Phosphatidylinositol 4,5-bisphosphate (PI4,5P2) is a key lipid signaling molecule that regulates a vast array of biological activities. PI4,5P2 can act directly as a messenger or can be utilized as a precursor to generate other messengers: inositol trisphosphate, diacylglycerol, or phosphatidylinositol 3,4,5-trisphosphate. PI4,5P2 interacts with hundreds of different effector proteins. The enormous diversity of PI4,5P2 effector proteins and the spatio-temporal control of PI4,5P2 generation allow PI4,5P2 signaling to control a broad spectrum of cellular functions. PI4,5P2 is synthesized by phosphatidylinositol phosphate kinases (PIPKs). The array of PIPKs in cells enables their targeting to specific subcellular compartments through interactions with targeting factors that are often PI4,5P2 effectors. These interactions are a mechanism to define spatial and temporal PI4,5P2 synthesis and the specificity of PI4,5P2 signaling. In turn, the regulation of PI4,5P2 effectors at specific cellular compartments has implications for understanding how PI4,5P2 controls cellular processes and its role in diseases.

Keywords:
- lipid messenger; phosphatidylinositol 4,5-bisphosphate; phosphatidylinositol phosphate kinase; PI4,5P2 effector

Introduction

Phosphatidylinositol 4,5-bisphosphate (PI4,5P2) occupies a central position in phosphoinositide signaling, as it can be used as a substrate to produce other second messengers, or can directly regulate a wide range of cellular functions (Fig. 1). Nearly 60 years ago, Lowell and Mabel Hokin discovered the phosphatidylinositol (PI) cycle [1], and soon after a series of discoveries revealed that PI could be sequentially phosphorylated on its myo-inositol ring to generate PI4,5P2 [2–4]. At that time, PI4,5P2 was thought to only serve as an intermediate in the PI cycle [5, 6]. Early studies identified a role for PI4,5P2 as a precursor of other signaling molecules, such as inositol trisphosphate (IP3) and diacylglycerol (DAG) [6–8]. In the 1980s, the role of PI4,5P2 as a precursor was further expanded via the discovery that PI4,5P2 could be used as substrate for PI3K to produce phosphatidylinositol 3,4,5-trisphosphate (PI3,4,5P3) [9].

In the mid 1980s, direct roles for PI4,5P2 signaling were defined. Anderson and Marchesi discovered that PI4,5P2 regulated the association of the cytoskeletal protein, band 4.1, with the membrane protein glycophorin, demonstrating a role for PI4,5P2 in regulating the interaction of cytoskeletal proteins with the plasma membrane [10]. Lassing and Lindberg discovered that PI4,5P2 directly interacts with, and inhibits, the actin-modifying proteins profilin and gelsolin, to promote actin assembly [11, 12]. These discoveries elevated PI4,5P2 from simply being a precursor of messengers, to a lipid messenger in its own right (Fig. 1), and initiated an explosion of research on PI4,5P2-binding proteins/effectors. Currently, hundreds of PI4,5P2-binding proteins/effectors have been identified and this number is increasing. PI4,5P2 effectors are distributed to diverse subcellular compartments and mediate distinct biological activities, including cell adhesion, cytoskeletal dynamics [13], cell polarity [14–16], secretion [17, 18], ion channel regulation [19], vesicular trafficking [20], nuclear signaling, and gene expression [21, 22] (Fig. 1).
Review essays

Alzheimer's disease, diabetes, cancer, and ciliopathies [35].

Understanding the mechanistic roles of PI4,5P2 signaling in human diseases is critical. This review, focusing on emerging proteins and this links PI4,5P2 synthesis to specific cellular compartments where they generate PI4,5P2 that regulates the local production of PI4,5P2 [24, 27] (Fig. 2). Thus, PIPKs along with enzymes that consume PI4,5P2, such as phospholipase C (PLC), PI3K, and PI4,5P2 5-phosphatases, are critical for the spatial and temporal regulation of PI4,5P2 levels [6, 13, 24, 28, 29].

Six genes encode the PIPKs that generate PI4,5P2. These are the PIP kinases types I and II (PIPKI and PIPKII, respectively). PIPKI and PIPKII synthesize the majority of PI4,5P2 in the cell [27]. PIPKI preferentially phosphorylates the 5-hydroxyl position on the myo-inositol ring of PI4P to generate PI4,5P2, while PIPKII uses PI5P as substrate and phosphorylates the 4-hydroxyl position to produce PI4,5P2 [27]. Both of the PIPK subfamilies have three isoforms α, β, and γ, which are functionally diversified into splice variants [24]. The PIPKs often directly interact with PI4,5P2 effector proteins and this links PI4,5P2 synthesis to specific cellular functions. In this way, targeted PI4,5P2 signaling modulates neuronal synaptic vesicle trafficking, epithelial morphogenesis, cell migration, phagocytosis, nuclear events, and gene expression [14, 16, 21, 30–32].

This review focuses on the emerging roles of PI4,5P2 in the cytosol. PI4,5P2 function in nuclear events has recently been reviewed [33, 34]. PI4,5P2 signaling or PI4,5P2-derived metabolites have been implicated in human disorders, including mental retardation, bipolar disorder, schizophrenia, Alzheimer’s disease, diabetes, cancer, and ciliopathies [35]. Understanding the mechanistic roles of PI4,5P2 signaling in human diseases is critical. This review, focusing on emerging areas, provides a synopsis of PI4,5P2 functions and how its signaling is modulated.

**PIPK isoforms synthesize PI4,5P2 at distinct cellular locations**

The PIPKI family is highly diverse. For example, there are at least six PIPKI splice variants expressed in humans, labeled PIPKIγ1 to PIPKIγ6 [36, 37] using the HUGO (Human Genome Organization) nomenclature [36]. PIPKI isoforms all contain a highly homologous kinase core domain with invariant catalytic residues that bind ATP or GTP and Mg2+, and residues that recognize the specific lipid substrate [27, 38, 39]. There are three domains in the PIPKI isoforms that are sequence divergent: the N-terminal, kinase insert and the C-terminal domains [24]. These regions of sequence divergence define cellular targeting and functional interactions [24]. The PIPKIγ splice variants emphasize this concept, as these isoforms have unique C-terminal sequence extensions from the PIPKIγ1 isoform [36]. The diversity of these sequences confers the ability to interact with different targeting proteins that are often PI4,5P2 effectors. These enzymes are then targeted to specific cellular compartments where they generate PI4,5P2 that specifically modulates effectors required for specific biological functions (Fig. 2).

Consistently, each splice variant of PIPKIγ localizes to select cellular locations. PIPKIγ1 can be targeted to focal adhesions by an interaction with talin [40, 41]. Conversely, PIPKIγ4 localizes to nuclear speckles and PIPKIγ5 is found at endosome compartments [36]. For historical reasons, the nomenclature for PIPKIα and PIPKIβ in the literature is complicated by the fact that PIPKIα in mouse corresponds to PIPKIβ in human and vice versa. To avoid confusion, we only use the human nomenclature when describing PIPKI isoforms in this review. PIPKIα is found at membrane ruffles [27] and nuclear

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**Figure 1.** Overview of PI4,5P2 functions in the cytosol and plasma membrane. PI4,5P2 is a polyphosphoinositide that is phosphorylated on the 4th and 5th hydroxyl group on the myo-inositol ring. Its greatest concentration is seen on the plasma membrane but it is also found on most cellular membrane compartments and in the nucleus. PI4,5P2 is utilized by PI3K or PLC to generate second messengers: PI3,4,5P3, DAG, and IP3. The generation of PI4,5P2 in a spatio-temporal manner is the basis for PI4,5P2 regulation of diverse cellular events, including endocytosis, exocytosis, vesicle trafficking, and cell migration.
speckles [21], whereas PIPKIβ is distinctively targeted to a perinuclear compartment [27].

Where is PI4,5P2?

The PI4,5P2-specific Pleckstrin homology (PH) domain from PLCδ fused to GFP (PH-PLCδ-GFP) has been used as a biosensor to examine the intracellular distribution of PI4,5P2 [42]. Although it is clear that PIPKs are localized to multiple subcellular compartments and generate PI4,5P2 at these sites [24, 33], the PH-PLCδ-GFP primarily detects PI4,5P2 at the plasma membrane [42]. This observation is in conflict with the fact that many PI4,5P2 effector proteins function at other compartments, such as focal adhesions, trafficking vesicles for integrin or E-cadherin, and the nucleus at sites of mRNA processing with Star-PAP [14, 21, 31, 40, 43], where the PH-PLCδ-GFP does not localize [42]. These results indicate that PH-PLCδ-GFP has a bias towards detection of plasma membrane PI4,5P2. It is possible that the levels of PI4,5P2 at these other compartments are low and/or that the PI4,5P2 generated there rapidly binds to effector proteins and cannot be detected by PLCδ-PH-GFP, or that PLCδ-PH-GFP access is prevented for steric reasons.

Besides the PLCδ-PH-GFP, the cellular distribution of PI4,5P2 beyond the plasma membrane has been demonstrated using other PI4,5P2-specific antibodies and probes. Using a purified GST-PLCδ-PH, PI4,5P2 was shown to be present at the plasma membrane, Golgi, endosomes, ER, and nucleus [44]. Additionally, PI4,5P2-specific antibodies have been shown to localize to the plasma membrane as well as to intracellular compartments [45]. More recently, these antibodies were used to show the presence of PI4,5P2 with PIPKIα and PIPKIβ on autolysosomes to regulate autophagic lysosome reformation [46]. Finally, the development of a specific small molecular probe, PHDM, that mimics the PLCδ PH domain, has the potential to expand our understanding of PI4,5P2 function, by binding to and inhibiting PI4,5P2-regulated events, including endocytosis and stress fiber formation [47].

Further evidence that PI4,5P2 functions throughout the cell is that PIPK-interacting proteins are also often PI4,5P2 effectors (Fig. 2), such as talin and Exo70, which form direct interactions with PIPKIγ2 [16, 40, 48, 49]. Presumably, the localized synthesis of PI4,5P2 by PIPKIγ2 modulates the function of effectors that are in close proximity, but without an obvious increase in PI4,5P2 levels that are detectable by current approaches. This represents an area of vigorous investigation and debate in the field.

Local production of PI4,5P2 regulates epithelial cell polarity and epithelial morphology

Epithelial cells are an essential cell type of many organs, including kidney, lung, and vasculature, and typically these cells maintain apical and basolateral polarity. In vitro studies of epithelial cells in 3D culture indicate that maintenance of PI4,5P2 and PI3,4,5P3 at distinct regions of the plasma membrane is crucial for cyst formation during morphogenesis [50]. PI4,5P2 concentrated at the apical region is important in promoting epithelial polarity through its recruitment of the scaffolding protein annexin-2, which then subsequently recruits the Cdc42 GTPase that binds to the Par6/aPKC complex [50]. The PTEN phosphatase is also
recruited to the apical region to dephosphorylate PI3,4,5P3 to PI4,5P2 [50]. These findings indicate that PI4,5P2 and PI3,4,5P3 function as molecular identifiers of these plasma membrane compartments during epithelial morphogenesis, since changes in the distribution of these phosphoinositides alters the polarity and orientation of epithelial cells. For example, the delivery of exogenous PI4,5P2 to the basolateral membrane is sufficient to induce a shift in proteins from apical to basolateral regions of the plasma membrane [50].

Despite its differences from 3D culture, several key findings regarding epithelial cell polarity were made using traditional 2D cell culture. PIPKIγ2 has emerged as an important player in maintaining the epithelial polarity via E-cadherin trafficking [14, 15] (Fig. 3). Simultaneous binding of PIPKIγ2 to E-cadherin molecules and the AP1B complex promotes E-cadherin transport to the basolateral surface to assemble adherens junctions. This process maintains epithelial polarity, while a loss of PIPKIγ2 inhibits E-cadherin transport to the basolateral membrane and disrupts epithelial polarity [14]. This dual interaction supports a mechanism in which the highly orchestrated genesis of PI4,5P2 drives the assembly of the trafficking machinery with E-cadherin for specific trafficking to adherens junctions (Figs. 3 and 4). Alternatively, the interaction and coordination of PIPKIγ2 with the exocyst complex mediates E-cadherin delivery to the basolateral membrane to facilitate adherens junction assembly and the establishment of epithelial polarity [15]. Consistently, neuroepithelial cells from pan-PIPKIγ knockout mice show clear defects in adherens junctions [51]. However, E-cadherin localization was normal in epithelial cells from other organs in PIPKIγ knockout mice [52], indicating that there are compensatory mechanisms in vivo. Potentially, a role specifically for PIPKIγ2 may be less critical in 3D culture and in vivo.

**PIPKIs regulate β-catenin transcriptional activity**

β-Catenin is a component of adherens junctions that can interact with E-cadherin or actin. Free β-catenin can also enter the nucleus where it regulates transcription through its association with transcription factors [53]. Stimuli, such as growth factors, can lead to the disassembly of adherens junctions and also enhance β-catenin translocation to the nucleus for regulation of transcription [54, 55]. Intriguingly, PIPKIk was...
recently found to also interact directly with β-catenin, independent of E-cadherin, and the increased expression of PIPKIγ (similar to expression levels in breast cancers [56]), but not a kinase dead form, was found to enhance β-catenin translocation to the nucleus and its transcriptional activity downstream of EGFR activation (Fig. 4) [57]. β-Catenin regulates a variety of target genes, including multiple genes that promote the epithelial to mesenchymal transition (EMT), such as Slug and ZEB1 [53, 58, 59]. During EMT, epithelial polarity is lost, and cells acquire a mesenchymal morphology. This role for PIPKIγ in β-catenin-regulated transcription represents a unique role for PI4,5P2 in regulating cell polarity. In normal epithelial cells, β-catenin transcriptional activity is limited by its interaction with E-cadherin and its degradation. The complex roles of PIPKIγ-regulated signaling in controlling both epithelial polarity and transcriptional activities of β-catenin remain an area of uncertainty. Therefore, the role of PIPKIγ and PI4,5P2 in β-catenin nuclear translocation requires further characterization, including its function in epithelial and mesenchymal cell types and its activation by specific growth factors.

**PI4,5P2 signaling regulates processes required for cell migration**

PI4,5P2 and PI3,4,5P3 are key regulators of cell migration under both physiological and pathological conditions [13, 60]. Migrating cells recruit different PIPKI enzymes and PI4,5P2 and PI3,4,5P3 effectors to modulate cellular processes required for cell migration. Broadly, PI4,5P2 regulates such processes as polarized vesicle trafficking, leading edge formation, cell polarity and directionality, and actin and tubulin cytoskeleton dynamics in migration [13, 16, 31, 51, 61–65].
PI4,5P₂ binds and/or regulates multiple actin-binding proteins, promoting cytoskeletal reorganization at a leading edge lamellipodium [63, 64]. This includes reciprocal regulation between PIPKIs, small GTPases (RhoA, Rac1, and Cdc42), and PI4,5P₂, during cell migration and in the targeting of signaling molecules to the plasma membrane [62, 66]. This phenomenon is illustrated in the N-WASP/Arp2/3 complex, where PI4,5P₂ directly binds to N-WASP to alleviate intramolecular constraints to activate the actin polymerization activity of Arp2/3 at leading edge lamellipodium [67]. Additionally, binding of both PI4,5P₂ and Cdc42 to distinct sites on N-WASP cooperatively activates the N-WASP/Arp2/3 complex required for cell migration [67, 68]. Finally, PI4,5P₂-rich micro-domains promote and sustain protrusive motility via capturing and stabilizing the plus-end of microtubules, which is crucial for cell polarization and migration [69].

Directionally migrating cells require the maintenance of cell polarity, polarized vesicle trafficking, and proper formation of a leading edge [16, 60], which likely requires synergism between PI4,5P₂ and PI3,4,5P₃ [70]. These phosphoinositides mediate the polarized recruitment and activation of different signaling molecules at the leading edge [60, 66]. However, the specific enzymes and accessory molecules that maintain PI4,5P₂ and PI3,4,5P₃ at the leading edge of migrating cell require further characterization. This is especially important for understanding the specific functions of the rapid and transient increase of PI3,4,5P₃ in response to extracellular stimulation, even though PI4,5P₂ levels remain relatively constant. Potentially, this is accomplished by de novo PI4,5P₂ synthesis or an infusion from an adjacent compartment such as a secretory vesicle. Ongoing studies of these events will further elucidate the precise role of PI4,5P₂ generation and signaling in cell migration.

Focal adhesion assembly at the leading edge is an essential process in cell migration [13], which requires the localized generation of PI4,5P₂ [40, 41, 71]. PIPKIγ2 is intensively linked with both integrin- and growth factor-stimulated cell migration [16, 31]. Polarized integrin trafficking and maintenance of cell polarity are essential for cell migration. PIPKIγ2 regulates these processes, through an interaction with the exocyst complex, a PI4,5P₂-effector and vesicle trafficking protein complex, that is necessary for the delivery of integrins to the plasma membrane [16] (Fig. 4). PIPKIγ2 also collaborates with talin to generate a localized PI4,5P₂ pool required for focal adhesion formation [40, 41] and this has been demonstrated by numerous studies showing defective cell adhesion and migration in PIPKIγ2 or talin knockdown cells [16, 31, 71]. The regulation of "inside-out" integrin activation by PIPKIγ2 and PI4,5P₂ is critical during cell migration. PI4,5P₂ induces the dissociation of the talin rod domain from its head domain and promotes the tethering of the talin head to PI4,5P₂-enriched membranes, exposing a hidden integrin-binding interface that is required for integrin clustering [72]. Also, the talin FERM domain binds to an NPxY motif in the cytoplasmic domain of the integrin beta chain, leading to "inside-out" integrin activation [72, 73]. Paradoxically, PIPKIγ2 competes with integrins for talin binding [73]. However, the presence of an additional talin-binding site in the integrin beta chain, and the homo-dimeric nature of talin, potentially allow for the formation of a single complex through simultaneous binding of integrin and PIPKIγ2 to talin, which would regulate focal adhesion assembly and signaling in migrating cells [16, 72]. The role of PIPKIγ2 and talin in polarized secretion of integrin suggests that concentrated PI4,5P₂ could be delivered to the leading edge via secretory vesicles (Fig. 4).

In addition to adherent cells, the roles of specific PIPKI isoforms and PI4,5P₂ signaling in regulation of chemotaxis and trans-endothelial migration has been extensively studied in non-adherent circulating leukocytes. PIPKIs and PI4,5P₂ signaling play critical roles in regulating uropods for leukocyte migration [61, 74]. Integrins recruit PIPKIγ2 in a polarized manner, leading to RhoA activation at uropods, which is required for neutrophil infiltration in vivo [61]. Similarly, PIPKIβ is recruited to the cell rear by its unique C terminus, where it interacts with EBPS and Rho-GDP to regulate cell polarity required for cell migration [74]. PIPKIγ is also reported to play a role in neutrophil chemotaxis via regulating uropod retraction [65]. All of these studies further illustrate the importance of PI4,5P₂ in backness response/signaling required for cell migration, spatially distinct from PI4,5P₂ signaling at the leading edge. In these studies, overexpression of PIPKIs may complicate the interpretation of the data, as in vivo synthesis of PI4,5P₂ may be spatially changed. Thus, studies employing only overexpression should be viewed with skepticism.

Endocytosis, exocytosis, and phagocytosis require PI4,5P₂

PI4,5P₂ signaling regulates the entry and exit of molecules at the plasma membrane from and to the extracellular space through endocytosis, exocytosis, and phagocytosis. The first step in endocytosis is the formation of the clathrin-coated pits that subsequently mature to form clathrin-coated endocytic vesicles [75]. PI4,5P₂ is a central component in the formation of clathrin-coated vesicles as it recruits various accessory molecules required for endocytic vesicle formation. These proteins include multiple phosphoinositide-binding proteins, such as the AP2 complex, AP180/CALM, epsin, Dab2, and HPIP1/IR [75]. Importantly, precise tuning of PI4,5P₂ turnover is critical during endocytosis, e.g. the increase in PI4,5P₂ that coincides with clathrin coat assembly or the corresponding decrease in PI4,5P₂ for clathrin coat disassembly [76]. Paradoxically, neither PI4,5P₂ nor PI4,5P₂-generating enzymes have been clearly observed on vesicle buds during clathrin-coat formation and maturation [75]. During late stages of endocytosis, the PI4,5P₂ phosphatases, OCRL and synaptojanin, are recruited to endocytic vesicles by direct interactions with clathrin and AP2, and subsequently reduce PI4,5P₂ levels, resulting in the dissociation of PI4,5P₂ effectors from endocytic vesicles, which is necessary for endosomal fusion [77, 78].

Neuronal cells are commonly used to study how PI4,5P₂ signaling regulates communication across the synapse, through its precise control over endocytic and exocytic events [79]. PIPKIγ is the predominant isoform for PI4,5P₂ synthesis in neuronal cells [30]. Following NMDA stimulation of neuronal cells, PIPKIγ2 is dephosphorylated by calcineurin, promoting the interaction of PIPKIγ2 with AP2 [80].
This interaction enhances PIPKι/γ2 kinase activity and, thus, the PI4,5P2 generation that is necessary for endocytosis during neuronal cell activation [30, 80]. Similarly, the interaction of PIPKι/γ2 with talin also regulates synaptic vesicle endocytosis, as inhibition of this interaction impairs endocytosis and exocytosis of synaptic vesicles [30]. This is further complicated by the phosphorylation status of the PIPKι/γ2 C terminus, which dictates its ability to bind to either talin or the AP2 complex [81, 82]. However, the precise mechanism that determines specific interactions and its significance in endocytosis remains to be defined.

Similar to endocytosis, PI4,5P2 regulates exocytosis in neuronal cells [83]. Exocytosis is especially important in neurons for the controlled release of neurotransmitters or hormones stored in secretory vesicles required for neuronal and hormonal communication [79]. Secretory vesicles docked near the plasma membrane are primed for rapid calcium-induced fusion and exocytosis [84, 85]. The membrane protein synaptotagmin-1, which is anchored on the surface of these synaptic vesicles, contains both calcium- and PI4,5P2-binding sites [85]. PI4,5P2 binding induces a conformational change in synaptotagmin-1 that promotes fusion of the vesicle with the plasma membrane [85, 86].

The study of bone marrow-derived macrophages obtained from PIPKιγ2 and PIPKια knockout mice has revealed a crucial role for both PIPKιγ2 and PIPKια in phagocytosis [32]. FcγR is important in regulating the phagocytosis of microbes by binding to immunoglobulins [87]. Loss of PIPKιγ2 impaired attachment of IgG-opsonized particles and clustering of FcγR. More specifically, the PIPKιγ2 isoform induced actin depolymerization to promote the particle attachment necessary for FcγR microclustering. Further, Syk-mediated phosphorylation of PIPKιγ2 during FcγR-mediated phagocytosis promotes PIPKιγ2 enzymatic activity. In addition, formation of the actin-driven structures required for optimal binding to targets of phagocytosis depends on PI4,5P2, PI3,4,5P3, and active Rac1 [88]. Alternatively, it is possible that PIPKια deficiency impairs particle ingestion without any effect on particle attachment. PIPKια regulates particle ingestion by activating WASP and Arp2/3-dependent actin polymerization at the nascent phagocytic cup [32].

Murine knockout studies demonstrate redundant and specific functions among PIPKIs

Although our precise understanding of tissue-specific expression of PI4,5P2-generating enzymes during embryonic development and postnatal life is lacking, genetic studies in mice indicate both redundant and distinct functions among PIPKIs (α, β, and γ using the nomenclature for human PIPKIs) [89]. Knockout of PIPKιγ2 in mice blocked embryonic development, resulting in prenatal death [30, 51], whereas mice lacking PIPKια or PIPKιβ survive to adulthood with some functional defects in specific cell types [90, 91]. It is clear that each PIPKι isoform has a role in generating distinct PI4,5P2 pools required for specific cellular processes, but functional redundancy among isoforms does exist [89, 90].

Platelets from PIPKια-knockout mice are deficient in PI4,5P2 synthesis and IP3 formation stimulated by thrombin [90]. PIPKιβ is also involved in this process since double knockout of PIPKια and PIPKιβ led to a complete loss of thrombin-induced IP3 synthesis in platelets [90]. Although PIPKιγ2 is the predominant PIPKι isoform in platelets, it does not appear to contribute to this process. Additionally, PIPKια, but not PIPKιβ, has a specific function in modulating platelet aggregation and in vivo thrombosis, further confirming the distinct roles of the different PIPKι isoforms [90].

PIPKιβ has been reported to negatively regulate mast cell functions and anaphylactic responses [91]. Knockout of PIPKιβ in mice enhances passive cutaneous and systemic anaphylaxis, which may be due to the function of PIPKιβ to negatively regulate FcεRI-mediated cellular responses and anaphylaxis [91]. Interestingly, although depletion of PIPKιβ decreases the total PI4,5P2 level by about 15% in mast cells, the production of the PI4,5P2-derived second messengers IP3 and PI3,4,5P3 is increased [91]. This indicates that PIPKιβ is not responsible for formation of PI4,5P2 pools required for IP3 and PI3,4,5P3 production.

The majority of PI4,5P2 in the brain is believed to be synthesized by PIPKιγ2 [89]. Knockout of PIPKιγ2 leads to a 30–50% reduction of PI4,5P2 in the brain and causes synaptic transmission defects [30], whereas knockout of PIPKια or PIPKιβ has no dramatic effect on levels of PI4,5P2 in the brain [89]. Truncation of the PIPKιγ2 gene in mice via deletion of exons 2–6, which encode most of the catalytic region, resulted in postnatal lethality within a single day, with mice displaying impaired motility and a lack of milk in their stomachs [30]. No other obvious anatomical anomalies were found in these mice, and the defects in nervous system function may have played an important role in their death [30]. Interestingly, another independent PIPKιγ-knockout mouse line, generated by inserting a β-geo gene trap within the first intron of the PIP5Kιγ gene, failed to develop beyond embryonic day 11.5, and these embryos had defects in myocardial development and neural tube closure [51]. The cause for the differences between these two knockout mouse lines remains unclear and may reflect the different genetic backgrounds or knockout approaches.

PIPKιγ has different splice variants and each variant can play distinct roles [24, 36]. While depletion of pan-PIPKιγ is lethal [30, 51, 52], mice with the specific knockout of PIPKιγ2 develop normally, and are indistinguishable from wild-type littermates [52]. This indicates that PIPKιγ activity, but not PIPKιγ2 is required for normal embryonic development. However, both pan-PIPKιγ- and the PIPKιγ2-knockout mice show defects in synaptic vesicle endocytosis, suggesting a specific and non-redundant role for PIPKιγ2 in this process [52]. Agonist-induced Ca2+ signaling is reduced in pan-PIPKιγ-knockout mice and enhanced in PIPKιγ2-knockout mice, indicating distinct roles of PIPKιγ variants in Ca2+ signaling [52].

**PI4,5P2-regulating enzymes are associated with human diseases**

Until now, only one disease-causing mutation has been reported for PIPKι genes. Genetic mutation in PIPKιγ (D253N) of a
required catalytic residue in the kinase domain diminishes its kinase activity and leads to lethal congenital contractural syndrome type 3 (LCCS3) [92]. Homozygosity of this mutation is lethal postnatally with respiratory insufficiency, fetal akinesis, limb contractures, and muscle atrophy [92]. These defects may be caused by disruption of PIPK1γ activity, leading to a defect of PI4,5P2 synthesis in the brain and neurological malfunction [92].

A potential link between PI4,5P2 signaling and Alzheimer’s disease was identified over two decades ago, where a reduced phosphoinositide content, including PI4,5P2, in the temporal cortex was suggested to impair receptor function in Alzheimer’s disease [93, 94]. Consistently, genetic mutations in presenilin1 and presenilin2 that cause familial Alzheimer’s disease perturb PI4,5P2 metabolism, which affects calcium currents and the biogenesis of amyloid-β-peptide [95, 96]. Further, treatment of primary cortical neurons with amyloid-β-peptide decreased PI4,5P2 levels and caused synaptic dysfunction [93, 96]. The amyloid-β-peptide-induced synaptic dysfunction was ameliorated by maintaining normal PI4,5P2 levels in the brain [93].

The importance of PI4,5P2 homeostasis is also evident in Lowe syndrome. Individuals with this condition exhibit several abnormalities, including cataracts, mental retardation, and defects in kidney function. The disease is caused by mutations in the Ocri1 gene, which encodes a PI4,5P2 5-phosphatase. Multiple different nonsense and missense mutations have been identified that result in truncated, degraded, or nonfunctional forms of the enzyme [78, 97]. A wide range of cellular defects have been identified in patients with Lowe syndrome. For example, loss of OCRL function causes ectopic accumulation of PI4,5P2 on early endosomes, which impairs the recycling of multiple receptors including megalin [78, 97]. This defect in megalin recycling in renal proximal tubular cells impairs the retrieval of proteins from kidney ultrafiltrates, which would contribute to the kidney dysfunction in patients with Lowe syndrome. These phenotypes indicate that aberrant generation or turnover of PI4,5P2 impacts normal function and leads to human diseases.

Summary and perspectives

PI4,5P2 is one of the most utilized signaling molecules in eukaryotic cells. Diverse PIPKs are targeted to distinct subcellular compartments via their targeting factors to establish specific local PI4,5P2 pools. These modulate the activity of PI4,5P2 effectors to mediate a vast array of biological functions. The discovery of an increasing number of new PIPK isoforms and splice variants, targeting factors, and novel PI4,5P2 effectors will expand our knowledge of PI4,5P2 biological functions. However, understanding the exact roles for PI4,5P2 signaling poses an enormous challenge due to the dynamic nature of PI4,5P2, the varying affinities and specificities of protein-lipid interactions, the diversity of PI4,5P2-generating and -utilizing enzymes, and their mechanisms of regulation [98].

PI4,5P2 can be utilized as a precursor to generate other phosphoinositides such as PI3,4,5P3 and PI4P. How the specific local PI4,5P2 pools affect the production of other phosphoinositides and modulate their signaling still needs to be clarified. In addition, some PI4,5P2 effectors also interact with, and are modulated by, additional phosphoinositide species. The specific roles of PI4,5P2 and its cooperativity with other phosphoinositides to modulate these effectors should be elucidated in future studies.

Continuing research is necessary to further understand how PI4,5P2 signaling functions in novel cellular pathways, and how these processes relate to known PI4,5P2-associated diseases. To accomplish this, it will be necessary to determine the expression of distinct PIPKs in pathological conditions, and further characterize the cellular localization of newly discovered isoforms and splice variants. Additionally, the identification of PI4,5P2 effectors will further explain how the unique distributions of PI4,5P2 can regulate specific processes. The characterization of animals with a knockout of specific PIPK splice variants or PI4,5P2 effectors will be important in determining the specific defects associated with each isoform and the associated effectors that mediate these pathways. It will be important to study knockout mice to define phenotypes that relate to neuronal diseases, cancer metastasis, and cardiovascular diseases. This will further our understanding of how PI4,5P2 can broadly regulate cellular functions with potential consequences in pathological conditions.

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