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Learning the lipid language of plant signalling

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Plant cells respond to different biotic and abiotic stresses by producing various uncommon phospholipids that are believed to play key roles in cell signalling. We can predict how they work because animal and yeast proteins have been shown to have specific lipid-binding domains, which act as docking sites. When such proteins are recruited to the membrane locations where these phospholipids are synthesized, the phospholipids activate them directly, by inducing a conformational change, or indirectly, by juxtaposing them with an activator protein. The same lipid-binding domains are present in *Arabidopsis* proteins. We believe that they represent an untapped well of information about plant lipid signalling.

We all assume that the organization of animal and plant cells have much in common, in the same way that all facilities in Amsterdam exist in a similar form in New York and are run along similar lines. Nonetheless, the two cities do not speak the same language, although there is a Dutch equivalent for every English word and some are even identical. It is like this with signalling systems in animal and plant cells. Take the two classic lipid-signalling systems in animal cells that depend on the lipid phosphatidylinositol-(4,5)-bisphosphate [PtdInsP_2]. The first uses phospholipase C (PLC) to hydrolyse PtdIns(4,5)P_2 to produce the second messengers inositol-(1,4,5)-trisphosphate [InsP_3] and diacylglycerol, which activate Ca^{2+} stores and protein kinase C, respectively. The second uses phosphoinositide 3-kinase to phosphorylate PtdIns(4,5)P_2 to produce phosphatidylinositol-(3,4,5)-trisphosphate [PtdIns(3,4,5)P_3]. Do these signalling languages exist in plants? Perhaps, but we wouldn't bet on it. The *Arabidopsis* genome does not contain either an Ins(1,4,5)P_3 receptor or a protein kinase C, and PtdIns(3,4,5)P_3 has never been found in plants [1]. Does this mean that PtdIns(4,5)P_2 has no signalling function in plants? No, of course not, but it does mean that you might have to learn another language. Let's first look at these PtdInsP_2-like words.

PtdInsP_2 is a polyphosphoinositide (PPI), a derivative of the membrane lipid phosphatidylinositol (PtdIns). However, PPIs can be phosphorylated at the D3, 4 or 5 position of the inositol ring, either alone [e.g. PtdIns(4)P] or in combination [e.g. PtdIns(4,5)P_2]. All mono- and bisphosphorylated combinations have been found in plants, yet altogether they represent less than 1% of the total phospholipids in a cell. Significantly, their synthesis or hydrolysis increases rapidly, within minutes when plants are subjected to agriculturally important stresses such as drought, salinity, temperature stress and pathogen attack [1–3]. This is what first suggested that PPIs were involved in plant signalling, just as in animal cells. Obviously, certain details differ. Instead of using Ins(1,4,5)P_3 to release Ca^{2+} from intracellular stores via a ligand-gated Ca^{2+} channel, plants and yeast seem to use inositol hexakisphosphate [1,4]. Similarly, instead of using diacylglycerol as a second messenger, plants use its phosphorylated derivative phosphatidic acid (PA) [5]. A way to switch off the PA signal also turned out to be plant specific: it is further phosphorylated to diacylglycerolpyrophosphate (DGPP), a molecule not found in animal cells [1]. Although it is an off-signal for PA, DGPP itself could be an on-signal, because it is practically absent from control cells yet is rapidly produced upon stimulation [1,5]. Although these are a few of the differences, let us now concentrate on what promises to be a basic similarity, one that embodies the essence of lipid signalling.

In animal cells, various PPI isomers have emerged as novel second messengers [6]. They function as docking sites that 'attract' signalling proteins to specific membrane locations. When bound, the lipid can activate the protein to transduce information, for example to phosphorylate another protein downstream in the signalling cascade. Alternatively, by concentrating signalling proteins on specific membrane surfaces, they function as molecular scaffolds for the assembly of multiprotein complexes, which again result in signal transduction or cellular reorganization. This signal transmission invariably changes the physiological status of the cell, often by changing cytoskeleton organization, protein stability or gene expression patterns [6].

Several PPI-binding domains have been discovered over the past few years [7,8]. The best known are the pleckstrin homology (PH), FYVE and Phox homology (PX) domains (Box 1). We discovered that *Arabidopsis* contains ~70 proteins with a PH, FYVE and/or PX domain (Figures 1–3). We believe that this large number of lipid-binding domains

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Box 1. Topology of the PH, FYVE and PX domains

The pleckstrin homology (PH) domain is a protein module of 100–120 amino acids. It consists of a seven-stranded β-sandwich of two orthogonal antiparallel β-sheets that is closed at one corner by a C-terminal α-helix (Figure Ia) [46]. Although the tertiary structure of PH domains is well conserved, their overall amino acid sequence homology is low. One tryptophan residue is found in all PH domains except for three plant proteins. In general, the PPI-binding specificity and affinity of PH domains cannot be predicted.

The FYVE domain consists of 60–80 amino acids, including eight conserved cysteine residues. It is composed of two β-hairpins plus a small C-terminal α-helix that are held together by two Zn^{2+}-binding clusters, forming a zinc-finger-like structure [48]. A basic motif in the first β-strand [R(R/K)HHCR] contributes to a shallow positively charged pocket that is crucial for PtdIns(3)*P* binding [49]. The crystal structure of EEA1 (Figure Ib) revealed that the C-terminal region forms an organized quaternary assembly, consisting of a parallel coiled coil and a FYVE domain homodimer [49]. Flanking each phosphoinositide-binding site, a short 'turret loop' tethers the protein to the membrane by protruding into the lipid bilayer.

The Phox homology (PX) domain consists of 100–120 amino acids and was first identified through detailed analysis of two of the cytosolic components of the NADPH oxidase complex, $p47^{phox}$ and $p40^{phox}$ [50]. The PX domain has an N-terminal three-stranded, meander topology β-sheet packed against a helical subdomain consisting of four α-helices. Its crystal structure (Figure Ic) shows that the PX domain embraces the 3-phosphate of the inositol on one side of a water-filled, positively charged pocket [51].

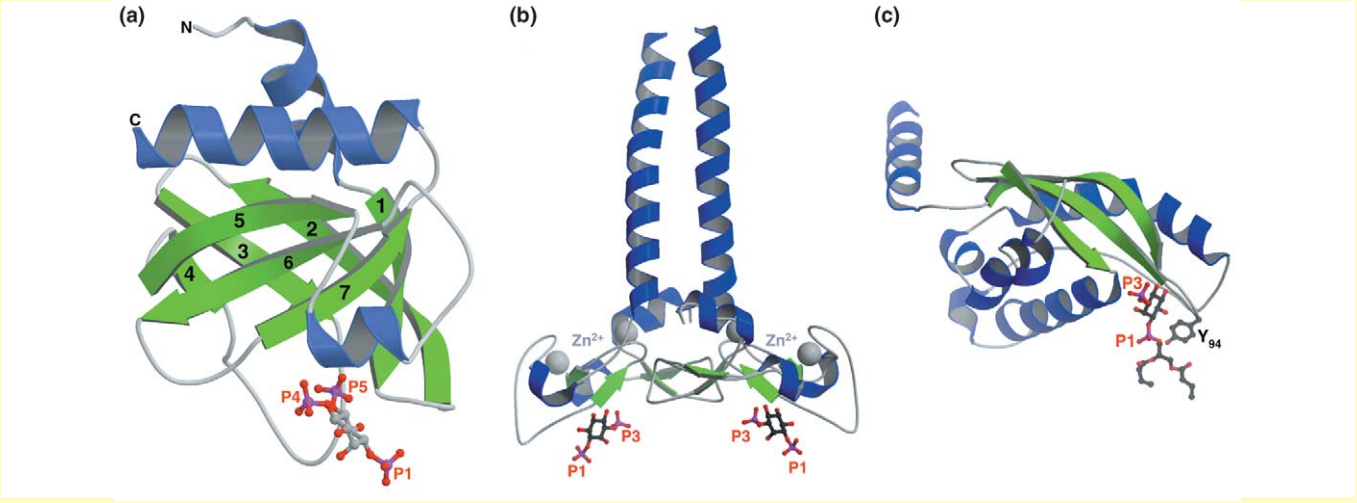

Figure I. (a) PH domain (PLCδ1) in complex with the headgroup of PtdIns(4,5)P_2. Reproduced, with permission, from Ref. [47]. **(b)** EEA1 homo-dimer with C-terminal FYVE-domains in complex with the headgroup of PtdIns(3)*P*. Reproduced, with permission, from Ref. [7]. **(c)** PX domain ($p40^{phox}$) in complex with the headgroup of PtdIns(3)*P*. Reproduced, with permission, from Ref. [7].

exemplifies a mechanism of how lipid signals talk to target proteins, yet hardly anyone has studied this language in plants.

PH-domain proteins in the *Arabidopsis* genome

Arabidopsis is predicted to contain 53 proteins with a PH domain (Box 1, Figure 1). Based on amino acid sequence homologies of the domain alone, they can be subdivided into 12 categories (Figures 1,4). Many of these categories are characterized by the presence of other exciting domains (e.g. ArfGAP, RhoGAP, oxysterol-binding protein, PtdIns 4-kinase or even FYVE or PX). Interestingly, identical phylogenetic trees were obtained irrespective of whether the whole PH-domain protein or only the domain (Figure 4) was aligned, except for two proteins that did not fit into any of the categories (data not shown). Although one cannot predict which PPI each PH domain binds to, the identical alignment indicates some form of specificity. Clearly, this requires further investigation, because it will have strong implications for the function of both PPIs and target proteins.

The PPI-binding specificity of PH domains can be determined using *in vitro* binding assays [9]. Well-studied examples include the PH domain from mammalian PLCδ1, which binds to PtdIns(4,5)P_2 or the PH domain from FAPP1, which binds to PtdIns(4)*P* [10]. However, there are also PH domains that bind PPIs nonspecifically (e.g. the PH domain from LL5β) [10], and even PH domains that do not seem to bind PPIs at all [11]. Crystal structures of PH domains in complex with bound PPIs have highlighted the various determinants of phosphoinositide recognition [8]. This diversity allows PH domains to control a wide range of cellular processes specifically [6,7].

The Arf GTPase-activating protein (ArfGAP) is involved in membrane trafficking by inactivating the small G-protein Arf. In mammal studies, PtdIns(4,5)P_2 has been shown to bind the PH domain of ArfGAP and to induce a conformational change [12], thereby stimulating the activity of the protein up to 30-fold [13]. In *Arabidopsis*, the regulation of ArfGAP (and, similarly, RhoGAP) [14,15] by PPIs is an exciting possibility but remains to be shown.

Another PH-domain subcategory is characterized by the presence of an oxysterol-binding protein (OBP) domain, which is implicated in sterol homeostasis in animals [16]. The PH domain of OBP specifically targets proteins to the *trans*-Golgi network of mammalian cells,

Name	Domains	AGI no.	MIPS annotation	Domain organization
PH1	PH	At2g30880	Unknown protein	
ArfGAP1	BAR + PH + ArfGAP + 2 ANK	At1g10870	BRCA1-associated RING domain protein [a]	
ArfGAP2	BAR + PH + ArfGAP + 2 ANK	At1g60860	GCN4-complementing protein – like [a]	
ArfGAP3	BAR + PH + ArfGAP + 2 ANK	At5g61980	GCN4-complementing protein – like [a]	
ArfGAP4	BAR + PH + ArfGAP + 3 ANK	At5g13300	Putative protein	
ADL6	DYN + PH + GED	At1g10290	Putative phragmoplastin [a]	
ADL3	DYN + PH + GED	At1g59610	Dynamin-like protein CF1 [a]	
PH2	PH	At2g29700	Unknown protein	
PH3	PH	At5g05710	AtPH1-like protein [a]	
PH4	PH	At1g77730	Hypothetical protein	
OBP1	PH + OBP	At2g31020	Putative oxysterol-binding protein	
OBP2	PH + OBP	At4g08180	Unknown protein	
OBP3	PH + OBP	At1g13170	Putative oxysterol-binding protein	
OBP4	PH + OBP	At4g12460	Putative SWH1 protein	
OBP5	PH + OBP	At4g22540	Unknown protein	
START1	PH + START	At3g54800	Unknown protein	
START2	PH + START	At4g19040	Hypothetical protein	
START3	PH + START	At5g45560	Unknown protein	
START4	PH + START	At5g35180	Unknown protein	
START5	PH + START	At2g28320	Unknown protein	
RhoGAP1	PH + RhoGAP	At4g24580	Putative protein	
RhoGAP2	PH + RhoGAP	At5g12150	Putative protein	
RhoGAP3	PH + RhoGAP	At5g19390	Unknown protein	
PH5	PH	At1g48090	Unknown protein	d
PH6	PH	At4g17140	Similar to sex muscle abnormal protein [b]	
PLD 1	PX + PH + 2 PLD	At3g16785-90	Phospholipase D	
PLD 2	PX + PH + 2 PLD	At3g05630	Phospholipase D	
PH7	PH	At1g17820	Unknown protein	
PH8	PH	At1g73200	Hypothetical protein	
Praf1	PH + 6 RCC1 + FYVE	At1g65920	Zinc-finger protein (PRAF1)	
Praf3	PH + 6 RCC1 + FYVE	At1g69710	Putative RCC protein	
Praf2	PH + 6 RCC1 + FYVE	At3g47660	Similar to rjs protein [c]	
Praf9	PH + 7 RCC1 + FYVE	At5g19420	Strong similarity to predicted RCC protein [a]	
Praf8	PH + 6 RCC1 + FYVE	At5g12350	UVB-resistance protein UVR8 [a]	
Praf6	PH + 6 RCC1 + FYVE	At3g23270	Putative protein	
Praf7	PH + 6 RCC1 + FYVE	At4g14370	Putative protein	
Praf4	PH + 6 RCC1 + FYVE	At1g76950	Zinc-finger protein (PRAF1)	
Praf5	PH + 6 RCC1 + FYVE	At5g42140	Putative protein	
PH9	PH	At4g32780-85	Unknown protein	
PH10	PH	At3g63300	Unknown protein	
PH11	PH	At5g43870	Putative protein	
PH12	PH	At3g22810	Unknown protein	
PH13	PH	At4g14740	Unknown protein	
PH14	PH	At4g16670	Unknown protein	
PH15	PH	At4g17350	Putative protein	
PH16	PH	At5g47440	Putative protein	
PDK1-1	S/T-Kinase + PH	At3g10540	Putative 3-PtdIns-dependent protein kinase-1	
PDK1-2	S/T-Kinase + PH	At5g04510	3-PtdIns-dependent protein kinase-1 (PDK1)	
PI3K/Vps34	PH + PI3/4Kinase	At1g60490	PtdIns 3-kinase (AtVps34)	
PI4K-1	PH + PI3/4Kinase	At1g51040	Putative protein	
PI4K-2	PH + PI3/4Kinase	At1g49340	Putative protein	
PI4K-3	PH + PI3/4Kinase	At5g09350	Strong similarity to PtdIns 4-kinase [a]	
PI4K-4	PH + PI3/4Kinase	At5g64070	PtdIns 4-kinase	

Figure 1. *Arabidopsis* pleckstrin homology domain proteins. Key to superscript letters: a, *Arabidopsis thaliana*; b, *Caenorhabditis elegans*; c, *Mus muculus*; d, At1g48090 is an unknown protein of 4099 amino acids of which the domain representation is discontinuous, as indicated by //. Abbreviations: OBP, oxysterol-binding protein; PH, pleckstrin homology; PLD, phospholipase D; PtdIns 3-kinase, phosphatidylinositol 3-kinase; PtdIns 4-kinase, phosphatidylinositol 4-kinase; RCC, regulator of chromosome condensation. Information in this table, plus links to MIPS, NCBI and SIGnAL, can be found at http://personal.rhul.ac.uk/ujba/110/lipid/lipid.htm.

depending on the presence of Golgi PPIs [17]. The function of OBP homologues in plants remains elusive.

In PtdIns 4-kinases (e.g. *At*PI4Kα1), the presence of the PH domain is required for optimal kinase activity and/or protein folding. It was found to bind PtdIns(4)*P* [18] and has also been implicated in binding *At*PI4Kα1 to F-actin, where it might facilitate the formation of fine F-actin filaments [18].

PDK1 is a protein kinase that integrates PtdIns(3,4,5)P_3 signalling in animal cells via its PH domain. Plants lack PtdIns(3,4,5)P_3 but do contain a PDK1 signalling cascade that has recently been shown to play a role in root hair development in *Arabidopsis* [19]. The PH domain of *At*PDK1 is more promiscuous in its lipid-binding specificity [20], controlling AtPDK1 via PtdIns(4,5)P_2 and PA [19].

FYVE-domain proteins in the *Arabidopsis* genome

The FYVE domain (Box 1) is named after the four proteins in which this zinc-finger domain was first identified in 1996: Fab1p, YOTB, Vac1p and EEA1 [21]. It plays an important role in endocytosis and vesicular trafficking [22] and, in contrast to PH domains, only binds one PPI, namely PtdIns(3)*P* [23]. The *Arabidopsis* genome contains 16 proteins with a predicted FYVE domain, in which three categories can be distinguished (Figures 2,4). One is the Fab family of phosphatidylinositol phosphate 5-kinases, which are thought to be responsible for making the novel

Name	Domains	AGI no.	MIPS annotation	Domain organization
Praf1	PH + 6 RCC1 + FYVE	At1g65920	Zinc-finger protein (PRAF1)	
Praf2	PH + 6 RCC1 + FYVE	At3g47660	Similar to rjs protein [c]	
Praf3	PH + 6 RCC1 + FYVE	At1g69710	Putative RCC protein	
Praf8	PH + 6 RCC1 + FYVE	At5g12350	UVB-resistance protein UVR8 [a]	
Praf9	PH + 7 RCC1 + FYVE	At5g19420	Strong similarity to predicted RCC protein [a]	
Praf4	PH + 6 RCC1 + FYVE	At1g76950	Zinc-finger protein (PRAF1)	
Praf5	PH + 6 RCC1 + FYVE	At5g42140	Putative protein	
Praf6	PH + 6 RCC1 + FYVE	At3g23270	Putative protein	
Praf7	PH + 6 RCC1 + FYVE	At4g14370	Putative protein	
FAB1	FYVE + Chaperonin + PIP5K	At3g14270	Unknown protein	
FAB2	FYVE + Chaperonin + PIP5K	At4g33240	Similar to FAB1 protein [e]	
FYVE1	FYVE	At1g20110	Unknown protein	
FYVE2	FYVE	At3g43230	Unknown protein	
FYVE3	FYVE	At1g29800	Unknown protein	
FYVE4	FYVE	At1g61690	Hypothetical protein	
RINGFYVE	RING + FYVE	At1g61620	Unknown protein	

Figure 2. *Arabidopsis* FYVE domain proteins. Key to superscript letters: a, *Arabidopsis thaliana*; c *Mus muculus*; e, *Saccharomyces cerevisiae*. Abbreviations: PH, pleckstrin homology; PRAF, PH domain, regulator of chromosome condensation (RCC) and FYVE; PX, Phox homology; PtdIns*P* 5-kinase, phosphatidylinositol phosphate 5-kinase, RCC, regulator of chromosome condensation. Information in this table, plus links to MIPS, NCBI and SIGnAL, can be found at http://personal.rhul.ac.uk/ujba/110/lipid/lipid.htm.

isomer PtdIns(3,5)P_2 in response to osmotic stress [2]. Another category, the largest, consists of so-called PRAF proteins [PH domain, Regulator of Chromosome Condensation (RCC) and FYVE], which contain both a PH and a FYVE domain. These PRAF proteins seem to be plant specific, because no mammalian homologues are known. RCC domains have been shown to catalyse guanine nucleotide exchange (the dissociation of GDP from a GTPase) of some of the small G-proteins of the Rab subfamily [24], but this function in PRAF proteins still remains to be shown. Several of the PRAF proteins have been incorrectly annotated because they were thought to be involved in disease resistance (e.g. Praf7 is a misannotated *in silico* fusion of a NBS-LRR (nucleotide-binding site-leucine-rich repeat) and an RCC-encoding gene, and Praf5 was misannotated as a consequence). The only reported function for a PRAF protein is for Praf8, which is the *Arabidopsis* ultraviolet-B resistance protein UVR8 [25].

In plants, PtdIns(3)*P* turns over rapidly and has been shown to accumulate in response to osmotic stress [3,26]. Recently, PtdIns(3)*P* was implicated in ABA-induced reactive oxygen species generation and stomatal closure [27], but it is still unknown whether these processes require FYVE-domain-containing proteins. Plant PtdIns 3-kinases have been shown to be associated with the nucleus and a causal link between its activity and nuclear transcription has been suggested [28].

PX-domain proteins in the *Arabidopsis* genome

In general, PX domains (Box 1) bind PtdIns(3)*P* [29], although that of $p47^{phox}$ (a component of the mammalian NADPH oxidase complex) preferentially binds PtdIns(3,4)P_2 and PA [30]. The *Arabidopsis* genome contains 11 proteins with a PX domain (Figures 3,4). Three of these are members of an interesting subcategory, the sorting nexin-like (SNX) proteins, which (in yeast and animals) are involved in endosomal trafficking [31], consistent with a function for PtdIns(3)*P* binding to the PX domain. Another subcategory consists of two phospholipases D (PLDζ1 and PLDζ2), which are interesting signalling enzymes themselves, because they produce the lipid second messenger PA [5]. PLDζ1 has recently been implicated in the initiation of root hair development via the homeobox gene product GLABRA2 [32]. *Arabidopsis* contains 12 PLDs, of which only PLDζ1 and PLDζ2 contain a PH and a PX domain. The others contain a C2 domain instead, which is believed to bind anionic phospholipids in a Ca^{2+}-dependent manner (and is therefore also called CalB, for calcium and lipid binding) [33]. Recently, the C2 domain of protein kinase Cε was shown to bind PA specifically [34], although, in general, it is unknown whether this specificity is retained. There are ~220 C2-domain-containing proteins in the *Arabidopsis* genome.

Name	Domains	AGI no.	MIPS annotation	Domain organization
PX1	PX	At3g48200	Similar to KIAA0226 gene product [f]	
PX-PXA1	PXA + PX	At2g15900	Hypothetical protein	
PX-PXA2	PXA + PX	At1g15240	Hypothetical protein	
PX2	PX	At3g15920	Hypothetical protein	
PX3	PX	At2g25350	Putative protein	
PX-SPEC	PX + SPEC	At4g32160	Unknown protein	
PX4	PX	At5g06140	Sorting nexin-like protein	
PX5	PX	At5g07120	Similar to sorting nexin 2 (SNX2) [f]	
PX6	PX	At5g58440	Unknown protein	
PLD 1	PX + PH + 2 PLD	At3g16785-90	Phospholipase D	
PLD 2	PX + PH + 2 PLD	At3g05630	Phospholipase D	

Figure 3. *Arabidopsis* Phox homology domain proteins. Key to superscript letter: f, *Homo sapiens*. Abbreviations: PH, pleckstrin homology; PLD, phospholipase D. Information in this table, plus links to MIPS, NCBI and SIGnAL, can be found at http://personal.rhul.ac.uk/ujba/110/lipid/lipid.htm.

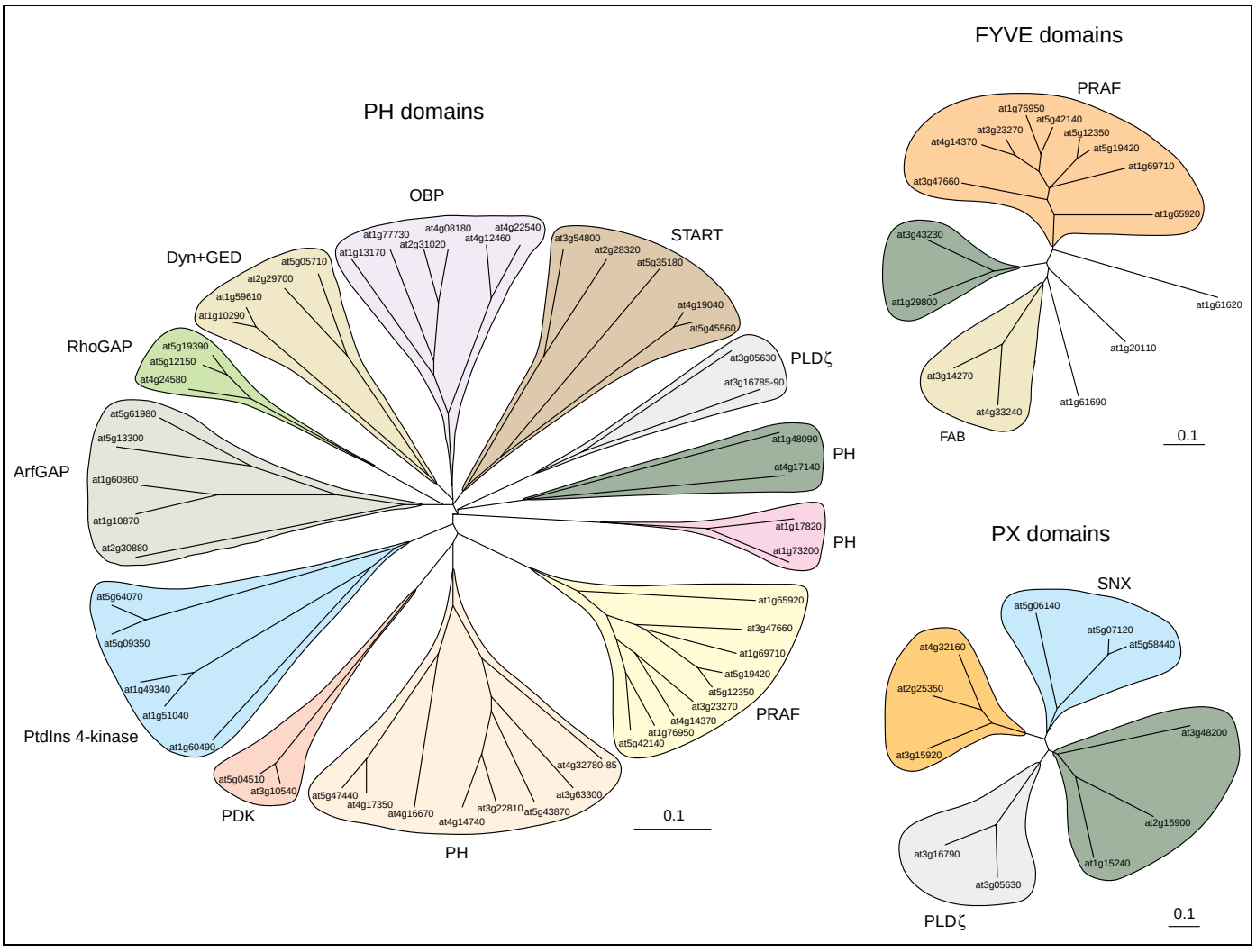

Figure 4. Radial phylogenetic trees of the pleckstrin homology, FYVE and Phox homology domains of *Arabidopsis* proteins. Domains were predicted using specific algorithms. Trees were made by sequence alignments of the domains only, using CLUSTAL W. Phylogenetic analyses were performed by using the PHYLIP program, with the neighbour-joining method. Bootstrap values obtained in 100 replications can be found in the phylogenetic trees at http://personal.rhul.ac.uk/ujba/110/lipid/lipid.htm. Coloured branches indicate different subcategories of lipid-binding-domain-containing proteins. Abbreviations: Arf, ADP-ribosylation factor; ArfGAP, Arf GTPase activating protein; Dyn + GED, Dynamin and GTPase effector domain; FAB, formation of aploid and binucleates; OBP, oxysterol-binding protein; PDK, 3-phosphoinositide dependent protein kinase; PH, pleckstrin homology; PLD, phospholipase D; PtdIns 4-kinase, phosphatidylinositol 4-kinase; PRAF, PH domain, regulator of chromosome condensation (RCC) and FYVE; PX, Phox homology; RhoGAP, Rho GTPase activating protein; SNX, sorting nexin-like; START, stereoidogenic acute regulatory protein and phosphatidylcholine transfer protein.

Conclusions and perspectives

The *Arabidopsis* genome contains ~70 proteins with a predicted PH, FYVE or PX domain. Such lipid-binding domains are likely to function as effectors of lipid signalling by recruiting proteins to membranes where specific PPIs are synthesized, or by activating proteins via a conformational change, induced upon lipid binding. Although FYVE, and most PX, domains will probably bind PtdIns(3)*P*, it is still unknown for most *Arabidopsis* lipid-binding domains which phospholipid they bind to. To gain further insight into plant lipid signalling, this needs to be characterized. This might also result in identifying targets for the novel PPIs, such as PtdIns(5)*P* or PtdIns(3,5)P_2, or even sphingosine-1-phosphate [2,5,35], about which we know practically nothing.

To demonstrate the role of a domain, null mutants should be created in which the protein without the domain is reintroduced behind its own promoter. Alternatively, mutated versions could be introduced, such that lipid binding is abolished. Once the lipid-binding specificity of a domain is determined, it can be used as a biosensor. This can be achieved by cloning the domain behind a green fluorescent protein reporter and expressing it in cells or whole plants. The advantage is that real-time dynamics and the topography of lipid signalling can be studied in much more detail. Such insights would significantly increase our understanding of how lipids function in plant cell signalling.

We believe that the lipid-binding domains described here are only the tip of the iceberg. Already, several other PtdIns(4,5)P_2-binding domains are known to have *Arabidopsis* homologues, including Tubby, ENTH (epsin N-terminal homology), ANTH (AP180 N-terminal homology) and FERM (4,1-ezrin-radixin-moesin) [7]. Another intriguing domain with lipid-binding potential is the plant homeodomain (PHD) [36], which is a conserved Cys_4–His–Cys_3 orphan zinc finger present throughout eukaryotic proteomes in many chromatin regulatory

factors [37]. This domain in ING2 (a candidate tumour suppressor protein) has recently been shown to bind the novel phosphoinositide PtdIns(5)*P* within the nucleus of human embryonic kidney (HEK293) cells [38]. It has been postulated that PtdIns(5)*P* levels therefore regulate the association of ING2 with chromatin and thereby modulate cell death [38]. As such, PHDs might represent new effectors of nuclear PPI signalling [39]. Interestingly, the *Arabidopsis* genome contains ~150 proteins with a PHD.

PA is another emerging lipid second messenger in plant and animal signalling [5]. Several PA-binding proteins have already been identified, although a universal PA-binding domain has yet to be recognized [40,41]. Only for Raf-1 kinase, mTOR kinase and the cAMP phosphodiesterase PDE4 have short amino acid regions been identified that interact with PA [42]. An intriguing addition is the recent finding that the PX domain of $p47^{phox}$ has two basic pockets, one of which binds PtdIns(3,4)P_2, whereas the other binds PA [43]. Simultaneous occupancy of both sites by PtdIns(3,4)P_2 and PA leads to a great synergistic increase in membrane affinity. Recently, the PH domain of *Arabidopsis* PDK1 was also found to bind PA, activating the protein kinase AGC2-1 in a PDK1-dependent manner [19]. AGC2-1 is identical to OXI1, a protein kinase thought to mediate general plant stress responses [44].

Five homologues of the PA-binding region of Raf-1 kinase can be found in *Arabidopsis* (C. Testerink and T. Munnik, unpublished), of which CTR1, a negative regulator of ethylene responses, is the best known [45]. However, a connection still remains to be shown between PA and ethylene signalling.

It is clear that lipid-binding domains form an interesting new dimension to our understanding of how plants use the lipid language of signalling and therefore deserve intensive study.

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