Phosphoinositide phosphatases and disease

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Abstract The field of inositol signaling has expanded greatly in recent years. Given the many reviews on phosphoinositide kinases, we have chosen to restrict our discussion to inositol lipid hydrolysis focused on the phosphatases and a brief mention of the lipase isoforms. We also discuss recent discoveries that link mutations in phosphoinositide phosphatases to disease.—Majerus, P. W., and J. D. York. **Phosphoinositide phosphatases and disease.** ***J. Lipid Res.*** **2009.** 50: **S249–S254.**

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There are eight known phosphoinositides (also known as phosphatidylinositol phosphates or PIPs) with phosphate linked to diacylglycerol (DAG) and monoester phosphates in every possible combination of the 3, 4, and 5 positions of the inositol ring as shown in **Fig. 1**. Budding yeast have most of the PIPs, including PI(3)P, PI(4)P, PI(3,5)P_2, and PI(4,5)P_2, consistent with their evolutionarily conserved functions. The inositol phospholipids are interconverted by kinases and phosphatases as well as cleaved by phospholipase C (PLC) enzymes.

A concept in the field has emerged in which each PIP has a distinct role: a so-called "lipid code" hypothesis. The lipid code postulates that distinct lipids mark each of the cellular membranes to maintain an orderly flow required for the complexities of membrane trafficking and the spatio-temporal signaling reaction. For example, Golgi membranes are enriched in PI(4)P, endosomal membranes are decorated with PI(3)P, and the plasma membrane with PI(4,5)P_2.

Among the most well-studied codes are the PIPs harboring a D-3 phosphate, including PI(3,4,5)P_3 (commonly referred to as PIP_3), PI(3,4)P_2, PI(3,5)P_2 and PI3P. Importantly, the D-3 class of lipids are not substrates for PLC enzymes. This, along with the work showing that stimulation of cells by many growth factors activates receptor-linked phosphatidylinositol 3-kinases to transiently generate PIP_3, helped secure the roles of inositol lipids as signaling molecules in their own right. While PI(3,4)P_2 was considered as the breakdown product of PI(3,4,5)P_3, evidence points to a distinct role for this lipid in cell signaling. Remarkably, interpretation of the inositol lipid code has rested on hundreds of recently discovered lipid binding protein domains, which are found attached to numerous signaling proteins. There are about a half-dozen classes of lipid binding domains, for example PH, FYVE, and PX domains. Overall, the complexity of lipid signaling has exceeded expectations, and its importance is underscored by the identification of disease states that arise from mutations in these enzymes. Many of these diseases result from defects in inositol lipid hydrolysis. In the following pages, we will outline the diseases and describe the hydrolytic enzymes responsible for regulating these important chemical messengers within cells.

PATHOLOGY RESULTING FROM DERANGED INOSITOL SIGNALING

The significance of inositol signaling with respect to human health is highlighted by the fact that mutations in enzymes of inositol signaling cause numerous diseases and pathologies (**Table 1**). Mutation of the gene encoding a PIP_2 5-phosphatase (5-ptase) (OCRL) in humans causes a severe X-linked disorder called Lowe syndrome, while the OCRL knockout mouse has no phenotype (1). Mutation of myotubularin (MTM) causes a severe and fatal disorder associated with a failure of skeletal muscle development. Subsequently, it has been shown that MTM is the founding member of a large family of phosphatidylinositol

Abbreviations: DAG, diacylglycerol; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; GFP, green fluorescent protein; ING2, inhibitor of growth protein-2; MTM, myotubularin; MTMR, myotubularin-related protein; 3PAP, 3-ptase adaptor protein; PHD, plant homeodomain; PIP, phosphoinositide; PLC, phospholipase C; 5-ptase, 5-phosphatase.

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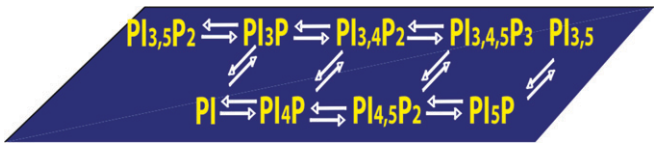

Fig. 1. Inositol lipid regulatory pathways. Seven individual phosphoinositides comprise a "lipid code". Forward and reverse arrows indicate kinase and phosphatase reactions. Nomenclature of PIxPn: x designates the inositol ring positions that are phosphorylated, whereas n refers to the total number phosphomonoesters.

3-phosphate phosphatases, designated now as MTM-related proteins (MTMR1-13). MTMR1-8 have 3-phosphatase activity, while MTMR9-13 lack the catalytic active site cysteine residue. The inactive subunits partner with active MTM proteins to form heteromers, which increase enzyme activity and in some cases alter cellular location. The importance of the inactive subunits is illustrated by the finding that mutations of MTMR13, the inactive partner of MTMR2, cause the same disorder as mutation of the active partner. Mutations in factor-induced gene 4 (FIG4) in mice cause the pale tremor mouse syndrome, while mutation of the human ortholog causes a form of Charcot Marie Tooth disease. Similarly, different types of mutation of the type IV inositol polyphosphate 5-ptase produce discrete disorders. Mutations that truncate the last 18 amino acids result in MORM syndrome, while mutations in the catalytic domain cause Jobert's syndrome.

ENZYMES THAT REGULATE LEVELS OF CELLULAR PIPS

PIP Selective Phospholipases

PLC enzymes are phosphodiesterases that upon stimulation cleave PIPs to produce soluble inositol phosphates and DAG. A number of PLCs have been identified in mammalian cells. These PLCs differ from a bacterial phosphatidylinositol (PI)-specific PLC in their absolute requirement for calcium and in their ability to hydrolyze phosphorylated forms of PI (PIP and PIP_2). Based on comparison of their sequences and structural studies, mammalian PLCs are divided into five families: PLCβ, PLCγ, PLCδ, PLCε, and PLCζ (2). The PLCδ class is conserved from yeast to man, whereas the others appear primarily in metazoans. The PLCγ-isoforms are activated by receptor tyrosine kinases, whereas the PLCβ-class are activated by trimeric G-protein coupled receptors, typically $G\alpha_q$.

PIP 5-ptase

The most abundant and perhaps best studied of the inositol lipid phosphatases are the 5-ptase family (3). 5-Ptases were first identified based on their ability to terminate $I(1,4,5)P_3$-mediated calcium release, because the product $I(1,4)P_2$ is unable to mobilize calcium. As more gene products were discovered, it became apparent that many, including all those in *Saccharomyces cerevisiae*, have a preference for breaking down PIPs rather than their soluble counterparts. Over 10 distinct mammalian and 4 yeast 5-ptases have been identified. All share magnesium-dependent phosphomonoesterase activities and contain two signature motifs: (F/I)WxGDxN(F/Y)R and (R/N)xP(S/A)(W/Y)(C/T)DR(I/V)(L/I). These motifs are critical for substrate binding and catalysis.

5-Ptases cay be divided into four groups according to their substrate selectivity. Group I 5-ptases hydrolyze only the water-soluble substrates $I(1,4,5)P_3$ and $I(1,3,4,5)P_4$. These enzymes are membrane-associated through isoprenylation and function to terminate calcium signaling. Group II enzymes hydrolyze both water-soluble and lipid substrates and they are also membrane-associated proteins. OCRL, the protein mutated in Lowe (oculocerebrorenal) Syndrome, is a member in this group (4). Another family member, synaptojanin (discussed below), is involved in synaptic vesicle trafficking (5). Group III enzymes hydrolyze mainly lipid substrates. SHIP1 and SHIP2 are members in this group and have been shown to regulate cytokine signaling in hematopoietic cells and insulin signaling (6). Group IV 5-ptases hydrolyze only PIP_3 and $PI(4,5)P_2$ and form complexes with PI 3-kinase.

TABLE 1. Disorders and pathologies associated with mutations in inositol lipid phosphatases

Enzyme	Disorder	Species	Symptoms	Reference
PTEN	Cancer	Human	Many tumor types	(10)
Inositolpolyphosphate 5-ptase (OCRL)	Lowe Syndrome	Human	X-linked renal failure, mental retardation, blindness	(4)
Inositol polyphosphate 4-phosphatase I	Weeble mouse	Mouse	Ataxia, cerebellar degeneration, early death	(7, 8)
Phosphatidyl inositol 3-phosphate phosphatase (MTM)	Myotubular myopathy	Human	Lack of muscle development, respiratory failure	(50)
MTMR2	Charcot Marie Tooth disease type 4B	Human	Neurodegeneration	(20)
MTMR13	Charcot Marie Tooth disease type 4B	Human	Neurodegeneration	(20)
FIG4 5-ptase on phosphatidyl inositol 3,5-bisphosphate	Pale tremor mouse	Mouse	Neuronal degeneration, early death	(19)
FIG4 5-ptase on phosphatidyl inositol 3,5-bisphosphate	Charcot Marie Tooth disease type 4J	Human	Neurodegeneration	(19)
Inositolpolyphosphate 5-ptase type IV	MORM syndrome	Human	Mental retardation, micropenis	(51)
Inositolpolyphosphate 5-ptase type IV	Jobert's syndrome	Human	Hypotonia, ataxia, retardation	(52, 53)

PIP 4-Phosphatases

These enzymes commonly referred to as 4-ptases were originally identified as hydrolases for inositol 1,3,4-trisphosphate and inositol 3,4-bisphosphate. Upon cloning and further biochemical characterization, it became apparent that the 4-ptase enzymatic activity may be most relevant to hydrolysis of the D-4 position phosphate from the second messenger PI(3,4)P_2, yielding PI(3)P as a product (7, 8). Therefore, it is now thought that a major function for 4-ptases is to terminate the PI(3,4)P_2 signal. Enzymes in the 4-ptase family, types I and II, all have a $Cx_5R(S/T)$ motif where the cysteine is critical for catalysis. All these enzymes are metal independent. Loss of 4-ptase activity results in neurological phenotypes highlighted by the discovery that the "weeble" mouse harbors a causative mutation in the type I gene (8).

The PTEN Class of Lipid Phosphatases

PTEN, a tumor suppressor, is found mutated in a variety of human cancers, including breast, prostate, and brain cancer. PTEN contains the $Cx_5R(S/T)$ and was originally thought to be a dual-specificity serine/threonine and tyrosine protein phosphatase. Later it was discovered that PTEN catalyzes the dephosphorylation of PIP_3 at the D-3 position to produce PI(4,5)P_2 as product (9). In this way, PTEN functions as a PIP_3 3-phosphatase to negatively control the PI 3-kinase signaling pathway. The $Cx_5R(S/T)$ active site motif shared with the 4-ptase family provides a strong evolutionary link to other lipid phosphatases and, as described below, the MTM and *Sac1*-like proteins. The biology and role of PTEN in human cancers has been reviewed in detail elsewhere (9, 10).

Promiscuous PIP Phosphatases: the Sac1-Like Proteins

The *Sac1*-like class of enzymes encode "multi" phosphatase activities that cleave the D-3, D-4, and D-5 position phosphates from PI(3)P, PI(4)P, PI(3,5)P_2, and PI(3,4)P_2 to produce PI (11). The promiscuity of the *Sac1*-like activities has confounded the interpretation of which lipid substrate is most physiologically relevant. However, the observed changes in specific PIPs in *Sac1*-like mutants, for example, selective elevations in PI(4)P and PI(3,5)P_2 in *sac1* and *fig4* mutants, indicates that individual proteins may prefer distinct lipid substrates in cells (11–14). Alternatively, it has also been proposed that the broad specificity of *Sac1*-like enzymes functions as a general mechanism to clear multiple signaling PIPs, thereby enabling lipid recycling, neutrality of membranes, and/or termination of many distinct signals.

There are several gene products designated to be *Sac1*-like, including three in mammals and four in budding yeast (11, 15).

The active site motif of the 400-amino acid *Sac1*-like domains is Cx_5R and they fall into two groups based on their domain structure. Group I gene products, including Sac1 and Fig4 and their homologs in higher eukaryotes, have a single *Sac1*-like domain (15). Group II proteins, including the synaptojanin class of proteins in mammals and yeast Sjl2/Inp52 and Sjl3/Inp53, are dual-functional lipid phosphatases that harbor both a *Sac1*-like domain and 5-ptase domain (discussed in more detail below).

Functional studies of the *Sac1*-like proteins suggest roles in a number of cellular processes. Sac1 is a transmembrane protein enriched in endoplasmic reticulum (ER)-Golgi fractions and is involved in ATP transport into the ER, growth factor signaling, and cell shape (13). Loss of *sac1* in yeast genetically links to overcoming defects in both actin and secretory mutants (16, 17) and in mice results in early embryonic lethality (18). Mutations in FIG4 in mice cause the pale tremor mouse syndrome, while mutation of the human ortholog causes a form of Charcot Marie Tooth disease (19, 20) (Table 1). Budding yeast Fig4 collaborates with the Fab1 lipid kinase and the regulatory proteins Vac7 and Vac14 to regulate lipid synthesis, vacuole biogenesis/dynamics, and endosomal transport (12, 14).

The PIP substrates hydrolyzed by *Sac1*-like domains have been implicated in the regulation of membrane trafficking (reviewed in (20–23). PI(4)P is synthesized by the PI 4-kinases, Pik1 in yeast and PI4Kβ in mammals, and is required for the formation of secretory vesicles from the late-Golgi (23, 24). Pik1 mutants are defective for protein secretion from the Golgi, vacuolar protein maturation, endocytosis, and lipid homeostasis. Pik1 also controls an essential nuclear pool of PIPs (23). A distinct non-Golgi pool of PI(4)P is produced by the PI 4-kinase, Stt4. Production of this PI(4)P is required for actin polarity, cell wall integrity, and phosphatidylserine metabolism (23, 25). Stt4 also fuels PI(4,5)P_2 production at the plasma membrane through the Stt4-Mss4 [a PI(4)P 5-kinase] pathway. The pleckstrin homology (PH) domain found in FAPP1 specifically binds PI(4)P and, along with other lipid binding domains, participates in decoding this messenger (26).

Components of the PI(3)P pathway are found in many eukaryotes, suggesting a conserved role of PI(3)P in protein transport from Golgi to the endosomal system (27). Interpretation of these signals occurs through FYVE domain containing proteins, which recognize PI(3)P, and the endosomal sorting complex required for transport (ESCRT) (22, 28). Additionally, the lipid binding properties of certain PX and PH domains mediate interactions of proteins with PI(3)P (29).

Another important role for PI(3)P is to serve as the precursor for PI(3,5)P_2, an osmotic stress-induced lipid regulator of multivesicular body endosomal pathways (14, 30) produced through the action of PI(3)P 5-kinases, Fab1 in yeast and PIKfyve in mammalian cells (31, 32).

The MTM 3-Phosphatases

The MTMs are a large family of 13 proteins, 8 of which have catalytic activity. These enzymes act to dephosphorylate PI(3)P and PI(3,5)P_2 (20, 33). Six members of the family are not active, because they lack the cysteine residue in the $Cx_5R(S/T)$ PTP motif. Despite having nearly identical catalytic activity, biochemical and genetic evidence supports the hypothesis that the MTMs are not redundant and have unique functions within cells. Current evidence suggests that each MTM protein regulates a specific

pool of PI(3)P and/or PI(3,5)P_2 that serves a variety of cellular functions.

The functions of MTM proteins are altered by the formation of heteromers between active and inactive subunits. The initial purification of MTM1 demonstrated the presence of MTM1 homodimers and MTM1/3-ptase adaptor protein (3PAP) heteromers (34). 3PAP was later designated as MTMR12. MTMR2 partners with MTMR5, and MTMR9 forms heteromers with MTMR6, MTMR7, and MTMR8. Heteromer formation has two major effects: *1*) increased activity of the enzymatic unit; and *2*) altered subcellular location. Both PI(4)P and PI(5)P, nonsubstrate inositides, cause an allosteric changes in the enzyme that increase activity (35).

Dual-Functional Lipid Phosphatases: the Synaptojanin Family

Synaptojanin was originally identified as a synaptic vesicle-localized PIP_2 5-ptase involved in synaptic vesicle recycling (5). Its name was derived from *Janus*, the God of two faces, based on links of the N-terminal *Sac1*-like domain to actin/secretion and a 5-ptase domain to PIPs. Subsequent studies showed that the synaptojanin-class of proteins had the ability to hydrolyze a variety of PIPs to PI (11, 36). The molecular basis for the "multi" phosphatase activity was revealed to be autonomously encoded by the 5-ptase and *Sac1*-like domains (11), which function independently to dephosphorylate PI(4,5)P_2 and PI(3,4,5)P3 (5-ptase substrates), as well as PI(3)P, PI(4)P, PI(3,4)P_2, and PI(3,5)P_2 (*Sac1*-like substrates).

Two isoforms of mammalian synaptojanin, synaptojanin-1 and -2, along with several splice variants have been characterized (21). Genetic studies in mice, worms, and yeast have established roles for synaptojanin-enzymes in vesicle recycling, endocytosis, and actin cytoskeleton. The PI(4,5)P_2 5-ptase activity of synaptojanin has been implicated in modulating the clathrin uncoating process during synaptic vesicle recycling (21, 37) and in control of lipid/actin regulatory protein interactions (38). A broader role for synaptojanin in multiple steps of synaptic vesicle recycling, budding, uncoating, and tethering to the cytoskeleton has been identified from studies in worms (37). A recent study has indicated a role for coordinated regulation of the two activities providing a basis for their tethering on a single peptide chain (39).

PI(4,5)P_2 4-Phosphatases

The most recently discovered of the seven known PIPs is phosphatidylinositol 5-ptase, PI(5)P. Analysis of the changes in the cellular levels of PI(5)P suggested that it is synthesized by the action of a phosphatase rather than by a kinase (40). The discovery of two 4-phosphatases that convert PI(4,5)P_2 to PI(5)P provides a molecular route for the synthesis of this lipid (41).

Recently, it was suggested that PI(5)P specifically interacts with a plant homeodomain (PHD) finger of inhibitor of growth protein-2 (ING2) protein and that this interaction is required for ING2-dependent acetylation of p53, which leads to increased apoptosis (42). This suggestion was based on the finding that RNAi of ING2 or overexpression of the phosphatidylinositol phosphate kinase (PIPK) type IIβ, an enzyme that converts PI(5)P to PI(4,5)P_2, decreases apoptosis. Thus, it was presumed that both ING2 and PI(5)P were required for acetylation of p53, although cellular PI(5)P was not measured in that study. Cells overexpressing type I 4-ptase have elevated levels of PI(5)P (43). When HeLa cells are treated with the DNA-damaging agents etoposide or doxorubicin, type I 4-ptase translocate to the nucleus and nuclear levels of PI(5)P increase. Overexpression of type I 4-ptase increased apoptosis, which is inhibited by cotransfection of PIPk IIβ. This enzyme therefore controls levels of PI(5)P and thereby p53-dependent apoptosis.

PIPS AND THEIR EFFECTOR PROTEINS

The decoding of PIP signals occurs through hundreds of lipid binding domains that are typically found attached to a variety of signaling modules, such as protein kinases, protein phosphatases, adaptor molecules, and small GTPases. Lipid binding domains are typically 50–120 amino acids in length and fall into a few common protein folds (reviewed in (29). Among the modules that have been characterized to bind inositol lipids are: pleckstrin-homology (PH), PX, C2, SH2, protein tyrosine binding, FYVE, PHD, GRAM, BAR, and ENTH/ANTH domains that collectively represent some of the largest families of domains within the proteome (29). While some of these domains are highly selective for a particular lipid, others bind a broad range of phospholipids with relatively weak affinity. Several examples exist in which various modules are fused, thereby generating a coincidence detector capable of being activated by combinations of lipids.

It is noteworthy that some of the lipid binding modules also bind the water-soluble inositol phosphates. For example, the PH domain of PLCδ binds PI(4,5)P_2 and its head group I(1,4,5)P_3 with high specificity. It has been proposed that such properties would enable another layer of regulation and/or termination by a competition model in which production of soluble molecules may compete the PH-containing protein off membranes. Lastly, when some modules dimerize, such as the BAR domains, they form a concave surface that may provide a template for membrane curvature. Different BAR domains have different curvature radii, possibly explaining in part their ability to discriminate among different sized vesicles or cellular membranes.

The lipid binding domain serves to impart a membrane-targeting signal that in many cases depends on the transient synthesis of a PIP, thereby enabling recruitment to specific locations within the cell and assuring a spatio-temporal mode to signaling biology. Two well-characterized lipid binding domains are the PH domain of Akt/PKB and the so-called FYVE-finger, named after the first four proteins identified to contain this structure: Fab1p, YOTB, Vac1p, and EEA1. The FYVE domains consist of 60–80 amino acids and harbor 2 zinc-finger motifs that specifically rec-

ognize PI(3)P [reviewed in (44)]. Several proteins involved in the ESCRT pathway that is required for multivesicular body formation utilize FYVE domains (22, 28).

Akt/PKB is critical for cell growth and survival through its binding to both $PI(3,4)P_2$ and PIP_3 in vitro, independent of $PI(4,5)P_2$ [reviewed in (45, 46)]. $PI(3,4)P_2$ and PIP_3 activate PKB/Akt by spatio-temporal synthesis of 3-phosphorylated lipids that leads to the translocation of Akt/PKB from the cytosol to the membrane in stimulated cells that primes Akt/PKB for its phosphorylation and activation by PIP-dependent kinase 1. Akt/PKB may also require intrinsic binding of these 3-phosphorylated lipids to be fully active.

Recent biochemical, electrophysiological, and molecular work has also shown that PIPs play a key role in regulating the intrinsic properties of transmembrane proteins, such as ion channels and vesicle fusion machinery (47, 48). Using molecular approaches, specific lipid phosphatases have been spatio-temporally activated to manipulate the levels of PIPs at specific membranes within cells. For example, methods in which $PI(4,5)P_2$ selective 5-ptase proteins have been inducibly targeted to cellular membranes have highlighted roles for $PI(4,5)P_2$ in membrane adhesion, ion channel gating activity, membrane trafficking, receptor internalization, and many other cellular processes (47, 49).

A technological benefit of the discovery of lipid binding domains has been their use as illuminators of lipid synthesis in living cells when fused to green fluorescent protein (GFP). GFP-tagged PKB/Akt PH and GFP-PH (PLCδ) are useful probes for marking PIP_3 and $PI(4,5)P_2$ levels in real time. One note of caution when using these probes is that some lipid binding modules appear to require more than PIP binding to translocate to membranes, such as protein-protein interactions. Nonetheless, the ability to monitor lipids in response to cellular signals has been a major advance.

CONCLUDING REMARKS

Overall, the complexity of PIP signaling has exceeded expectations. The link of in-born errors in PIP metabolism to vertebrate disease states highlights the relevance and significance of the field to human health. Studies of proteins dedicated to the regulation and interpretation of PIP signals have provided several new insights into fundamental questions in biology related to signaling specificity. While this review covers only a small portion of the field, we hope the readers glean a sense that inositol lipid metabolism and signaling remains a tremendously active area of research full of many surprises.

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