COMMENTARY

Phosphoinositides regulate chloroplast processes

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Phosphoinositides (PIs), the phosphorylated derivatives of the membrane glycerophospholipid phosphatidylinositol (PtdIns), are minor constituents of eukaryotic cell membranes that play an important role as signaling molecules (1). The inositol ring can be phosphorylated at three positions and the seven resulting phosphorylated forms are dynamically interconverted and differentially distributed among cellular membranes. Important regulators of PI phosphorylation and distribution are proteins harboring a CRAL_TRIO (cellular retinaldehyde binding-triple response) domain, for which yeast Sec14 is the prototype (2, 3). Sec14-like proteins bind PI in a hydrophobic cavity in the CRAL_TRIO domain. In that cavity, another hydrophobic ligand can be bound alternatively. The nature of this alternative ligand is characteristic of a Sec14-like protein (4) and may link activities of the metabolic pathway producing this hydrophobic ligand with PI signaling (5, 6). The second ligand is phosphatidylcholine in the case of Sec14 (7); squalene and sterols in the case of yeast Sec14 homologs SFH2 and SFH3, respectively (4); α-tocopherol (vitamin E) in the case of the α-tocopherol transfer protein (8); or retinaldehyde in the case of the cellular retinaldehyde-binding protein (9). According to the nanoreactor model (3, 6, 10), the hydrophobic cavity is loaded with the alternative ligand at the source membrane and, after transfer of the Sec14-like protein to the target membrane, exchange of the ligand occurs through an inefficient heterotypic exchange reaction. During multiple abortive exchange trials, PtdIns headgroups become accessible to PtdIns kinases for phosphorylation. PIs formed at the target membrane are then recognized by PI-binding effector proteins such as small GTPases mediating membrane traffic.

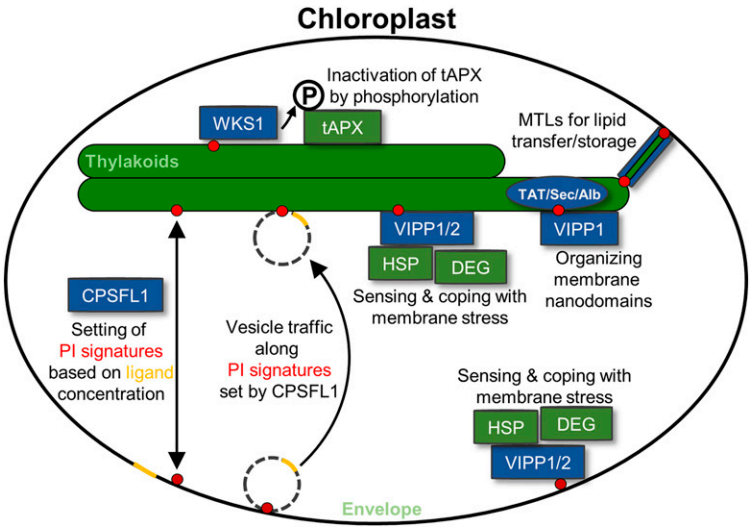

Fig. 1. Processes potentially regulated by PIs in the chloroplast. CPSFL1 senses concentrations of its hydrophobic ligand (e.g., carotenoids, orange) and converts this information into PI signatures at envelope and thylakoid membranes (red circles). PI signatures set by CPSFL1 drive vesicle traffic from the envelope to thylakoids, mediating the transport of the ligand (orange). PI signatures might target the resistance protein WKS1 to thylakoid membranes, where it reduces tAPX activity by phosphorylation, leading to the accumulation of H_2O_2 and cell death. PI signatures might also target VIPP1 and VIPP2 to envelope and thylakoids. There they play roles in the sensing of lipid packing stress caused, e.g., by misfolded proteins and recruit chaperones (HSP) and proteases (DEG) to cope with it. By organizing membrane nanodomains at thylakoid membranes (blue ellipse) VIPP1 might support the function of transporters and integrases. Rods formed by VIPP1 (blue tube) can engulf PI-containing membranes (green), potentially serving for lipid transfer or storage, as proposed for microtubule-like structures (MTLs).

Vascular plants encode particularly many Sec14-like proteins, as exemplified by the 32 Sec14 family members in *Arabidopsis thaliana* (5, 11). Members of two subfamilies adopt a multidomain structure in which the N-terminal Sec14 domain is linked to a C-terminal Nlj16-like "nodulin" or a GOLD (Golgi dynamics) domain, while members of a third subfamily are single-domain proteins. In PNAS, Hertle et al. (12) report on a chloroplast-targeted, single-domain Sec14-like protein termed CPSFL1 (chloroplast-localized Sec14-like protein 1) from *Arabidopsis*. The *cpsfl1* knockout mutant was incapable of photoautotrophic growth, which correlated with a strongly reduced accumulation of all photosynthetic complexes in the thylakoid membranes (less than 25% of wild-type levels). Chlorophyll and carotenoid levels were reduced, particularly those of α- and β-carotene. The mutant had fewer, smaller, and

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irregularly shaped chloroplasts and the thylakoid membrane network was underdeveloped. Strands of thylakoid membranes remained connected with the inner envelope, which is never observed in wild-type chloroplasts. Vesicles budding off from the inner envelope in wild-type chloroplasts were not observed in the *cpsfl1* mutant. Instead, mature chloroplasts of the mutant formed large balloon-like invaginations at the inner envelope. CPSFL1 localized to vesicles and the overexpression of CPSFL1 resulted in more vesicles. CPSFL1 bound to phosphatidic acid (PA) and was able to extract PI4P from PA-containing donor liposomes and to transfer it to PA-containing acceptor liposomes in vitro. The efficiency of this transfer increased when acceptor liposomes contained more PA. CPFSL1 bound PA even in the absence of its CRAL_TRIO domain, implying that PA is not the ligand competing with PIs for binding to the cavity formed by this domain. Rather, PA might serve in recruiting CPSFL1 to membranes. CPFSL1 partially colocalized with PA and PI4P in chloroplast membranes. Finally, CPSFL1 was able to complement the growth defect of a yeast *sec14* mutant.

Based on these results, Hertle et al. (12) propose that CPSFL1 plays a role in chloroplast vesicle formation, in line with a role of yeast Sec14 in membrane trafficking through late Golgi/endosomal compartments. Since no vesicles were formed in the mutant but thylakoids still were formed, vesicle traffic appears not to be required for the biogenesis of thylakoid membranes. Obviously, they can form also via direct contact sites with the inner envelope. However, vesicle traffic appears important for the formation and/or maintenance of functional thylakoids, for example by transferring carotenoids from their site of synthesis at the inner envelope to thylakoid membranes. Hence, CPSFL1 might link carotenoid metabolism with PI signaling in chloroplasts (Fig. 1).

On the one hand, the work by Hertle et al. (12) undoubtedly will launch a quest for PI-related effector proteins involved in chloroplast vesicle traffic. On the other hand, their work underpins the existence of PI signaling in chloroplasts, which was implied by the presence of 2 to 3% PtdIns in chloroplast envelope and thylakoid membranes (13) and the identification of chloroplast PI-binding proteins WKS1 (14), VIPP1 (15), and VIPP2 (16).

WKS1 (WHEAT KINASE START1) confers partial resistance to *Puccinia striiformis,* causing stripe rust of wheat. WKS1 harbors an N-terminal serine/threonine kinase domain and a C-terminal steroidogenic acute regulatory protein-related lipid transfer (START) domain, through which WKS1 binds to PA and PIs and phosphorylates a thylakoid-associated ascorbate peroxidase (tAPX) (14). Phosphorylation decreases tAPX activity, resulting in reduced detoxification of H_2O_2 generated during photosynthesis. This initiates a progressive cell death response characteristic of the WKS1-mediated partial resistance reaction to *P. striiformis*. Possibly, WKS1 targeting to the thylakoid membrane is mediated via PI signatures set by CPSFL1 (Fig. 1).

VIPP proteins are conserved in all organisms performing oxygenic photosynthesis (16) and *Arabidopsis vipp1* knockout mutants lack thylakoid membranes (17). VIPP proteins can oligomerize into rings and rods (18, 19) and bind to membranes via an N-terminal amphipathic α-helical domain (20). *Chlamydomonas* chloroplast VIPP1 and its paralog VIPP2 bind to PIs and VIPP1 rods can engulf PI-containing liposomes in vitro (15, 16). Since cyanobacteria possess neither PtdIns (21) nor Sec14-like proteins (12), the adaptation of VIPP1 to PI signaling appears to have evolved during domestication of the cyanobacterial endosymbiont. VIPP1 was proposed to organize membrane nanodomains resembling eisosomes (22). Like eisosomes, such nanodomains might ensure the full functionality of membrane transporters and integrases such as Sec, TAT, and Alb involved in the biogenesis and repair of thylakoid membrane protein complexes (15). Rods formed by VIPP1 resemble microtubule-like structures (19) with possible functions in lipid transfer or storage (15) (Fig. 1). VIPP2 plays a role in the sensing and signaling of membrane packing stress induced by lipid peroxidation or misfolded/aggregated proteins (16) (Fig. 1). It will be interesting to see whether CPSFL1 connects the metabolism of hydrophobic ligands such as carotenoids with functions of VIPP proteins in thylakoid membrane protein complex assembly and protein quality control through chloroplast PI signaling.

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