Review: PIP kinases and their role in plant tip growing cells.

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Abstract

Phosphatidylinositol (4,5) bisphosphate, [PtdIns(4,5)P_2], is a signalling lipid involved in many important processes in animal cells such as cytoskeleton organization, intracellular vesicular trafficking, secretion, cell motility, regulation of ion channels, and nuclear signalling pathways. In the last years PtdIns(4,5)P_2 and its synthesizing enzyme, phosphatidylinositol phosphate kinase (PIPK), has been intensively studied in plant cells, revealing a key role in the control of polar tip growth. Analysis of the PIPK members from *Arabidopsis thaliana*, *Oryza sativa* and *Physcomitrella patens* showed that they share some regulatory features with animal PIPKs but also exert plant-specific modes of regulation. This review aims at giving an overview on the PIPK family from *Arabidopsis thaliana* and *Physcomitrella patens*. Even though their basic structure, modes of activation and physiological role is evolutionary conserved, modules responsible for plasma membrane localization are distinct for different PIPKs, depending on differences in physiological and/or developmental status of cells, such as polarized and non-polarized.

The *A. thaliana* PIPK family: Basis of structural properties in plant PIPKs

PtdIns(4,5)P_2 is synthesized by phosphorylation in the D-5 position of the inositol ring of phosphatidylinositol-4-phosphate (PtdIns4*P*), by PtdIns4*P* 5-kinases (PIPKs).[1] The basic structure shared by animal, yeast and plant PIPKs consists of a dimerization domain and a highly conserved kinase domain located at the C terminus (Fig 1A).[1] In addition, most plant PIPKs contain a unique conserved domain at the N terminus, the MORN domain (Membrane Occupation and Recognition Nexus) that is characterized by repetitions of MORN motifs[1] and followed by a non-conserved linker region (Fig 1A). MORN motifs that do not contain a PIPK catalytic domain have been found in several animal and plant proteins, such as junctophilins which participate in endomembrane to plasma membrane attachment;[2] the MORN1 protein of *Toxoplasma gondii* involved in cell-division;[3] and the *A. thaliana* accumulation and replication of chloroplasts 3 protein (ARC3) involved in plastidial fission.[4]

As shown in Figure 1B, *A. thaliana* contains eleven genes encoding type I/II A and B *PIPKs*. Subfamily A consists of two members, *AtPIP5K10* and *AtPIP5K11*, which lack the MORN domain and exhibit a domain structure similar to human type I PIPKs, whereas the other nine isoforms (*AtPIP5K1- 9*) in subfamily B contain the N-terminal MORN domain.[1]

***Physcomitrella patens* PIPK family**

Recently we have also proceeded with the characterization of the PIPK family in the moss *Physcomitrella patens*. *P. patens* has emerged as a model system in plant biology mainly due to its high frequency of homologous recombination which allows gene targeting, thus studying gene function by direct generation of loss-of-function and point mutations on the gene of interest.[5, 6] In contrast to the eleven PIPKs encoded by the *A. thaliana* genome, only two isoforms are present in *P. patens*, *PpPIPK1* and *PpPIPK2*, indicating a smaller gene family and less redundancy compared with flowering plants. Both PIPKs correspond to the subfamily B and no members for the A subfamily are present.[7] PpPIPKs amino acid sequences share 84.5% of identity, and their structure consist of eight MORN motifs at the N terminus, a linker region, and a dimerization domain followed by the catalytic kinase domain. Their kinase catalytic kinase domain is highly conserved when compared with PIPKs of other organisms, including flowering plant, human and yeast members. PpPIPK1 and PpPIPK2 belong to the clade containing AtPIP5K9 (Fig. 1B) and an identity of 55% and 57% with AtPIP5K9, respectively, among their kinase domains.

Activation modes of plant PIPKs

It has been shown that PtdIns4*P* is the preferred substrate *in vitro* for the synthesis of PtdIns(4,5)P_2 by all AtPIPKs; [8-12] this is also the case for PpPIPK1, but not for PpPIPK2, which *in vitro* prefers PI to produce PtdIns3*P*.[7] However, we have shown that *in vivo* both PpPIPK1 and PpPIPK2 catalyze the synthesis of PtdIns(4,5)P_2, since the knockout lipid profile showed a reduction only in PtdIns(4,5)P_2 in both *pipk1* and *pipk2* mutants.[13]

Likewise to animal PIPKs, AtPIP5K1 and PpPIPK1 are activated by phosphatidic acid (PA) *in vitro*.[7, 14, 15] Whereas it has been shown that the MORN domain of AtPIP5K1 binds PtdOH and is essential

for PtdOH activation,[14] the MORN domain does not affect PtdOH activation for PpPIPK1.[16] Another characteristic of type I PIPKs is their susceptibility to phosphorylation by protein kinase A, which has been shown for AtPIP5K1 and PpPIPK1.[7, 12]

All PIPKs have a region within the kinase domain known as the activation loop, which contains a conserved glutamic acid residue (Fig. 1C), responsible for the substrate specificity of animal type I PIPKs.[17] Similarly, the corresponding PpPIPK1E885A or AtPIP5K1E715A mutants showed an almost completely abolished activity towards PtdIns4*P* and PtdIns3*P*, but resulted in some activity with PtdIns5*P*, *in vitro*.[7] These results were confirmed *in vivo* by the fact that the phenotype of *P. patens pipk1* knockout could not be completely complemented by overexpression of *PpPIPK1E885A*.[13] In addition, the dibasic amino acid pair KR in the activation loop (Fig 1C) is also essential for the lipid kinase activity of PpPIPK1, as the mutation of KR to ND abolished activity towards PtdIns3*P* and PtdIns4*P*.[18]

What drives PIPKs to the plasma membrane?

PIPKs are recruited to membranes but they are not integral membrane proteins. Based on the structural characteristics, one can suggest that the MORN domain is responsible for the membrane localization. For example, the MORN domain is thought to be the plasma membrane localizing module for OsPIPK1, AtPIP5K1 and AtPIP5K3.[14, 19, 20]

Nevertheless, results obtained for AtPIP5K1, AtPIP5K2, AtPIP5K5, NtPIP5K6-1 and both PpPIPKs showed that there are other modules important for correct subcellular localization. [18, 21] In the case of PpPIPK1, we shown that the lipid kinase domain confers the plasma membrane localization through two positively charged amino acids (KR) conserved in the activation loop (Fig 1C).[18] Point mutations of the dibasic amino acid pair KR to ND in the activation loop of PpPIPK1 resulted in alternation of localization from the plasma membrane to the cytosol in *P. patens* protoplasts.[18] Importance of the kinase domain of AtPIP5K1 in plasma membrane localization was also demonstrated.[18] Interestingly, the kinase domain of AtPIP5K2 directs plasma membrane localization but not its apical localization in pollen tubes.[21] This suggests that more than one regulatory component determines apical plasma membrane localization in polarized cells. Whereas the kinase domain plays a role in recruitment of

the protein to every area of the plasma membranes, the apical localization needs an additional regulatory system collaborating with the kinase domain. In contrast, AtPIP5K5 and NtPIP5K6-1 require non-conserved linker (LIM) domain for correct localization in pollen tubes, as the deletion of the N-terminal and MORN domain did not affect their apical plasma membrane localization.[21] The kinase domain of these two PIPKs has no function in the regulation of subcellular localization in pollen tubes.

According to the findings described previously, it is possible that protein modules responsible for plasma membrane localization are distinct in each PIPK that depends on differences in physiological and/or developmental status of cells, such as polarized and non-polarized. It is therefore necessary to compare the function of the MORN, LIM and kinase domains of every PIPKs in cells exhibiting the same stage in development. However, one should also be aware of the difficulty to compare and generalise results obtained with different experimental as well as plant systems.

The activity of PIPKs and levels of PtdIns(4,5)P_2 in tip growing cells

In contrast to animal cells, cellular levels of PtdIns4*P* are much higher compared to PtdIns(4,5)P_2 in plants, highlighting a restriction step controlling PtdIns(4,5)P_2 levels by PIPKs, and thereby indicating the importance of PIPK regulation in physiological processes requiring PtdIns(4,5)P_2.[22]

In *A. thaliana* vegetative tissues under standard conditions, PtdIns(4,5)P_2 is hard to detect which may account for the scarce information available for such tissues. Specialized plant cells such as root hairs, pollen tubes and protonemal cells of mosses (all sharing the process of cell expansion by tip growth), have been used as preferable models for studying PIPK function for several reasons. PtdIns(4,5)P_2 was found to accumulate at the tip of these cells [9, 11, 13, 23] making its detection easier by the use of fluorescence markers fused to the pleckstrin homology (PH) domain of the human PLCd1, that specifically recognizes PtdIns(4,5)P_2.[24] These cells may have higher (and thus measurable) levels of PtdIns(4,5)P_2 under standard conditions due to their high signalling activities (perceiving and transducing extracellular stimuli). Furthermore, this kind of cells can be easily followed at a single cell level. The first reports indicating the presence of PtdIns(4,5)P_2 in membrane microdomains of pollen tubes or in the plasma membrane of root hair cell tips date from 1999.[25, 26] Since then it has

been demonstrated that different members of the *A. thaliana* and moss PIPKs family play a key role in tip growth.[9-11, 13, 19, 23, 27]

From the eleven PIPK isoforms present in *A. thaliana*, AtPIP5K3 is specific for root hairs. Multiple lines of *pip5k3* mutants exhibited reduced root hair growth, and overexpression of the gene in a wild type background resulted in deformed root hairs.[11] Interestingly, a *AtPIP5K3* mutated version lacking the N-terminal MORN domain, but with full catalytic activity *in vitro*, did not complement the phenotype of *pip5k3* mutants, and the overexpression of this construct resulted in deformed root hairs, meaning that AtPIP5K3 functionality in root hair development requires other factors in addition to the catalytic activity.[11] In pollen tubes, six different PIPK isoforms, *AtPIP5K10*, *AtPIP5K11*, *AtPIP5K2*, *AtPIP5K4*, *AtPIP5K5*, and *AtPIP5K6* are expressed. Despite their high similarity, different roles in tip growth have been attributed to them. For example, *AtPIP5K4* and *AtPIP5K5* are both expressed at the apical region of the plasma membrane of pollen tubes, and *pip5k4 -pip5k5* double mutants exhibited reduced pollen germination and defects in pollen tube elongation.[9, 23] Overexpression of *AtPIP5K4* or *AtPIP5K5* in tobacco pollen tubes cause severe growth defects, which were attributed to increased apical pectin deposition.[9, 23] AtPIP5K6 is localized at the subapical region of the plasma membrane of pollen tubes, and the suppression of *AtPIP5K6* expression by RNAi resulted in impaired tip growth and inhibited clathrin-dependent endocytosis.[27] In contrast to AtPIP5K4, AtPIP5K5 and AtPIP5K6 which belongs to the B subfamily, AtPIP5K10 and AtPIP5K11 of the A subfamily are localized to lateral subapical plasma membrane in tobacco pollen tubes. Phenotypes observed for these latter isoforms are remarkably different from those mentioned above. Pollen tubes of *pip5k10-pip5k11* double mutants, exhibited increased sensitivity to the actin polymerization inhibitor, latrunculin B, whereas overexpression of both enzymes in tobacco pollen tubes resulted in aggregation of the apical actin fine structure and a tip-swelling phenotype.[10] Overexpression of *AtPIP5K2:EYFP*, another type B isoform, resulted also in severe tip swelling of pollen tubes.[21] Thus the mechanisms of action of PtdIns(4,5)P_2 produced by PIPKs are different, some members affect membrane trafficking and secretion, whereas others affect the actin cytoskeleton. It has been suggested that the distinct localization patterns of the enzymes may be the consequence of interactions with specific partner lipids or proteins, which recruit the enzymes to different functional microdomains.[10] It is clear that it

is not the N-terminal MORN domain responsible for these differences in phenotypes observed between type A and B subfamilies, since overexpression of mutated isoforms lacking the MORN domain from AtPIP5K3 or AtPIP5K5 resulted in the same phenotypes as that of full-length proteins.[9, 11]

In *P. patens*, both PIPKs are expressed in protoplasts, protonema and gametophores.[7] The disruption of both genes by gene targeting showed that both enzymes are involved in tip growth. Wild type moss treated with F-actin drugs resulted in a phenotype that mimicked the *pipk* knockout phenotype, suggesting a role of PtdIns(4,5)P_2 in the cytoskeleton organization. This role was confirmed by *in vivo* imaging of the cytoskeleton network, which revealed that the shortened caulonemal cells in the *pipk1* mutant was the result of the absence of the apicobasal gradient of cortical F-actin cables normally observed in wild type caulonemal cells.[13] Despite the high similarity between both proteins, a strong phenotype for *pipk1* but not for *pipk2* single knockout was obtained.[13] *pipk1* knockout lines showed a dramatic growth reduction of protonema as well as of rhizoids. *pipk1-2* double knockouts showed a stronger phenotype compared to *pipk1*; protonemal filaments exhibited an extremely compact structure and lacked the caulonemal cell type; gametophores were much shorter than the wild type with very short rhizoids and could not produce sporophytes.[13] Phosphoinositide analysis of *pipk* mutants demonstrated that a reduction of PtdIns(4,5)P_2 levels was responsible for the phenotypes observed.

Future perspectives

During the last years, research about plant PIPKs has developed significantly. However, our knowledge about PIPKs and the function of its main product, PtdIns(4,5)P_2 is still limited. Despite the high similarity between PIPKs and their preference for PtdIns4*P*, at least *in vitro*, other roles aside from tip growth have been described. AtPIP5K9, was shown to interact with the cytosolic invertase CINV1 to regulate sugar-mediated root cell elongation negatively.[28] It has recently been shown that AtPIP5K2 is involved in regulating lateral root formation and root gravity response, through regulation of PIN proteins, providing a link between the phosphatidylinositol signaling pathway and auxin response.[29]

The function of the MORN domain still remains elusive[23], but it is not excluded that it could be important for interaction with other signalling components and aid in creating signalling complexes involving PIPKs. Supporting this idea, it was shown that AtPIP5K2 interacts with small GTPases of the RabE family through the MORN domain, which may stimulate temporally or spatially localized PtdIns(4,5)P_2 production at the plasma membrane.[30]

The different functions so far ascribed to plant PIPKs points to the following open questions demanding further investigation: 1) Is a specific function related to (or dependent on) the cell-type?; 2) Is it obtained through the interaction of individual PIPKs with different interaction partners?, or 3) Is function dependent on different pools of PtdIns(4,5)P_2 derived from different microdomains of the cell?

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Figure legends.

Figure 1.

(A) Modular structure of plant type I/II B PpPIPK1. N-terminal (N-ter), MORN motifs (1-8), linker (Lin), dimerization domain (Dim), PIPK catalytic kinase domain (PIPKc) and activation loop (al).

(B) Phylogenetic analysis of PIPKs. Maximum likelihood (ML) tree created with the full-length PIPKs sequences of *P. patens* and *A. thaliana.*

(C) Amino acid sequence alignment of the activation loop of *P. patens*¸ *A. thaliana*, and type I and type II *H. sapiens* PIPKs. The asterisks indicates conserved amino acids mentioned in this review. First, two conserved positively charged amino acids (KR or KK); second, (E or A), which are involved in substrate specificity; third, (K) which is involved in plasma localization of animal type I PIPKs.