (PATL) proteins in *Arabidopsis thaliana* and representative identified phosphorylation sites. (A) Generalized model of the PATL protein structure with conserved domain architecture. Cyan, CRAL/TRIO N-terminal (CTN) domain; red, SEC14 lipid-binding domain; yellow, Golgi dynamics (GOLD) domain. Below are depicted the different interactions and functional roles of the domains. (B) Protein structures of PATL1 (AT1G72150) and PATL2 (AT3G22530) with identified phosphorylation sites. Phosphorylation (phospho-, p) sites are indicated by colored arrowheads. Black, serine (pS); purple, threonine (pT); blue, tyrosine (pY); filled, experimentally verified through detection of phosphopeptides in phosphoproteomics, mapped based on data from the PhosPhAt server (https://phosphat.uni-hohenheim.de/phosphat.html, accessed on 19.09.2024); hollow, putative, exact sites in the phosphopeptides not determined. A full list of phosphopeptides is provided in Table S1 (see the supplemental information online). Variations in phosphorylation patterns between different PATL paralogs suggest functional divergence or regulation under different physiological conditions (compare with Figure S1 in the supplemental information online). See Peterman *et al*. [8], Montag *et al*. [9], Suzuki *et al*. [10], Wu *et al*. [11], Chu *et al*. [14], Zhou *et al*. [15], Hornbergs *et al*. [16], Schaaf *et al*. [37], Mohr *et al*. [64], Saito *et al*. [65]. Abbreviations: LBS, lipid-binding site; MPK4, Map kinase 4; PI, phosphatidylinositol; PM, plasma membrane; Toc, alpha-tocopherol.

[peptide sequence (s)(s)FVFVSDFR] residues 365–374 in PATL1 and residues 473–482 in PATL2, at serines 428/536 in PATL1 and PATL2, respectively, as well as in the C-terminal region of the SEC14, either right after the S428 in PATL1, or in the region that links the SEC14 and GOLD domains as in PATL2 (Figure 1B). For PATL4, but not PATL3, PATL5, and PATL6, a single phosphosite was identified in the GOLD domain (Figures S1 and S2 in the supplemental information online).

PATL protein phosphorylation occurs in response to nutrient deficiency, stresses, and developmental conditions

The identified phosphopeptides can be sorted according to their proportions based on detection in response to plant treatments and cellular fractionation (100% corresponding to the sum of all detected PATL phosphopeptides, Figure 2A). The largest proportion of the detected phosphopeptides was identified under particular experimental control growth conditions, indicating that PATL phosphorylation is playing a major role in these growth conditions (Figure 2A), also depending on genetic composition [26,27]. A large proportion of phosphopeptides was detected in nutrient treatments (nitrate starvation/nitrate resupply, sucrose treatment, or iron deficiency, 19.8%) and hormone treatment [abscisic acid (ABA), ethylene (ET), 18.6%] (Figure 2A). Biotic stress was reflected by flagellin 22 (flg22) treatment and also resulted in a considerable proportion of phosphopeptides (9.6 %) (Figure 2A). Further, a large proportion of phosphopeptides was detected after treatment with ionizing radiation (16.4%) and changes in the circadian rhythm (14.1%) (Figure 2A). These observations agree with functional connections of PATLs with environment signaling responses and the cell cycle [8,10,12,15,16,28]. This is interesting because the environment signaling pathways are interlocked (e.g., iron deficiency and light signaling) [29–33]. Germination and seedling development involve breakdown of storage molecules and hormone signaling [34–36]. These processes are connected with the highest counts of PATL phosphorylation sites (seeding or seedlings; Figure 2B), which is expected because PATLs partake in cell plate assembly and cell division [8,10,12]. Although cell culture is no natural state of plants, a high proportion of phosphorylation sites in cell culture condition may reflect a role in cell division (cell culture; Figure 2B).

Fewer PATL phosphopeptides are detectable in roots compared with leaves. This observation cannot be explained by the lack of investigation of root tissue in the studies, as almost all studies investigated roots. Hence, root tissue may require less diversity in PATL PTM than photosynthetically active tissue or, alternatively, only few phosphorylation sites may steer the PATL activities in roots.

Fractionation studies provide information on the cellular localization of PATLs from which the phosphopeptides are derived, such as membrane-associated and soluble fractions as compared with total protein fractions (Figure S3A in the supplemental information online). Not surprisingly, total protein fractions display most phosphorylation sites and membrane-associated fractions also detect many phosphorylation sites (Figure S3B). Eighteen phosphorylation sites are unique for the total protein fraction which shares an additional eight sites exclusively with the soluble fraction. Four sites are shared exclusively with the membrane-associated fraction and 25 with both of them (Figure S3B). The flexibility of the PATLs to localize either to the soluble or the membrane-associated fractions indicates their nature as peripheral membrane proteins that are present in cytoplasm and at membranes. Remarkably, our investigation finds nine and six sites linked with either soluble protein or membrane fractions (Figure S3B). Potentially, the ability of PATL proteins to associate with membranes is controlled by protein phosphorylation.

Most phosphopeptide annotations corresponded to PATL1 and PATL2 maybe because of their high protein abundancies, compared with PATL3, PATL4, PATL5, and PATL6 (Figure S4 in the supplemental information online). The presence of PATL1 and PATL2 proteins in specific cellular protein fractions may be associated with their phosphorylation status. Roughly two-thirds of the PATL1 phosphorylation sites are found in either the total or soluble protein fractions. The remaining ones are either present in soluble and total protein fractions or in the soluble, total, and membrane protein fractions (Figure 2C, Cell fraction). No phosphorylation site occurred only in the membrane-associated fraction (Figure 2C, Cell fraction). Phosphorylation sites unique to the 'total protein' fraction may come from studies that used specific treatments but did not distinguish the cellular fractions. When compared with the treatments, it appears that phosphorylation

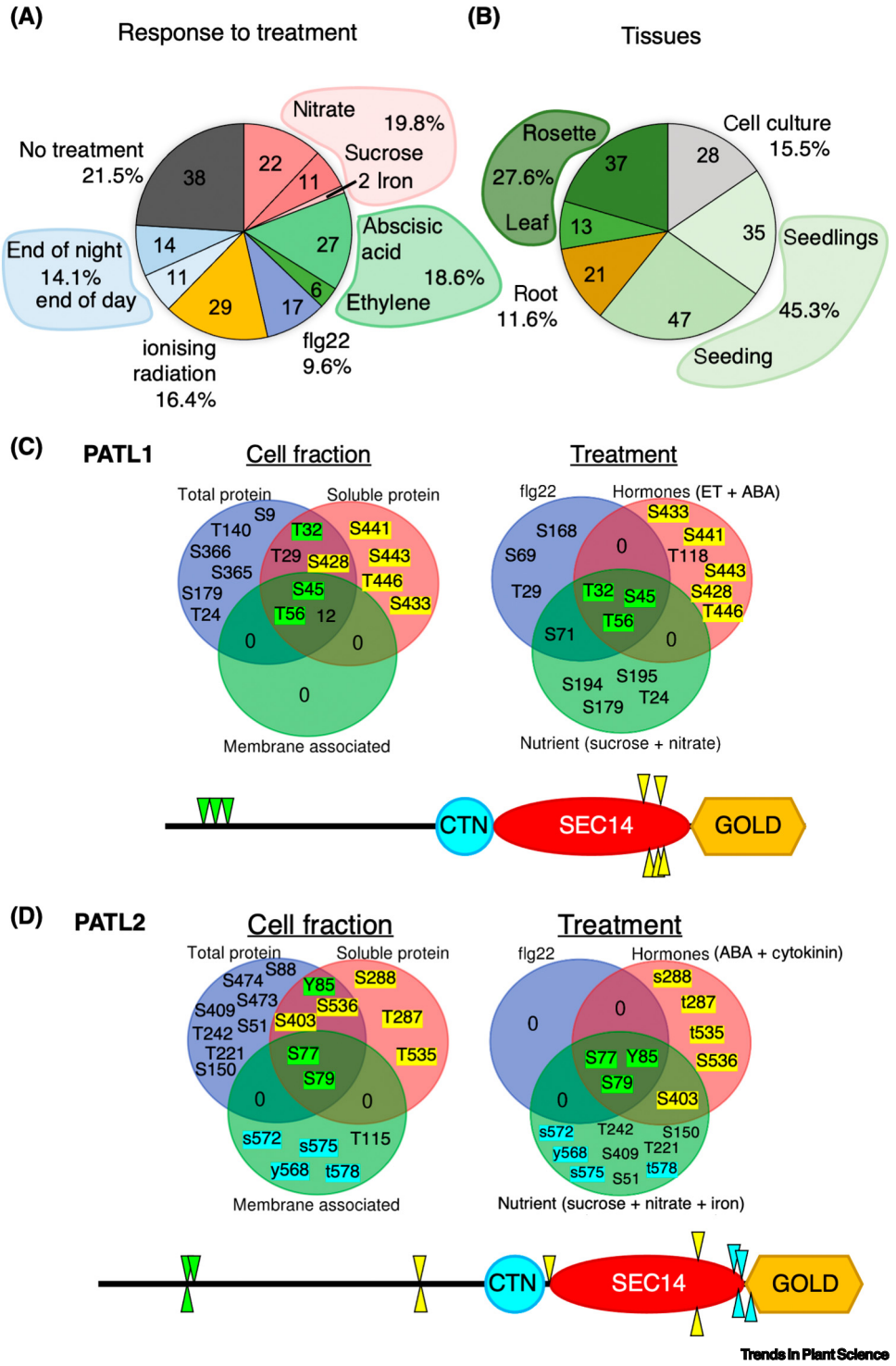

Figure 2. Occurrence of PATELLIN (PATL) phosphopeptides and phosphorylation sites according to treatment, tissue, and cell fractions in *Arabidopsis thaliana*. (A,B) Pie charts representing the % proportion of detected PATL phosphopeptides (listed in Table S1 in the supplemental information online) according to (A) treatment;

(Figure legend continued at the bottom of the next page.)

sites from the intersection of the three fractions occur in response to flg22, hormone, and nutrient treatment (Figure 2C, Treatment). These phosphorylation sites are located in the first quarter of the PATL1 N-region (Figure 2C, bottom). However, the soluble fraction contains phosphorylated PATL1 upon hormone treatment (Figure 2C, Treatment). Interestingly, these phosphorylation sites are present in the SEC14 domain (Figure 2C, bottom). These findings indicate that the phosphorylation sites are affected by different triggers.

Similarly, PATL2 has three phosphorylation sites near the start of its N-region. They are found in all the analyzed subcellular fractions and treatment categories (Figure 2D, Cell fraction and Treatment). Hormones act upon specific phosphorylation sites that are found in the soluble protein fraction and located towards the SEC14 domain (Figure 2D). Phosphorylation in the SEC14 region may affect the molecular dynamics of membrane association. Remarkably, some phospho-sites are situated in the cleft between CTN and SEC14 domain (Figure 2D, bottom), which mediates PI and PC headgroup binding in yeast Sec14p and Sfh1, especially overlapping with the position of the PI headgroup [37] (Figure S5A in the supplemental information online). Thus, phosphorylation in this specific area may result in changes of the affinity of the LBS for the ligand, possibly altering the protein lipid-binding function. Indeed, changes in S536 targeted by MAP kinase 4 (MPK4) resulted in changes of PI affinity in protein–lipid overlay assays which gives an example for the impact of phosphorylation in the SEC14 domain [10]. Interestingly, there are membrane-associated phosphorylation sites responsive to nutrient treatment in between the SEC14 and GOLD domains (Figure 2D, Treatment). It may be that the phosphorylation in this region alters the three-dimensional mobility of the GOLD domain, as it was found to be flexible towards the CTN-SEC14 domain in molecular dynamics simulation [16] (Figure S5B). In addition, the GOLD domain negatively affected α-tocopherol binding. Thus, control of the GOLD domain may be a necessary step to adjust membrane dynamics to certain nutrient stresses, including iron deficiency [16,20]. Alternatively, localization at the PM versus the endomembrane system might be controlled by such phosphorylation [9].

PATL phosphorylation can be integrated in various ways in cell signaling pathways

Surface charges, including the ones conferred by phosphates due to protein phosphorylation, are highly relevant to facilitate or block interactions between proteins and membranes. This is relevant in the context where SEC14 proteins act to alter membrane identity and dynamics. The multiple phosphorylation sites in SEC14 proteins imply different functional aspects (summarized in Figure 3, Key figure), pointing to three remarkable findings. First, PhosPhAt mining showed evidence for few SEC14 proteins to be phosphorylated in plants, primarily the PATLs. Further experimental studies are yet needed since low protein abundance might have hampered the detection of other phosphorylated SEC14 fragments. Second, the phosphorylated SEC14 proteins had phosphorylation marks in multiple functional sites, such as the N-region for protein interaction with transporters and components of vesicular trafficking, and the SEC14 domain nearby the lipid-binding and membrane association regions. Phosphorylation in the region

reddish, nutrient treatments; green, hormone treatments; dark blue, biotic stress and flg22 treatments; yellow, ionizing radiation treatments; light blue, light/dark treatments; grey, no indicated treatments; and (B) tissue types as indicated in the figure. (C,D) Venn diagrams showing which phosphorylation sites in *A. thaliana* PATL1 and PATL2 were detected according to cellular fraction (left) and plant treatment (right). Schematic structures of PATL proteins are shown below the Venn diagrams; with arrowheads pointing to phosphorylation sites represented in the colors as used for highlighting in the Venn diagrams. Further information: PATL1, AT1G72150; PATL2, AT1G22530; with conserved domain architecture: cyan, CRAL/TRIO N-terminal (CTN) domain; red, SEC14 lipid-binding domain; yellow, Golgi dynamics (GOLD) domain. Abbreviations: ABA, abscisic acid; ET, ethylene; flg22, flagellin 22. Venn diagrams were prepared using https://bioinformatics.psb.ugent.be/webtools/Venn/.

Key figure

Summarizing model of protein phosphorylation in the PATELLIN (PATL) protein family and possible functional roles

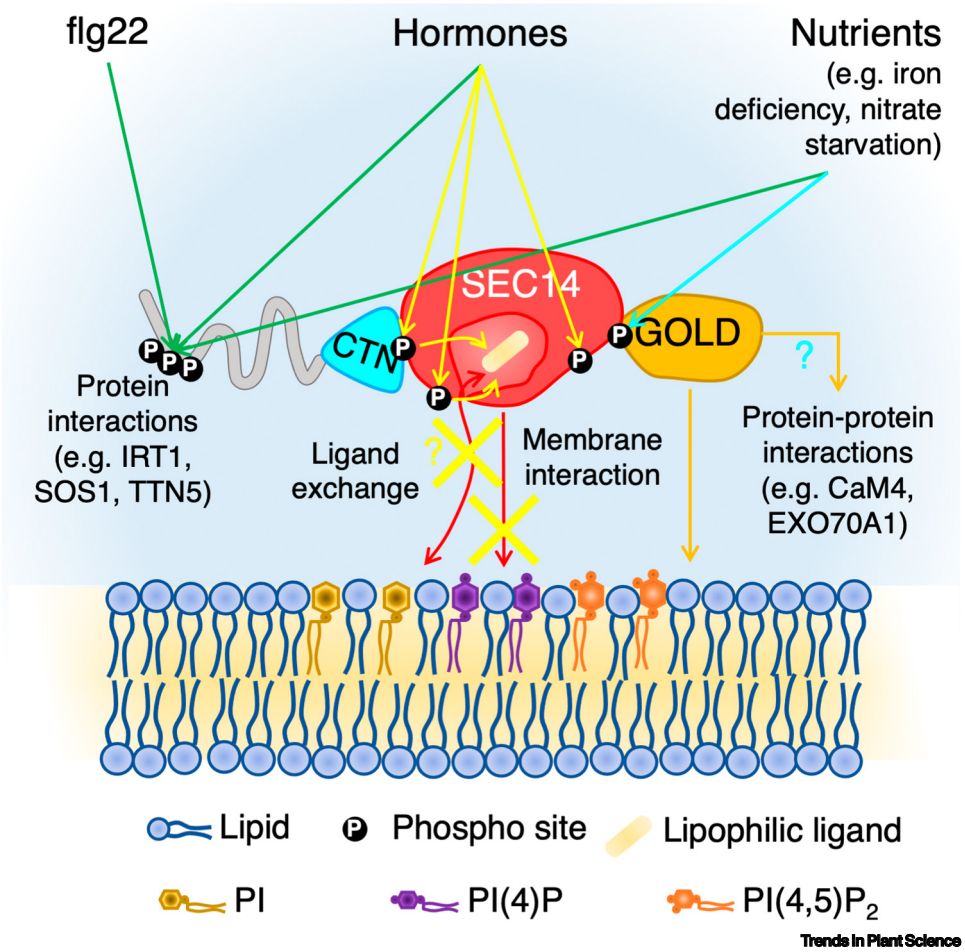

Figure 3. The figure illustrates a generalized model of a PATL, interacting with phospholipids and lipophilic substrates in the plasma membrane and in the lipid-binding site, which is facilitated or inhibited depending on the phosphorylation status. Exemplified phosphorylation is represented in the N-region affecting protein–protein interaction and in the SEC14 domain, affecting interactions with lipophilic surfaces and ligands. PATL protein phosphorylation (represented by black circles with (P) is triggered by hormone and nutrient cues. Schematic representations of the structures of a PATL with conserved domain architecture: cyan, CRAL/TRIO N-terminal (CTN) domain; red, SEC14 lipid-binding domain; yellow, Golgi dynamics (GOLD) domain. Phospholipids and lipophilic substrates are explained at the bottom.

between the CTN and SEC14 domains (e.g., pS428 in PATL1 and pS403/pS536 in PATL2, Figure S5 in the supplemental information online) could directly affect ligand exchange by altering ligand affinity or accessibility to the LBS (Figures 3 and S5). A change in the electrostatic potential in this region can support orientation towards the membrane [38,39]. This could explain the altered membrane affinity observed for PATL2 upon phosphorylation at S536 by kinase MPK4 [10]. Interestingly, also other MPKs may be suitable for phosphorylation of S536 in PATL2, or S428 in PATL1, as they share overlapping phosphorylation sites but may vary in phosphorylation

efficiency [40]. Third, phosphorylation can be triggered by environment cues, indicating that SEC14 protein phosphorylation is part of regulatory cell signaling with regard to hormone response, nutrient deficiency, and abiotic stress. Phosphorylation in the N-regions of PATL1 and PATL2 may guide interactions with transporters. However, phosphorylation at the SEC14 domain is facilitated by hormones and nutrients, suggesting that the basic SEC14 lipid binding and transfer or membrane association can be controlled through such signals (Figure 3).

An Interesting aspect is that the highlighted treatments are interconnected, as both nutrient availability and flg22 treatment engage hormone signaling pathways. For instance, PATL2 is phosphorylated at S77, S79, and Y85 across all three treatments, and CYTOSOLIC ABA RECEPTOR KINASE 7 (CARK7) has been identified as an interactor of PATL2 under iron-sufficient conditions in previous studies [16], potentially linking ABA perception with tyrosine phosphorylation. Furthermore, biotic stress through caterpillar elicitors depends on PATL2-mediated endocytosis and is agonized by jasmonate [41]. A conserved (pS)F/VKEE motif was identified in multiple PATL proteins – PATL1 (S71), PATL2 (S79), PATL3 (S108), PATL4 (S53), and PATL5 (S290) – in response to treatments such as flg22, iron deficiency, nitrate starvation/resupply, ABA, ET, and sucrose/rapamycin treatment [25,42–54] (Table S1 and Figure S2 in the supplemental information online). This shared motif indicates a potentially conserved mechanism by which PATLs modulate their responses to environmental and hormonal signals, involving kinases such as CASEIN KINASE II (CK2), SUCROSE NON-FERMENTING RELATED KINASEs (SnrKs), or CALCIUM-DEPENDENT PROTEIN KINASES (CDPKs) [55,56]. Moreover, PATLs may adjust cell division, depending on their phosphorylation [8,10,12]. Thus, PATL phosphorylation is intricately linked to developmental processes, particularly during seedling growth and early plant development, which aligns with the observed embryo lethality in PATL knockout mutants [12].

Concluding remarks and future perspectives

Phosphorylation emerges as a pivotal regulatory mechanism in the PATL family, particularly through the diverse N-terminal regions as well as the SEC14 domains, which could be phosphorylation-dependent protein interaction hubs. Further, the observed variability in PATL localization between soluble and membrane-associated fractions highlights a potential role as peripheral membrane proteins, capable of shuttling between compartments based on phosphorylation status. A recent study on intrinsically disordered regions (IDR) in membrane proteins implies membrane curvature induction using the entropy of IDRs [57], which can be a mechanism for the PATL N-region. Yet, targeted studies and physiological and genetic proofs for the relevance of PATL phosphorylation is lacking. Such studies can answer the open questions outlined (see Outstanding questions) and lead to far-reaching practical applications in crop improvement. Recent studies identified *SEC14* genes to be related with quantitative trait loci of drought resistance and development cues [58–60]. SEC14 proteins are also involved in chloroplast metabolism [61–63]. Engineering plants with modified PATL phosphorylation sites has potential to enhance stress tolerance, nutrient uptake efficiency, or developmental adaptability.

Outstanding questions

Can PATL protein phosphorylation in response to nutrient, stress, and hormone cues be validated in targeted studies?

Is protein phosphorylation of PATL proteins functionally relevant and do phosphomicking and phosphodefective mutants of PATLs have functional consequences in plants?

Does protein phosphorylation in the N-region modulate PATL protein interactions with other proteins?

Does phosphorylation of the N-region alter its disordered state and, if so, does it affect membrane curvature?

Does protein phosphorylation in the SEC14-GOLD domains lead to different abilities for ligand binding and membrane association?

Which protein kinase pathways act upon the different PATL phosphorylation sites in plants?

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Declaration of interests

The authors declare no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Chat GPT 4 in order to improve the text and summarize content. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplemental information

Supplemental information associated with this article can be found online https://doi.org/10.1016/j.tplants.2025.07.013.

References

1. Di Paolo, G. and De Camilli, P. (2006) Phosphoinositides in cell regulation and membrane dynamics. *Nature* 443, 651–657
2. Bunney, T.D. and Katan, M. (2010) Phosphoinositide signalling in cancer: beyond PI3K and PTEN. *Nat. Rev. Cancer* 10, 342–352
3. Balla, T. (2013) Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol. Rev.* 93, 1019–1137
4. Heilmann, I. (2016) Phosphoinositide signaling in plant development. *Development* 143, 2044–2055
5. Bilanges, B. *et al.* (2019) PI3K isoforms in cell signalling and vesicle trafficking. *Nat. Rev. Mol. Cell Biol.* 20, 515–534
6. Heilmann, M. and Heilmann, I. (2022) Regulators regulated: different layers of control for plasma membrane phosphoinositides in plants. *Curr. Opin. Plant Biol.* 67, 102218
7. Jaillais, Y. *et al.* (2024) Guidelines for naming and studying plasma membrane domains in plants. *Nat Plants* 10, 1172–1183
8. Peterman, T.K. *et al.* (2004) Patellin1, a novel Sec14-like protein, localizes to the cell plate and binds phosphoinositides. *Plant Physiol.* 136, 3080–3094
9. Montag, K. *et al.* (2020) Phylogenetic analysis of plant multidomain SEC14-like phosphatidylinositol transfer proteins and structure–function properties of PATELLIN2. *Plant Mol. Biol.* 104, 665–678
10. Suzuki, T. *et al.* (2016) Identification of phosphoinositide-binding protein PATELLIN2 as a substrate of *Arabidopsis* MPK4 MAP kinase during septum formation in cytokinesis. *Plant Cell Physiol.* 57, 1744–1755
11. Wu, C. *et al.* (2017) *Arabidopsis* EXO70A1 recruits Patellin3 to the cell membrane independent of its role as an exocyst subunit. *J. Integr. Plant Biol.* 59, 851–865
12. Tejos, R. *et al.* (2018) PATELLINS are regulators of auxin-mediated PIN1 relocation and plant development in *Arabidopsis thaliana*. *J. Cell Sci.* 131, jcs204198
13. Mendes, L.F.S. and Costa-Filho, A.J. (2022) A gold revision of the Golgi Dynamics (GOLD) domain structure and associated cell functionalities. *FEBS Lett.* 596, 973–990
14. Chu, M. *et al.* (2018) AtCaM4 interacts with a Sec14-like protein, PATL1, to regulate freezing tolerance in *Arabidopsis* in a CBF-independent manner. *J. Exp. Bot.* 69, 5241–5253
15. Zhou, H. *et al.* (2018) Patellin1 negatively modulates salt tolerance by regulating PM Na+/H+ antiport activity and cellular redox homeostasis in *Arabidopsis*. *Plant Cell Physiol.* 59, 1630–1642
16. Hornbergs, J. *et al.* (2023) SEC14-GOLD protein PATELLIN2 binds IRON-REGULATED TRANSPORTER1 linking root iron uptake to vitamin E. *Plant Physiol.* 192, 504–526
17. Melicher, P. *et al.* (2024) Methyl viologen-induced changes in the *Arabidopsis* proteome implicate PATELLIN 4 in oxidative stress responses. *J. Exp. Bot.* 75, 405–421
18. Heazlewood, J.L. *et al.* (2008) PhosPhAt: a database of phosphorylation sites in *Arabidopsis thaliana* and a plant-specific phosphorylation site predictor. *Nucleic Acids Res.* 36, D1015–D1021
19. Durek, P. *et al.* (2010) PhosPhAt: the *Arabidopsis thaliana* phosphorylation site database. An update. *Nucleic Acids Res.* 38, D828–D834
20. Lan, P. *et al.* (2012) Quantitative phosphoproteome profiling of iron-deficient *Arabidopsis* roots. *Plant Physiol.* 159, 403–417
21. Tang, W. *et al.* (2008) Proteomics studies of brassinosteroid signal transduction using prefractionation and two-dimensional DIGE. *Mol. Cell. Proteomics* 7, 728–738
22. Hsu, J.L. *et al.* (2009) Functional phosphoproteomic profiling of phosphorylation sites in membrane fractions of salt-stressed *Arabidopsis thaliana*. *Proteome Sci.* 7, 42
23. Chang, I.F. *et al.* (2012) Comparative phosphoproteomic analysis of microsomal fractions of *Arabidopsis thaliana* and *Oryza sativa* subjected to high salinity. *Plant Sci.* 185–186, 1–13
24. Mattei, B. *et al.* (2016) Comprehensive analysis of the membrane phosphoproteome regulated by oligogalacturonides in *Arabidopsis thaliana*. *Front. Plant Sci.* 7, 1107
25. Van Leene, J. *et al.* (2019) Capturing the phosphorylation and protein interaction landscape of the plant TOR kinase. *Nat Plants* 5, 316–327
26. Wu, X. *et al.* (2014) A kinase-phosphatase signaling module with BSK8 and BSL2 involved in regulation of sucrose-phosphate synthase. *J. Proteome Res.* 13, 3347–3355
27. Roitinger, E. *et al.* (2015) Quantitative phosphoproteomics of the ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia-mutated and rad3-related (ATR) dependent DNA damage response in *Arabidopsis thaliana*. *Mol. Cell. Proteomics* 14, 556–571
28. Montag, K. *et al.* (2023) Role of SEC14-like phosphatidylinositol transfer proteins in membrane identity and dynamics. *Front. Plant Sci.* 14, 1181031
29. Vert, G.A. *et al.* (2003) Dual regulation of the *Arabidopsis* high-affinity root iron uptake system by local and long-distance signals. *Plant Physiol.* 132, 796–804
30. Santi, S. and Schmidt, W. (2009) Dissecting iron deficiency-induced proton extrusion in *Arabidopsis* roots. *New Phytol.* 183, 1072–1084
31. Hong, S. *et al.* (2013) Reciprocal interaction of the circadian clock with the iron homeostasis network in *Arabidopsis*. *Plant Physiol.* 161, 893–903
32. Xu, G. *et al.* (2019) The central circadian clock proteins CCA1 and LHY regulate iron homeostasis in *Arabidopsis*. *J. Integr. Plant Biol.* 61, 168–181
33. Trofimov, K. *et al.* (2025) Shedding light on iron nutrition: exploring intersections of transcription factor cascades in light and iron deficiency signaling. *J. Exp. Bot.* 76, 787–802
34. Weitbrecht, K. *et al.* (2011) First off the mark: early seed germination. *J. Exp. Bot.* 62, 3289–3309
35. Han, C. and Yang, P. (2015) Studies on the molecular mechanisms of seed germination. *Proteomics* 15, 1671–1679
36. Smolikova, G. *et al.* (2021) Transition from seeds to seedlings: hormonal and epigenetic aspects. *Plants* 10, 1884
37. Schaaf, G. *et al.* (2008) Functional anatomy of phospholipid binding and regulation of phosphoinositide homeostasis by proteins of the sec14 superfamily. *Mol. Cell* 29, 191–206
38. Sugiura, T. *et al.* (2019) Biophysical parameters of the Sec14 phospholipid exchange cycle. *Biophys. J.* 116, 92–103
39. Tripathi, A. *et al.* (2019) Functional diversification of the chemical landscapes of yeast Sec14-like phosphatidylinositol transfer protein lipid-binding cavities. *Lipids* 50, 19081–19098
40. Sörensson, C. *et al.* (2012) Determination of primary sequence specificity of Arabidopsis MAPKs MPK3 and MPK6 leads to identification of new substrates. *Biochem. J.* 446, 271–278
41. Yan, Z.W. *et al.* (2023) Endocytosis-mediated entry of a caterpillar effector into plants is countered by jasmonate. *Nat. Commun.* 14, 6551
42. Al-Momani, S. *et al.* (2018) Comparative qualitative phosphoproteomics analysis identifies shared phosphorylation motifs and associated biological processes in evolutionary divergent plants. *J. Proteome* 186, 1–14
43. Benschop, J.J. *et al.* (2007) Quantitative phosphoproteomics of early elicitor signaling in *Arabidopsis*. *Mol. Cell. Proteomics* 6, 1198–1214

44. Chen, H. *et al.* (2017) Quantitative phosphoproteomic analysis reveals shared and specific targets of *Arabidopsis* mitogen-activated protein kinases (MPKs) MPK3, MPK4, and MPK6. *Mol. Cell. Proteomics* 16, 1795–1810
45. Choudhary, M.K. *et al.* (2015) Quantitative circadian phosphoproteomic analysis of *Arabidopsis* reveals extensive clock control of key components in physiological, metabolic, and signaling pathways. *Mol. Cell. Proteomics* 14, 2243–2260
46. Rayapuram, N. *et al.* (2013) Identification of novel PAMP-triggered phosphorylation and dephosphorylation events in *Arabidopsis thaliana* by quantitative phosphoproteomic analysis. *J. Proteome Res.* 13, 2137–2153
47. Reiland, S. *et al.* (2009) Large-scale *Arabidopsis* phosphoproteome profiling reveals novel chloroplast kinase substrates and phosphorylation networks. *Plant Physiol.* 150, 889–903
48. Reiland, S. *et al.* (2011) Comparative phosphoproteome profiling reveals a function of the STN8 kinase in fine-tuning of cyclic electron flow (CEF). *Proc. Natl Acad. Sci. U. S. A.* 108, 12955–12960
49. Umezawa, T. *et al.* (2013) Genetics and phosphoproteomics reveal a protein phosphorylation network in the abscisic acid signaling pathway in *Arabidopsis thaliana*. *Sci. Signal.* 6, rs8
50. Wang, X. *et al.* (2013) A large-scale protein phosphorylation analysis reveals novel phosphorylation motifs and phosphoregulatory networks in *Arabidopsis*. *J. Proteome* 78, 486–498
51. Wang, P. *et al.* (2017) Protein phosphorylation orchestrates acclimations of *Arabidopsis* plants to changing pH conditions. *Plant Cell* 29, 2936–2957
52. Whiteman, S.A. *et al.* (2008) Identification of novel proteins and phosphorylation sites in a tonoplast-enriched membrane fraction of *Arabidopsis thaliana*. *Proteomics* 8, 3536–3547
53. Xue, L. *et al.* (2013) Quantitative measurement of phosphoproteome response to osmotic stress in *Arabidopsis* based on Library-Assisted eXtracted Ion Chromatogram (LAXIC). *Mol. Cell. Proteomics* 12, 3274–3285
54. Yang, Z. *et al.* (2013) Stable isotope metabolic labeling-based quantitative phosphoproteomic analysis of *Arabidopsis* mutants reveals ethylene-regulated time-dependent phosphoproteins and putative substrates of constitutive triple response 1 kinase. *Mol. Cell. Proteomics* 12, 3559–3572
55. Hrabak, E.M. *et al.* (2003) The *Arabidopsis* CDPK-SnRK superfamily of protein kinases. *Plant Physiol.* 132, 666–680
56. Salinas, P. *et al.* (2006) An extensive survey of CK2 α and β subunits in *Arabidopsis*: multiple isoforms exhibit differential subcellular localization. *Plant Cell Physiol.* 47, 1295–1308
57. Poveda-Cuevas, S.A. *et al.* (2024) Intrinsically disordered region amplifies membrane remodeling to augment selective ER-phagy. *Proc. Natl. Acad. Sci. U. S. A.* 121, e2408071121
58. Ergon, Å. *et al.* (2022) Identification of loci controlling timing of stem elongation in red clover using genotyping by sequencing of pooled phenotypic extremes. *Mol. Gen. Genomics.* 297, 1587–1600
59. Afonnikova, S.D. *et al.* (2024) Identification of novel loci precisely modulating pre-harvest sprouting resistance and red color components of the seed coat in *T. aestivum* L. *Plants (Basel)* 13, 1309
60. Dang, P. *et al.* (2024) Genome-wide association analysis identified quantitative trait loci (QTLs) underlying drought-related traits in cultivated peanut (*Arachis hypogaea* L.). *Genes (Basel)* 15, 868
61. Hertle, A.P. *et al.* (2020) A Sec14 domain protein is required for photoautotrophic growth and chloroplast vesicle formation in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. U. S. A.* 117, 9101–9111
62. Kim, E.H. *et al.* (2022) Chloroplast-localized PITP7 is essential for plant growth and photosynthetic function in *Arabidopsis*. *Physiol. Plant.* 174, e13760
63. Yao, H.Y. *et al.* (2023) Arabidopsis Sec14 proteins (SFH5 and SFH7) mediate interorganelle transport of phosphatidic acid and regulate chloroplast development. *Proc. Natl Acad. Sci. U. S. A.* 120, e2221637120
64. Mohr, I. *et al.* (2024) Small ARF-like 2 GTPase TITAN 5 is linked with the dynamic regulation of IRON-REGULATED TRANSPORTER 1. *J. Cell Sci.* 137, jcs263645
65. Saito, K. *et al.* (2007) The lipid-binding SEC14 domain. *Biochim. Biophys. Acta* 1771, 719–726
66. Panagabko, C. *et al.* (2003) Ligand specificity in the CRAL-TRIO protein family. *Biochemistry* 42, 6467–6474
67. Huang, J. *et al.* (2016) Two-ligand priming mechanism for potentiated phosphoinositide synthesis is an evolutionarily conserved feature of Sec14-like phosphatidylinositol and phosphatidylcholine exchange proteins. *Mol. Biol. Cell* 27, 2317–2330
68. Bankaitis, V.A. *et al.* (2012) The Sec14 superfamily and mechanisms for crosstalk between lipid metabolism and lipid signaling. *Trends Biochem. Sci.* 37, 185–195
69. Ghosh, R. *et al.* (2015) Sec14-nodulin proteins and the patterning of phosphoinositide landmarks for developmental control of membrane morphogenesis. *Mol. Biol. Cell* 26, 1764–1781
70. de Campos, M.K.F. and Schaaf, G. (2017) The regulation of cell polarity by lipid transfer proteins of the SEC14 family. *Curr. Opin. Plant Biol.* 40, 158–168
71. Xu, W. *et al.* (2024) OsSNDP4, a Sec14-nodulin domain protein, is required for pollen development in rice. *Rice (N Y)* 17, 54