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Metabolism and roles of phosphatidylinositol 3-phosphate in pollen development and pollen tube growth in Arabidopsis

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Phosphoinositides play important roles in eukaryotic cells, although they constitute a minor fraction of total cellular lipids. Specific kinases and phosphatases function on the regulation of phosphoinositide levels. Phosphatidylinositol 3-phosphate (PtdIns3*P*), a molecule of phosphoinositides regulates multiple aspects of plant growth and development. In this article, we introduce and discuss the kinases and phosphatases involved in PtdIns3*P* metabolism and their roles in pollen development and pollen tube growth in Arabidopsis.

Introduction

Phosphatidylinositols (PtdIns) are minor components in the eukaryotic cell membrane. The inositol ring of PtdIns can be phosphorylated at D3, D4, and D5 by specific phosphoinositide kinases, resulting in formation of phosphatidylinositol phosphate (PtdIns*P*), phosphatidylinositol bisphosphate (PtdInsP_2) and phosphatidylinositol trisphosphate (PtdInsP_3). These inositol phospholipids are called phosphoinositides (PIs). The lipid phosphatases remove the D3, D4, or D5 phosphates from these PIs to establish PI homeostatic levels in cell. Each PI isoform shows a specific pattern of intracellular distribution.

Fluorescence protein labeling has revealed that phosphatidylinositol 3-phosphate (PtdIns3*P*) mainly distributes in late endosomes, multivesicular bodies, and prevacuolar membranes.[1,2] PIs themselves or serving as precursors for second messengers regulate plant growth and development, such as pollen development, pollen hydration and germination, pollen tube growth, root hair elongation, and stomatal movement modulation.[3-12] Here, we focus on reviewing the functions of kinases and phosphatases in the PtdIns3*P* metabolism involved in pollen development, germination, and pollen tube growth in Arabidopsis.

The conversion between PtdIns and PtdIns3*P*

PtdIns can be phosphorylated to PtdIns3*P* by class III PtdIns 3-kinases (PtdIns3K) (**Fig. 1**). VPS34 is the first identified class III PtdIns3K in yeast with lipid kinase activity.[13] Two kinds of PtdIns3K complexes were identified in yeast and mammals. Complex I is composed of vacuolar protein sorting-associated protein (VPS) 34, VPS15, VPS30/autophagy-related protein (ATG) 6/coiled-coil myosin-like BCL2-interacting protein (BECLIN) 1 and ATG14, and regulates vesicle nucleation in autophagy. Complex II consists of VPS34, VPS15, VPS30/ATG6/BECLIN 1 and VPS38/UV irradiation resistance-associated protein (UVRAG), and functions on hydrolase sorting through the vacuolar protein sorting (VPS) pathway.[14,15] By interacting directly with VPS34, VPS15 regulates the membrane targeting and activity of VPS34.[16-18] VPS30/ATG6 was identified by searching AuTophaGy-related mutants in yeast. Mammalian Beclin1 is a homolog of yeast ATG6.[19] Although Vps30/Atg6/Beclin1 is found to be involved in autophagy and vesicular trafficking, its roles in PtdIns3K complexes are little known.[20,21] ATG14 together with ATG6 mediates the localization of complex I and other ATG proteins on phagophore assembly site (PAS).[22] VPS38 serves as a bridge between VPS34-VPS15 and VPS30, and localizes complex II to endosome.[22,23]

AtVPS34, AtVPS15 and AtVPS30 in Arabidopsis are the homologs of yeast VPS34, VPS15 and VPS30 (**Table 1**). The knockout of *AtVPS34* in Arabidopsis plants leads to pollen failure in undergoing normal fission after the first mitotic division. Although the morphology of the *atvps34* mutant is normal, its pollens display defects in vacuolar morphology and cannot germinate.[8] Either *atvps15* or *atvps30* mutant also shows abnormal pollen germination. The defect of *atvps15* pollen germination can be restored partially after applification of PtdIns3*P* in vitro.[11,24-26] Therefore, AtVPS34, AtVPS15 and AtVPS30 might be the components of PtdIns3K complex in plant cells as in yeast, and function in PtdIns3*P* generation and pollen tube growth. PtdIns3*P* is mainly localized in endosomes and vacuolar membrane in plant cells.[2,7] Although both *atvps15* and *atvps30* mutants displays abnormal germination of pollen, no vacuolar

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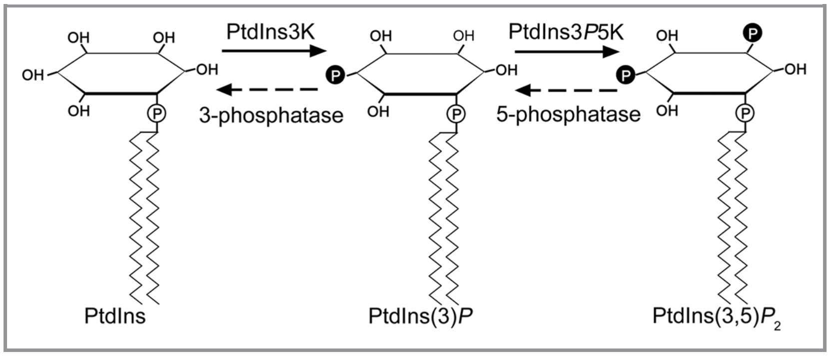

Figure 1. Metabolism of Phosphatidylinositol 3-phosphate. PtdIns3K, PtdIns 3-kinase; PtdIns3*P*5K, PtdIns3*P* 5-kinase.

phenotype in *atvps30* pollen was observed.[24-26] It is likely that AtVPS30 in the PtdIns3K complex are not involved in PtdIns3*P* metabolism. Alternatively, AtVPS15 and AtVPS34, but not AtVPS30, may regulate vacuolar development in pollen development. It was reported that AtVPS30 is co-localized with ATATG8, a PAS marker protein in Arabidopsis.[24] AtVPS30 may be involved in vesicular trafficking by recruiting the PtdIns3K complex to the putative PAS in Arabidopsis plant cells, which is required for pollen germination and pollen tube growth. Three putative homologs of *ATG14/UVRAG* were identified in Arabidopsis (gene ID: AT2G32760, AT4G08540, AT1G77890; **Table 1**) according to their putative protein containing the conserved ATG14 domain. These indicate that class III PtdIns3K complex is conserved in animals, yeast and plants. Two genes (ID: AT4G08540, AT1G77890) are predicated to encode the DNA-directed RNA polymerase. Another gene's (ID: AT2G32760) function is unknown although its expression is detected during pollen development (bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi). The components of class III PtdIns3K complex and their functions remain to be investigated.

PtdIns3*P* 3-phosphatases can cleave the 3-phosphate from PtdIns3*P* and form PtdIns (**Fig. 1**).[27] The level of PtdIns3*P* decreases in yeast that expresses myotubularin-related protein (MTMR) 3, a human PtdIns3*P* 3-phosphatases belonging to the myotubularin family.[28] Myotubularins (MTMs) is a lipid phosphatase family with specificity for PtdIns3*P* and phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P_2) as substrates.[29] Yeast myotubularin-related protein (YMR) 1, the only myotubularin 3-phosphatase in yeast, corporately functions with synaptojanin-like phosphatase synaptojanin-like protein (SJL) 3 in regulating the localization and level of PtdIns3*P*.[30] In human, 14 members of myotubularin family PtdIns3*P* 3-phosphatases were identified, and their mutation led to human diseases.[31] ATMTM1 and ATMTM2 in Arabidopsis are myotubularin family homologs (**Table 1**).[32,33] ATMTM1 dephosphorylates for PtdIns3*P*, however its phosphatase activity is higher with PtdIns(3,5)P_2 as substrate than with PtdIns3*P* in vitro. Gene expression analysis reveals that the transcription of *AtMTM1* increases significantly after drought stress, indicating its function might be involved in a drought responsive pathway.[32] *ATMTM2* shows high expression level in pollen (www.bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi), but its roles in pollen development and pollen dehydration remains to be established.

PTEN/MMAC (a phosphatase and tensin homolog / mutated in multiple advanced cancers), dephosphorylating the D3 phosphate group of phosphatidylinositol 3,4,5-trisphosphate (PtdIns (3,4,5)P_3), functions in autophagy of mammalian.[34,35] In Arabidopsis genome, there are three putative *PTEN* homologs, *AtPTEN1*, *AtPTEN2A* and *AtPTEN2B*, and they show high expressions in pollen.[36,37] The silencing of *AtPTEN1* by RNA interference led to aborted pollen grains.[36] Additionally, the overexpression of either *AtPTEN1* or *AtPTEN2A* induces male sterility.[12,37] Although AtPTEN1 can dephosphorylate PtdIns(3,4,5)P_3 in vitro, it still can bind PtdIns3*P*. The overexpression of *AtPTEN1* caused the accumulation of autophagic bodies in tobacco pollen tube, which can be inhibited by exogenous PtdIns3*P* or by the expression of *AtVPS34*.[12] Thus, AtPTEN1 regulating PtdIns3*P* metabolism is involved in the autophagy in pollen tube.

The Conversion between PtdIns3*P* and PtdIns(3,5)P_2

Class III PtdIns3*P* 5-kinases (PtdIns3*P*5K) catalyze the phosphorylation of PtdIns3*P* to PtdIns(3,5)P_2 (**Fig. 1**) that plays vital roles in endosomal trafficking.[38] FYVE (FAB1, YGL023, VPS27 and EEA1) domain-containing proteins, yeast formation of haploid and binucleate cells 1 (FAB1) and mammal FYVE finger-containing phosphoinositide kinase (PIKfyve), exhibit PtdIns3*P*5K activity.[39] Mutation of FAB1 reduces PtdIns(3,5)P_2 level in yeast. The PtdIns3*P*5K activity of FAB1 is required for cargo-selective sorting into the vacuolar lumen in yeast. PIKfyve regulates the retrograde traffic from endosome to trans-Golgi-network.[40,41] Arabidopsis genome contains two putative *FAB1/PIKfyve* homologs, *AtFAB1A* and *AtFAB1B*.[42] The pollen of the double mutant *atfab1a atfab1b* is lethal and shows severe defects in vacuolar organization.[43] The enhanced or reduced levels of *AtFAB1A/B* expression interfere the endomembrane homeostasis including endocytosis, vacuolar formation and acidification.[44] In yeast, hyperosmotic stress increases the level of PtdIns(3,5)P_2 that plays roles in membrane trafficking in the endosomal/lysosomal system.[38] Endocytosis is also involved in pollen tube growth.[45] Therefore, PtdIns(3,5)P_2 might play roles through similar mechanism in pollen tube growth with yeast hyperosmotic tolerance.

FAB1/PIKfyve forms a protein complex with factor-induced protein (FIG) 4/suppressor of actin (SAC) 3, vacuolar segregation protein (VAC) 7, ATG18 and VAC14 in yeast.[46,47] Homologs of the subunits of the FAB1/PIKfyve complex except VAC7 were identified in Arabidopsis (**Table 1**). Although VAC7 is a FAB1 activator in PtdIns(3,5)P_2-synthesizing complex, hyperosmotic shock still induces the increase in PtdIns(3,5)P_2 levels in the absence of VAC7 in yeast.[48] Thus, VAC7 is not necessary in this complex for PtdIns(3,5)P_2 synthesis and turnover. Whereas, FIG4 itself can function in both PtdIns(3,5)P_2 synthesis and turnover in yeast. Function of the most Arabidopsis homologs of

Table 1. Enzymes and regulators for PI3*P* metabolism in *Saccharomyces cerevisiae* and *Homo sapiens*, and their homologs in Arabidopsis.

	S. cerevisiae	*H. sapiens*	Arabidopsis			
			Protein	**Gene ID***	**Physiological functions**	**References**
PtdIns3K complex	VPS34	hVPS34	AtVPS34	**AT1G60490**	Pollen development and germination	5, 7, 8, 61
	VPS15	hVPS15	AtVPS15	**AT4G29380**	Pollen development and germination	11
	VPS30/ATG6	BECLIN 1	AtBECLIN/AtATG6/AtVPS30	**AT3G61710**	Pollen germination	24, 25, 26
	ATG14	hATG14	AtATG14	**AT2G32760**	ND	
	VPS38	UVRAG				
FAB1/PIKfyve complex	FAB1	PIKfyve	AtFAB1A AtFAB1B	**AT4G33240** **AT3G14270**	Pollen development	43, 44
	VAC14	hVAC14	AtVAC14	AT2G01690	ND	62
	ATG18	hATG18/ WIPI	AtATG18A AtATG18B AtATG18C AtATG18D AtATG18E AtATG18F AtATG18G AtATG18H	AT3G62770 AT4G30510 AT2G40810 **AT3G56440** AT5G05150 AT5G54730 AT1G03380 **AT1G54710**	Nutrient stress and senescence	63
	FIG4					
	VAC7	ND	ND			
PtdIns3*P* 3-phosphatases	YMR1	MTMs MTMRs	ATMTM1 ATMTM2	AT3G10550 AT5G04540	Dehydration stress	32, 64
	TEP1 CDC14	hPTEN/MMAC1	AtPTEN1 AtPTEN2A AtPTEN2B	**AT5G39400** **AT3G19420** **AT3G50110**	Pollen development and pollen tube growth	12, 36, 37
PtdIns(3,5)P_2 5-phosphatases and regulator	FIG4 SAC1 SJL1 SJL2 SJL3	hSAC1 hSAC2 hSAC3 Synaptojanin1 Synaptojanin2	AtSAC1 AtSAC2 AtSAC3 AtSAC4 AtSAC5 AtSAC6 AtSAC7 AtSAC8 AtSAC9	**At1G22620** **At3G14205** At3G43220 **At5G20840** At1G17340 **At5G66020** **At3G51460** At3G51830 **At3G59770**	Pollen development; Cell wall thickening; Root hair development; Stress response	49, 54, 55, 56, 57, 58
	VAC14	hVAC14	AtVAC14	AT2G01690	ND	62

*The bold gene ID indicating the gene has high expression in pollen according to bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi. ND, not determined.

FAB1/PIKfyve complex components are not investigated, except that the FIG4 homolog (gene ID: At5G66020, **Table 1**) was found to be involved in pollen development.[49]

PtdIns(3,5)P_2 5-phosphatase hydrolyzes the 5-phosphate of PtdIns(3,5)P_2 to form PtdIns3*P* (**Fig. 1**). In yeast, the level of PtdIns(3,5)P_2 significantly increases after hyperosmotic stress, and then, it decreases to the basal level after 30 min. It was found that yeast FIG4, a SAC phosphatase domain-containing protein, functions in this process.[50] In the cells lacking FIG4, PtdIns(3,5)P_2 level shows a little decrease after hyperosmotic shock. Other proteins, SAC1, SJL1, SJL2 and SJL3, also show similarity with FIG4 in yeast.[51] Synaptojanin is the mammalian phosphoinositide 5-phosphatases, such as hSAC1, hSAC2, hSAC3, Synaptojanin 1 and Synaptojanin 2 in human. The loss of function of *hSAC3*, a homolog of *FIG4* in human, reduces the level of PtdIns(3,5)P_2 and leads to neuronal degeneration.[52,53] Arabidopsis genome encodes nine putative SAC domain-containing proteins (AtSACs) that can be classified into three classes: AtSAC1–5 are similar to yeast FIG4, AtSAC6–8 are similar to yeast SAC1, and AtSAC9 contains unique domains (**Table 1**).[54] AtSAC1 exhibits phosphatase activity with PtdIns(3,5)P_2 as the substrate. Mutation of *AtSAC1* causes a decrease in the wall thickness, and results in a weak stem phenotype.[49] However, AtSAC7 (ROOT HAIR DEFECTIVE 4) and AtSAC9 all display a preference for phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) as substrate in vitro.[55,56] Mutation of both *AtSAC7* and *AtSAC9* lead to over-accumulation of PtdIns(4,5)P_2, resulted in root hair-defect and constitutive expression of the stress-response genes. Five *SAC*-like genes (gene ID At1G22620, At3G51460, At3G59770, At3G14205, At5G20840 and At5G66020) show high expression level in Arabidopsis pollen (**Table 1**).[57] Only the function of *AtSAC6* was found to be involved in β-aminobutyric acid-induced pollen sterility.[58]

Conclusion and Perspective

Although some information has been accumulated on understanding for PtdIns3*P* metabolism in Arabidopsis, the functions of itself and its products remain to be investigated in the development and transmission of male gametophyte.[59] Arabidopsis genome encodes the homologs of the binding proteins of PtdIns(3,5)P_2 and PtdIns3*P*, such as Arabidopsis β-propellers that binds phosphoinositides, epsin-like protein EpsinR2, and sorting nexin 2b, however, their function is still unknown in plant.[60] Further investigations on PtdIns3*P* metabolism will provide a deeper insight for the function of phosphoinositides in plant reproductive development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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