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Phosphatidylinositol-4,5-bisphosphate: Targeted Production and Signaling

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Summary

Phosphatidylinositol-4,5-bisphosphate (PI4,5P₂) is a key lipid signaling molecule that regulates a vast array of biological activities. PI4,5P₂ can directly act as a messenger or can be utilized as a precursor to generate other messengers: inositol trisphosphate (IP₃), diacylglycerol (DAG) or phosphatidylinositol-3,4,5-trisphosphate (PI3,4,5P₃). 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 allows 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 sub-cellular 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

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

Introduction

PI4,5P₂ occupies a central position in phosphoinositide signaling, as it is used as a substrate to produce other second messengers, or PI4,5P₂ can directly regulate a wide range of cellular functions (Fig. 1). Nearly 60 years ago, Lowell and Mabel Hokin discovered the phosphatidylinositol cycle [1], and soon after a series of discoveries revealed that phosphatidylinositol (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 phosphatidylinositol cycle [5,6]. Early studies identified a role for PI4,5P₂ as a precursor of other signaling molecules, such as IP₃ and 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 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

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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 a precursor of messengers, to a lipid messenger in its own right (see Fig. 1). These findings initiated an explosive discovery of 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 sub-cellular 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).

Now there is large and diverse array of PI4,5P₂ effectors many of which are in the same membrane compartment, for example the plasma membrane. Herein lies the dilemma, the cellular or membrane content of most second messengers dramatically fluctuate upon agonist stimulation [6]. However, the cellular or membrane content of PI4,5P₂ is relatively constant [23–25] and undergoes only modest changes upon stimulation [26,27]. This begs the question: How does PI4,5P₂ specific modulate cellular events?

In the past decades, the discovery and study of the phosphatidylinositol-phosphate kinases (PIPKs), enzymes producing PI4,5P₂, revealed a mechanism for the targeted production of PI4,5P₂. Distinct isoforms of PIPKs are targeted to specific sub-cellular locations via association with unique interacting proteins and this controls 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 type I and II PIP kinases (PIPKI and PIPKII, respectively). PIPKI and PIPKII synthesize the majority of PI4,5P₂ in the cell [27]. PIPKI preferentially phosphorylate the 5-hydroxyl position on the myo-inositol ring of PI4P to generate PI4,5P₂, while PIPKII use PI5P as substrate and phosphorylate the 4-hydroxyl position to produce PI4,5P₂ [27]. Both of the PIPKs 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 will focus on the emerging roles of PI4,5P₂ in the cytosol. PI4,5P₂ function in nuclear events has been recently reviewed recently [33,34]. Recent studies have implicated PI4,5P₂ signaling or PI4,5P₂-derived metabolites 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 a critical focus. This review will provide a synopsis of PI4,5P₂ functions and how its signaling is modulated, focusing on emerging areas.

PIP kinase isoforms synthesize PI4,5P₂ at distinct cellular locations

The PIPKI family is highly diverse, for example, there are at least six PIPKI γ splice variants expressed in humans, labeled PIPKI γ 1 to PIPKI γ 6 [36,37] using the HUGO (Human Genome Organization) nomenclature [36]. PIPKI isoforms are highly homologous within the kinase domains they 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. These are the N-terminus, kinase insert and the C-terminal domains [24]. These regions of sequence divergence define cellular targeting and functional interactions [24]. The PIPKI γ splice variants emphasize this concept, as these isoforms have unique C-terminal sequence extensions from the PIPKI γ 1 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 (see Fig. 2).

Consistently, each splice variant of PIPKI γ localizes to select cellular locations. PIPKI γ 2 can be targeted to focal adhesions by an interaction with talin [40,41]. Conversely, PIPKI γ 4 localizes to nuclear speckles and PIPKI γ 5 is found at endosome compartments [36]. For historical reasons, the nomenclature for PIPKI α and PIPKI β in the literature is complicated by the fact that PIPKI α in mouse corresponds to PIPKI β in human and vice versa. To avoid confusion, we will only use the human nomenclature when describing PIPKI isoforms in this review. PIPKI α is found at membrane ruffles [27] and nuclear speckles [21]. While PIPKI β is distinctively targeted to a perinuclear compartment [27].

Where is PI4,5P₂?

The PI4,5P₂ specific PH domain from PLC δ fused to GFP (PH-PLC δ -GFP) has been used as a biosensor to examine the intracellular distribution of PI4,5P₂ [42]. Though it is clear that PIPKs are localized to multiple sub-cellular compartments and generate PI4,5P₂ at these sites [24,33], the PH-PLC δ -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 compartments, such as focal adhesions, trafficking vesicles for integrin or E-cadherin, and the nucleus at sites of mRNA processing with Star-PAP [14,16,21,31,40,43], where the PH-PLC δ -GFP does not localize [42]. These results indicate that PH-PLC δ -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 PLC δ -PH-GFP, or that PLC δ -PH-GFP access is prevented for steric reasons.

Besides the PLC δ -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-PLC δ -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 intracellular compartments [45]. More recently, these antibodies were used to show the presence of PI4,5P₂ with PIPKI α and PIPKI β on autolysosomes to regulate autophagic lysosome reformation [46]. Finally, the development of a specific small molecular probe, PHDM, that mimics the PLC δ 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 often PIPKs interacting proteins are also PI4,5P₂ effectors (Fig. 2), such as talin and Exo70, which form direct interactions with PIPKI γ 2 [16,40,48,49]. Presumably, the localized synthesis of PI4,5P₂ by PIPKI γ 2 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 where the highly orchestrated genesis of PI4,5P₂ drives the assembly of the trafficking machinery with E-cadherin for specific trafficking to adherens junctions (Fig. 3 and Fig. 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 PIPKIγ2 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. Alternatively, 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 enhances β-catenin translocation to the nucleus for regulation of transcription [54,55]. Intriguingly, PIPKIγ was recently found to also interact directly with β-catenin, independent of E-cadherin, and 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 PIPKI γ and PI4,5P $_2$ in β -catenin nuclear translocation requires further characterization, including its function in epithelial and mesenchymal cell types and its activation by specific growth factors.

PI4,5P $_2$ signaling regulates processes required for cell migration

PI4,5P $_2$ and PI3,4,5P $_3$ are key regulators of cell migration in both physiological and pathological conditions [13,60]. Migrating cells recruit different PIPKIs enzymes and PI4,5P $_2$ and PI3,4,5P $_3$ effectors to modulate cellular processes required for cell migration. Broadly, PI4,5P $_2$ 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].

PI4,5P $_2$ binds and/or regulates multiple actin binding proteins, promoting cytoskeletal reorganization at leading edge lamellipodium [63,64]. This includes reciprocal regulation between PIPKIs, small GTPases (RhoA, Rac1 and Cdc42), and PI4,5P $_2$, 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 PI4,5P $_2$ directly binds to N-WASP to alleviate intra-molecular constraints to activate the actin polymerization activity of Arp2/3 at leading edge lamellipodium [67]. Additionally, binding of both PI4,5P $_2$ and Cdc42 to distinct sites on N-WASP cooperatively activates the N-WASP/Arp2/3 complex required for cell migration [67,68]. Finally, PI4,5P $_2$ 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 PI4,5P $_2$ and PI3,4,5P $_3$ [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 PI4,5P $_2$ and PI3,4,5P $_3$ at the leading edge of migrating cell require further characterization. This is especially important to understand the specific functions of the rapid and transient increase of PI3,4,5P $_3$ in response to extracellular stimulation, even though PI4,5P $_2$ levels remain relatively constant. Potentially, this is accomplished by de novo PI4,5P $_2$ 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 PI4,5P $_2$ 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 PI4,5P $_2$ [40,41,71]. PIPKI γ 2 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. PIPKI γ 2 regulates these processes, through an interaction with the exocyst complex, a PI4,5P $_2$ -effector and vesicle trafficking protein complex, which is necessary for the delivery of integrins to the plasma membrane [16] (Fig. 4). PIPKI γ 2 also collaborates with talin to generate a localized PI4,5P $_2$ pool required for focal adhesion formation [40,41] and this has been demonstrated by numerous studies showing defective cell adhesion and migration in PIPKI γ 2 or talin knockdown cells [16,31,71]. The regulation of “inside-out” integrin activation by PIPKI γ 2 and PI4,5P $_2$ is critical during cell migration. PI4,5P $_2$ induces the dissociation of the talin rod domain from its head domain and promotes the tethering of the talin head to PI4,5P $_2$ -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, PIPKI γ 2 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 PIPKI γ 2 to talin, which would regulate focal adhesion assembly and signaling in migrating cells [16,72]. The role of PIPKI γ 2 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 PIPKI γ 2 in a polarized manner, leading to RhoA activation at uropods, which is required for neutrophil infiltration in vivo [61]. Similarly, PIPKI β is recruited to the cell rear by its unique C-terminus, where it interacts with EBP50 and Rho-GDP to regulate cell polarity required for cell migration [74]. PIPKI γ 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 PIP kinases may complicate the interpretation of the data as in vivo synthesis of PI4,5P₂ may be spatially changed. Thus, studies employing only over expression 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 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 via recruiting 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 HIP1/1R [75]. Importantly, precise tuning of PI4,5P₂ turnover is critical during endocytosis, for example, 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]. PIPKI γ is the predominant isoform for PI4,5P₂ synthesis in neuronal cells [30]. Following NMDA stimulation of neuronal cells, PIPKI γ 2 is dephosphorylated by calcineurin, promoting the interaction of PIPKI γ 2 with AP2. [80]. This interaction enhances PIPKI γ 2 kinase activity and, thus, PI4,5P₂ generation that is necessary for endocytosis during neuronal cell activation [30,80]. Similarly, the interaction of PIPKI γ 2 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 PIPKI γ 2 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]. On the surface of these synaptic vesicles is the anchored membrane protein, synaptotagmin-1, which 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 derived from PIPKI γ and PIPKI α knockout mice has revealed a crucial role for both PIPKI γ and PIPKI α in phagocytosis [32]. Fc γ R is important in regulating the phagocytosis of microbes by binding to immunoglobulins [87]. Loss of PIPKI γ impaired attachment of IgG-opsonized particles and clustering of Fc γ R. More specifically, the PIPKI γ 1 isoform induced actin depolymerization to promote particle attachment necessary for Fc γ R microclustering. Further, Syk-mediated phosphorylation of PIPKI γ 1 during Fc γ R-mediated phagocytosis promotes PIPKI γ 1 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, PIPKI α deficiency impaired particle ingestion without any effect on particle attachment. PIPKI α 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 PIPKI γ in mice blocked embryonic development, resulting in prenatal death [30,51]. While, mice lacking PIPKI α or PIPKI β survive to adulthood with some functional defects in specific cell types [90,91]. It is clear that each PIPKI 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 PIPKI α -knockout mice are deficient in PI4,5P₂ synthesis and IP₃ formation stimulated by thrombin [90]. PIPKI β also plays some roles in this process since double knockout of PIPKI α and PIPKI β led to a complete loss of thrombin-induced IP₃ synthesis in platelets [90]. Though PIPKI γ is the predominant PIPKI isoform in platelets, it does not appear to contribute to this process. Additionally, PIPKI α , but not PIPKI β , has a specific function in modulating platelet aggregation and in vivo thrombosis further confirming the distinct roles of different PIPKI isoforms [90].

PIPKI β is reported to negatively regulate mast cell functions and anaphylactic responses [91]. Knockout of PIPKI β in mice enhances passive cutaneous and systemic anaphylaxis [91]. It may due to the function of PIPKI β to negatively regulate Fc ϵ RI-mediated cellular responses and anaphylaxis [91]. Interestingly, though depletion of PIPKI β 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 PIPKI β 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 PIPKI γ [92]. Knockout of PIPKI γ leads to 30–50% reduction of PI4,5P₂ in the brain and causes synaptic transmission defects [30], whereas knockout of PIPKI α or PIPKI β has no dramatic effect on

levels of PI4,5P₂ in the brain [92]. Truncation of the PIPKI γ 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]. There are no other obvious anatomical anomalies found in these mice and the defects in nervous system function may play an important role in their death [30]. Interestingly, another independent PIPKI γ -knockout mouse line, generated by inserting a β -geo gene trap within the first intron of the PIP5KI γ gene, fails to develop beyond embryonic day 11.5 and these embryos have myocardial developmental and neural tube closure defects [51]. The cause for the differences between these two knockout mice remains unclear and may reflect the different genetic backgrounds or knockout approaches.

PIPKI γ has different splice variants and each variant can play distinct roles [24,36]. While depletion of pan-PIPKI γ is lethal [30,51,93], mice with the specific knockout of PIPKI γ i2-develop normally and are indistinguishable from wild type littermates [93]. This indicates that PIPKI γ activity, but not PIPKI γ i2 is required for normal embryonic development. However, both pan-PIPKI γ and the PIPKI γ i2-knockout mice show defects in synaptic vesicle (SV) endocytosis suggesting a specific and non-redundant role for PIPKI γ i2 in this process [93]. Agonist-induced Ca²⁺ signaling is reduced in pan-PIPKI γ -knockout mice and enhanced in PIPKI γ i2-knockout mice indicating distinct roles of PIPKI γ variants in Ca²⁺ signaling [93].

PI4,5P₂ regulating enzymes are associated with human diseases

Until now, only one disease causing mutation has been reported for PIPKI genes. Genetic mutation in PIPKI γ (D253N) of a required catalytic residue in the kinase domain diminishes its kinase activity and leads to lethal congenital contractural syndrome type 3 (LCCS3) [94]. Homozygosity of this mutation is postnatal lethal with respiratory insufficiency, fetal akinesia, limb contractures, and muscle atrophy [94]. These defects may be caused by disruption of PIPKI γ activity leading to defect of PI4,5P₂ synthesis in brain and neurological malfunction [94].

A potential link between PI4,5P₂ signaling and Alzheimer's disease was identified over two decades ago, where reduced phosphoinositide content, including PI4,5P₂, in the temporal cortex were suggested to impair receptor function in Alzheimer's disease [95,96]. 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 [97,98]. Further, treatment of primary cortical neurons with amyloid- β -peptide decreased PI4,5P₂ levels and synaptic dysfunction [95,98]. The amyloid- β -peptide-induced synaptic dysfunction was ameliorated by maintaining normal PI4,5P₂ levels in the brain [95].

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 Ocr11 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] [99]. 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 that impairs the recycling of multiple receptors including megalin [78,99]. This defect in megalin recycling in renal proximal tubular cells impairs the retrieval of proteins from kidney ultra-filtrates, 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 perspective

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, which 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 [100].

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 contributed by 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 unique distributions of PI4,5P₂ can regulate specific processes. The characterization of animals with 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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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 and is in the greatest concentration on the plasma membrane but is 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.

Figure 2.

The localized production of PI4,5P₂ modulates specific PI4,5P₂ effectors. Extracellular signals and regulators regulate the interaction of PIPKs with targeting factors that recruit PIPKs to specific sub-cellular compartments. By generating PI4,5P₂ on site, PIPKs establish a local PI4,5P₂ pool to modulate the function of local PI4,5P₂ effectors necessary for site-specific biological activities.

PI4,5P₂ modulates E-cadherin trafficking. PIPKI γ interacts with both E-cadherin and AP complexes. Through this dual interaction, PIPKI γ acts as a scaffold between AP complexes and E-cadherin to facilitate E-cadherin endocytosis and trafficking to the basolateral membrane. In addition, AP complexes are a PI4,5P₂ effectors. The localized generation of PI4,5P₂ via PIPKI γ activates AP complexes. PIPKI γ also modulates exocyst-mediated E-cadherin trafficking. PIPKI γ generates local PI4,5P₂ pools on the basolateral membrane via interacting with E-cadherin, which direct the targeting of exocyst. The direct interaction between exocyst and PIPKI γ further facilitates the recruitment of exocyst to adherens junctions, where exocyst-bound lateral cargoes, such as E-cadherin, can be delivered.

Figure 4.

PIPKI γ regulates the assembly of E-cadherin into adherens junctions in polarized epithelial cells. Stimuli, such as growth factors, can promote disassembly and phosphorylation of adherens junction components. PIPKI γ can promote nuclear translocation of β -catenin, leading to enhanced transcriptional activity. In migrating tumor cells, which have lost E-cadherin expression (after EMT), PIPKI γ 2 coordinates with exocyst complex and talin to mediate polarized delivery of integrins at the leading edge membrane. PI4,5P $_2$ generation by PIPKI γ 2 in plasma membrane or in recycling endosomes near the plasma membrane facilitates the assembly of the exocyst complex and talin. The coordinated activity of PIPKI γ 2 and the exocyst complex in concert with talin at the leading edge promotes the polarized recruitment and trafficking of integrin molecules. Loss of PIPKI γ 2, the exocyst complex or talin impairs the polarized recruitment/trafficking of integrins required for cell migration.