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Tsujita, Kazuya

Itoh, Toshiki

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Phosphoinositides at the interface between the plasma membrane and actin cortex

Kazuya Tsujita and Toshiki Itoh

Biosignal Research Center, Organization of Advanced Science and Technology, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo, 657-8501, Japan

Correspondence to: Kazuya Tsujita (tsujita@people.kobe-u.ac.jp)

Toshiki Itoh (titoh@people.kobe-u.ac.jp)

Abstract

In order for the cell to function well within a multicellular system, the mechanical properties of the plasma membrane need to meet two different requirements: cell shape maintenance and rearrangement. To achieve these goals, phosphoinositides play key roles in the regulation of the cortical actin cytoskeleton. PI(4,5)P_2 is the most abundant phosphoinositide species in the plasma membrane. It maintains cell shape by linking the actin cortex to the membrane via interactions with ERM proteins and class 1 myosins. Although the role of D3-phosphoinositides, such as PI(3,4,5)P_3 in actin-driven cell migration, has been a subject of controversy, it becomes evident that the dynamic turnover of the phosphoinositide by the action of metabolizing enzymes, such as 5-phosphatases, is necessary. Recent studies have revealed an important role of PI(3,4)P_2 in podosome/invadopodia formation, shedding new light on the actin-based organization of membrane structures regulated by phosphoinositide signaling.

In most eukaryotic cells, the plasma membrane (PM) closely adheres to the actin cortex, a thin network of actin filaments. It has become evident that this PM–actin cortex interaction is central to the regulation of the mechanical properties of the cell membrane. The membrane mechanics are important for the generation and maintenance of the overall cell architecture, as well as local membrane domains such as the apical microvilli [1]. On the other hand, the actin cortex needs to rapidly rearrange or locally dissociate from the membrane during cell shape changes such as membrane protrusions for cell motility or membrane invagination during endocytosis. Furthermore, these phenomena are accompanied by dynamic deformations of the PM that are resisted by the force produced by PM–actin cortex adhesions [2]. Phosphoinositides, in particular phosphatidylinositol 4,5-bisphosphate ($PI[4,5]P_2$), have been recognized as crucial interfaces between the PM and the cytosolic proteins that link the cytoskeleton to the membrane. Over the last three decades, considerable progress has been made for the identification of actin-binding proteins. It is known that the activities of these proteins are fine-tuned by direct interaction with $PI(4,5)P_2$ as well as other phosphoinositides [3-5]. Importantly, recent studies have revealed that $PI(4,5)P_2$ and its binding proteins are key regulators of the PM mechanical characteristics [6-10]. Given that the phosphoinositide cellular levels are modified by phosphoinositide-metabolizing enzymes, the phospholipid turnover enables cells to change and maintain their shape according to the extracellular environment, through the regulation of reversible associations between the PM and actin cortex. In this review, we focus on recent progress in the understanding of how phosphoinositides regulate actin-based cellular dynamics. First, we provide an overview of the proposed mechanisms on how

phosphoinositide-binding “linker” proteins create PM-actin cortex adhesions that play key roles in cell migration driven by actin polymerization. We also discuss the roles of D3-phosphoinositides in directed cell migration during chemotaxis. Finally, the specific involvement of PI(3,4)P_2 in actin-based membrane structures such as circular dorsal ruffles and podosomes/invadopodia is introduced.

1. Regulation of PM–actin cortex adhesion by PI(4,5)P_2

The close interaction between the PM and actin cortex produces adhesion energy between the membrane and cytoskeleton, which is a critical determinant of cell morphology. Defining the force required for membrane deformation, the adhesion energy regulates several mechanical cellular functions, including cell motility, endocytosis/exocytosis, and cytokinesis. Tether force measurements using optical tweezers revealed that membrane–actin cytoskeleton adhesion energy is primary regulated by PI(4,5)P_2, as both sequestration and depletion of PI(4,5)P_2 led to a decrease in the adhesion energy [6]. Consistently, megakaryocytes from PIPKIγ-deficient mice, a key enzyme that produces PI(4,5)P_2, exhibited a decrease in PM–cytoskeletal adhesion energy [11]. Because cells employ a subset of phosphoinositide kinases/phosphatases to control the amount of PI(4,5)P_2, phosphoinositide metabolism seems to be important for the regulation of PM–actin cortex adhesion. In addition, it is likely that phospholipase C (PLC), which irreversibly hydrolyze PI(4,5)P_2 into diacylglycerol (DG) and inositol 1,4,5-trisphosphate (Ins[1,4,5]P_3), play an active role in the regulation of membrane–cytoskeleton adhesion. In fact, a decrease in the adhesion energy level following

platelet-derived growth factor (PDGF) stimulation is inhibited by the PLC inhibitor U73122 [6], suggesting that dissociations of the “linker” proteins such as those of the Ezrin/Radixin/Moesin (ERM) family (see following section) from the PM by PLC-mediated $PI(4,5)P_2$ hydrolysis contribute to rearrangement of the membrane–cytoskeleton interactions.

1.1 ERM-mediated PM–actin cortex adhesions

ERM proteins are major linkers between the PM and actin cytoskeleton, playing important roles in cell shape changes such as directed cell migration and cell polarity formation [12] (Fig. 1). ERM proteins contain the band 4.1, ezrin, radixin and moesin (FERM) domains that directly bind to $PI(4,5)P_2$ with high affinity [13], as well as a C-terminal ERM-association domain (C-ERMAD) containing an F-actin-binding site, that collectively enable them to connect between the PM and the actin cortex [12]. It is believed that the ERM proteins are regulated by an intramolecular interaction between FERM and C-ERMAD domains that is relieved by $PI(4,5)P_2$-binding and phosphorylations at the C-terminal region [12, 14]. Importantly, direct interaction with $PI(4,5)P_2$ is a requisite for the PM localization of ERM proteins, as mutants of ERM proteins with a FERM domain defective in $PI(4,5)P_2$-binding could not be localized at the PM [15]. Hao *et al.* [16] demonstrated in lymphocytes that the PLC inhibitor inhibits chemokine-induced dissociation of ERM proteins from the PM. The study also showed that acute depletion of $PI(4,5)P_2$ is sufficient for the dissociation of ERM proteins from the PM, indicating that $PI(4,5)P_2$ metabolism regulates the PM–actin cortex adhesion via ERM [16]. Such a mechanism is important for cell shape changes in lymphocytes during chemotaxis, because the dissociation of ERM proteins from the PM

is correlated with the transition of cell morphology from a quiescent spherical state to a moving one with membrane protrusions [17]. It was also observed that cell spreading is enhanced by the reduction in membrane–cytoskeleton adhesion [18]. Indeed, atomic force microscopy (AFM) revealed that membrane–cytoskeleton adhesion energy is significantly reduced in ERM-deficient cells when compared to the control cells [7]. This observation was confirmed by a subsequent study that demonstrated a marked PM tension increase in lymphocytes expressing constitutively active ezrin [19], an increase that is significantly influenced by the increase in membrane–cytoskeleton adhesion energy [2]. Interestingly, ERM-deficient cells, as well as $PI(4,5)P_2$-depleted cells, exhibited the formation of more membrane blebs than wild type cells did [6, 7]. Membrane blebs are formed by the local detachment of the PM and actin cytoskeleton adhesion [2, 20]. Therefore, PLC-mediated decrease of $PI(4,5)P_2$ results in a release of ERM proteins, and thus the actin cortex from the PM, which leads to a cell shape change in response to extracellular stimuli.

1.2 Role of Class 1 myosins

Another class of linker proteins between the PM and actin cortex is class 1 myosin family proteins (Fig. 1). Class 1 myosin is a member of the single-head myosin superfamily that acts as a membrane–cytoskeleton linker [8]. Similar to the ERM proteins, class 1 myosins have an acidic phospholipid-binding motif, called the C-terminal tail homology 1 (TH1) domain [8]. Originally, the TH1 domain was reported to bind to phosphatidylserine (PS) through electrostatic interactions [21]. Subsequent studies revealed that the TH1 domain shows higher affinity for $PI(4,5)P_2$ than PS [22], due to a putative PH domain structure [23]. PM-localization of class 1 myosins appears

to be mediated by $PI(4,5)P_2$, as a mutant defective in $PI(4,5)P_2$-binding cannot be localized in the PM [23]. Importantly, class 1 myosins are thought to be critical regulators of PM tension by linking the actin cytoskeleton to the PM. PM tension in the brush border membrane of the small intestine isolated from myosin-1c knockout mice was significantly lower than that in wild type samples, whereas overexpression of myosin-1c resulted in an increase in membrane tension [24], due to the enhancement of PM–actin cortex adhesions. An independent study confirmed that membrane–cytoskeleton adhesion energy is reduced in myosin 1b-deficient mesendoderm cells [7]. Interestingly, the TH1 domain alone exhibits a dominant negative effect, because overexpression of the TH1 domain led to a PM tension decrease [24]. This effect might be due to a masking of $PI(4,5)P_2$ by the TH1 domain. Although it is not known whether the depletion of $PI(4,5)P_2$ leads to the dissociation of class 1 myosins from the membrane, it is possible that $PI(4,5)P_2$ contributes to the membrane–cortex adhesion energy, and thus membrane tension, via interactions with class 1 myosins.

2. Role of $PI(4,5)P_2$ in cell migration: activator or inhibitor?

2.1 Positive effect of $PI(4,5)P_2$ on actin polymerization for cell migration

A vast number of biochemical studies have demonstrated that *in vitro* activities of most of the actin-binding proteins can be regulated by direct interaction with phosphoinositides, in particular $PI(4,5)P_2$ [3, 5]. Consequently, it has been generally assumed that $PI(4,5)P_2$ is the central lipid molecule in cell migration, which is driven by

the rearrangement of the actin cortex under the PM. However, knowledge about the roles of this lipid–cytoskeletal interaction *in vivo* has been rather limited. Recent advances in live imaging techniques and the development of acute manipulation of $PI(4,5)P_2$ levels have progressed our understanding about its role in actin-based cellular functions such as cell migration. Evidence for the role of $PI(4,5)P_2$ in cell migration has been provided by studies demonstrating that PIPKIα and Iγ are localized at the leading edge of migrating fibroblasts [25-28].

One of the best illustrated examples of the activator of $PI(4,5)P_2$-dependent actin polymerization is the N-WASP–Arp2/3 complex [29]. Initially, it was proposed that an autoinhibited, and thus inactive, state of N-WASP can be relieved by direct interaction with $PI(4,5)P_2$, in concert with small GTPase Cdc42, leading to actin polymerization at the PM [30, 31]. However, subsequent studies have revised this model: (i) N-WASP is inhibited by its binding proteins such as WIP, rather than by autoinhibition *in vivo*; and (ii) the F-BAR domain-containing Toca family proteins, rather than $PI(4,5)P_2$, are essential activators for N-WASP [32]. $PI(4,5)P_2$ plays only a supplementary role, because N-WASP does not show clear specificity for $PI(4,5)P_2$ and could be efficiently activated by Toca proteins in the presence of other acidic phospholipids such as PS [33]. In addition, dimerization/oligomerization of WASP family proteins is important for the Arp2/3-dependent actin polymerization [34]. This is supported by the importance of Toca proteins in N-WASP activation, whose oligomerization can be achieved by the F-BAR domain proteins' self-assembly at the membrane [33]. However, a recent study has provided evidence for the role of $PI(4,5)P_2$ in cell migration through interaction with IQGAP1 [28]. IQGAP1 binds to $PI(4,5)P_2$ via a polybasic motif, whose interaction

leads to the release of its autoinhibited state, promoting direct interaction with N-WASP to activate actin nucleation [28, 35].

2.2 Role of PI(4,5)P_2 at the rear of migrating cells

There has been disagreement regarding the role of PI(4,5)P_2 as an instructive signal to promote actin polymerization at the leading edge, since PI(4,5)P_2 levels are already high in the resting cells and are even transiently dropped following stimulation with chemoattractants [36]. Given the fact that actin polymerization always occurs just beneath the PM, the primary role of PI(4,5)P_2 appears to be the global organization of the actin cortex. However, it is possible that PI(4,5)P_2 is involved in the formation of the leading edge by regulation of focal adhesion as well as polarized vesicular trafficking [37, 38]. Importantly, recent studies revealed that in rapidly moving cells such as leukocytes, PI(4,5)P_2 could function as the direct signal for cell migration by regulating actin cortex organization at the rear of cells rather than at the front (Fig. 2). During leukocytes chemotaxis, PIPKIβ and Iγ are localized in a polarized manner at the rear tail (uropod) [39-41], which is reinforced by the PM–actin cortex linkers, including ERM proteins [42, 43]. Live imaging experiments confirmed that a GFP-tagged version of the PLCδ1 PH domain, a probe for PI(4,5)P_2, is concentrated at the uropod [39]. Although it is not yet clear if PI(4,5)P_2 is involved in the localization of ERM proteins at uropods [15, 16], these results indicate that PI(4,5)P_2 contributes to the front-rear polarity formation during chemotaxis. Furthermore, PIPKIβ and Iγ have been shown to be involved in the activation of RhoA [40, 41], which is important for the rear membrane retraction by activating contractile actomyosin structures [44, 45]. Importantly, the ERM proteins are known to be the upstream regulators of Rho signaling [46]. It seems

likely that $PI(4,5)P_2$ restriction to the cell rear is also facilitated by the segregation of phosphatase and tensin homolog deleted from chromosome 10 (PTEN), a phosphoinositide 3-phosphatase that converts $PI(3,4,5)P_3$ to $PI(4,5)P_2$. PTEN is confined to the lateral and rear membranes of migrating cells, probably keeping the $PI(3,4,5)P_3$ level low and $PI(4,5)P_2$ levels high at these sides of the PM [39, 47, 48]. An inverse correlation between $PI(4,5)P_2$ levels and membrane protrusion has been also reported for carcinoma cells as well as fibroblasts, in which EGF-stimulated PLC activity induces lamellipodia formation while an acute depletion of $PI(4,5)P_2$ promotes a global increase in membrane protrusion [49, 50]. Interestingly, endocytic proteins such as clathrin and AP2 are accumulated at the rear membrane of migrating leukocytes [41, 51], suggesting that polarized distribution of $PI(4,5)P_2$ enhances endocytosis at the cell rear while reducing surface area and promoting membrane retraction (Fig. 2).

3. PI3K signaling in cell migration

D3-phosphoinositides, i.e., lipid products of PI 3-kinases (PI3Ks), have long been recognized as “compass lipids”, which decide the direction of cell migration. It was first shown that the $PI(3,4,5)P_3$/$PI(3,4)P_2$ probe Akt PH-GFP expressed in *Dictyostelium discoideum* cells was localized at the PM with a polarized pattern in a cAMP stimulation-dependent manner [52]. Polarized formation of $PI(3,4,5)P_3$ has also been observed in PDGF-stimulated NIH3T3 cells by evanescent-field optical microscopy [53]. In chemotaxing neutrophils, Akt PH-GFP is significantly localized at the leading edge, suggesting that the gradient of chemoattractant concentration in the extracellular

environment can be efficiently amplified by PI3K-mediated intracellular signaling pathways [54]. The inevitable role of PI3K in cell migration has been confirmed by numerous studies using animal models. PI3Kγ-deficient mice show higher numbers of circulating neutrophils and significantly reduced migration of neutrophils as well as macrophages [55, 56]. A mutant of *D. discoideum* cells that simultaneously lacks two PI3K isoforms also showed slow migration toward the chemoattractant cAMP [57]. All these observations lead to the conclusion that localized accumulation of downstream target(s) of PI3K, which interact(s) with D3-phosphoinositides at the plasma membrane, play(s) essential roles in polarity formation during cell migration.

However, there are questions about this simple model that places $PI(3,4,5)P_3$ at the center of the mechanism underlying directed cell migration. Andrew and Insall [58] demonstrated that only the frequency, and not the direction, of pseudopod formation requires PI3K activity in *D. discoideum* chemotaxis. According to the study, the direction is created by the selection of one pseudopod that is located toward the attractant, rather than by others that emerge randomly. Hoeller and Kay [59] knocked out all PI3K isoforms in *D. discoideum* cells, and showed that polarized formation of membrane $PI(3,4,5)P_3$ is not necessary for chemotaxis purposes, but it can also decrease cell speed during random migration. When the lipid-metabolizing enzymes in the PI3K pathway were deleted, the cell formed multiple pseudopodia and became less motile [59]. This means that the PI3K activity itself underpins the promotion of actin polymerization, which pushes the PM forward while the direction of cell migration can be decided by other factors.

What is the precise role of PI3K in cell migration? From a biochemical point of view, the immediate function of the PI3K product, PI(3,4,5)P_3, is to activate Rac, one of the Rho family small GTPases, by recruiting their GTP/GDP exchange factors (GEFs) such as P-Rex, PIX, and Dock2 to the PM [60-62]. As a membrane-localized activator for Rac, PI3K may contribute to “effective” or “efficient” cell migration. Yoo *et al.* [63] proposed a two-tiered role for PI3K in cell motility, in which PI3K is mostly involved in the promotion (but not the initiation) of Rac-mediated actin polymerization at the leading edge, generating the antero-posterior polarity during migration of zebrafish neutrophils. In fibroblasts, Welf *et al.* [64] showed that PI3K signaling is triggered only after the induction of Rac activation. This result confirms the idea that PI3K is not required for the initiation of newly branched pseudopodia, but is rather involved in the stabilization and competition between pseudopodia for cell polarity during chemotaxis. Recent studies have revealed a self-organized dynamic of PI(3,4,5)P_3 synthesis and degradation [65, 66]. In latrunculin-treated *D. discoideum* cells, PI3K (or PI[3,4,5]P_3) and PTEN localize exclusively to each other at the PM. The lipid kinase and phosphatase behave as rotational traveling waves at the PM, which is explained by a self-organization mechanism based on the combination of negative and positive feedback loops composed of PI3K, PTEN, and their substrates PI(3,4,5)P_3 and PI(4,5)P_2 [65, 66]. Interestingly, this phenomenon can also be observed in the absence of an external chemoattractant gradient, indicating the spontaneous generation of signals that govern random migration. These findings seem to support the idea that the rapid cycle of phosphorylation–dephosphorylation of the D3-phosphoinositide regulates the formation of the pseudopodium as a self-organizing structure, which may be the key to determining the cell movement directionality during chemotaxis [67].

4. Emerging roles of PI(3,4)P_2 in actin-based membrane structures

Nishio *et al.* [68] reported that Src homology 2 (SH2) domain-containing inositol-5-phosphatase 1 (SHIP1) is necessary for polarity formation in neutrophil chemotaxis. While *ship1-/-* neutrophils showed normal directionality toward the chemoattractant fMLP, the migration speed was significantly reduced. The defect in efficient chemotaxis in the *ship1-/-* neutrophils is mainly caused by the induction of multiple pseudopods, indicating a role for the 5-phosphatase in the establishment of front-rear polarity [68]. The consequence of SHIP1 activity is different from that of PTEN, as SHIP1 does not simply "down-regulate" PI(3,4,5)P_3, but can convert PI(3,4,5)P_3 into another form of D3-phosphoinositide, PI(3,4)P_2 (Fig. 2). The above results indicate two important aspects: (i) previous studies that utilized AktPH-GFP, recognizing both PI(3,4,5)P_3 and PI(3,4)P_2, may not have discriminated the two D3-phosphoinositides, and may have thus overlooked specific roles of PI(3,4)P_2; and (ii) PI(3,4)P_2 may exert its own effects on the actin cytoskeleton through interactions with downstream targets, as exemplified in the following sections.

4.1 Tandem PH domain-containing protein 1 and 2 (TAPP1 and TAPP2)

The first evidence for PI(3,4)P_2 as a phosphoinositide that can be recognized by downstream target proteins was provided by Dowler *et al.* [69]. The study succeeded in identifying two proteins with PH domains named "tandem PH domain-containing

protein 1" (TAPP1) and TAPP2 that specifically interact with $PI(3,4)P_2$, but not with $PI(3,4,5)P_3$ or other phosphoinositides. It has been reported that TAPP1 and TAPP2 are involved in actin-related functions. For example, TAPP1 is localized to the circular dorsal ruffle (CDR) that is formed in response to PDGF stimulation in NIH3T3 cells [70] (Fig. 3). Exogenous overexpression of TAPP1, as well as knockdown of endogenous TAPP1, effectively suppresses CDR formation [70, 71]. The C-terminus of TAPP1 interacts with several kinds of PDZ domain-containing proteins such as MUPP1 and γ1-syntrophin [70, 72], thus has strong affinity to the protein scaffolds beneath the PM. Because $PI(3,4)P_2$ is produced by dephosphorylation of $PI(3,4,5)P_3$, TAPP1 and TAPP2 are considered to act in a negative feedback reaction mediated by SHIP2, a ubiquitous form of $PI(3,4,5)P_3$ 5-phosphatase. It is noteworthy that TAPP1 and TAPP2 bind to a tyrosine phosphatase, PTP-L1, an enzyme that could act as a negative regulator for the actin polymerization and cell adhesion mediated by tyrosine phosphorylated proteins [73].

4.2 Lamellipodin (Lpd)

Lamellipodin (Lpd), also designated as RIAM, was originally identified as a binding partner of Ena/VASP, an actin-associated protein that regulates lamellipodia protrusion [74-76]. Lpd is thus named because it is not only specifically localized at the edge of the lamellipodium but is also necessary for the formation of lamellipodia [74]. Lpd also associates with the WAVE/Scar complex via an active form of Rac as well as Abi [77]. The essential role of Lpd in cell migration was demonstrated in *in vivo* situations, such as mouse neural crest-derived melanoblasts and collective epithelial border cell migration in *Drosophila* [77]. Lpd protein is composed of a central Ras-association

(RA) domain, a PH domain, as well as proline-rich regions, which are also referred to as "FPPPP" motifs. One of the most interesting characteristics of Lpd is its PH domain, which specifically recognizes PI(3,4)P_2 [74]. Importantly, profilin, a G-actin-binding protein that is regulated by phosphoinositides, was reported to inhibit lamellipodia formation by reducing PI(3,4)P_2 localized at the leading edge [78], suggesting an important role of PI(3,4)P_2 in lamellipodia formation via interaction with Lpd (Fig. 3).

4.3 Sorting nexin (SNX) 9

Recently, Sorting nexin 9 (SNX9), a protein with tandem PX domains and a BAR domain, has emerged as a novel downstream target of PI(3,4)P_2 that links the phosphoinositide to actin-mediated functions. SNX9 belongs to a branch of PX domain family proteins with an amino-terminal Src homology 3 (SH3) domain that binds and activates N-WASP [79]. The plasma membrane recruitment of SNX9, as well as N-WASP-mediated actin polymerization by SNX9, plays inevitable roles in PDGF-induced CDR formation and subsequent clathrin-independent endocytosis/macropinocytosis [79] (Fig. 3). While most PX domains preferentially interact with PI(3)P [80], there are some PX domains known to bind PI(3,4)P_2, such as Tyrosine kinase substrate with five SH3 domains (Tks5) (described below) and p47phox [81, 82]. In an *in vitro* binding assay with purified recombinant proteins, the phosphoinositide specificity of SNX9 has been observed to be only moderate, and most of the highly phosphorylated phosphoinositide species, such as PI(4,5)P_2 or PI(3,4,5)P_3, are recognized [83]. However, recent work has revealed that SNX9 protein purified from brain extracts (thus considered to be in complex with physiological binding partners) interacts preferentially with PI(3,4)P_2 over other phosphoinositides [84].

While the exact binding mechanism is unknown as well as the identity of the protein-binding interface, the idea that SNX9 acts downstream of $PI(3,4)P_2$ as an activator of N-WASP-driven actin polymerization could provide useful insights into the regulation of cytoskeletal organization under the control of this newly evaluated phosphoinositide species.

4.4 Tyrosine kinase substrate with five SH3 domains (Tks5)

As mentioned above, Tks5 is one of the $PI(3,4)P_2$ effectors with established function related to actin-based structure. It is composed of a single amino-terminal PX domain and five SH3 domains at its central to carboxy-terminal region. Tks5 has been identified as a substrate for Src tyrosine kinase [85], and plays an essential role in podosome formation in Src-transformed NIH3T3 cells [86]. The PX domain of Tks5 specifically binds to $PI(3,4)P_2$, which is concentrated at the podosomal membrane [82] (Fig. 3). The mechanism is based on the recruitment of the Tks5–Grb2–N-WASP complex to the site of $PI(3,4)P_2$ synthesis in a manner dependent on Src-activated PI3K activity and synaptojanin 2, a phosphoinositide 5-phosphatase. The protein complex then triggers Arp2/3-mediated actin polymerization at the basal plasma membrane, leading to the formation of circular podosomes [82]. As for the 5-phosphatase that may act upstream of Tks5, the involvement of SHIP2 has been also reported [87]. Tks5 and SHIP2 are localized at invadopodia, actin-rich membrane protrusions developed in cancer cells. Live-imaging analysis indicates that the conversion from $PI(3,4,5)P_3$ to $PI(3,4)P_2$ coincides with the arrival of Tks5 to invadopodia, at their maturation steps [87]. Thus, phosphoinositide 5-phosphatases that produce $PI(3,4)P_2$ are common key enzymes for the formation of podosome/invadopodia through the membrane recruitment of the

adaptor protein Tks5.

5. Concluding Remarks

Studies on phosphoinositide-binding proteins have provided an enormous amount of knowledge about each player that functions at the interface between the cortical actin cytoskeleton and the PM. In this brief review, we have attempted to offer an integrated view on the phosphoinositides involved in the induction and regulation of cellular morphogenesis based on actin-driven membrane dynamics. $PI(4,5)P_2$ should be regarded as relatively abundant “points of attachment” for bundled actin filaments that reinforce the mechanical property of the PM (Fig. 1). The conversion of $PI(4,5)P_2$ into $PI(3,4,5)P_3$ by PI3K may weaken the stiffness of the membrane, allowing the rearrangement of actin cables into branches (Fig. 2), which pushes the PM toward the extracellular space for cell migration. The newly evaluated roles for $PI(3,4)P_2$ (Fig. 3) will provide more insight into the organized distribution of phosphoinositide and associated actin polymerization at the PM in response to changes in the extracellular environment or oncogenic signals. In order to obtain a more comprehensive understanding of the mechanism by which phosphoinositides control membrane mechanics, biophysical techniques combined with mathematical modeling will be one of the most powerful approaches for future studies.

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Figure legends

Fig. 1 Generation of the plasma membrane–actin cortex adhesion by PI(4,5)P_2.

Phosphatidylinositol-4,5-bisphosphate (PI[4,5]P_2) connects “linker proteins” such as ERMs and class 1 myosins with membrane, generating adhesion energy between the plasma membrane and actin cytoskeleton.

Fig. 2 Phosphoinositide metabolism and actin organization in migrating cells.

PI(4,5)P_2 (red) at the rear side of PM induces F-actin bundles (straight black lines), while PI(3,4,5)P_3 (blue) at the migration front supports the formation of branched actin structures (meshed black lines). Phosphoinositide-metabolizing enzymes such as PI3K and PTEN promote the transitions between these two phosphoinositide species. SHIP1 produces PI(3,4)P_2 (purple) by dephosphorylating PI(3,4,5)P_3. The direction of cell migration is indicated by a green arrow.

Fig. 3 Actin-based structures underpinned by PI(3,4)P_2 and downstream targets.

Membrane areas positive for PI(3,4)P_2 is colored in magenta, and its downstream targets involved in each actin-based structure are indicated by indigo boxes. Circular dorsal ruffles are formed at the dorsal side of PM by which a cell incorporates outer materials through macropinocytosis. Podosomes/invadopodia are involved in the exocytosis of various enzymes in order to degrade extracellular matrix (yellow).

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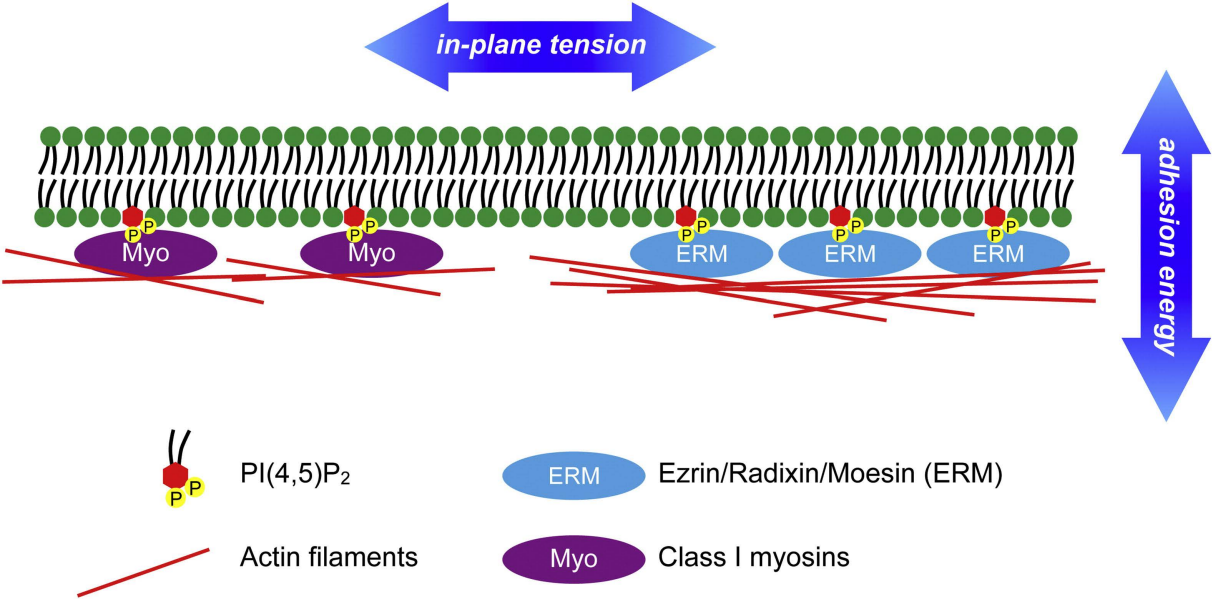
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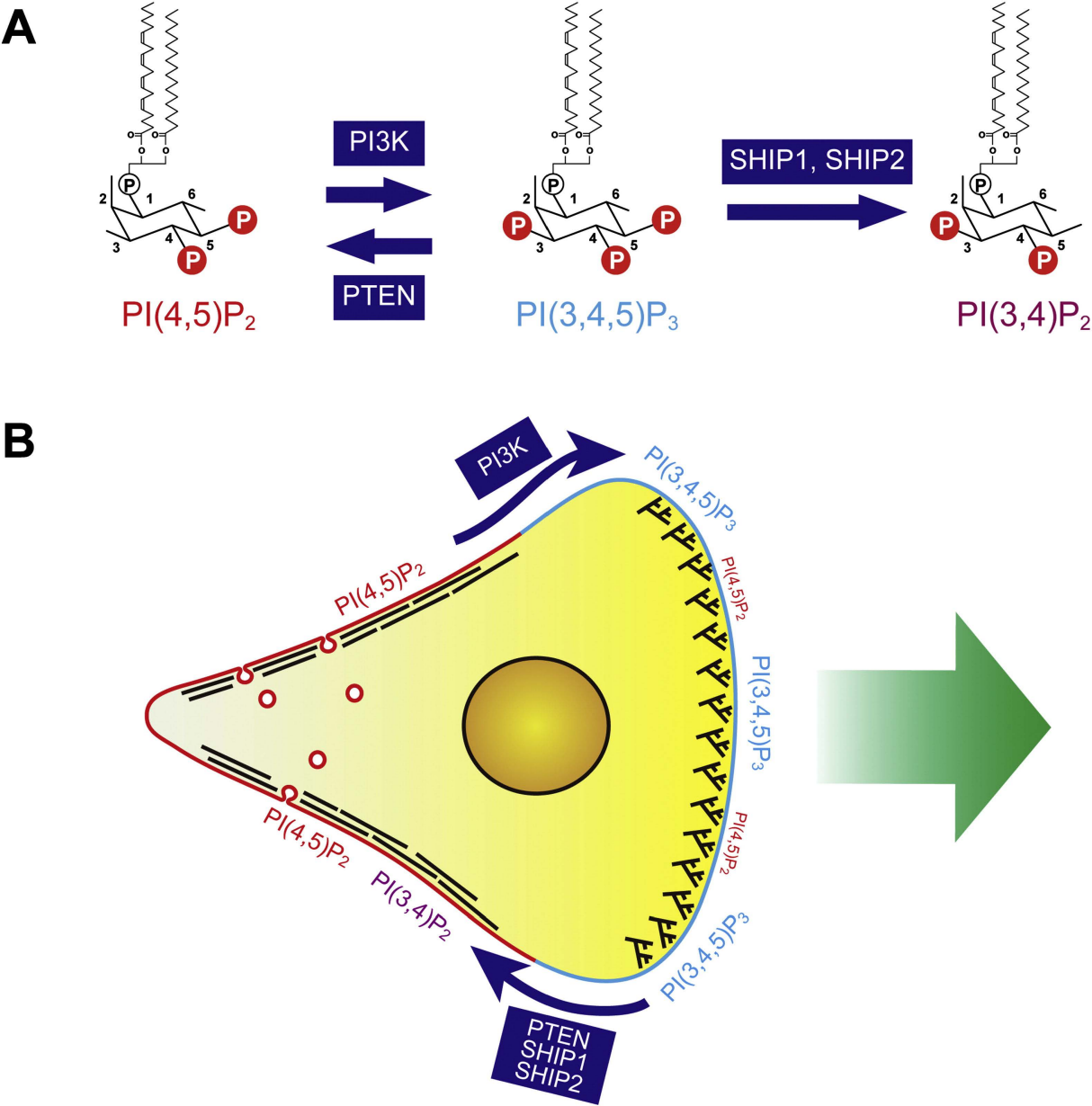
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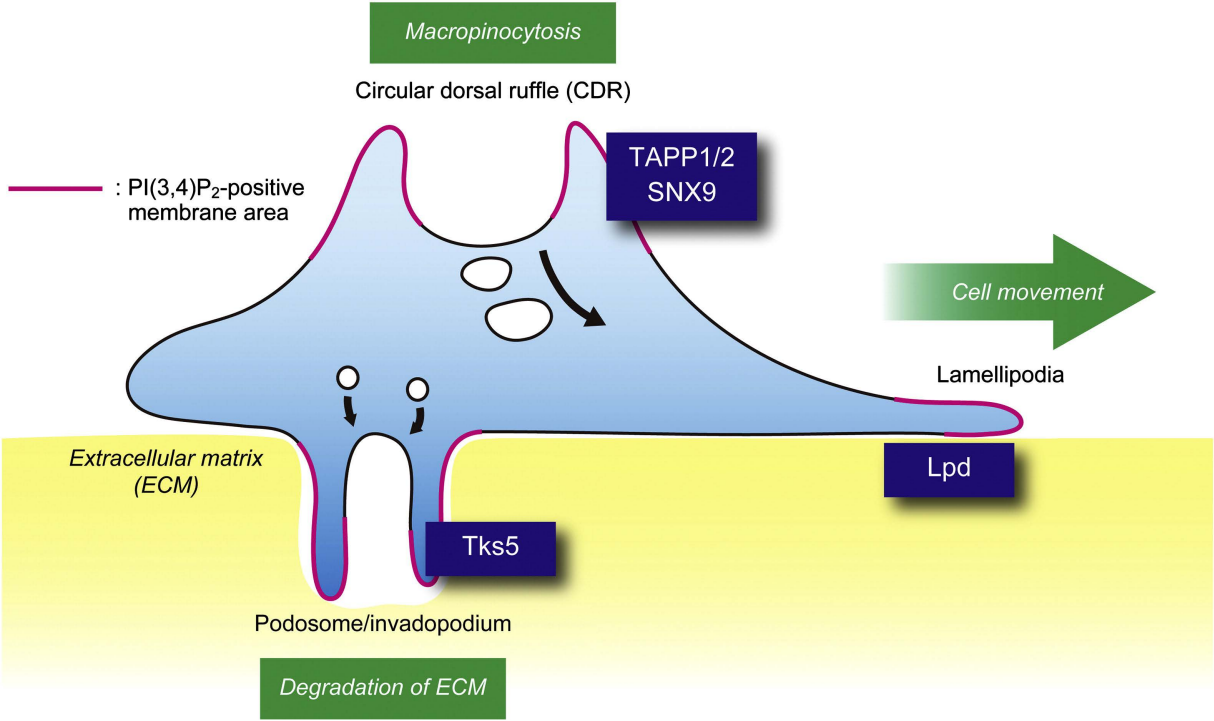
Macropinocytosis
Circular dorsal ruffle (CDR)
: PI(3,4)P2-positive membrane area
TAPP1/2
SNX9
Cell movement
Lamellipodia
Extracellular matrix (ECM)
Lpd
Tks5
Podosome/invadopodium
Degradation of ECM