© 2016. Published by The Company of Biologists Ltd | Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

PRIMER

Phosphoinositide signaling in plant development

Ingo Heilmann*

ABSTRACT

The membranes of eukaryotic cells create hydrophobic barriers that control substance and information exchange between the inside and outside of cells and between cellular compartments. Besides their roles as membrane building blocks, some membrane lipids, such as phosphoinositides (PIs), also exert regulatory effects. Indeed, emerging evidence indicates that PIs play crucial roles in controlling polarity and growth in plants. Here, I highlight the key roles of PIs as important regulatory membrane lipids in plant development and function.

KEY WORDS: Lipids, Membranes, Phosphoinositides, Plant, Polarity, Recruitment

Introduction

Biological membranes act as a barrier limiting the free exchange of molecules and information. They also serve as a primary contact site between intracellular and extracellular spaces and, in eukaryotic cells, between subcellular compartments. Proteins embedded in, or associated with, membranes enable membranes or membrane areas to serve particular functions (Nicolson, 2014). These functions include the controlled exchange of substance and information across membranes, but also the attachment of proteins and cytoskeletal structures to membranes, and the insertion or recycling of membrane components (Fig. 1A). Membranes are composed of a diverse array of lipids (van Meer et al., 2008). Owing to their structural complexity and various membrane systems, eukaryotic cells require an extra level of control of subcellular membrane traffic and have evolved to contain specialized classes of membrane lipids that exert regulatory influences on membrane-associated processes (Lee, 2004). Such regulatory lipids can exert their influence by acting as ligands for membrane-associated proteins (Eyster, 2007) or by influencing the biophysical properties (Lundbaek et al., 2010) of the membrane itself, or both (Fig. 1B).

Phosphoinositides (PIs) are an example of such regulatory membrane lipids found in eukaryotic membranes. Unlike the majority of membrane lipids, which serve a structural role, PIs are of only minor abundance and their dynamic formation occurs within narrow spatial and temporal limits. Although plant PIs were discovered two or three decades ago, their function long remained elusive. However, in recent years, PIs have been shown to have crucial roles in plant development and function (Boss and Im, 2012; Heilmann and Heilmann, 2015; Munnik and Nielsen, 2011; Thole and Nielsen, 2008). Here, I provide an overview of PI structure, biogenesis and modes of action, and I discuss the key functions of PIs in plants.

Department of Cellular Biochemistry, Institute for Biochemistry and Biotechnology, Martin-Luther-University Halle-Wittenberg, Kurt-Mothes-Str. 3, Halle (Saale) 06114, Germany.

*Author for correspondence (ingo.heilmann@biochemtech.uni-halle.de)

I.H., 0000-0002-2324-1849

PI structure and biogenesis

As in other eukaryotes, all plant PIs are formed via phosphorylation of the head group of the membrane phospholipid, phosphatidylinositol (PtdIns). The PtdIns head group is composed of D-myo-inositol, which is a cyclic polyol linked via a phosphodiester bond in the C1 position to the glycerin backbone of the lipid {Fig. 2A; this figure depicts the structure of the key phosphorylated PI, phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]}. The hydroxyl groups in positions 3, 4 and 5 of the lipid head group are accessible for phosphorylation. There are notable differences in the complement of PI-based species found in plants and animals, and in plants only five of the seven PI species known from other eukaryotic model systems have been detected, namely the PtdIns monophosphates PtdIns3*P*, PtdIns4*P* and PtdIns5*P*, and the PtdIns bisphosphates PtdIns(3,5)P_2 and PtdIns(4,5)P_2 (Fig. 2B). The presence of PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3 in plants has not been reliably confirmed.

PtdIns3*P* and PtdIns4*P* are formed from PtdIns by specific PI-kinases (Fig. 2B): the PI 3-kinase VPS34 and at least two subfamilies of PI 4-kinases, namely PI4Kα and PI4Kβ, respectively (Mueller-Roeber and Pical, 2002). To date, no PI-kinase has been demonstrated to be capable of generating PtdIns5*P* from PtdIns, leaving the biogenesis of this lipid unclear. PtdIns3*P* and PtdIns4*P* can be further phosphorylated by PIP-kinases, resulting in the formation of PtdIns(3,5)P_2 and PtdIns(4,5)P_2, respectively. The formation of PtdIns(3,5)P_2 from PtdIns3*P* is catalyzed by four PI3P 5-kinases that bear similarity to yeast Fab1, while the conversion of PtdIns4*P* to PtdIns(4,5)P_2 is carried out by PI4P 5-kinases, which are encoded in the *Arabidopsis* genome in subfamilies A, comprising isoforms PIP5K1-PIP5K9, and B, comprising isoforms PIP5K10 and PIP5K11 (Mueller-Roeber and Pical, 2002). As a side reaction, both these subfamilies also convert PtdIns3*P* to PtdIns(3,5)P_2. Some plant PI4P 5-kinases display particular domain structures and features not encountered in related enzymes from other model systems, suggesting that the plant enzymes contain additional mechanisms for regulation. Examples are the highly variable linker domains of PI4P 5-kinases of subfamily B in *Arabidopsis*, which do not resemble any known protein sequence and have been shown to be involved in the specification of downstream regulatory effects of PtdIns(4,5)P_2 formed by these enzymes (Stenzel et al., 2012). The same enzymes also contain multiple membrane occupation and recognition nexus (MORN) repeats not found in PI4P 5-kinases from other organisms (Mueller-Roeber and Pical, 2002).

In comparison to the well-studied and highly specific enzymes of PI biosynthesis, there is only limited information about the enzymes involved in PI degradation (Fig. 2C). The *Arabidopsis* genome encodes a number of PI phosphatases and phospholipases (Mueller-Roeber and Pical, 2002). However, partly due to their low degree of substrate specificity, it has been difficult to assign particular catalytic or physiological functions to many of these candidates. Important families of PI phosphatases include suppressor of actin (SAC) phosphatases (Zhong and Ye, 2003), which are involved in

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

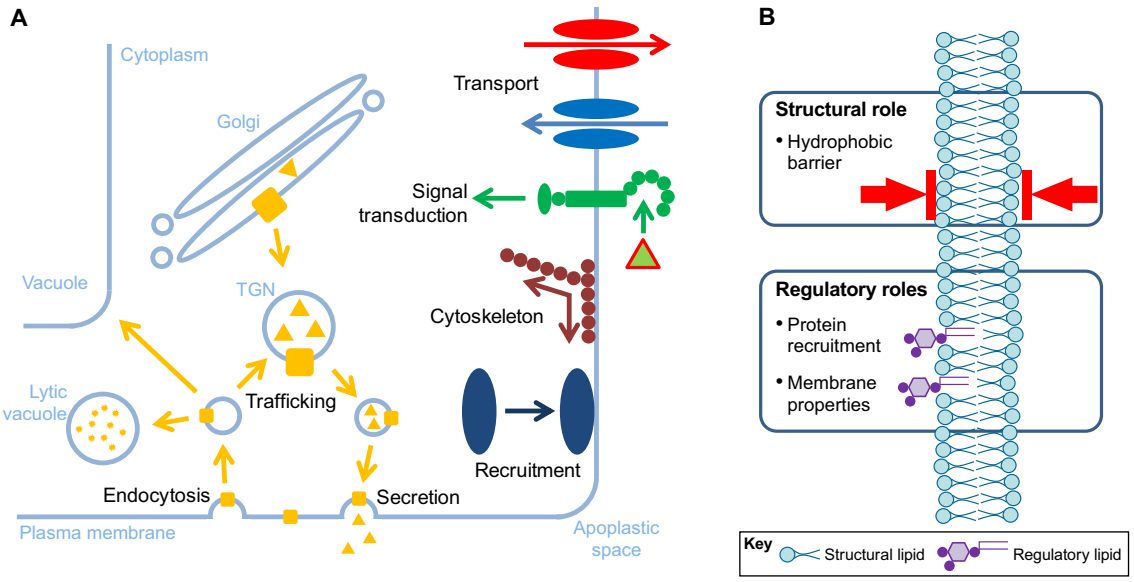


Fig. 1. Cellular membranes and their functions. (A) Cellular membranes restrict the exchange of substance and information between cells and, in eukaryotic cells, between subcellular compartments. The properties of membranes are defined by embedded or associated proteins that carry out various functions, allowing several membrane-associated cellular processes to take place including transport, signal transduction, cytoskeletal attachment, protein recruitment and dynamic membrane trafficking. (B) Membranes can play both structural and regulatory roles. They consist of bilayer-forming lipids that generate a hydrophobic barrier. Although the body of the membrane comprises structural lipids, there are also membrane lipids of low abundance that exert predominantly regulatory effects on membrane functions. These regulatory effects can be mediated by lipids acting as ligands for protein partners or through direct effects on the biophysical properties of membranes. TGN, trans-Golgi network; yellow, cargo; yellow triangles, soluble cargo.

the degradation of PtdIns bisphosphates at the plasma membrane or the tonoplast, and phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-related enzymes, which mediate the degradation of 3-phosphorylated PtdIns monophosphates and PtdIns bisphosphates (Pribat et al., 2012). Further relevant phosphatases are enzymes of the *Arabidopsis* PI 5-phosphatase (5PTase) family, which exhibit varying specificities and may also act on soluble inositol polyphosphates (IPPs) (Gunesekera et al., 2007). PIs can also be degraded by PI-specific phospholipase C (PI-PLC) enzymes, which are encoded in the *Arabidopsis* genome as a family of nine isoforms (Mueller-Roeber and Pical, 2002). All plant PI-PLCs reported so far are activated by Ca^{2+} and display similarity to the human ζ-family of PLCs that cannot be activated by heterotrimeric G-proteins (Pokotylo et al., 2014).

The enzymes listed above together mediate the biosynthesis and degradation of PIs. PIs are constantly formed and degraded, resulting in rapid and dynamic turnover (reviewed by Heilmann, 2016). This high turnover contributes to the dynamic changes in PI levels that have been observed in numerous studies in different plant models. For instance, the levels of PtdIns(4,5)P_2 change in response to environmental stresses, including wounding (Mosblech et al., 2008), salt or osmotic stress (DeWald et al., 2001; Einspahr et al., 1988a,b; Heilmann et al., 1999, 2001; König et al., 2008, 2007; Pical et al., 1999) or heat (Mishkind et al., 2009). PI formation also changes transiently during gravitropic curvature (Perera et al., 1999, 2001), and it has also been shown that PI levels respond to exogenously applied auxin (Tejos et al., 2014) or salicylic acid (Krinke et al., 2007b). Notably, *Arabidopsis* PLC2 has been reported to act in the endoplasmic reticulum (ER) stress response (Kanehara et al., 2015). The modes of regulation underlying the dynamic interplay between PI biosynthesis and degradation in plants remain unclear. The enzymes catalyzing PI interconversions are expressed in different patterns (see Table 1), with some being expressed ubiquitously and at all developmental stages analyzed, whereas others are restricted in their expression patterns to certain organs or cell types (Heilmann, 2016). The transcription of some enzymes of PI metabolism is also induced upon perception of environmental stresses. For instance, in *Arabidopsis* transcription of the *PLC1* gene is induced upon dehydration and salt stress (Hirayama et al., 1995) and that of *PIP5K1* by exogenous auxin (Tejos et al., 2014). However, a review of publicly available transcriptomic data (Zimmermann et al., 2004) indicates that the transcription of the majority of PI-related genes does not change dramatically upon challenge by a wide range of stresses, suggesting that transcriptional control might in sum contribute only little to the dynamic changes in PI levels observed during plant stress responses (Heilmann, 2016). The control of PI dynamics might thus not take place at the transcriptional level, but rather via post-translational modifications such as phosphorylation (Westergren et al., 2001). However, to date the relevance of post-translational modifications of PI pathway enzymes during plant stress responses has not been addressed in detail.

Overall, plants contain a complex network of enzymes that mediate the dynamic formation and degradation of PIs. As in animals, plant PIs appear to be continuously formed and degraded with a high rate of turnover, resulting in a dynamic equilibrium that is easily perturbed when plants are exposed to stress conditions. This dynamic equilibrium enables fast and transient or localized signaling events to be mediated by PIs in response to developmental cues or stimulation.

PI modes of action

PIs exert their functions via various mechanisms. For example, they can act as intact lipids, binding to target proteins, or they can influence the properties of the membrane in which they are embedded. Alternatively, they can act as precursors for the formation of soluble IPPs (Fig. 3).

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

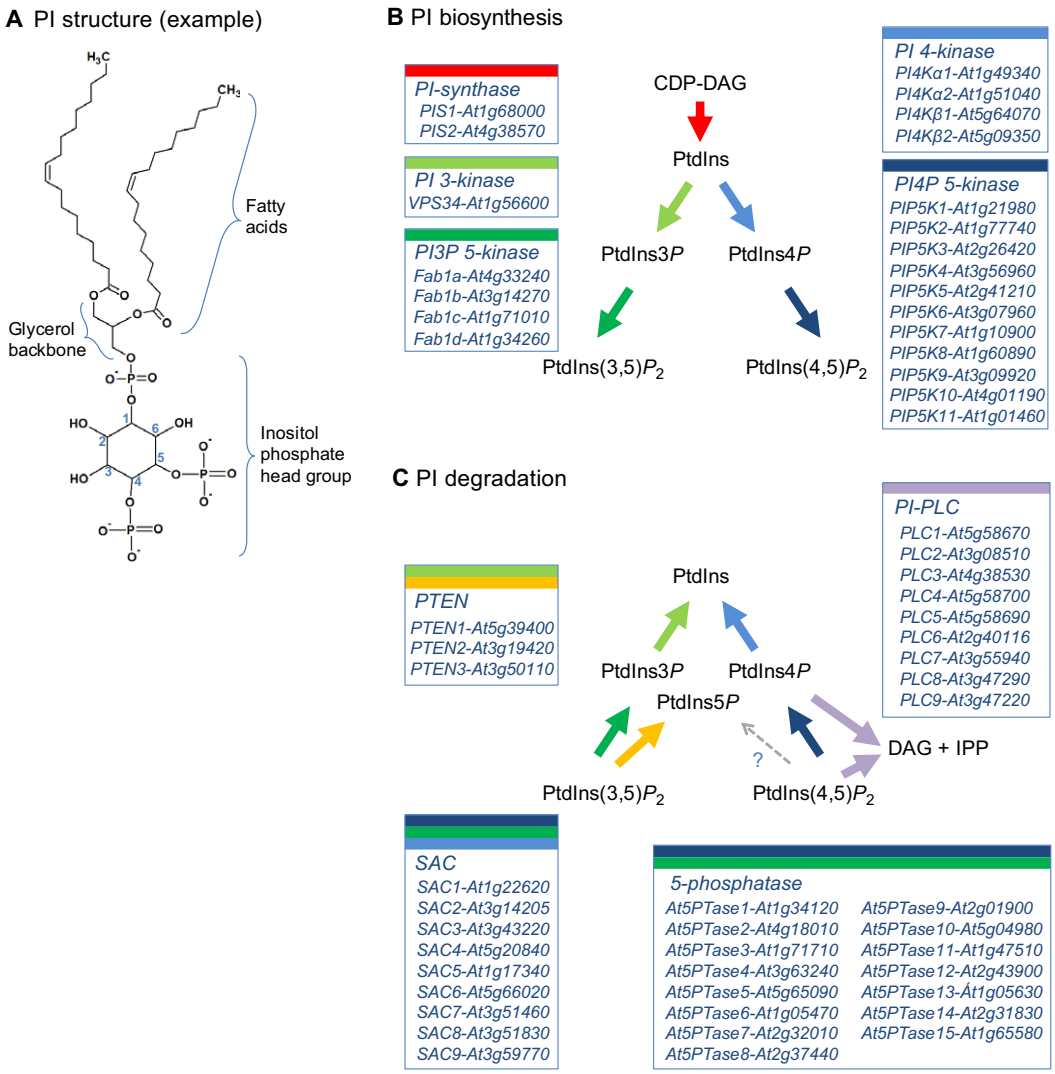


Fig. 2. The plant phosphoinositide network. (A) Structure of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], a key regulatory phosphoinositide (PI). The molecule contains two fatty acids esterified to a glycerol backbone that convey its hydrophobic properties. The inositol-phosphate head group is hydrophilic and mediates the amphiphilic properties of the membrane lipid. The inositol-phosphate head group is the characteristic moiety that can be recognized and bound by PI-binding protein domains. (B,C) Schematic representation of the PI biosynthesis (B) and degradation (C) pathways in *Arabidopsis*. Arrows indicate the individual enzymatic steps. The *Arabidopsis* genes encoding the enzymes that catalyze each step are highlighted in boxes matching the colors of the arrow. Please note that the specificities for some of the degrading enzymes are not clear in all cases and that not all isoforms represented in each box might perform the reactions specified by the colors. The formation of phosphatidylinositol 5-phosphate (PtdIns5*P*) is still a matter of debate. CDP-DAG, cytidine diphosphodiacylglycerol; DAG, diacylglycerol; IPP, inositol polyphosphate; PI-PLC, PI-specific phospholipase C; PtdIns, phosphatidylinositol; PtdIns3*P*, PtdIns 3-phosphate; PtdIns(3,5)P_2, PtdIns 3,5-bisphosphate; PtdIns4*P*, PtdIns 4-phosphate; PtdIns(4,5)P_2, PtdIns 4,5-bisphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SAC, suppressor of actin.

Intact PIs are found predominantly at the cytosolic face of cellular membranes. It has also been reported that under certain conditions PIs may occur in the luminal/extracellular leaflets of vesicles or the plasma membrane (Gonorazky et al., 2012, 2008; Kale et al., 2010), although it is currently unclear how PIs would manifest in these locations. Within the membrane, PIs act as regulators of integral membrane proteins or mark membrane areas for the recruitment of peripheral membrane proteins. In this context, PIs act mainly through their head groups, which are characterized by their phosphorylation patterns and protrude from the plane of the membrane further into the cytosol than the head groups of other, structural lipids (Takenawa, 2010). These distinctive phosphorylated PI head groups can be recognized and bound by PI-binding protein domains, such as Pleckstrin homology (PH) domains, Fab1 YOTB Vac1 EEA1 (FYVE) domains and phagocytic oxidase (PX) domains (Lemmon, 2003). The interactions between PIs and such domains can result in the recruitment of particular proteins to membrane areas enriched in PIs.

A number of proteins regulated by PI binding have been identified; in some studies, these proteins have been termed PI-'modulins' (McLaughlin et al., 2002). It should be noted that not all PI-binding domains of such modulins display a high degree of specificity towards a particular PI species (Fig. 3A). In fact, only a select few PI-binding domains have been found to be highly specific, including the PH domain of human PLCδ1, which preferentially binds PtdIns(4,5)P_2, and the PH domain of human four-phosphate adaptor protein 1 (FAPP1, also known as PLEKHA3), which preferentially binds PtdIns4*P* (Takenawa and Itoh, 2006). Nonetheless, even though most PI-binding proteins do not display exquisite specificity, protein recruitment to PIs does

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

Table 1. Phenotypes reported upon perturbation of the PI signaling system in *Arabidopsis thaliana*

Enzyme	Isoform	Expression pattern	Subcellular distribution	Experimental perturbation	Reported phenotype	References
PI-synthase	PIS1	Ubiquitous	ER/Golgi	PIS1 OE	Enhanced growth	(Löfke et al., 2008)
	PIS2	Ubiquitous	ER/Golgi	PIS2 OE	Enhanced growth, pollen tube defects	(Ischebeck et al., 2010; Löfke et al., 2008)
PI 3-kinase	VPS34	Ubiquitous	Endomembranes	*pi3k* (T-DNA)	Homozygous lethal, heterozygous defective pollen development, root hair defects, vacuolar defects	(Lee et al., 2008a,b; Welters et al., 1994)
PI 4-kinase	PI4Kα1	Ubiquitous	Plasma membrane/ plastid outer envelope	Mutant; knockdown	Homozygous lethal, knockdown increased plastid division	(Delage et al., 2013; Okazaki et al., 2015)
	PI4Kα2	Ubiquitous	–	–	–	–
	PI4Kβ1	Ubiquitous	TGN			(Antignani et al., 2015; Preuss et al., 2006)
	PI4Kβ2	Ubiquitous	TGN			(Antignani et al., 2015; Preuss et al., 2006)
				pi4kβ1 pi4kβ2 double mutant (T-DNA)	Reduced growth, root hair defects, enhanced defense, increased plastid division	(Antignani et al., 2015; Preuss et al., 2006)
PI3P 5-kinase	FAB1A	Ubiquitous	–	*fab1a* (T-DNA)	Vacuolar defects in pollen	(Whitley et al., 2009)
	FAB1B	Ubiquitous	–	*fab1b* (T-DNA)	Vacuolar defects in pollen, reduced stomatal closure	(Bak et al., 2013)
	FAB1C	Ubiquitous	–	*fab1c* (T-DNA)	Reduced stomatal closure	(Bak et al., 2013)
	FAB1D	Ubiquitous	–	–	No reports	–
PI4P 5-kinase	PIP5K1	Ubiquitous	Plasma membrane/ vesicles/nucleus	*pip5k1* (T-DNA)	Reduced growth	(Ischebeck et al., 2013; Tejos et al., 2014)
				PIP5K1 OE	Wavy root growth, defects in PIN trafficking	(Ischebeck et al., 2013)
	PIP5K2	Ubiquitous	Plasma membrane/ vesicles/nucleus	*pip5k2* (T-DNA)	Reduced growth, defects in PIN trafficking	(Camacho et al., 2009; Ischebeck et al., 2013; Mei et al., 2012; Tejos et al., 2014)
				PIP5K2 OE	Wavy root growth, defects in PIN trafficking	(Ischebeck et al., 2013)
				pip5k1 pip5k2 (T-DNA)	Defects in early embryo development; defects in vascular development; defects in PIN trafficking and clathrin-mediated endocytosis	(Ischebeck et al., 2013; Tejos et al., 2014)
	PIP5K3	Root epidermis and cortex	Plasma membrane/ vesicles	*pip5k3* (T-DNA)	Root hair defects	(Kusano et al., 2008; Stenzel et al., 2008)
				PIP5K3 OE	Root hair defects	(Kusano et al., 2008; Stenzel et al., 2008)
	PIP5K4	Flowers/pollen	Plasma membrane/ vesicles	*pip5k4* (T-DNA)	Reduced pollen germination	(Ischebeck et al., 2008; Sousa et al., 2008)
				PIP5K4 OE	Pollen tube defects, enhanced pectin secretion	(Ischebeck et al., 2008)
	PIP5K5	Flowers/pollen	Plasma membrane/ vesicles	*pip5k5* (T-DNA)	Reduced pollen germination	(Ischebeck et al., 2008; Sousa et al., 2008)
				PIP5K5 OE	Pollen tube defects, enhanced pectin secretion	(Ischebeck et al., 2008)
				pip5k4 pip5k5 double mutant (T-DNA)	Reduced pollen germination	(Ischebeck et al., 2008; Sousa et al., 2008)
	PIP5K6	Ubiquitous	Plasma membrane/ vesicles	*pip5k6* (T-DNA)	Defects in clathrin-mediated endocytosis	(Zhao et al., 2010)
	PIP5K7	Ubiquitous	–	–	–	–
	PIP5K8	Ubiquitous	–	–	–	–
	PIP5K9	Ubiquitous	Plasma membrane/ nucleus	*pip5k9* (T-DNA; resulting in OE)	Altered sugar metabolism	(Lou et al., 2007)

Continued

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

Table 1. Continued

Enzyme	Isoform	Expression pattern	Subcellular distribution	Experimental perturbation	Reported phenotype	References
	PIP5K10	Pollen	Plasma membrane/ nucleus	*pip5k10* (T-DNA)	Increased sensitivity to LatB	(Ischebeck et al., 2011)
				pip5k10 OE	Pollen tube defects, stabilized actin cytoskeleton	(Ischebeck et al., 2011)
	PIP5K11	Pollen	Plasma membrane/ nucleus	*pip5k11* (T-DNA)	Increased sensitivity to LatB	(Ischebeck et al., 2011)
				pip5k11 OE	Pollen tube defects, stabilized actin cytoskeleton	(Ischebeck et al., 2011)
				pip5k10 pip5k11 double mutant (T-DNA)	Increased sensitivity to LatB	(Ischebeck et al., 2011)
PI-PLC	PLC1	Roots, leaves	Plasma membrane	–	–	(Pokotylo et al., 2014)
	PLC2	Roots, leaves, stems, flowers	Plasma membrane	*plc2* (T-DNA)	Growth defects	(Kanehara et al., 2015; Pokotylo et al., 2014)
	PLC3	Roots, leaves	Plasma membrane	*plc3* (T-DNA)	–	(Pokotylo et al., 2014; Zheng et al., 2012)
	PLC4	Roots, stems, flowers	Plasma membrane	–	–	(Pokotylo et al., 2014)
	PLC5	Roots, siliques	–	–	–	(Pokotylo et al., 2014)
	PLC6	Flowers				(Pokotylo et al., 2014)
	PLC7	–	Plasma membrane	*plc7* (T-DNA)	–	(Pokotylo et al., 2014; Zheng et al., 2012)
	PLC8	Leaves, siliques, flowers	–	*plc8* (T-DNA)	–	(Pokotylo et al., 2014; Zheng et al., 2012)
	PLC9	Roots, siliques	–	*plc9* (T-DNA)	Impaired thermotolerance	(Pokotylo et al., 2014; Zheng et al., 2012)
SAC phosphatase	SAC1/FRA7	Ubiquitous	Golgi	*sac1/fra7* (T-DNA)	Cell wall defects	(Zhong et al., 2005)
	SAC2	Ubiquitous	Tonoplast			(Novakova et al., 2014)
	SAC3	Ubiquitous	Tonoplast			(Novakova et al., 2014)
	SAC4	Ubiquitous	Tonoplast			(Novakova et al., 2014)
	SAC5	Ubiquitous	Tonoplast			(Novakova et al., 2014)
				sac2 sac3 sac4 sac5 quadruple mutant (T-DNA)	Vacuolar defects	(Novakova et al., 2014)
	SAC6/SAC1b	No report	ER	–	–	(Despres et al., 2003)
	SAC7/RHD4/ SAC1c	Ubiquitous	ER/TGN	*sac7/rhd4/sac1c* (EMS; T-DNA)	Root hair defects	(Despres et al., 2003; Thole et al., 2008)
	SAC8/SAC1a	No report	ER	–	–	(Despres et al., 2003)
	SAC9	Ubiquitous	No report	*sac9* (T-DNA)	Growth defects, constitutive stress response	(Williams et al., 2005)
PTEN	PTEN1	Flowers/pollen	Vesicles	PTEN1 OE	Pollen defects	(Zhang et al., 2011)
	PTEN2a	Ubiquitous	–	–	–	(Pribat et al., 2012)
	PTEN2b	Ubiquitous	–	–	–	(Pribat et al., 2012)
5PTase	At5PTase1	Ubiquitous	No report	*5ptase1-1*, *5ptase1-2* (T-DNA)	Defective seedling development, hypocotyl defects	(Gunesekera et al., 2007)
	At5PTase2	Ubiquitous	No report	*5ptase2-1* (T-DNA)	Defective seedling development, hypocotyl defects	(Gunesekera et al., 2007)
				5ptase1-1 5ptase2-1 double mutant (T-DNA)	Defective seedling development, hypocotyl defects	(Gunesekera et al., 2007)
	At5PTase3	Ubiquitous	–	–	–	
	At5PTase4	Shoot apex, ubiquitous	–	–	–	
	At5PTase5/ BST1	Pollen, weak ubiquitous	–	*bst1* (EMS)	Root hair defects	(Parker et al., 2000)
	At5PTase6/ CVP2	Ubiquitous, vascular tissue	No report	*cvp2-1*, *cvp2-2*, *cvp2-3* (EMS)	Vascular defects	(Carland and Nelson, 2004; Rodriguez-Villalon et al., 2015)

Continued

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

Table 1. Continued

Enzyme	Isoform	Expression pattern	Subcellular distribution	Experimental perturbation	Reported phenotype	References
	At5PTase7/ CVL1	Ubiquitous, weak in shoots and siliques	No report	*cvl1-1*, *cvl1-2* (T-DNA)	Vascular defects, altered ROS production and salt tolerance	(Carland and Nelson, 2009; Kaye et al., 2011)
	At5PTase8	Young roots, weak ubiquitous	–	–	–	
	At5PTase9	Young roots, weak ubiquitous	No report	*At5ptase9* (T-DNA), *At5ptase9* OE	Defects in salt tolerance and endocytosis	(Golani et al., 2013)
	At5PTase10	Late flowers, senescent leaves, weak ubiquitous	–	–	–	
	At5PTase11	Developing seeds, seedlings	Cytosol, plasma membrane	*5ptase11-1*, *5ptase11-2* (T-DNA)	Defective seedling development, hypocotyl growth	(Ercetin et al., 2008)
	At5PTase12	Ubiquitous (leaves weak)	No report	*5pt12* (T-DNA)	Altered pollen dormancy/ germination	(Wang et al., 2012; Zhong and Ye, 2004)
	At5PTase13	Ubiquitous	–	*Atptase13* (T-DNA)	Vascular defects, altered Ca^{2+} signatures, altered phototropin signaling	(Chen et al., 2008; Lin et al., 2005; Zhong and Ye, 2004)
	At5PTase14	Seedlings, shoot apices	–	–	–	(Zhong and Ye, 2004)
	At5PTase15/ FRA3	Ubiquitous	–	*fra3* (T-DNA)	Cell wall defects	(Zhong et al., 2004)
Sec14-like	SFH1	Ubiquitous	Plasma membrane	*sfh1* (T-DNA)	Root hair defects	(Ghosh et al., 2015; Vincent et al., 2005)

ER, endoplasmic reticulum; LatB, latrunculin B; OE, overexpression; ROS, reactive oxygen species; TGN, trans-Golgi network.

exhibit substantial specificity depending on the local abundance of certain PIs and on coincident binding to other (protein) factors and coincidence detection (Carlton and Cullen, 2005), a concept discussed in more detail below. It should also be noted that PI-binding domains, such as PH domains, are not well conserved with regards to primary sequence and are instead characterized by their three-dimensional fold. These domains are therefore not readily identified by mere sequence analysis. Extensive bioinformatics analyses, however, have revealed that the *Arabidopsis* genome encodes numerous proteins with recognizable PI-binding domains (van Leeuwen et al., 2004), including: the PI 3-kinase VPS34 (At1g60490), the PI 4-kinases α1 (At1g49340) and α2 (At1g51040), the 3-phosphoinositide-dependent protein kinase 1 (PDK1; At3g10540), the dynamin-related proteins 2A (DRP2A; At1g10290) and B (DRP2B; At1g59610) and the Rho-of-plants GTPase-activating proteins 1-3 (ROP-GAPs 1-3; At4g24580, At5g12150 and At5g19390), all of which contain a PH domain; FAB1A (At4g33240) and FAB1B (At3g14270), which contain a FYVE domain; and the sorting nexin-like protein 2 (SNX2B; At5g07120), which contains a PX domain (van Leeuwen et al., 2004). Interestingly, there are several instances in which proteins contain more than one PI-binding domain, for example the phospholipases D ζ1 (PLDζ1; At3g16785-90) and ζ1 (PLDζ2; At3g05630), which contain both a PH domain and a PX domain (van Leeuwen et al., 2004). It appears likely that the number of proteins with PI-binding domains is still underestimated, as PI-binding domains may form transiently upon dynamic interaction of protein partners, thus escaping detection via sequence annotation (van Rossum et al., 2005). Furthermore, despite the seeming abundance of PI-modulins, the direct experimental identification of PI-binding proteins has so far not yielded a substantial amount of data. A notable exception is the identification of numerous candidate binding partners for 3-phosphorylated PIs from *Arabidopsis*, which were determined by agarose-phosphatidylinositol-phosphate affinity chromatography and subsequent mass spectrometric analysis (Oxley et al., 2013).

Intact PIs can also influence a membrane directly by modifying its biophysical properties, inducing membrane curvature or stabilizing curved membrane areas (Lundbaek et al., 2010). Thus, PIs can both exert an intrinsic influence on the membrane and mediate the recruitment of proteins that stabilize certain membrane structures, although the relative contributions of these individual effects remain unclear.

Besides their roles as intact lipids, PIs can serve as precursors for PLC-mediated cleavage into diacylglycerol (DAG) and soluble IPPs (Gillaspy, 2013). In mammals, DAG acts as a potent activator of protein kinase C (Amadio et al., 2006), but this enzyme is missing in plants (Mueller-Roeber and Pical, 2002) and a signaling function for plant DAG has not been reported. Instead, DAG in plants is rapidly phosphorylated to phosphatidic acid (PtdOH), which has been shown to have a number of regulatory functions (Meijer and Munnik, 2003). PtdOH can be further phosphorylated to diacylglycerol pyrophosphate (DGPP), a lipid of unknown relevance (van Schooten et al., 2006). Soluble IPPs, such as inositol hexakisphosphate (InsP_6) or inositol pentakisphosphate (InsP_5), are present within the crystal structures of receptor proteins for auxin (Tan et al., 2007) or jasmonic acid (Sheard et al., 2010). However, the role of these IPPs in the perception of auxin or jasmonic acid as structural or possibly functional co-factors is currently unclear. It has been reported that *Arabidopsis ipk1-1* mutants with decreased levels of InsP_6 and increased levels of InsP_5 display an enhanced wounding response and enhanced resistance to herbivory

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

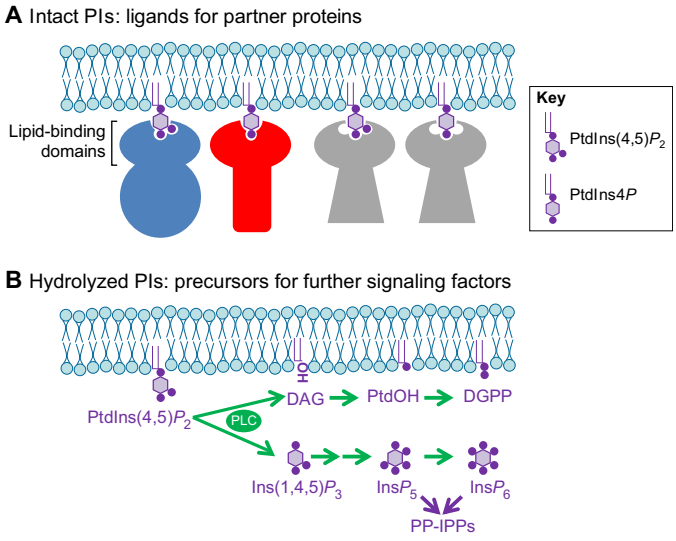


Fig. 3. Modes of PI action. PIs can act through the binding of their characteristic head groups to partner proteins containing specific PI-recognition domains (A), or as substrates for hydrolyzing enzymes that generate a range of lipid messengers and soluble inositol polyphosphates (IPPs) (B). (A) The binding of a PI ligand to a protein may serve as a recruitment signal or have effects on the activity or function of the target protein. Some PI-recognition domains display a high degree of specificity for particular PIs. For example, some pleckstrin homology (PH) domain-containing proteins (dark blue) have a strong preference for PtdIns(4,5)P_2, whereas others (red) bind preferentially to PtdIns4P. However, most PH domains do not exhibit such preferences, and proteins harboring these domains (gray) can bind to any given PI. The specificity of PI function is thus likely to be exerted by additional factors, including locally increased levels of certain PIs and coincidence detection (see Fig. 4). (B) The hydrolysis of PtdIns(4,5)P_2 by PLC yields DAG and Ins(1,4,5)P_3. In plants, DAG is rapidly phosphorylated to PtdOH and possibly further to DGPP. An Ins(1,4,5)P_3-sensitive Ca^{2+} channel is absent in plants and Ins(1,4,5)P_3 instead appears to act as a precursor for IPPs, including InsP_5, InsP_6 or pyrophosphorylated IPPs (PP-IPPs). DAG, diacylglycerol; DGPP, diacylglycerol pyrophosphate; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; InsP_5, inositol pentakisphosphate; InsP_6, inositol hexakisphosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PtdIns4P, PtdIns 4-phosphate; PtdOH, phosphatidic acid.

(Mosblech et al., 2011). More recently, other IPPs, such as the pyrophosphorylated IPPs (PP-IPPs) InsP_7 or InsP_8, have also been implicated in modulating the function of the jasmonic acid receptor (Laha et al., 2015), possibly suggesting a role for IPPs in phytohormone perception and the control of hormone-induced gene expression patterns. Other IPPs, such as inositol 1,4,5-trisphosphate (InsP_3), are also implicated in mediating Ca^{2+} responses, although an InsP_3-sensitive Ca^{2+} channel has not been reported in plants (Krinke et al., 2007a).

PIs and membrane microdomains

From the current data it is evident that plant PIs exert a number of different functions by interacting with a variety of binding partners. However, it is also clear that not all cellular processes that can in principle be controlled by PIs will manifest whenever PIs are formed. In fact, the physiological consequences of enhanced PI formation tend to be rather limited and highly specific, raising the question of how the specificity of PI functions is achieved (Heilmann and Heilmann, 2013). A key piece of information for understanding PI function is that these lipids do not display uniform distribution within membranes (Ischebeck et al., 2008, 2011, 2013; Kost et al., 1999; Kusano et al., 2008; Sousa et al., 2008; Tejos et al., 2014; van Leeuwen et al., 2007; Vermeer et al., 2009; Vincent et al., 2005). Instead, PIs are enriched in certain membrane domains, and it is likely that such microdomains play a part in mediating the specific effects of PIs (Fig. 4A). Plants form particular molecular species of PIs under certain conditions (e.g. when challenged by salt) that are characterized by an increased degree of unsaturation in their associated fatty acids (König et al., 2007). The degree to which lipids are unsaturated is known to influence their lateral mobility within the plane of the membrane and to guide their association with particular planar or curved membrane domains (Cho et al., 2006; Mukherjee et al., 1999). The degree of unsaturation of PI-associated fatty acids might, therefore, be an as yet underestimated factor governing the lateral mobility of PIs within membranes, with possible implications for their association with certain partner proteins and for defining the specificity of PI effects. The lateral distribution of PIs within a membrane is also likely to be influenced by other membrane lipids, such as sphingolipids and sterols (Furt et al., 2010).

An additional aspect contributing to the specificity of PI effects is the concept of coincidence detection (Carlton and Cullen, 2005). Although a number of plant proteins have been demonstrated to bind PIs, as discussed above, it is known from other model systems that there can be additional factors guiding the effects of PIs (Balla, 2005; Várnai et al., 2005). Regulation by coincidence detection (Fig. 4B) suggests that PI binding is necessary for the regulation of certain processes but might not be sufficient by itself, and other factors must coincide for an effect to manifest. Thus, in addition to the binding of a PI, a membrane-associated protein partner, for example, must also be bound and only membrane areas containing both recruitment factors will be targeted by modulins that require coincidence detection to function. An interesting aspect of

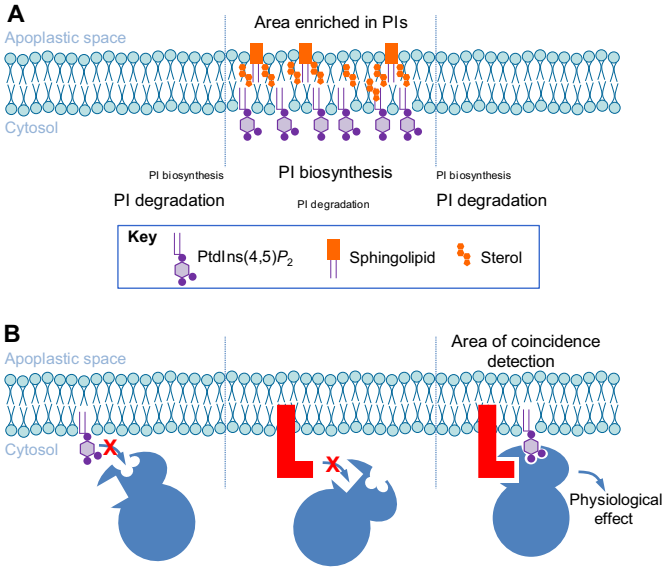


Fig. 4. PI membrane domains and localized potentiation of PI function. (A) PIs are not uniformly distributed within membranes; instead, concentrated areas of increased PI abundance are observed and are associated with specific PI functions. This localized accumulation of PIs in a membrane might be due to increased PI biosynthesis but is also possibly linked to the presence of other membrane lipids, such as sphingolipids with very long-chain fatty acids or sterols. (B) The presence of a PI ligand (left) or a membrane-associated factor (red, middle) that can bind to a target protein (dark blue) might each by themselves not be sufficient to recruit the target protein and exert a regulatory effect. Only when the PI ligand and the membrane-associated factor coincide at a membrane domain (the 'area of coincidence detection') and both bind to the target protein will a regulatory effect be exerted.

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

coincidence detection involves the possible role of proteins that potentiate PI functionality by facilitating their interaction with certain target proteins. For instance, recent evidence in *Arabidopsis* indicates that Sec14-like lipid transfer proteins aid PI-protein interaction in a specific manner (Bankaitis et al., 2010; Ghosh et al., 2015; Schaaf et al., 2008; Vincent et al., 2005). As a consequence, membrane areas that contain Sec14-like proteins will exhibit potentiated PI effects, possibly similar to the effects of localized PI overproduction.

PI functions in plants

In recent years, PIs have emerged as central regulators of plant function and development, and a number of important and severe plant phenotypes have been reported when plant PI metabolism is perturbed (Carland and Nelson, 2009, 2004; Chen et al., 2008; Ercetin et al., 2008; Golani et al., 2013; Gunesekera et al., 2007; Ischebeck et al., 2008, 2011, 2013; Kaye et al., 2011; Kusano et al., 2008; Löfke et al., 2008; Mei et al., 2012; Novakova et al., 2014; Parker et al., 2000; Preuss et al., 2006; Rodriguez-Villalon et al., 2015; Sousa et al., 2008; Stenzel et al., 2008; Tejos et al., 2014; Wang et al., 2012; Williams et al., 2005; Zhong et al., 2004, 2005; Zhong and Ye, 2004). The perturbation of PI signaling components in *Arabidopsis*, for example, gives rise to a variety of phenotypes affecting multiple tissue types and aspects of plant development (summarized in Table 1). A number of studies utilizing model systems other than *Arabidopsis* have also highlighted a role for PIs in plant development and function. For instance, the overexpression of PI-synthase in maize results in altered drought tolerance (Liu et al., 2013). Work on petunia, tobacco and the moss *Physcomitrella patens* supports an important role for PIs in the control of polar tip growth (Dowd et al., 2006; Helling et al., 2006; Saavedra et al., 2011, 2015). Although some of the reported phenotypes are severe, the molecular mechanisms underlying PI functions in plants remain mostly unclear.

In the absence of detailed information on the molecular targets of PIs in plants, the interpretation of reported phenotypes poses a challenge. Based on the available data, the phenotypes can be categorized according to the defects observed, enabling a first approximation of how the elements of the plant PI network might be interlinked at the cellular level (Fig. 5). For example, phenotypes resulting from the perturbation of PI-synthases (Löfke et al., 2008), PI4Kβ1/PI4Kβ2 (Preuss et al., 2006), PIP5K1-PIP5K6 (Ischebeck et al., 2008, 2013; Kusano et al., 2008; Sousa et al., 2008; Stenzel et al., 2008; Tejos et al., 2014; Zhao et al., 2010) or of SAC1 (Zhong et al., 2005) or SAC7 (Thole and Nielsen, 2008) are all related to functional changes in membrane trafficking between the Golgi and the plasma membrane. Based on the subcellular localization of the individual enzymes, it appears that an increasing degree of phosphorylation [PtdIns→PtdIns4*P*→PtdIns(4,5)P_2] is associated with the progressive delivery or retrieval of vesicles to and from the plasma membrane. Another category of phenotypes indicates the control of cytoskeletal dynamics by PIs. For instance, the perturbation of PIP5K2 (Stenzel et al., 2012) or PIP5K10 and PIP5K11 (Ischebeck et al., 2011) results in altered actin dynamics and the loss of cellular polarity. Yet a different category of phenotypes indicates that 3-phosphorylated PIs, which are perturbed by altered levels of VPS34 (Lee et al., 2008a,b), Fab1-like proteins (Bak et al., 2013) or SAC2-SAC5 (Novakova et al., 2014), might be involved in endomembrane trafficking and the control of vacuolar functions. The overall scheme suggests that PIs are involved in controlling the central machinery for membrane trafficking and protein sorting in plants. As a consequence, PIs have an impact on several key cellular processes, such as the establishment of cell polarity (Ischebeck et al., 2008, 2011, 2013; Kusano et al., 2008; Mei et al., 2012; Preuss et al., 2006; Sousa et al., 2008; Stenzel et al., 2008; Tejos et al., 2014) and cell wall deposition (Krishnamoorthy et al., 2014; Zhong et al., 2004, 2005). These processes influence embryo development, vascular development and, ultimately, plant growth.

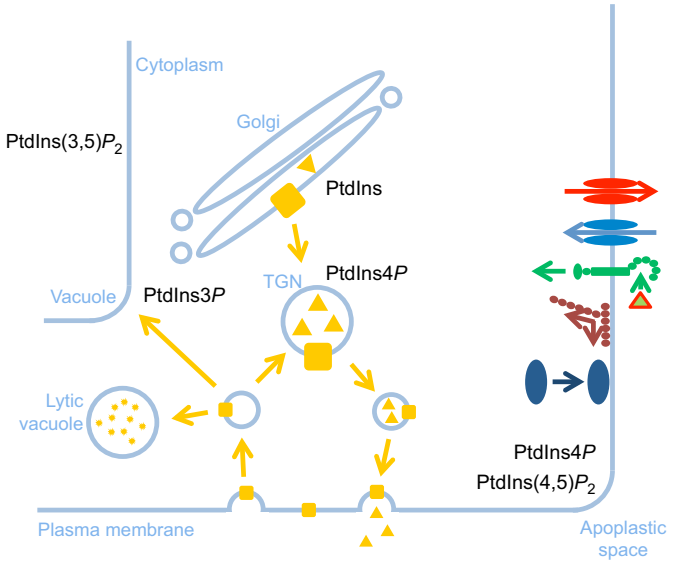
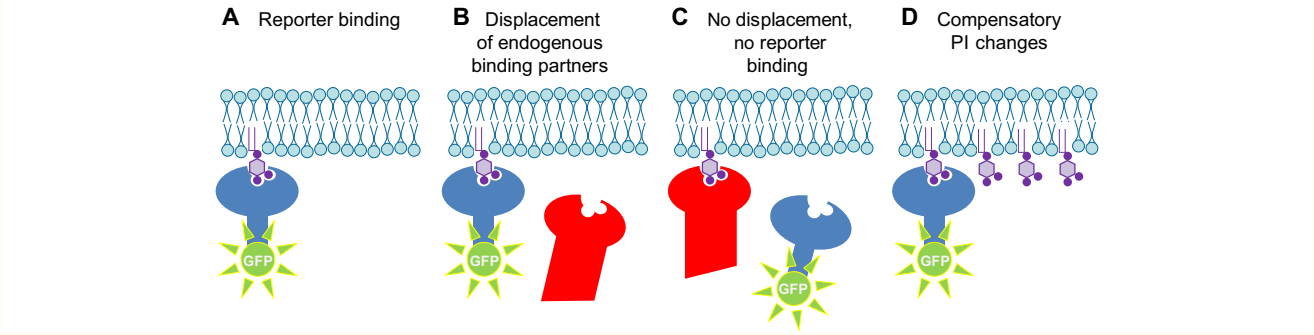


Fig. 5. Model of subcellular PI distribution. Based on the effects of perturbed PI metabolism, a number of conclusions can be drawn with regard to the subcellular organization of PIs and their function in plants. PtdIns, the precursor lipid of all PIs, is formed in the ER or Golgi. PtdIns can be phosphorylated to PtdIns4*P* on vesicles of the trans-Golgi network (TGN) en route to the plasma membrane. PtdIns4*P* is further phosphorylated at the plasma membrane to PtdIns(4,5)P_2. PtdIns(4,5)P_2 influences membrane trafficking events at the plasma membrane, such as exocytosis and endocytosis. It is also likely to have regulatory effects on membrane recruitment of proteins controlling cytoskeletal dynamics and on transporters. The 3-phosphorylated PIs PtdIns3*P* and PtdIns(3,5)P_2 are likely to be involved in the control of endomembrane trafficking in the context of autophagy and the control of vacuolar/tonoplast functions.

In addition to their cytosolic effects on trafficking and membrane recruitment, emerging evidence indicates that PIs may also have a direct or indirect influence on nuclear function in plants. PI reporters (see Box 1) have been observed in the nuclei of plant cells (Mishkind et al., 2009), as have fluorescently tagged variants of enzymes involved in PI metabolism (Ischebeck et al., 2011, 2013; Lou et al., 2007; Tejos et al., 2014). However, so far it is unclear whether these observations have biological relevance or if they simply reflect fluorescence patterns ensuing after fluorescent fusion proteins have been degraded. The expression of human PI4P 5-kinase in plant cells resulted in changes to the cell cycle control machinery and histone modifications (Dieck et al., 2012b), but it is unclear whether this finding has relevance for endogenous plant PI-based processes. Further information comes from the analysis of factors involved in the control of stem cell populations in plant meristems. For instance, the protein phosphatase POLTERGEIST, an enzyme that functionally links plasma membrane perception of CLAVATA peptides and nuclear regulation, has been shown to bind PtdIns4*P* (Gagne and Clark, 2010). This binding to PtdIns4*P* results in the activation of phosphatase activity *in vitro*, suggesting that PtdIns4*P* might be involved in the signaling pathways controlling stem cell maintenance. So far, there is only very limited information about the effects of PIs on nuclear function in plants, and a role for PIs in plant nuclei (Dieck et al., 2012a), as has been proposed for PIs in animal cells, is currently unclear.

Development (2016) 143, 2044-2055 doi:10.1242/dev.136432

Box 1. PI reporters: now you see me, now you don't



The highly specific binding of PIs can be visualized using genetically encoded reporters consisting of PI-binding domains fused to fluorescent proteins (Hammond et al., 2014; Kost et al., 1999; Simon et al., 2014; van Leeuwen et al., 2007; Várnai and Balla, 1998; Vermeer et al., 2009, 2006). Although at first this appears to be a straight-forward approach, there are a number of catches that render it difficult to interpret data obtained using these reporters (Balla, 2007; Balla et al., 2000). The difficulty arises largely from the fact that in living cells the binding of the reporters to PIs occurs in competition with the binding of endogenous binding partners. Consequently, the reporters might not bind to their target PIs, or they may perturb PI-dependent processes, as illustrated above. The desired effect is that a fluorescent reporter specifically binds its PI and relocalizes from the cytosol to a cellular membrane (A). However, an inherent problem of the reporter binding to a PI is that the reporter will compete for PI occupancy with endogenous proteins. If the apparent affinity of the reporter for a PI is higher than that of the endogenous protein, the reporter will displace the endogenous protein from the membrane (B), abolishing the function of the endogenous protein and causing undesirable side effects that might complicate interpretation. On the other hand, an endogenous binding protein with a high affinity for a PI might not be displaced by the reporter, resulting in the absence of any informative fluorescence distribution pattern (C). A further complication of reporter expression is that cells might respond to the displacement of endogenous proteins with compensatory upregulation of PI biosynthesis, resulting in an overall change to PI levels (D). The relative affinities of reporters and endogenous partner proteins for their specific ligands are thus of great importance and reporters should be used at the minimal expression levels required for adequate imaging. It is also important to note that PIs bound to overexpressed PI reporters no longer contribute to a functional PI system; this has led researchers to harness reporter expression to functionally inhibit certain PIs (Lee et al., 2008a). All these caveats have to be considered when interpreting data obtained using genetically encoded PI reporters, with particular care required when expression levels and/or apparent affinities of the reporters are high, for instance in reporters using more than one lipid-binding domain for enhanced affinity (Simon et al., 2014).

Conclusions and future perspectives

The perturbation of PI metabolism in plants results in severe phenotypes, highlighting the fact that PIs play central roles in plant development and plant adaptation to the environment. The profound influences of PIs on diverse cellular processes indicate that the plant PI network is an important field for future studies. Our current understanding of the plant PI network is still limited and the precise molecular targets of PI action in plants are largely unknown. A massive amount of data has already accumulated on the plant PI system, but to date it seems that not all dots have been connected and the available information has not been used to the fullest extent. For instance, numerous proteins have been proposed to contain PI-binding domains, making these proteins prime candidates for regulation by PIs (van Leeuwen et al., 2004). However, only a minority of these potential PI-modulins have been experimentally tested for PI binding by *in vitro* lipid overlay assays (Munnik and Wierzchowiecka, 2013) or liposome binding tests (Julkowska et al., 2013), leaving the field open for further scrutiny. Whereas many of the potential PI-modulins are proteins of unknown function, others are well annotated, linking potential PI binding to relevant cellular processes. In combination with the review of PI-dependent plant phenotypes related to membrane trafficking or cytoskeletal control, this information might indicate future avenues of research to elucidate how and when PIs are formed and what their respective modulins in each context might be. The study of the molecular mechanisms by which PIs act will require a combination of complementary *in vitro* and *in vivo* data to demonstrate a precise mode of action. In particular, information on the specificity and affinity of PI-protein binding is currently missing. Moreover, the study of PI-protein binding should expand to include other lipid classes known to influence membrane biophysics, such as sphingolipids and sterols, because PIs are unlikely to act individually in a cell but rather in the context of membrane areas defined by a mixture of different lipids (van Meer et al., 2008). In this sense, it will be a major goal of future studies to determine the coincident properties of mixed membrane microdomains with regard to protein recruitment and binding. On the *in vivo* side, plant researchers have started using fluorescent probes for the detection of membrane lipid-ordered domains (Frescatada-Rosa et al., 2014; Gerbeau-Pissot et al., 2016), which might prove a powerful tool to link *in vitro* and *in vivo* studies. Considering the current status of sequence information and the range of available *in vitro* and *in vivo* methods, I am confident that the coming years will bring substantial advances in the field of plant PI signaling.

Acknowledgements

The author apologizes to all authors whose relevant contributions might not have been cited owing to space restrictions and wishes to thank Dr Mareike Heilmann and Dr Praveen Krishnamoorthy (both Martin-Luther-University Halle-Wittenberg) for helpful discussion.

Competing interests

The author declares no competing or financial interests.

Funding

Funding from the German Research Foundation (Deutsche Forschungsgemeinschaft) [grants He3424/1, He3424/2, He3424/3 and He3424/6] and the European Regional Development Fund of the European Commission (EFRE) is gratefully acknowledged.

References

Amadio, M., Battaini, F. and Pascale, A. (2006). The different facets of protein kinases C: old and new players in neuronal signal transduction pathways. *Pharmacol. Res.* **54**, 317-325.

Antignani, V., Klocko, A. L., Bak, G., Chandrasekaran, S. D., Dunivin, T. and Nielsen, E. (2015). Recruitment of PLANT U-BOX13 and the PI4Kbeta1/beta2

phosphatidylinositol-4 kinases by the small GTPase RabA4B plays important roles during salicylic acid-mediated plant defense signaling in Arabidopsis. *Plant Cell* **27**, 243-261.

Bak, G., Lee, E.-J., Lee, Y., Kato, M., Segami, S., Sze, H., Maeshima, M., Hwang, J.-U. and Lee Y. (2013). Rapid structural changes and acidification of guard cell vacuoles during stomatal closure require phosphatidylinositol 3,5-bisphosphate. *Plant Cell* **25**, 2202-2216.

Balla, T. (2005). Inositol-lipid binding motifs: signal integrators through protein-lipid and protein-protein interactions. *J. Cell Sci.* **118**, 2093-2104.

Balla, T. (2007). Imaging and manipulating phosphoinositides in living cells. *J. Physiol.* **582**, 927-937.

Balla, T., Bondeva, T. and Várnai, P. (2000). How accurately can we image inositol lipids in living cells? *Trends Pharmacol. Sci.* **21**, 238-241.

Bankaitis, V. A., Mousley, C. J. and Schaaf, G. (2010). The Sec14 superfamily and mechanisms for crosstalk between lipid metabolism and lipid signaling. *Trends Biochem. Sci.* **35**, 150-160.

Boss, W. F. and Im, Y. J. (2012). Phosphoinositide signaling. *Annu. Rev. Plant Biol.* **63**, 409-429.

Camacho, L., Smertenko, A. P., Perez-Gomez, J., Hussey, P. J. and Moore, I. (2009). Arabidopsis Rab-E GTPases exhibit a novel interaction with a plasma-membrane phosphatidylinositol-4-phosphate 5-kinase. *J. Cell Sci.* **122**, 4383-4392.

Carland, F. M. and Nelson, T. (2004). Cotyledon vascular pattern2–mediated inositol (1,4,5) triphosphate signal transduction is essential for closed venation patterns of Arabidopsis foliar organs. *Plant Cell* **16**, 1263-1275.

Carland, F. and Nelson, T. (2009). CVP2- and CVL1-mediated phosphoinositide signaling as a regulator of the ARF GAP SFC/VAN3 in establishment of foliar vein patterns. *Plant J.* **59**, 895-907.

Carlton, J. G. and Cullen, P. J. (2005). Coincidence detection in phosphoinositide signaling. *Trends Cell Biol.* **15**, 540-547.

Chen, X., Lin, W.-H., Wang, Y., Luan, S. and Xue, H.-W. (2008). An inositol polyphosphate 5-phosphatase functions in PHOTOTROPIN1 signaling in Arabidopis by altering cytosolic Ca^{2+}. *Plant Cell* **20**, 353-366.

Cho, H., Kim, Y. A. and Ho, W. K. (2006). Phosphate number and acyl chain length determine the subcellular location and lateral mobility of phosphoinositides. *Mol. Cells* **22**, 97-103.

Delage, E., Puyaubert, J., Zachowski, A. and Ruelland, E. (2013). Signal transduction pathways involving phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate: convergences and divergences among eukaryotic kingdoms. *Prog. Lipid Res.* **52**, 1-14.

Despres, B., Bouissonnie, F., Wu, H.-J., Gomord, V., Guilleminot, J., Grellet, F., Berger, F., Delseny, M. and Devic, M. (2003). Three SAC1-like genes show overlapping patterns of expression in Arabidopsis but are remarkably silent during embryo development. *Plant J.* **34**, 293-306.

DeWald, D. B., Torabinejad, J., Jones, C. A., Shope, J. C., Cangelosi, A. R., Thompson, J. E., Prestwich, G. D. and Hama, H. (2001). Rapid accumulation of phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate correlates with calcium mobilization in salt-stressed *Arabidopsis*. *Plant Physiol.* **126**, 759-769.

Dieck, C. B., Boss, W. F. and Perera, I. Y. (2012a). A role for phosphoinositides in regulating plant nuclear functions. *Front. Plant Sci.* **3**, 50.

Dieck, C. B., Wood, A., Brglez, I., Rojas-Pierce, M. and Boss, W. F. (2012b). Increasing phosphatidylinositol (4,5) bisphosphate biosynthesis affects plant nuclear lipids and nuclear functions. *Plant Physiol. Biochem.* **57**, 32-44.

Dowd, P. E., Coursol, S., Skirpan, A. L., Kao, T. H. and Gilroy, S. (2006). Petunia phospholipase c1 is involved in pollen tube growth. *Plant Cell* **18**, 1438-1453.

Einspahr, K. J., Maeda, M. and Thompson, G. A., Jr (1988a). Concurrent changes in Dunaliella salina ultrastructure and membrane phospholipid metabolism after hyperosmotic shock. *J. Cell Biol.* **107**, 529-538.

Einspahr, K. J., Peeler, T. C. and Thompson, G. A., Jr. (1988b). Rapid changes in polyphosphoinositide metabolism associated with the response of Dunaliella salina to hypoosmotic shock. *J. Biol. Chem.* **263**, 5775-5779.

Ercetin, M. E., Ananieva, E. A., Safaee, N. M., Torabinejad, J., Robinson, J. Y. and Gillaspy, G. E. (2008). A phosphatidylinositol phosphate-specific myo-inositol polyphosphate 5-phosphatase required for seedling growth. *Plant Mol. Biol.* **67**, 375-388.

Eyster, K. M. (2007). The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. *Adv. Physiol. Educ.* **31**, 5-16.

Frescatada-Rosa, M., Stanislas, T., Backues, S. K., Reichardt, I., Men, S., Boutté, Y., Jürgens, G., Moritz, T., Bednarek, S. Y. and Grebe, M. (2014). High lipid order of Arabidopsis cell-plate membranes mediated by sterol and DYNAMIN-RELATED PROTEIN1A function. *Plant J.* **80**, 745-757.

Furt, F., Konig, S., Bessoule, J. J., Sargueil, F., Zallot, R., Stanislas, T., Noirot, E., Lherminier, J., Simon-Plas, F., Heilmann, I. et al. (2010). Polyphosphoinositides are enriched in plant membrane rafts and form microdomains in the plasma membrane. *Plant Physiol.* **152**, 2173-2187.

Gagne, J. M. and Clark, S. E. (2010). The Arabidopsis stem cell factor POLTERGEIST is membrane localized and phospholipid stimulated. *Plant Cell* **22**, 729-743.

Gerbeau-Pissot, P., Der, C., Grebe, M. and Stanislas, T. (2016). Ratiometric fluorescence live imaging analysis of membrane lipid order in Arabidopsis mitotic cells using a lipid order-sensitive probe. *Methods Mol. Biol.* **1370**, 227-239.

Ghosh, R., de Campos, M. K. F., Huang, J., Huh, S. K., Orlowski, A., Yang, Y., Tripathi, A., Nile, A., Lee, H.-C., Dynowski, M. et al. (2015). Sec14-nodulin proteins and the patterning of phosphoinositide landmarks for developmental control of membrane morphogenesis. *Mol. Biol. Cell* **26**, 1764-1781.

Gillaspy, G. E. (2013). The role of phosphoinositides and inositol phosphates in plant cell signaling. *Adv. Exp. Med. Biol.* **991**, 141-157.

Golani, Y., Kaye, Y., Gilhar, O., Ercetin, M., Gillaspy, G. and Levine, A. (2013). Inositol polyphosphate phosphatidylinositol 5-phosphatase9 (At5ptase9) controls plant salt tolerance by regulating endocytosis. *Mol. Plant* **6**, 1781-1794.

Gonorazky, G., Laxalt, A. M., Testerink, C., Munnik, T. and de la Canal, L. (2008). Phosphatidylinositol 4-phosphate accumulates extracellularly upon xylanase treatment in tomato cell suspensions. *Plant Cell Environ.* **31**, 1051-1062.

Gonorazky, G., Laxalt, A. M., Dekker, H. L., Rep, M., Munnik, T., Testerink, C. and de la Canal, L. (2012). Phosphatidylinositol 4-phosphate is associated to extracellular lipoproteic fractions and is detected in tomato apoplastic fluids. *Plant Biol.* **14**, 41-49.

Gunesekera, B., Torabinejad, J., Robinson, J. and Gillaspy, G. E. (2007). Inositol polyphosphate 5-phosphatases 1 and 2 are required for regulating seedling growth. *Plant Physiol.* **143**, 1408-1417.

Hammond, G. R. V., Machner, M. P. and Balla, T. (2014). A novel probe for phosphatidylinositol 4-phosphate reveals multiple pools beyond the Golgi. *J. Cell Biol.* **205**, 113-126.

Heilmann, I. (2016). Plant phosphoinositide signaling - dynamics on demand. *Biochim. Biophys. Acta* (in press).

Heilmann, M. and Heilmann, I. (2013). Arranged marriage in lipid signalling? The limited choices of PtdIns(4,5)P 2 in finding the right partner. *Plant Biol.* **15**, 789-797.

Heilmann, M. and Heilmann, I. (2015). Plant phosphoinositides—complex networks controlling growth and adaptation. *Biochim. Biophys. Acta* **1851**, 759-769.

Heilmann, I., Perera, I. Y., Gross, W. and Boss, W. F. (1999). Changes in phosphoinositide metabolism with days in culture affect signal transduction pathways in *Galdieria sulphuraria*. *Plant Physiol.* **119**, 1331-1340.

Heilmann, I., Perera, I. Y., Gross, W. and Boss, W. F. (2001). Plasma membrane phosphatidylinositol 4,5-bisphosphate levels decrease with time in culture. *Plant Physiol.* **126**, 1507-1518.

Helling, D., Possart, A., Cottier, S., Klahre, U. and Kost, B. (2006). Pollen tube tip growth depends on plasma membrane polarization mediated by tobacco PLC3 activity and endocytic membrane recycling. *Plant Cell* **18**, 3519-3534.

Hirayama, T., Ohto, C., Mizoguchi, T. and Shinozaki, K. (1995). A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **92**, 3903-3907.

Ischebeck, T., Stenzel, I. and Heilmann, I. (2008). Type B phosphatidylinositol-4-phosphate 5-kinases mediate Arabidopsis and Nicotiana tabacum pollen tube growth by regulating apical pectin secretion. *Plant Cell* **20**, 3312-3330.

Ischebeck, T., Vu, L. H., Jin, X., Stenzel, I., Löfke, C. and Heilmann, I. (2010). Functional cooperativity of enzymes of phosphoinositide conversion according to synergistic effects on pectin secretion in tobacco pollen tubes. *Mol. Plant* **3**, 870-881.

Ischebeck, T., Stenzel, I., Hempel, F., Jin, X., Mosblech, A. and Heilmann, I. (2011). Phosphatidylinositol-4,5-bisphosphate influences Nt-Rac5-mediated cell expansion in pollen tubes of Nicotiana tabacum. *Plant J.* **65**, 453-468.

Ischebeck, T., Werner, S., Krishnamoorthy, P., Lerche, J., Meijon, M., Stenzel, I., Löfke, C., Wiessner, T., Im, Y. J., Perera, I. Y. et al. (2013). Phosphatidylinositol 4,5-bisphosphate influences PIN polarization by controlling clathrin-mediated membrane trafficking in Arabidopsis. *Plant Cell* **25**, 4894-4911.

Julkowska, M. M., Rankenberg, J. M. and Testerink, C. (2013). Liposome-binding assays to assess specificity and affinity of phospholipid–protein interactions. *Methods Mol. Biol.* **1009**, 261-271.

Kale, S. D., Gu, B., Capelluto, D. G. S., Dou, D., Feldman, E., Rumore, A., Arredondo, F. D., Hanlon, R., Fudal, I., Rouxel, T. et al. (2010). External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. *Cell* **142**, 284-295.

Kanehara, K., Yu, C.-Y., Cho, Y., Cheong, W.-F., Torta, F., Shui, G., Wenk, M. R. and Nakamura, Y. (2015). Arabidopsis AtPLC2 is a primary phosphoinositide-specific phospholipase C in phosphoinositide metabolism and the endoplasmic reticulum stress response. *PLoS Genet.* **11**, e1005511.

Kaye, Y., Golani, Y., Singer, Y., Leshem, Y., Cohen, G., Ercetin, M., Gillaspy, G. and Levine, A. (2011). Inositol polyphosphate 5-phosphatase7 regulates the production of reactive oxygen species and salt tolerance in Arabidopsis. *Plant Physiol.* **157**, 229-241.

König, S., Mosblech, A. and Heilmann, I. (2007). Stress-inducible and constitutive phosphoinositide pools have distinctive fatty acid patterns in *Arabidopsis thaliana*. *FASEB J.* **21**, 1958-1967.

König, S., Ischebeck, T., Lerche, J., Stenzel, I. and Heilmann, I. (2008). Salt-stress-induced association of phosphatidylinositol 4,5-bisphosphate with clathrin-coated vesicles in plants. *Biochem. J.* **415**, 387-399.

Kost, B., Lemichez, E., Spielhofer, P., Hong, Y., Tolias, K., Carpenter, C. and Chua, N.-H. (1999). Rac homologues and compartmentalized phosphatidylinositol 4, 5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *J. Cell Biol.* **145**, 317-330.
Krinke, O., Novotna, Z., Valentova, O. and Martinec, J. (2007a). Inositol trisphosphate receptor in higher plants: is it real? *J. Exp. Bot.* **58**, 361-376.
Krinke, O., Ruelland, E., Valentova, O., Vergnolle, C., Renou, J.-P., Taconnat, L., Flemr, M., Burketova, L. and Zachowski, A. (2007b). Phosphatidylinositol 4-kinase activation is an early response to salicylic acid in *Arabidopsis* suspension cells. *Plant Physiol.* **144**, 1347-1359.
Krishnamoorthy, P., Sanchez-Rodriguez, C., Heilmann, I. and Persson, S. (2014). Regulatory roles of phosphoinositides in membrane trafficking and their potential impact on cell-wall synthesis and re-modelling. *Ann. Bot.* **114**, 1049-1057.
Kusano, H., Testerink, C., Vermeer, J. E. M., Tsuge, T., Shimada, H., Oka, A., Munnik, T. and Aoyama, T. (2008). The *Arabidopsis* phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. *Plant Cell* **20**, 367-380.
Laha, D., Johnen, P., Azevedo, C., Dynowski, M., Weiss, M., Capolicchio, S., Mao, H., Iven, T., Steenbergen, M., Freyer, M. et al. (2015). VIH2 regulates the synthesis of inositol pyrophosphate InsP8 and jasmonate-dependent defenses in Arabidopsis. *Plant Cell* **27**, 1082-1097.
Lee, A. G. (2004). How lipids affect the activities of integral membrane proteins. *Biochim. Biophys. Acta* **1666**, 62-87.
Lee, Y., Bak, G., Choi, Y., Chuang, W.-I., Cho, H.-T. and Lee, Y. (2008a). Roles of phosphatidylinositol 3-kinase in root hair growth. *Plant Physiol.* **147**, 624-635.
Lee, Y., Kim, E.-S., Choi, Y., Hwang, I., Staiger, C. J., Chung, Y.-Y. and Lee, Y. (2008b). The Arabidopsis phosphatidylinositol 3-kinase is important for pollen development. *Plant Physiol.* **147**, 1886-1897.
Lemmon, M. A. (2003). Phosphoinositide recognition domains. *Traffic* **4**, 201-213.
Lin, W.-H., Wang, Y., Mueller-Roeber, B., Brearley, C. A., Xu, Z.-H. and Xue, H.-W. (2005). At5PTase13 modulates cotyledon vein development through regulating auxin homeostasis. *Plant Physiol.* **139**, 1677-1691.
Liu, X., Zhai, S., Zhao, Y., Sun, B., Liu, C., Yang, A. and Zhang, J. (2013). Overexpression of the phosphatidylinositol synthase gene (ZmPIS) conferring drought stress tolerance by altering membrane lipid composition and increasing ABA synthesis in maize. *Plant Cell Environ.* **36**, 1037-1055.
Löfke, C., Ischebeck, T., König, S., Freitag, S. and Heilmann, I. (2008). Alternative metabolic fates of phosphatidylinositol produced by PI-synthase isoforms in *Arabidopsis thaliana*. *Biochem. J.* **413**, 115-124.
Lou, Y., Gou, J.-Y. and Xue, H.-W. (2007). PIP5K9, an *Arabidopsis* phosphatidylinositol monophosphate kinase, interacts with a cytosolic invertase to negatively regulate sugar-mediated root growth. *Plant Cell* **19**, 163-181.
Lundbaek, J. A., Collingwood, S. A., Ingolfsson, H. I., Kapoor, R. and Andersen, O. S. (2010). Lipid bilayer regulation of membrane protein function: gramicidin channels as molecular force probes. *J. R. Soc. Interface* **7**, 373-395.
McLaughlin, S., Wang, J., Gambhir, A. and Murray, D. (2002). PIP(2) and proteins: interactions, organization, and information flow. *Annu. Rev. Biophys. Biomol. Struct.* **31**, 151-175.
Mei, Y., Jia, W.-J., Chu, Y.-J. and Xue, H.-W. (2012). Arabidopsis phosphatidylinositol monophosphate 5-kinase 2 is involved in root gravitropism through regulation of polar auxin transport by affecting the cycling of PIN proteins. *Cell Res.* **22**, 581-597.
Meijer, H. J. G. and Munnik, T. (2003). Phospholipid-based signaling in plants. *Annu. Rev. Plant Biol.* **54**, 265-306.
Mishkind, M., Vermeer, J. E. M., Darwish, E. and Munnik, T. (2009). Heat stress activates phospholipase D and triggers PIP 2 accumulation at the plasma membrane and nucleus. *Plant J.* **60**, 10-21.
Mosblech, A., König, S., Stenzel, I., Grzeganek, P., Feussner, I. and Heilmann, I. (2008). Phosphoinositide and inositolpolyphosphate signalling in defense responses of *Arabidopsis thaliana* challenged by mechanical wounding. *Mol. Plant* **1**, 249-261.
Mosblech, A., Thurow, C., Gatz, C., Feussner, I. and Heilmann, I. (2011). Jasmonic acid perception by COI1 involves inositol polyphosphates in Arabidopsis thaliana. *Plant J.* **65**, 949-957.
Mueller-Roeber, B. and Pical, C. (2002). Inositol phospholipid metabolism in *Arabidopsis*. Characterized and putative isoforms of inositol phospholipid kinase and phosphoinositide-specific phospholipase C. *Plant Physiol.* **130**, 22-46.
Mukherjee, S., Soe, T. T. and Maxfield, F. R. (1999). Endocytic sorting of lipid analogues differing solely in the chemistry of their hydrophobic tails. *J. Cell Biol.* **144**, 1271-1284.
Munnik, T. and Nielsen, E. (2011). Green light for polyphosphoinositide signals in plants. *Curr. Opin. Plant Biol.* **14**, 489-497.
Munnik, T. and Wierzchowiecka, M. (2013). Lipid-binding analysis using a fat blot assay. *Methods Mol. Biol.* **1009**, 253-259.
Nicolson, G. L. (2014). The Fluid—Mosaic Model of Membrane Structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years. *Biochim. Biophys. Acta* **1838**, 1451-1466.
Novakova, P., Hirsch, S., Feraru, E., Tejos, R., van Wijk, R., Viaene, T., Heilmann, M., Lerche, J., De Rycke, R., Feraru, M. I. et al. (2014). SAC phosphoinositide phosphatases at the tonoplast mediate vacuolar function in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **111**, 2818-2823.
Okazaki, K., Miyagishima, S.-y. and Wada, H. (2015). Phosphatidylinositol 4-phosphate negatively regulates chloroplast division in Arabidopsis. *Plant Cell* **27**, 663-674.
Oxley, D., Ktistakis, N. and Farmaki, T. (2013). Differential isolation and identification of PI(3)P and PI(3,5)P2 binding proteins from Arabidopsis thaliana using an agarose-phosphatidylinositol-phosphate affinity chromatography. *J. Proteomics* **91**, 580-594.
Parker, J. S., Cavell, A. C., Dolan, L., Roberts, K. and Grierson, C. S. (2000). Genetic interactions during root hair morphogenesis in Arabidopsis. *Plant Cell* **12**, 1961-1974.
Perera, I. Y., Heilmann, I. and Boss, W. F. (1999). Transient and sustained increases in inositol 1,4,5-trisphosphate precede the differential growth response in gravistimulated maize pulvini. *Proc. Natl. Acad. Sci. USA* **96**, 5838-5843.
Perera, I. Y., Heilmann, I., Chang, S. C., Boss, W. F. and Kaufman, P. B. (2001). A role for inositol 1,4,5-trisphosphate in gravitropic signaling and the retention of cold-perceived gravistimulation of oat shoot pulvini. *Plant Physiol.* **125**, 1499-1507.
Pical, C., Westergren, T., Dove, S. K., Larsson, C. and Sommarin, M. (1999). Salinity and hyperosmotic stress induce rapid increases in phosphatidylinositol 4,5-bisphosphate, diacylglycerol pyrophosphate, and phosphatidylcholine in *Arabidopsis thaliana* cells. *J. Biol. Chem.* **274**, 38232-38240.
Pokotylo, I., Kolesnikov, Y., Kravets, V., Zachowski, A. and Ruelland, E. (2014). Plant phosphoinositide-dependent phospholipases C: variations around a canonical theme. *Biochimie* **96**, 144-157.
Preuss, M. L., Schmitz, A. J., Thole, J. M., Bonner, H. K. S., Otegui, M. S. and Nielsen, E. (2006). A role for the RabA4b effector protein PI-4Kbeta1 in polarized expansion of root hair cells in *Arabidopsis thaliana*. *J. Cell Biol.* **172**, 991-998.
Pribat, A., Sormani, R., Rousseau-Gueutin, M., Julkowska, M. M., Testerink, C., Joubes, J., Castroviejo, M., Laguerre, M., Meyer, C., Germain, V. et al. (2012). A novel class of PTEN protein in Arabidopsis displays unusual phosphoinositide phosphatase activity and efficiently binds phosphatidic acid. *Biochem. J.* **441**, 161-171.
Rodriguez-Villalon, A., Gujas, B., van Wijk, R., Munnik, T. and Hardtke, C. S. (2015). Primary root protophloem differentiation requires balanced phosphatidylinositol-4,5-biphosphate levels and systemically affects root branching. *Development* **142**, 1437-1446.
Saavedra, L., Balbi, V., Lerche, J., Mikami, K., Heilmann, I. and Sommarin, M. (2011). PIPKs are essential for rhizoid elongation and caulonemal cell development in the moss Physcomitrella patens. *Plant J.* **67**, 635-647.
Saavedra, L., Catarino, R., Heinz, T., Heilmann, I., Bezanilla, M. and Malhó, R. (2015). Phosphatase and tensin homolog is a growth repressor of both rhizoid and gametophore development in the moss Physcomitrella patens. *Plant Physiol.* **169**, 2572-2586.
Schaaf, G., Ortlund, E. A., Tyeryar, K. R., Mousley, C. J., Ile, K. E., Garrett, T. A., Ren, J., Woolls, M. J., Raetz, C. R. H., Redinbo, M. R. et al. (2008). Functional anatomy of phospholipid binding and regulation of phosphoinositide homeostasis by proteins of the sec14 superfamily. *Mol. Cell* **29**, 191-206.
Sheard, L. B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T. R., Kobayashi, Y., Hsu, F.-F., Sharon, M., Browse, J. et al. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. *Nature* **468**, 400-405.
Simon, M. L. A., Platre, M. P., Assil, S., van Wijk, R., Chen, W. Y., Chory, J., Dreux, M., Munnik, T. and Jaillais, Y. (2014). A multi-colour/multi-affinity marker set to visualize phosphoinositide dynamics in Arabidopsis. *Plant J.* **77**, 322-337.
Sousa, E., Kost, B. and Malho, R. (2008). *Arabidopsis* phosphatidylinositol-4-monophosphate 5-kinase 4 regulates pollen tube growth and polarity by modulating membrane recycling. *Plant Cell* **20**, 3050-3064.
Stenzel, I., Ischebeck, T., König, S., Holubowska, A., Sporysz, M., Hause, B. and Heilmann, I. (2008). The type B phosphatidylinositol-4-phosphate 5-kinase 3 is essential for root hair formation in *Arabidopsis thaliana*. *Plant Cell* **20**, 124-141.
Stenzel, I., Ischebeck, T., Quint, M. and Heilmann, I. (2012). Variable regions of PI4P 5-kinases direct PtdIns(4,5)P2 toward alternative regulatory functions in tobacco pollen tubes. *Front. Plant Sci.* **2**, 114.
Takenawa, T. (2010). Phosphoinositide-binding interface proteins involved in shaping cell membranes. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **86**, 509-523.
Takenawa, T. and Itoh, T. (2006). Membrane targeting and remodeling through phosphoinositide-binding domains. *IUBMB Life* **58**, 296-303.
Tan, X., Calderon-Villalobos, L. I. A., Sharon, M., Zheng, C., Robinson, C. V., Estelle, M. and Zheng, N. (2007). Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* **446**, 640-645.
Tejos, R., Sauer, M., Vanneste, S., Palacios-Gomez, M., Li, H., Heilmann, M., van Wijk, R., Vermeer, J. E. M., Heilmann, I., Munnik, T. et al. (2014). Bipolar plasma membrane distribution of phosphoinositides and their requirement for auxin-mediated cell polarity and patterning in Arabidopsis. *Plant Cell* **26**, 2114-2128.
Thole, J. M. and Nielsen, E. (2008). Phosphoinositides in plants: novel functions in membrane trafficking. *Curr. Opin. Plant Biol.* **11**, 620-631.
Thole, J. M., Vermeer, J. E. M., Zhang, Y., Gadella, T. W. J., Jr. and Nielsen, E. (2008). ROOT HAIR DEFECTIVE4 encodes a phosphatidylinositol-4-phosphate

phosphatase required for proper root hair development in *Arabidopsis thaliana*. *Plant Cell* **20**, 381-395.
van Leeuwen, W., Ökrész, L., Bögre, L. and Munnik, T. (2004). Learning the lipid language of plant signalling. *Trends Plant Sci.* **9**, 378-384.
van Leeuwen, W., Vermeer, J. E. M., Gadella, T. W. J., Jr. and Munnik, T. (2007). Visualization of phosphatidylinositol 4,5-bisphosphate in the plasma membrane of suspension-cultured tobacco BY-2 cells and whole *Arabidopsis* seedlings. *Plant J.* **52**, 1014-1026.
van Meer, G., Voelker, D. R. and Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **9**, 112-124.
van Rossum, D. B., Patterson, R. L., Sharma, S., Barrow, R. K., Kornberg, M., Gill, D. L. and Snyder, S. H. (2005). Phospholipase Cgamma1 controls surface expression of TRPC3 through an intermolecular PH domain. *Nature* **434**, 99-104.
van Schooten, B., Testerink, C. and Munnik, T. (2006). Signalling diacylglycerol pyrophosphate, a new phosphatidic acid metabolite. *Biochim. Biophys. Acta* **1761**, 151-159.
Várnai, P. and Balla, T. (1998). Visualization of phosphoinositides that bind pleckstrin homology domains: calcium- and agonist-induced dynamic changes and relationship to *myo*-[^{3}H]inositol-labeled phosphoinositide pools. *J. Cell Biol.* **143**, 501-510.
Várnai, P., Bondeva, T., Tamás, P., Tóth, B., Buday, L., Hunyady, L. and Balla, T. (2005). Selective cellular effects of overexpressed pleckstrin-homology domains that recognize PtdIns(3,4,5)P_3 suggest their interaction with protein binding partners. *J. Cell Sci.* **118**, 4879-4888.
Vermeer, J. E. M., van Leeuwen, W., Tobeña-Santamaria, R., Laxalt, A. M., Jones, D. R., Divecha, N., Gadella, T. W. J., Jr. and Munnik, T. (2006). Visualization of PtdIns3P dynamics in living plant cells. *Plant J.* **47**, 687-700.
Vermeer, J. E. M., Thole, J. M., Goedhart, J., Nielsen, E., Munnik, T. and Gadella, T. W. J.Jr. (2009). Imaging phosphatidylinositol 4-phosphate dynamics in living plant cells. *Plant J.* **57**, 356-372.
Vincent, P., Chua, M., Nogue, F., Fairbrother, A., Mekeel, H., Xu, Y., Allen, N., Bibikova, T. N., Gilroy, S. and Bankaitis, V. A. (2005). A Sec14p-nodulin domain phosphatidylinositol transfer protein polarizes membrane growth of *Arabidopsis thaliana* root hairs. *J. Cell Biol.* **168**, 801-812.
Wang, Y., Chu, Y.-J. and Xue, H.-W. (2012). Inositol polyphosphate 5-phosphatase-controlled Ins(1,4,5)P_3/Ca^{2+} is crucial for maintaining pollen dormancy and regulating early germination of pollen. *Development* **139**, 2221-2233.
Welters, P., Takegawa, K., Emr, S. D. and Chrispeels, M. J. (1994). AtVPS34, a phosphatidylinositol 3-kinase of Arabidopsis thaliana, is an essential protein with homology to a calcium-dependent lipid binding domain. *Proc. Natl. Acad. Sci. USA* **91**, 11398-11402.
Westergren, T., Dove, S. K., Sommarin, M. and Pical, C. (2001). AtPIP5K1, an *Arabidopsis thaliana* phosphatidylinositol phosphate kinase, synthesizes PtdIns (3,4)P(2) and PtdIns(4,5)P(2) in vitro and is inhibited by phosphorylation. *Biochem. J.* **359**, 583-589.
Whitley, P., Hinz, S. and Doughty, J. (2009). Arabidopsis FAB1/PIKfyve proteins are essential for development of viable pollen. *Plant Physiol.* **151**, 1812-1822.
Williams, M. E., Torabinejad, J., Cohick, E., Parker, K., Drake, E. J., Thompson, J. E., Hortter, M. and Dewald, D. B. (2005). Mutations in the *Arabidopsis* phosphoinositide phosphatase gene *SAC9* lead to overaccumulation of PtdIns (4,5)P_2 and constitutive expression of the stress-response pathway. *Plant Physiol.* **138**, 686-700.
Zhang, Y., Li, S., Zhou, L.-Z., Fox, E., Pao, J., Sun, W., Zhou, C. and McCormick, S. (2011). Overexpression of Arabidopsis thaliana PTEN caused accumulation of autophagic bodies in pollen tubes by disrupting phosphatidylinositol 3-phosphate dynamics. *Plant J.* **68**, 1081-1092.
Zhao, Y., Yan, A., Feijó, J. A., Furutani, M., Takenawa, T., Hwang, I., Fu, Y. and Yang, Z. (2010). Phosphoinositides regulate clathrin-dependent endocytosis at the tip of pollen tubes in Arabidopsis and tobacco. *Plant Cell* **22**, 4031-4044.
Zheng, S.-Z., Liu, Y.-L., Li, B., Shang, Z.-l., Zhou, R.-G. and Sun, D.-Y. (2012). Phosphoinositide-specific phospholipase C9 is involved in the thermotolerance of Arabidopsis. *Plant J.* **69**, 689-700.
Zhong, R. and Ye, Z.-H. (2003). The SAC domain-containing protein gene family in Arabidopsis. *Plant Physiol.* **132**, 544-555.
Zhong, R. and Ye, Z. H. (2004). Molecular and biochemical characterization of three WD-repeat-domain-containing inositol polyphosphate 5-phosphatases in Arabidopsis thaliana. *Plant Cell Physiol.* **45**, 1720-1728.
Zhong, R., Burk, D. H., Morrison, W. H., III and Ye, Z. H. (2004). FRAGILE FIBER3, an *Arabidopsis* gene encoding a type II inositol polyphosphate 5-phosphatase, is required for secondary wall synthesis and actin organization in fiber cells. *Plant Cell* **16**, 3242-3259.
Zhong, R., Burk, D. H., Nairn, C. J., Wood-Jones, A., Morrison, W. H., III and Ye, Z. H. (2005). Mutation of SAC1, an *Arabidopsis* SAC domain phosphoinositide phosphatase, causes alterations in cell morphogenesis, cell wall synthesis, and actin organization. *Plant Cell* **17**, 1449-1466.
Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L. and Gruissem, W. (2004). GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. *Plant Physiol.* **136**, 2621-2632.