Phosphatidylinositol 4,5-bisphosphate: Targeted production and signaling

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Phosphatidylinositol 4,5-bisphosphate ($PI4,5P_2$) is a key lipid signaling molecule that regulates a vast array of biological activities. $PI4,5P_2$ 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. $PI4,5P_2$ interacts with hundreds of different effector proteins. The enormous diversity of $PI4,5P_2$ effector proteins and the spatiotemporal control of $PI4,5P_2$ generation allow $PI4,5P_2$ signaling to control a broad spectrum of cellular functions. $PI4,5P_2$ 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 $PI4,5P_2$ effectors. These interactions are a mechanism to define spatial and temporal $PI4,5P_2$ synthesis and the specificity of $PI4,5P_2$ signaling. In turn, the regulation of $PI4,5P_2$ effectors at specific cellular compartments has implications for understanding how $PI4,5P_2$ controls cellular processes and its role in diseases.

Keywords:
lipid messenger; phosphatidylinositol 4,5-bisphosphate; phosphatidylinositol phosphate kinase; $PI4,5P_2$ effector

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Abbreviations:
DAG, diacylglycerol; **IP_3**, inositol trisphosphate; **$PI3,4,5P_3$**, phosphatidylinositol 3,4,5-trisphosphate; **$PI4,5P_2$**, phosphatidylinositol 4,5-bisphosphate; **PIPK,** phosphatidylinositol phosphate kinase; **PLC**, phospholipase C.

Introduction

Phosphatidylinositol 4,5-bisphosphate ($PI4,5P_2$) 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 $PI4,5P_2$ [2–4]. At that time, $PI4,5P_2$ was thought to only serve as an intermediate in the PI cycle [5, 6]. Early studies identified a role for $PI4,5P_2$ as a precursor of other signaling molecules, such as inositol trisphosphate (IP_3) and diacylglycerol (DAG) [6–8]. In the 1980s, the role of $PI4,5P_2$ as a precursor was further expanded via the discovery that $PI4,5P_2$ could be used as substrate for PI3K to produce phosphatidylinositol 3,4,5-trisphosphate ($PI3,4,5P_3$) [9].

In the mid 1980s, direct roles for $PI4,5P_2$ signaling were defined. Anderson and Marchesi discovered that $PI4,5P_2$ regulated the association of the cytoskeletal protein, band 4.1, with the membrane protein glycophorin, demonstrating a role for $PI4,5P_2$ in regulating the interaction of cytoskeletal proteins with the plasma membrane [10]. Lassing and Lindberg discovered that $PI4,5P_2$ directly interacts with, and inhibits, the actin-modifying proteins profilin and gelsolin, to promote actin assembly [11, 12]. These discoveries elevated $PI4,5P_2$ from simply being a precursor of messengers, to a lipid messenger in its own right (Fig. 1), and initiated an explosion of research on $PI4,5P_2$-binding proteins/effectors. Currently, hundreds of $PI4,5P_2$-binding proteins/effectors have been identified and this number is increasing. $PI4,5P_2$ 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).

There is now known to be a large and diverse array of $PI4,5P_2$ effectors, many of which are found in the same membrane compartment, e.g. the plasma membrane. This presents a dilemma, the cellular or membrane contents of most second messengers dramatically fluctuate upon agonist stimulation

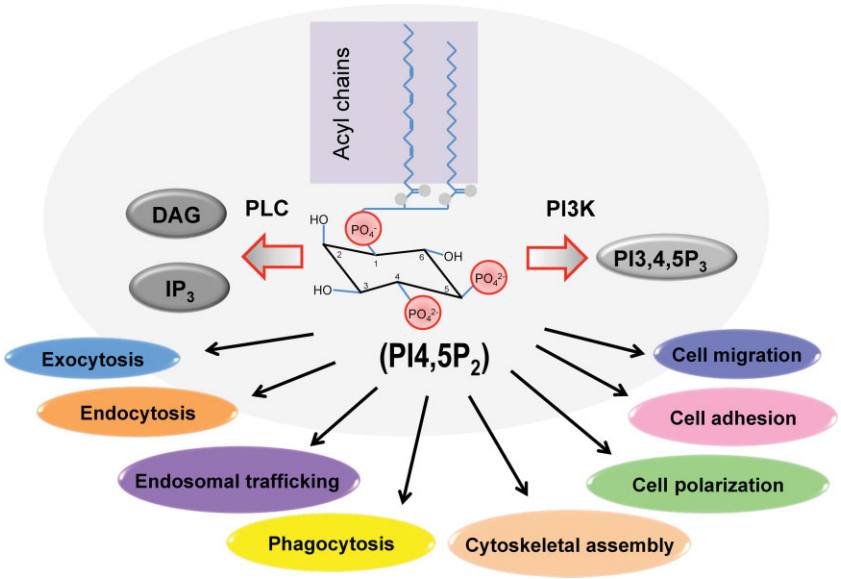

Figure 1. Overview of $PI4,5P_2$ functions in the cytosol and plasma membrane. $PI4,5P_2$ is a polyphosphoinositide that is phosphorylated on the 4th and 5th hydroxyl group on the myo-inositol ring. Its greatest concentration is seen on the plasma membrane but it is also found on most cellular membrane compartments and in the nucleus. $PI4,5P_2$ is utilized by PI3K or PLC to generate second messengers: $PI3,4,5P_3$, DAG, and IP_3. The generation of $PI4,5P_2$ in a spatio-temporal manner is the basis for $PI4,5P_2$ regulation of diverse cellular events, including endocytosis, exocytosis, vesicle trafficking, and cell migration.

[6]. However, the cellular or membrane content of $PI4,5P_2$ is relatively constant [23–25] and undergoes only modest changes upon stimulation [26, 27]. This raises the question: how does $PI4,5P_2$ specifically modulate cellular events?

Over the last few decades, the discovery and study of the phosphatidylinositol phosphate kinases (PIPKs), the enzymes that produce $PI4,5P_2$, revealed a mechanism for the targeted production of $PI4,5P_2$. Distinct isoforms of PIPKs are targeted through association with unique interacting proteins to specific subcellular locations, and this controls the local production of $PI4,5P_2$ [24, 27] (Fig. 2). Thus, PIPKs along with enzymes that consume $PI4,5P_2$, such as phospholipase C (PLC), PI3K, and $PI4,5P_2$ 5-phosphatases, are critical for the spatial and temporal regulation of $PI4,5P_2$ levels [6, 13, 24, 28, 29].

Six genes encode the PIPKs that generate $PI4,5P_2$. These are the PIP kinases types I and II (PIPKI and PIPKII, respectively). PIPKI and PIPKII synthesize the majority of $PI4,5P_2$ in the cell [27]. PIPKI preferentially phosphorylates the 5-hydroxyl position on the myo-inositol ring of PI4P to generate $PI4,5P_2$, while PIPKII uses PI5P as substrate and phosphorylates the 4-hydroxyl position to produce $PI4,5P_2$ [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 $PI4,5P_2$ effector proteins and this links $PI4,5P_2$ synthesis to specific cellular functions. In this way, targeted $PI4,5P_2$ 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 $PI4,5P_2$ in the cytosol. $PI4,5P_2$ function in nuclear events has recently been reviewed [33, 34]. $PI4,5P_2$ signaling or $PI4,5P_2$-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 $PI4,5P_2$ signaling in human diseases is critical. This review, focusing on emerging areas, provides a synopsis of $PI4,5P_2$ functions and how its signaling is modulated.

PIPK isoforms synthesize $PI4,5P_2$ at distinct cellular locations

The PIPKI family is highly diverse. For example, there are at least six PIPKIγ splice variants expressed in humans, labeled PIPKIγi1 to PIPKIγi6 [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 Mg^{2+}, 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 PIPKIγi1 isoform [36]. The diversity of these sequences confers the ability to interact with different targeting proteins that are often $PI4,5P_2$ effectors. These enzymes are then targeted to specific cellular compartments where they generate $PI4,5P_2$ that specifically modulates effectors required for specific biological functions (Fig. 2).

Consistently, each splice variant of PIPKIγ localizes to select cellular locations. PIPKIγi2 can be targeted to focal adhesions by an interaction with talin [40, 41]. Conversely, PIPKIγi4 localizes to nuclear speckles and PIPKIγi5 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

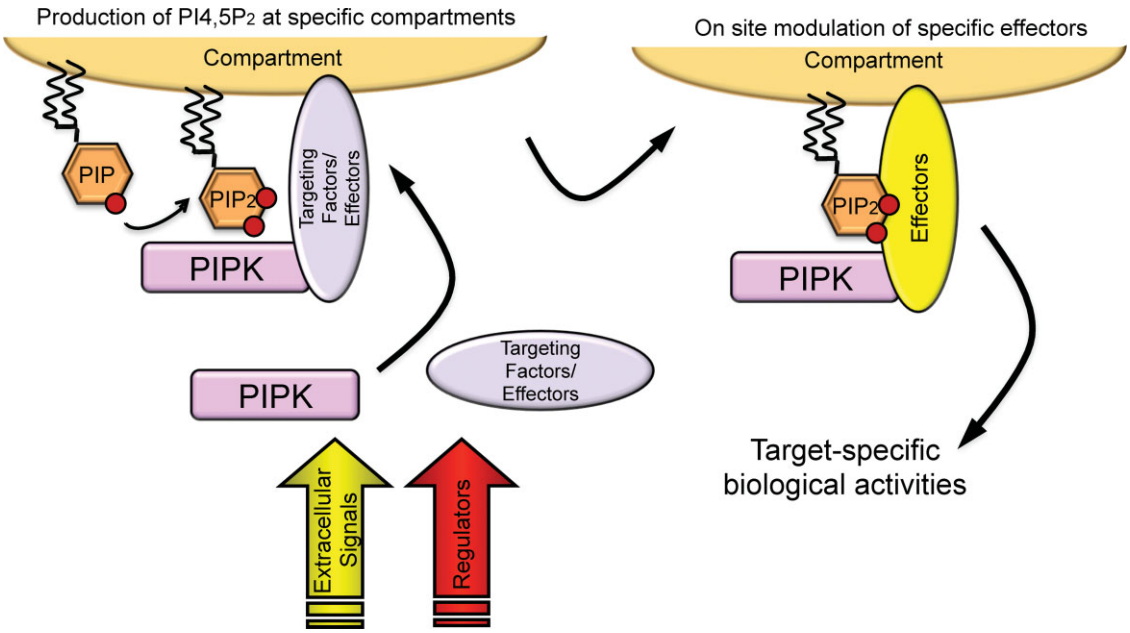

Figure 2. The localized production of $PI4,5P_2$ modulates specific $PI4,5P_2$ effectors. Extracellular signals and regulators regulate the interaction of PIPKs with targeting factors that recruit PIPKs to specific subcellular compartments. By generating $PI4,5P_2$ on site, PIPKs establish a local $PI4,5P_2$ pool to modulate the function of local $PI4,5P_2$ effectors necessary for site-specific biological activities.

speckles [21], whereas PIPKIβ is distinctively targeted to a perinuclear compartment [27].

Where is $PI4,5P_2$?

The $PI4,5P_2$-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 $PI4,5P_2$ [42]. Although it is clear that PIPKs are localized to multiple subcellular compartments and generate $PI4,5P_2$ at these sites [24, 33], the PH-PLCδ-GFP primarily detects $PI4,5P_2$ at the plasma membrane [42]. This observation is in conflict with the fact that many $PI4,5P_2$ 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, 16, 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 $PI4,5P_2$. It is possible that the levels of $PI4,5P_2$ at these other compartments are low and/or that the $PI4,5P_2$ 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 $PI4,5P_2$ beyond the plasma membrane has been demonstrated using other $PI4,5P_2$-specific antibodies and probes. Using a purified GST-PLCδ-PH, $PI4,5P_2$ was shown to be present at the plasma membrane, Golgi, endosomes, ER, and nucleus [44]. Additionally, $PI4,5P_2$-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 $PI4,5P_2$ 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 $PI4,5P_2$ function, by binding to and inhibiting $PI4,5P_2$-regulated events, including endocytosis and stress fiber formation [47].

Further evidence that $PI4,5P_2$ functions throughout the cell is that PIPK-interacting proteins are also often $PI4,5P_2$ effectors (Fig. 2), such as talin and Exo70, which form direct interactions with PIPKIγi2 [16, 40, 48, 49]. Presumably, the localized synthesis of $PI4,5P_2$ by PIPKIγi2 modulates the function of effectors that are in close proximity, but without an obvious increase in $PI4,5P_2$ levels that are detectable by current approaches. This represents an area of vigorous investigation and debate in the field.

Local production of $PI4,5P_2$ 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 $PI4,5P_2$ and $PI3,4,5P_3$ at distinct regions of the plasma membrane is crucial for cyst formation during morphogenesis [50]. $PI4,5P_2$ 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

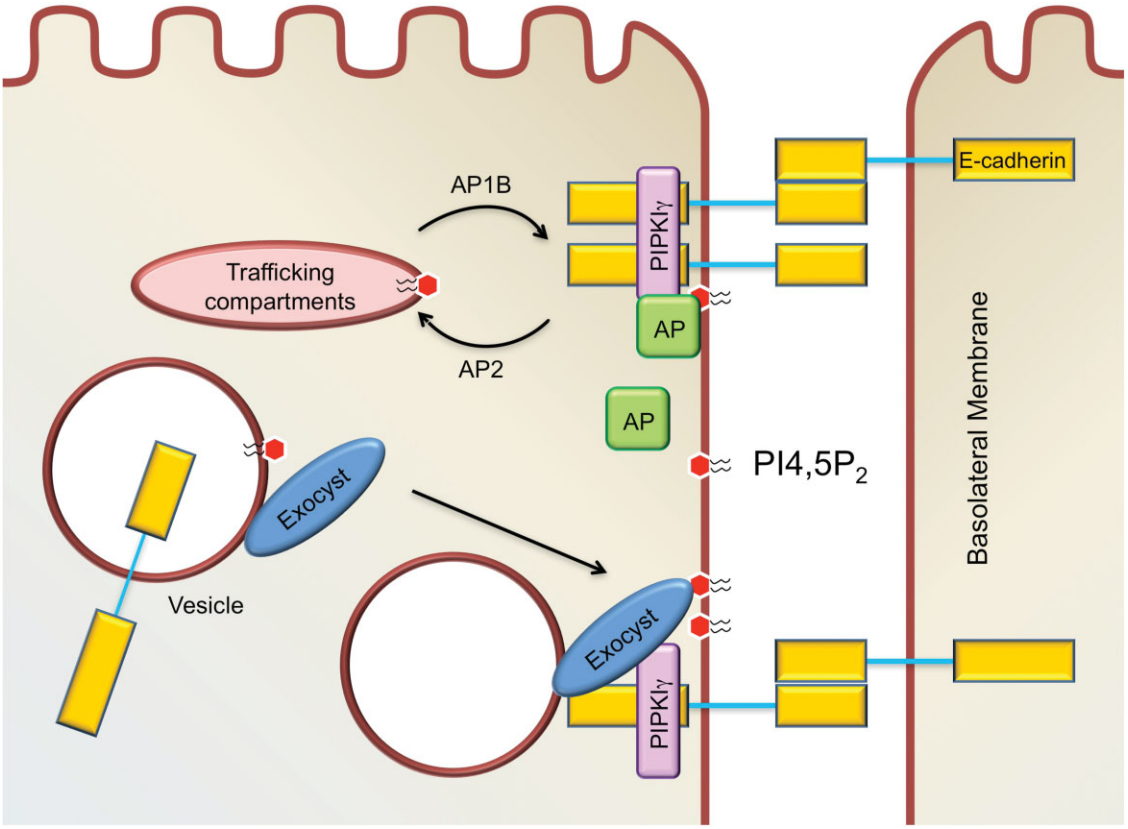

Figure 3. $PI4,5P_2$ modulates E-cadherin trafficking. PIPKIγ interacts with both E-cadherin and AP complexes. Through this dual interaction, PIPKIγ acts as a scaffold between AP complexes and E-cadherin to facilitate E-cadherin endocytosis and trafficking to the basolateral membrane. In addition, AP complexes are $PI4,5P_2$ effectors. The localized generation of $PI4,5P_2$ via PIPKIγ activates AP complexes. PIPKIγ also modulates exocyst-mediated E-cadherin trafficking. PIPKIγ generates local $PI4,5P_2$ pools on the basolateral membrane via interaction with E-cadherin, which direct the targeting of the exocyst. The direct interaction between the exocyst and PIPKIγ further facilitates the recruitment of the exocyst to adherens junctions, where exocyst-bound lateral cargoes, such as E-cadherin, can be delivered.

recruited to the apical region to dephosphorylate $PI3,4,5P_3$ to $PI4,5P_2$ [50]. These findings indicate that $PI4,5P_2$ and $PI3,4,5P_3$ 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 $PI4,5P_2$ 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. PIPKIγi2 has emerged as an important player in maintaining the epithelial polarity via E-cadherin trafficking [14, 15] (Fig. 3). Simultaneous binding of PIPKIγi2 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 PIPKIγi2 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 $PI4,5P_2$ 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 PIPKIγi2 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 PIPKIγi2 knockout mice [52], indicating that there are compensatory mechanisms in vivo. Potentially, a role specifically for PIPKIγi2 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, PIPKIγ was

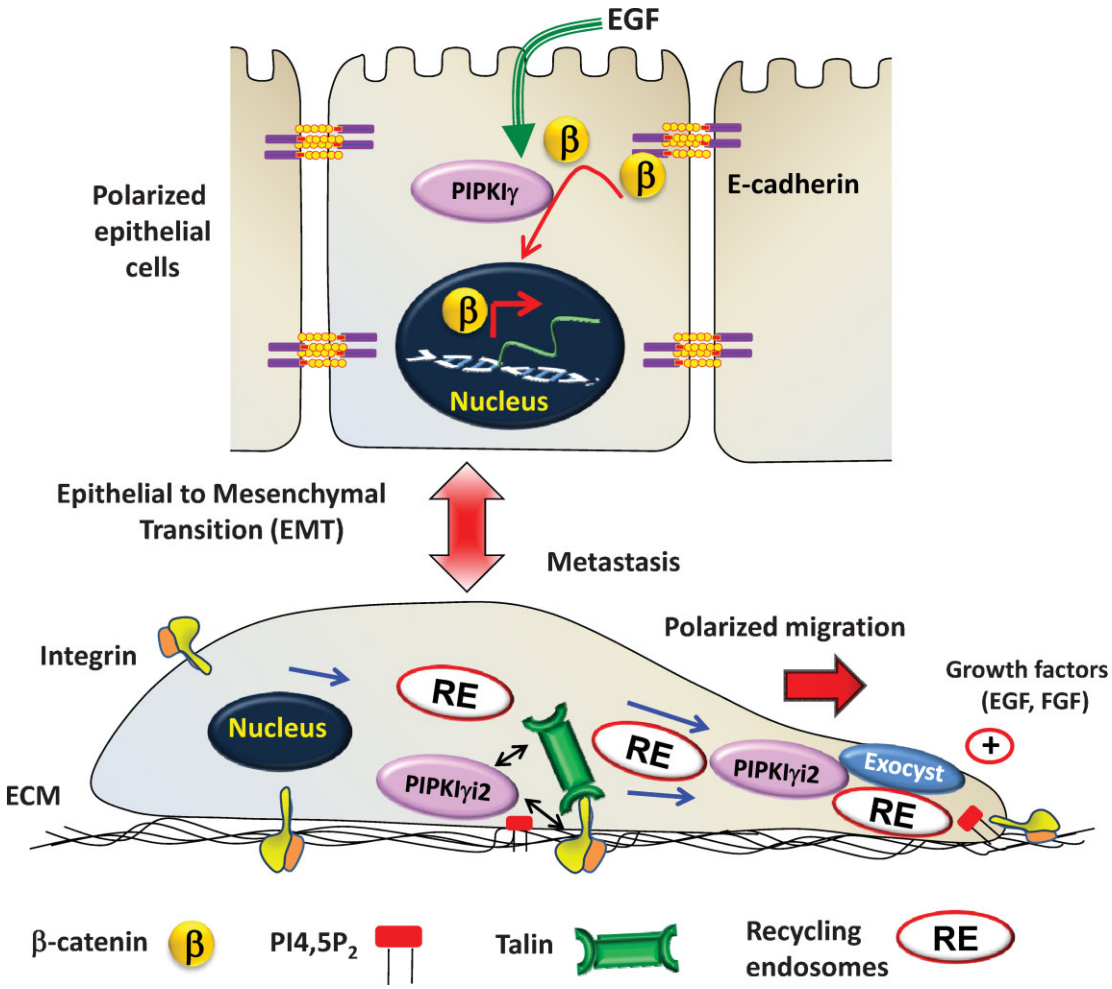

Figure 4. PIPKIγ regulates the assembly of E-cadherin into adherens junctions in polarized epithelial cells. Stimuli, such as growth factors, can promote disassembly and phosphorylation of adherens junction components. PIPKIγ can promote nuclear translocation of β-catenin, leading to enhanced transcriptional activity. In migrating tumor cells, which have lost E-cadherin expression (after epithelial to mesenchymal transition, EMT), PIPKIγi2 coordinates with the exocyst complex and talin to mediate polarized delivery of integrins at the leading edge membrane. $PI4,5P_2$ generation by PIPKIγi2 in the plasma membrane or in recycling endosomes near the plasma membrane facilitates the assembly of the exocyst complex and talin. The coordinated activity of PIPKIγi2 and the exocyst complex in concert with talin at the leading edge promotes the polarized recruitment and trafficking of integrin molecules. Loss of PIPKIγi2, the exocyst complex or talin impairs the polarized recruitment/trafficking of integrins required for cell migration.

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 $PI4,5P_2$ 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 $PI4,5P_2$ 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 under both physiological and pathological conditions [13, 60]. Migrating cells recruit different PIPKI 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 intramolecular 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 for understanding 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]. PIPKIγi2 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. PIPKIγi2 regulates these processes, through an interaction with the exocyst complex, a $PI4,5P_2$-effector and vesicle trafficking protein complex, that is necessary for the delivery of integrins to the plasma membrane [16] (Fig. 4). PIPKIγi2 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 PIPKIγi2 or talin knockdown cells [16, 31, 71]. The regulation of ''inside-out'' integrin activation by PIPKIγi2 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, PIPKIγi2 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 PIPKIγi2 to talin, which would regulate focal adhesion assembly and signaling in migrating cells [16, 72]. The role of PIPKIγi2 and talin in polarized secretion of integrin suggests that concentrated $PI4,5P_2$ could be delivered to the leading edge via secretory vesicles (Fig. 4).

In addition to adherent cells, the roles of specific PIPKI isoforms and $PI4,5P_2$ signaling in regulation of chemotaxis and trans-endothelial migration has been extensively studied in non-adherent circulating leukocytes. PIPKIs and $PI4,5P_2$ signaling play critical roles in regulating uropods for leukocyte migration [61, 74]. Integrins recruit PIPKIγi2 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 EBP50 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 $PI4,5P_2$ in backness response/signaling required for cell migration, spatially distinct from $PI4,5P_2$ signaling at the leading edge. In these studies, overexpression of PIPKs may complicate the interpretation of the data, as in vivo synthesis of $PI4,5P_2$ may be spatially changed. Thus, studies employing only overexpression should be viewed with skepticism.

Endocytosis, exocytosis, and phagocytosis require $PI4,5P_2$

$PI4,5P_2$ 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]. $PI4,5P_2$ 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 HIP1/1R [75]. Importantly, precise tuning of $PI4,5P_2$ turnover is critical during endocytosis, e.g. the increase in $PI4,5P_2$ that coincides with clathrin coat assembly or the corresponding decrease in $PI4,5P_2$ for clathrin coat disassembly [76]. Paradoxically, neither $PI4,5P_2$ nor $PI4,5P_2$-generating enzymes have been clearly observed on vesicle buds during clathrin-coat formation and maturation [75]. During late stages of endocytosis, the $PI4,5P_2$ phosphatases, OCRL and synaptojanin, are recruited to endocytic vesicles by direct interactions with clathrin and AP2, and subsequently reduce $PI4,5P_2$ levels, resulting in the dissociation of $PI4,5P_2$ effectors from endocytic vesicles, which is necessary for endosomal fusion [77, 78].

Neuronal cells are commonly used to study how $PI4,5P_2$ signaling regulates communication across the synapse, through its precise control over endocytic and exocytic events [79]. PIPKIγ is the predominant isoform for $PI4,5P_2$ synthesis in neuronal cells [30]. Following NMDA stimulation of neuronal cells, PIPKIγi2 is dephosphorylated by calcineurin, promoting the interaction of PIPKIγi2 with AP2 [80].

This interaction enhances PIPKIγi2 kinase activity and, thus, the $PI4,5P_2$ generation that is necessary for endocytosis during neuronal cell activation [30, 80]. Similarly, the interaction of PIPKIγi2 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 PIPKIγi2 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, $PI4,5P_2$ 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 $PI4,5P_2$-binding sites [85]. $PI4,5P_2$ 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 PIPKIγ and PIPKIα knockout mice has revealed a crucial role for both PIPKIγ and PIPKIα in phagocytosis [32]. FcγR is important in regulating the phagocytosis of microbes by binding to immunoglobulins [87]. Loss of PIPKIγ impaired attachment of IgG-opsonized particles and clustering of FcγR. More specifically, the PIPKIγi1 isoform induced actin depolymerization to promote the particle attachment necessary for FcγR microclustering. Further, Syk-mediated phosphorylation of PIPKIγi1 during FcγR-mediated phagocytosis promotes PIPKIγi1 enzymatic activity. In addition, formation of the actin-driven structures required for optimal binding to targets of phagocytosis depends on $PI4,5P_2$, $PI3,4,5P_3$, and active Rac1 [88]. Alternatively, it is possible that PIPKIα deficiency impairs particle ingestion without any effect on particle attachment. PIPKIα 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 $PI4,5P_2$-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 PIPKIγ in mice blocked embryonic development, resulting in prenatal death [30, 51], whereas mice lacking PIPKIα or PIPKIβ 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 $PI4,5P_2$ pools required for specific cellular processes, but functional redundancy among isoforms does exist [89, 90].

Platelets from PIPKIα-knockout mice are deficient in $PI4,5P_2$ synthesis and IP_3 formation stimulated by thrombin [90]. PIPKIβ is also involved in this process since double knockout of PIPKIα and PIPKIβ led to a complete loss of thrombin-induced IP_3 synthesis in platelets [90]. Although PIPKIγ is the predominant PIPKI isoform in platelets, it does not appear to contribute to this process. Additionally, PIPKIα, but not PIPKIβ, has a specific function in modulating platelet aggregation and in vivo thrombosis, further confirming the distinct roles of the different PIPKI isoforms [90].

PIPKIβ has been reported to negatively regulate mast cell functions and anaphylactic responses [91]. Knockout of PIPKIβ in mice enhances passive cutaneous and systemic anaphylaxis, which may be due to the function of PIPKIβ to negatively regulate FcεRI-mediated cellular responses and anaphylaxis [91]. Interestingly, although depletion of PIPKIβ decreases the total $PI4,5P_2$ level by about 15% in mast cells, the production of the $PI4,5P_2$-derived second messengers IP_3 and $PI3,4,5P_3$ is increased [91]. This indicates that PIPKIβ is not responsible for formation of $PI4,5P_2$ pools required for IP_3 and $PI3,4,5P_3$ production.

The majority of $PI4,5P_2$ in the brain is believed to be synthesized by PIPKIγ [89]. Knockout of PIPKIγ leads to a 30–50% reduction of $PI4,5P_2$ in the brain and causes synaptic transmission defects [30], whereas knockout of PIPKIα or PIPKIβ has no dramatic effect on levels of $PI4,5P_2$ in the brain [89]. Truncation of the PIPKIγ 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 PIPKIγ-knockout mouse line, generated by inserting a β-geo gene trap within the first intron of the PIP5KIγ 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.

PIPKIγ has different splice variants and each variant can play distinct roles [24, 36]. While depletion of pan-PIPKIγ is lethal [30, 51, 52], mice with the specific knockout of PIPKIγi2 develop normally, and are indistinguishable from wild-type littermates [52]. This indicates that PIPKIγ activity, but not PIPKIγi2 is required for normal embryonic development. However, both pan-PIPKIγ- and the PIPKIγi2-knockout mice show defects in synaptic vesicle endocytosis, suggesting a specific and non-redundant role for PIPKIγi2 in this process [52]. Agonist-induced Ca^{2+} signaling is reduced in pan-PIPKIγ-knockout mice and enhanced in PIPKIγi2-knockout mice, indicating distinct roles of PIPKIγ variants in Ca^{2+} signaling [52].

$PI4,5P_2$-regulating enzymes are associated with human diseases

Until now, only one disease-causing mutation has been reported for PIPKI genes. Genetic mutation in PIPKIγ (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 akinesia, limb contractures, and muscle atrophy [92]. These defects may be caused by disruption of PIPKIγ activity, leading to a defect of $PI4,5P_2$ synthesis in the brain and neurological malfunction [92].

A potential link between $PI4,5P_2$ signaling and Alzheimer's disease was identified over two decades ago, where a reduced phosphoinositide content, including $PI4,5P_2$, 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 $PI4,5P_2$ 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 $PI4,5P_2$ levels and caused synaptic dysfunction [93, 96]. The amyloid-β-peptide-induced synaptic dysfunction was ameliorated by maintaining normal $PI4,5P_2$ levels in the brain [93].

The importance of $PI4,5P_2$ 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 Ocrl1 gene, which encodes a $PI4,5P_2$ 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 $PI4,5P_2$ 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 $PI4,5P_2$ impacts normal function and leads to human diseases.

Summary and perspectives

$PI4,5P_2$ 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 $PI4,5P_2$ pools. These modulate the activity of $PI4,5P_2$ 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 $PI4,5P_2$ effectors will expand our knowledge of $PI4,5P_2$ biological functions. However, understanding the exact roles for $PI4,5P_2$ signaling poses an enormous challenge due to the dynamic nature of $PI4,5P_2$, the varying affinities and specificities of protein-lipid interactions, the diversity of $PI4,5P_2$-generating and -utilizing enzymes, and their mechanisms of regulation [98].

$PI4,5P_2$ can be utilized as a precursor to generate other phosphoinositides such as $PI3,4,5P_3$ and PI4P. How the specific local $PI4,5P_2$ pools affect the production of other phosphoinositides and modulate their signaling still needs to be clarified. In addition, some $PI4,5P_2$ effectors also interact with, and are modulated by, additional phosphoinositide species. The specific roles of $PI4,5P_2$ and its cooperativity with other phosphoinositides to modulate these effectors should be elucidated in future studies.

Continuing research is necessary to further understand how $PI4,5P_2$ signaling functions in novel cellular pathways, and how these processes relate to known $PI4,5P_2$-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 $PI4,5P_2$ effectors will further explain how the unique distributions of $PI4,5P_2$ can regulate specific processes. The characterization of animals with a knockout of specific PIPK splice variants or $PI4,5P_2$ 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 $PI4,5P_2$ can broadly regulate cellular functions with potential consequences in pathological conditions.

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