Invited Mini Review

Biochemistry and structure of phosphoinositide phosphatases

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Phosphoinositides are the phosphorylated derivatives of phosphatidylinositol, and play a very significant role in a diverse range of signaling processes in eukaryotic cells. A number of phosphoinositide-metabolizing enzymes, including phosphoinositide-kinases and phosphatases are involved in the synthesis and degradation of these phospholipids. Recently, the function of various phosphatases in the phosphatidylinositol signaling pathway has been of great interest. In the present review we summarize the structural insights and biochemistry of various phosphatases in regulating phosphoinositide metabolism. [BMB Reports 2013; 46(1): 1-8]

INTRODUCTION

Phosphoinositides, the phosphorylated derivatives of phosphatidylinositol (PtdIns) (Fig. 1), are negatively charged phospholipids that comprise less that 10% of the total phospholipids in eukaryotic cells (1). PtdIns in mammalian cells shows molecular diversity, and includes PtdIns-3-P, PtdIns-4-P, PtdIns-5-P, phosphatidylinositol-3,5-bisphosphate (PtdIns-3,5-P_2), PtdIns-4,5-P_2, PtdIns-3,4-P_2, and phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P_3). These govern a range of cellular processes, including signal transduction, intracellular membrane trafficking, cytoskeleton remodeling, nuclear events, cell growth, survival, etc. (1, 2). In the resting state of cells, PtdIns-3-P, PtdIns-4-P, and PtdIns-4,5-P_2 are regulated tightly by different kinases and phosphatases. PtdIns-5-kinase and PtdIns-4-kinase use PtdIns-4-P and PtdIns-5-P as substrate to produce PtdIns-4,5-P_2, which serves as a substrate for activated phospholipase C, and the secondary messenger molecules, diacylglycerol and Ins-1,4,5-P_3 (IP$_3$), are generated, which are important to induce receptor-mediated signal transduction. PtdIns-3-P, PtdIns-3,4-P_2, and PtdIns-3,4,5-P_3 are produced by the action of a large protein family of class I, II and III phosphoinositide-3-kinases in response to certain signals (2-4, Fig. 2). In this review, recent progresses in biochemical and structural studies on phosphoinositide phosphatases are discussed.

PHOSPHOINOSITIDE 3-PHOSPHATE PHOSPHATASES

Phosphatase and tensin homologue, deleted on chromosome 10 (PTEN)

Tumor suppressor PTEN, also known as MMAC or TEP-1, is located in a region of chromosome 10q23, which shows loss-of-heterozygosity (LOH) in human tumors (1, 5). PTEN can also be inactivated by other mechanisms in somatic cancers (for review, see ref. 6). It shows a dual-specificity protein/lipid phosphatase. The lipid phosphatase activity of PTEN is observed toward PtdIns-3-P, PtdIns-3,4-P_2, and
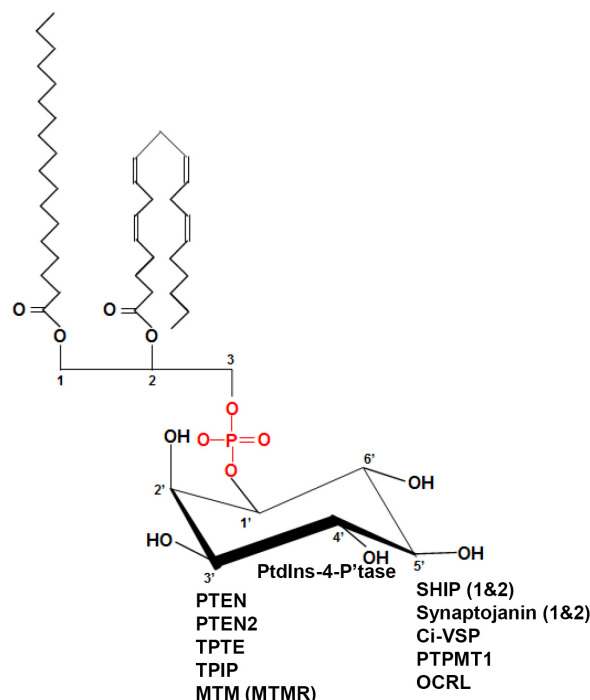
Fig. 1. Chemical structure of PtdIns. The hydroxyls at positions D-3, D-4, and D-5 of the inositol head-group can be phosphorylated separately and in all combinations to generate seven PtdIns. Under each hydroxyl groups, typical phosphatases specific for the phosphorylated form are represented.

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PtdIns- 3,4,5-P₃, and hydrolyzes the D-3 position phosphate of these lipid molecules (1, 3, 5). Considerable biochemical and genetic studies have demonstrated that PTEN is the central negative regulator of PI3K/AKT-mediated signaling. PTEN functions as a highly effective tumor suppressor in a wide range of tissues (7). PTEN binds directly to plasma membrane lipids, and performs different cellular functions, including cell proliferation, cell survival, and cell migration in animal cells, regulation of cell size in Drosophila eye development, and it is involved in sporulation in *Saccharomyces cerevisiae* (5, 8). Study on the crystal structure has revealed a two-domain organization of PTEN, with an N-terminal domain (five standard central β-sheets surrounded by six α-helices) containing both enzymatic and cellular localization activities, which is connected via a flexible internal loop to the C-terminal C2 domain (two antiparallel β-sheets linked by two short α-helices) with a class I PDZ binding motif at the C-terminal tail (Fig. 2) (5, 9). This N-terminal domain contains an HCXXGXXR motif in a P loop (123-130 residues) at the bottom of the active site (5, 9). Another anion-binding site, called the TI loop in the phosphatase domain, may also be required for phosphatase activity (5, 9).

The C2 domain facilitates effective membrane association and activation of the phosphatase by presenting a short stretch of amino acids in the N-terminus (5, 9, 10). The C-terminal tail mainly contains three phosphorylation sites, a PDZ binding motif, and two PEST sequences (5, 7). In addition, two mechanisms of PTEN degradation corresponding to PTEN protein stability have been identified: cleavage by caspase 3 and ubiquitination by NEDD4-1 (11, 12). Initial studies have shown that the expression of PTEN induces a decrease in proliferation because of cell cycle arrest in the G1 phase, which is attributed to an increase of p27[Kip1] and decreased level and nuclear localization of cycline D1 (13). PTEN also inhibits the migration of cells, likely by the involvement of Rac and cdc42, but not of RhoA (14, 15). However, PTEN is not dedicated to inhibiting the PI3K/Akt pathway, but also inhibits other growth pathways through protein-protein interactions, thus becoming a major growth-signaling inhibitor. Recently, Bahk et al. demonstrated the proteome profile changes evoked by the expression of PTEN in NIH/3T3 and U87-MG cells and the discrimination of the lipid and protein phosphatase activity by proteomic techniques (16, 17).
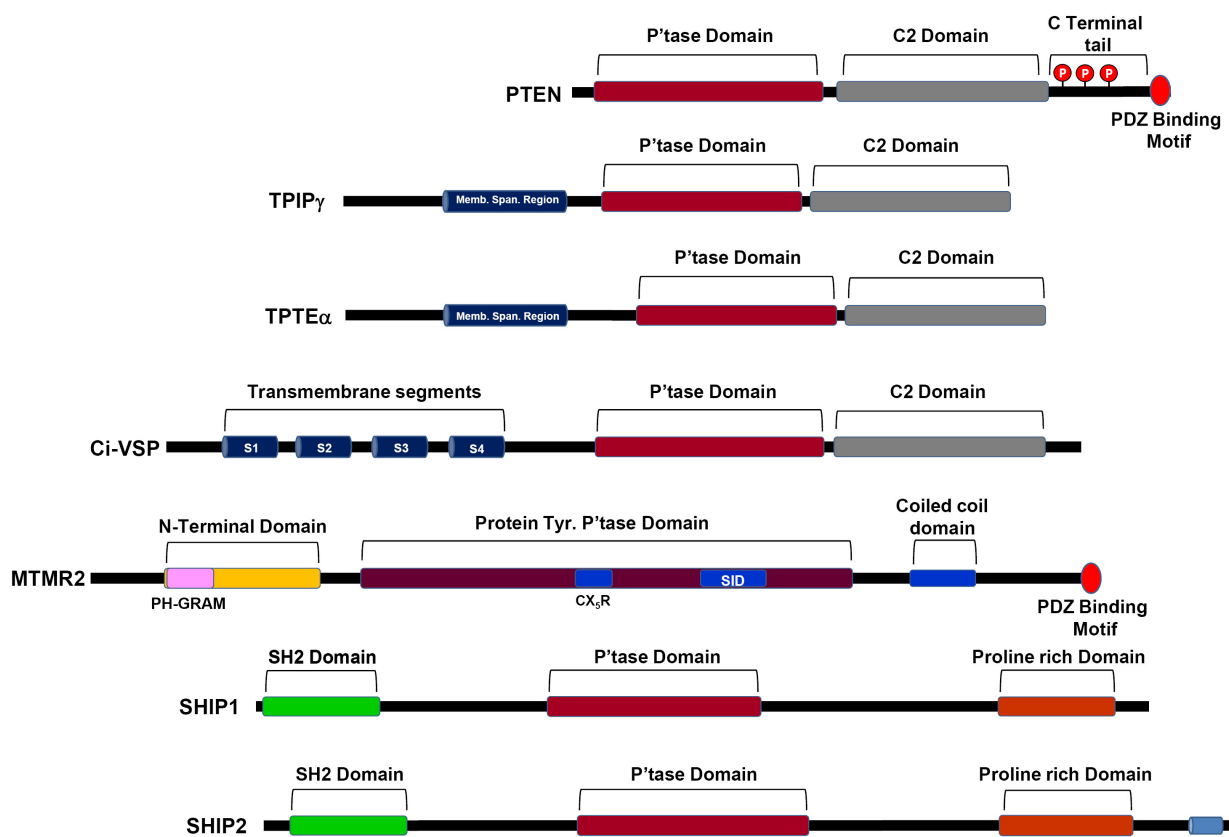

Fig. 2. Domain organization of PtdIns phosphatases. The domain structures of PTEN, TPIPγ, TPTEα, Ci-VSP, MTMP2, SHIP1, and SHIP2.

PTEN2, trans-membrane phosphatase with tensin homologue (TPTE) and TPTE and PTEN homologous inositol-lipid phosphatase (TPIP)

PTEN2, trans-membrane phosphatase with tensin homologue (TPTE), and TPTE and PTEN homologous inositol-lipid phosphatase (TPIP), are mammalian homologue proteins with primary sequence similarity to PTEN (1, 18). Murine PTEN2 shows 39% sequence homology to PTEN, and a testis-specific protein primarily in secondary spermatocytes and early spermatids. It is a membrane-associated molecule that passes through the secretory pathway and is localized to the Golgi apparatus via N-terminal membrane-spanning regions. Many of the catalytic residues and substrate-specific residues, which are important in maintaining the catalytic and substrate-recognition functions of PTEN, are conserved in PTEN2, retain the same substrate specificity and binding mode as that proposed for PTEN (19). TPTE, which maps to the short arm of chromosome 21 in humans, is a testis-specific protein. There are four isoforms of TPTEα, β, γ, and δ. TPTEα, TPTEβ, and TPTEγ contain four transmembrane domains, followed by the catalytic domain (20). The TPIP proteins, another functional TPTE gene mapped on chromosome 13, are expressed as three predominant isoforms, designated as TPIPα, TPIPβ, and TPIPγ. TPIPα shares homology to PTEN within the phosphatase and C2 domains, whereas TPIPβ has only a partial C2 domain. TPIPα has three putative transmembrane domains in the N-terminal, but TPIPβ retains no transmembrane domain, and thus cannot attach firmly to membranes (20). TPIP has a wider tissue distribution than the testis-specific TPTE (21). PTEN2 shows strong phosphatase towards PtdIns-3,5-P_2 > PtdIns-3,4,5-P_3, and weak activity towards PtdIns-3-P and PtdIns-3,4-P_2. TPIPα exhibits clear enzyme activity towards PtdIns-3,4,5-P_3 > PtdIns-3,5-P_2 > PtdIns-3,4-P_2 > PtdIns-3-P, whereas TPIPβ does not show any preferable phosphoinositide phosphatase activity (1, 18). Although TPIPα has phosphoinositide 3-phosphatase activity, it does not regulate the PtdIns-3,4,5-P_3-dependent phosphorylation of PKB (22). Although Chen *et al.* referred to TPTEα as the orthologue of murine PTEN2, it does not have phosphoinositide phosphatase activity or specificity (23). This is probably due to the location of Thr-Asp in TPTE in place of Lys-Gly residues of the active site of PTEN, since mutation in these residues in PTEN alters its substrate specificities (1, 18).

Myotubularins

The myotubularin family of phosphoinositide phosphatases includes 15 members (namely MTM1 and MTMRs 1-14) in humans that are mutated in neuromuscular diseases, or associated with metabolic syndrome, obesity, and cancer (22, 24). They share the Cys(Xxx)$_5$Arg motif with the tyrosine/dual- specificity phosphatase, with peculiar specificities towards PtdIns- 3-P and PtdIns-3,5-P_2, which in turn regulates endocytosis and membrane trafficking (25-27). Recent findings suggest that they regulate additional processes, including cell proliferation and differentiation, autophagy, cytokinesis, and cytoskeletal and cell junction dynamics (22). The initial member of this group is the MTM1 gene, the mutation of which causes X-linked myotubular myopathy, a severe congenital disorder in muscle cell development. Among these 15 members, six genes (*MTMR5, MTMR9-13*) encode catalytically inactive protein, as the protein contains substitutions in the Cys and Arg residues within the Cys(Xxx)$_5$Arg (25, 28). All MTM proteins except MTMR14 have four functional domains that mediate protein-protein and protein-lipid interactions: PH-GRAM (Pleckstrin homology-glucosyltransferases, rab-like GTPase activators and myotubularins), RID (Rac-induced recruitment), PTD (protein tyrosine phosphatase domain), and SID (SET-interacting domain). The PH-GRAM domain binds to phosphoinositides. The RID domain has a membrane-targeting motif, whereas the SID domain mediates protein-protein interactions. The Cys(Xxx)$_5$Arg protein tyrosine phosphatase active site is found within the PTD domain. In addition, most MTM proteins also contain a coiled-coil domain and a PDZ-binding motif (25, 28). The crystal structure of MTMR2 reveals a domain organization consisting of a 112-residue N-terminal domain and a 376-residue C-terminal domain. The N-terminal domain contains a GRAM domain sequence as a part of a larger motif with a pleckstrin homology (PH) domain fold, and was characterized as a putative novel domain in glycosyltransferase, rab-like GTPase activator, ABA-responsive element binding protein, and myotubularins. The structural features suggested that GRAM as a 70-residue motif consists of four β strands followed by an α helix (29). Although the function of the PH-GRAM domain of myotubularins is not clear, it may play a role in membrane targeting by specifically binding to PtdIns-5-P and PtdIns-3,5-P_2. Another function of the PH-GRAM domain may be in PtdIns-3-P/PtdIns-5-P-mediated oligomarization and PtdIns-5-P specific allosteric activation of the myotubularin family, and the binding of the PH-GRAM domain with phosphoinositides has been proposed to intercede the effect (25, 29). The C-terminal of MTMR2 containing a phosphatase domain also known as the PTP domain has structural similarity to that of other PTP superfamily members, but is much larger, with about 375 residues. Two additional structural elements, the SID of a putative protein-protein interaction domain and the RID of a putative membrane-binding motif, are unique features of myotubularins, and any alteration of these regions causes both disruption of the core structure and functional integrity of the protein (25, 29). The entire PTP domain structure of MTMR2 is similar to that of PTP super family members. However, it differs in that the substrate binding pocket of MTMR2 is significantly large in both depth and width, and lacks an aspartic acid in the 'WPD' loop, which is found in PTEN and is necessary for catalytic function. These structural features suggest that they may utilize a distinctive, unique mechanism during catalysis (29). The positively charged membrane-proximal surface can create electrostatic interaction with negatively charged PtdIns, and the larger substrate binding pocket of the protein allows contact with the larger head group of PtdIns (25). Most myotubularins may contain some additional domains, for which relatively little is

known about their function, but they are probably required for function of the protein. Some examples of such domains are coiled-coil motifs, which may be involved in arbitrating interaction between active and inactive family members; FYVE domains in MTMR3 and MTMR4 are a binding module of PtdIns3P and may be involved in the localization of host protein; MTMR5 and MTMR13 contain DENN and PH domain, which probably acts as a GTPase effector domain, and as a PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 binding domain, respectively (25). The inactive form of myotubuarins was previously thought to act as anti-phosphatases, which might prevent the phosphorylation of substrate via binding with them. The identification of some inactive and active family members together in a number of studies revealed the function of inactive myotubularins in the localization and enhancement of catalytic activity of active myotubularins, which suggests that both family members are probably involved in the same signaling pathway (25). Myotubularins also utilize PtdIns-3-P and PtdIns-3,5-P_2 as a substrate.

A number of studies have shown the relevance of myotubularin gene mutation with different human diseases and mouse phenotypes. Mutated MTM1 was identified in XLMTM disease, which may be caused by a defect either in late muscle maturation or in the structural organization of the fibers (26, 28). Truncating mutation in MTMR2 causes CMT4B1, a disease characterized by the presence of foci of abnormally folded myelin sheaths and Schwann cell proliferation in peripheral nerves. *Mtmr2*-deficient mice showed a very similar phenotype to CMT4B1 (26, 28). As the mutated disease-causing MTMR2 lost its phosphatase activity, CMT4B1 probably resulted from the tainted regulation of PtdIns-3-P and/or PtdIns-3,5-P_2 (28, 29). Overexpression of MTMR6 hinders the activity of calcium-activated potassium channels (type Kca3.1) that are normally activated by PtdIns-3-P in lymphocytes, epithelia, and other cells, suggesting the involvement of mutated MTMR6 in autoimmune disease (28).

PHOSPHOINOSITIDE 4-PHOSPHATE PHOSPHATASES

PtdIns-4-P has been considered for many years to be just the precursor of PtdIns-4,5-P_2. Over the last decade, however, evidence has accumulated that shows that PtdIns-4-P is, in its own right, a key regulator of pivotal cell functions (29). The PtdIns-4-P phosphatase family has been implicated in the regulation of PI3-kinase, and comprises two widely expressed isoforms (type I and II) (30). Two types of PtdIns-4-P phosphatase transcripts are alternatively spliced, resulting in the expression of proteins with putative transmembrane domains near their C-termini (31). The activity of PtdIns-4-P-phosphatases toward PtdIns-4-P is greater than that towards PtdIns-3,4-P_2, and to a much lesser extent, towards Ins-3,4-P_2 and Ins-1,3,4-P_3. A single base deletion in the gene inositol polyphosphate-4- phosphatase type I (*Inpp4a*) causes extensive neurodegeneration in the cerebellum, especially in Purkinje cells. The *weeble* mu-

tant mouse has a frame shift mutation in this gene, which is characterized by early-onset cerebellar ataxia and neurodegeneration (32). In addition, some investigation suggests a role for inositol phosphate phosphatase in the virulence of bacterial infections. In *Shigella flexneri*, one of these virulence factors is invasion plasmid gene D (*IpgD*), which contains two motifs related to the mammalian inositol 4-phosphatase. *Salmonella Dublin* and *S.typhimurium* secrete SopB and SigD, respectively, which are homologues of IpgD (3). In mammals, two orthologues of *IpgD* have been identified. Although the human phosphatases and *IpgD* do not show marked similarity, both have PtdIns-4,5-P_2 4-phosphatases activity.

PHOSPHOINOSITIDE 5-PHOSPHATE PHOSPHATASES

This enzyme family consists of 10 mammalian and four yeast members, which are involved in a variety of cellular events, such as protein trafficking, phagocytosis, and synaptic vesicle recycling (1, 33-35), and contains a central catalytic '5- phosphatase' domain with two motifs: (F/I)WXGDXN(F/Y)R and (R/N)XP(S/A)(W/Y)-(C/T)DR(I/V)(L/I), except for 5-phosphatase I (1, 3, 36).

The SH2 domain containing 5-phosphatase (SHIP1 & SHIP2)
This enzyme is a hemopoietic-specific inhibitory molecule that plays a regulatory role in B cells, T cells, macrophages, and mast cells (37, 38). This group of phosphatase shows Ins-1,3,4, 5-P_4, PtdIns-4,5-P_2, and PtdIns-3,4,5-$P_3$5-phosphatase activity (39). SHIP1 is a 145-kDa protein that was first purified from a Grb2- and Shc-rich complex of murine hematopoietic cells (1, 3, 40, 41). A related 142-kDa protein SHIP2 is expressed in different tissues in humans and rats (1, 3, 42). SHIP1 contain an N-terminal SRC homology 2 (SH2) domain, a central catalytic 5-phosphatase domain that contains the two highly conserved signatures of inositol polyphosphate 5-phosphatases, a C-terminal proline-rich domain (PRD) with consensus sites for SH3 domain interactions, and an N-terminal proline-rich sequences and two potential phosphotyrosine binding (PTB) domain-binding sites (NPXY) (1, 3, 43). The Pro-rich domains also contain glutamate within PEST sequences, which are believed to be involved in the putative signal for rapid intracellular proteolytic degradation (43). SHIP2 shares the same domain structure of SHIP1, with the exception that SHIP2 contains one potential phosphotyrosine-binding (PTB) domain-binding site (NPXY) instead of two NPXY, and holds a distinctive C-terminal sterile α motif (SAM) domain (1, 3, 44). The sterile α motif (SAM) domain found in numerous signaling proteins, like tyrosine and serine/threonine protein kinases, are involved in protein-protein interaction (45). SHIP1 plays an important role in the immune system and in platelet function, where they are involved in B cell negative signaling by recognizing the tyrosine-based inhibitor motif (ITIM) of immmunoreceptor FcγRIIB via target motif (pY) and SH2 domain interaction, where it converts PtdIns-3,4,5-P_3 to PtdIns-3,4-P_2. This

results in the prevention of PtdIns-3,4,5-P$_3$ induced Btk activation at the membrane activation site, and thus both intracellular Ca$^+$ influx and B cell growth and development (43, 45, 46). *In vitro* studies showed that the overexpression of wild-type SHIP2 hinders insulin signaling in 3T3-L1 adipocytes and L6 myocytes (43, 47-50).

The Sac domain containing 5-phosphatases (synaptojanin 1 & 2)
The synaptojanins are dual-function Sac-domain phosphatases that are evolutionarily conserved from yeast to humans, including cytosolic protein Sac2/INPP5f, Sac3 and synaptojanin 1/2 in mammals, and Inp51p, Inp52 p, and Inp53p in yeast (51, 52). Synaptojanins contain Sac1 (suppressor of actin) domain at their N-terminus, and 5-phosphatase domain at their center, but not the diverged proline-rich domain at the C-terminal (32, 53). This Sac1 domain contains two modules, the SacN region and the catalytic region containing the CX$_5$R(T/S) motif. Synaptojanins can degrade PtdIns-4,5-P$_2$, PtdIns-3,4,5-P$_3$,Ins-1,4,5-P$_3$, and Ins-1,3,4,5-P$_4$ (53-55). The synaptojanin 1 is largely expressed at the postsynaptic nerve terminal in the brain, where it interacts with the Sac homology 3 (SH3) domain containing proteins including Grb2, amphiphysin, Syndapin, and endophilins (56, 57). The phosphatase activity of synaptojanin 1 towards PtdIns-4,5-P$_2$ is important in regulating clathrin coat assembly and disassembly (33). Synaptojanin 2 is found in non-neuronal cells, and is involved in regulating the early step in the clathrin-mediated endocytic pathway.

Ciona intestinalis voltage-sensor-containing phosphatase (Ci-VSP)
The Ci-VSP displaying channel-like gating currents and changing in membrane potential into the turnover of PtdIns was identified from ascidian *Cinona intestinalis* (58, 59). The PTP active motif, HCXXGXXR, which shares structural features with PTEN, was found at the C-terminus region of this protein, suggesting the protein may have the activity of both tyrosine-phosphatase and PtdIns phosphatase. Ci-VSP is a dual-function PtdIns phosphatase, displaying 5 phosphatase activity towards both PtdIns-4,5-P$_2$ and PtdIns-3,4,5-P$_3$ (60). The activity of PtdIns phosphatase of Ci-VSP is turned within a physiological range of membrane potential. This protein is expressed in *Ciona* sperm tail membranes, suggesting a possible role in sperm function or morphology (18, 61).

PTP localized to the mitochondrion 1 (PTPMT1)
PTPMT1 was initially identified as a PTP that contains a PTEN-like active site, and is a mitochondrion lipid phosphatase that belongs to the PTP superfamily. It has been found to be highly conserved in most eukaryotic lineages and in a few bacteria, such as *Pirellula* (62). PTPMT1 localizes primarily to mitochondria on the inner membrane facing the mitochondrial matrix (63). PTPMT1 has been identified in the β-cell of pancreatic islets, and knockdown of its expression resulted in a dramatic increase of cellular ATP levels and insulin secretion,

suggesting that PTPMT1 may be a potential target for the treatment of type II diabetes (64). From the lipid analysis of *Ptpmt1*-deficient fibroblasts, phosphatidylglycerol phosphate (PGP), an intermediate in the biosynthesis of cardiolipin found in the inner mitochondrial membrane, has been established as a substrate of PTPMT1 *in vitro* (65).

PTPMT1 encodes a 261-amino-acid protein in mice. The crystal structure of PTPMT1 demonstrates the presence of a PTP domain fold comprising a β-sheet formed by five β-strands (β1-β5) with α helices (α1-α2) on one side, and α3-α6 on the other side of the sheet. Although PTPMT1 shows only limited protein sequence similarity to other PTPs, its overall structure is similar to that of many dual-specificity phosphatases (66).

Oculocerebrorenal Lowe syndrome phosphatase (OCRL)
Inositol 5-phosphatases have been implicated in a wide range of disorders, including various cancers, obesity, type 3 diabetes neurodegenerative diseases, and rare genetic disorders. Patients with Lowe syndrome suffer primarily from congenital cataracts, neonatal hypotoia, intellectual disability, and renal proximal tubule dysfunction (67). OCRL is located on vesicular structures throughout the endosomal system and the Golgi complex, and is present at the plasma membrane in membrane ruffles and at late-stage endocyticclathrin-coated pits (68). OCRL exhibits three conserved domains: the central inositol polyphosphate 5-phosphatase domain, an ASH domain, and a C-terminal catalytically inactive Rho guanosine triphosphatase-activating protein (GAP)-like domain (69). OCRL1 shares 51% primary sequence identity with INPP5B, the other inositol polyphosphate 5-phosphatase with GAP-like domain in humans and mice (70). Both OCRL1 and INPP5B hydrolyze PtdIns-4,5-P$_2$ and PtdIns-3,4,5-P$_3$, as well as the soluble inositol polyphosphate Ins-1,4,5-P$_3$ and Ins-1,3,4,5-P$_4$, but with distinct substrate preferences. OCRL1 is suggested to regulate actin dynamics on the basis that actin organization is defective in Lowe syndrome fibroblasts (71).

CONCLUSION

Several studies have confirmed the involvement of phosphoinosites in cellular trafficking and signaling events. These cellular functions of PtdIns required specific localization of the lipid molecules and association of numerous metabolizing enzymes that govern phosphoinositide synthesis and degradation in a spatially and temporally controlled mode. This is particularly true for phosphatases dedicated to phosphoinositide catabolism, and any mutation or deficiencies in which lead to human diseases. The domain arrangements of these phosphatases are fascinating in understanding their interaction with other proteins or lipid molecules, and reflect some fundamental property of these enzymes. This review intends to bridge the structural detail with the functional facts of phosphatases in the metabolism of PtdIns. Understanding the functional specificity of this large and complex PtdIns phosphatase

family is a very important task. Information about the expression, the localization, and the regulation of these enzymes will reveal great insight into their disease association.

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