Regulation of actin dynamics by PI(4,5)P_2 in cell migration and endocytosis

Yosuke Senju and Pekka Lappalainen*

Institute of Biotechnology, University of Helsinki, FI-00014 Helsinki, Finland

***Correspondence to:**
Dr. Pekka Lappalainen,
Institute of Biotechnology, P.O. Box 56,
00014 University of Helsinki, Finland.

Email: pekka.lappalainen@helsinki.fi
Phone: +358-405941533

ABSTRACT

The actin cytoskeleton is indispensable for several cellular processes, including migration, morphogenesis, polarized growth, endocytosis, and phagocytosis. The organization and dynamics of the actin cytoskeleton in these processes are regulated by Rho family small GTPases and kinase-phosphatase pathways. Moreover, membrane phospholipids, especially the phosphatidylinositol phosphates have emerged as important regulators of actin dynamics. From these, PI(4,5)P_2 is the most abundant at the plasma membrane, and directly regulates the activities and subcellular localizations of numerous actin-binding proteins. Here, we discuss recent studies demonstrating that actin-binding proteins interact with PI(4,5)P_2-rich membranes through drastically different affinities and dynamics correlating with their roles in cytoskeletal dynamics. Moreover, by using mesenchymal cell migration and clathrin-mediated endocytosis as examples, we present a model for how interplay between PI(4,5)P_2 and actin-binding proteins control the actin cytoskeleton in cells.

Introduction

Coordinated polymerization of actin filaments against the plasma membrane produces forces for the generation of membrane protrusions for cell migration and morphogenesis, as well as plasma membrane invaginations for endocytotic processes. Actin filaments, together with myosin II filaments, also assemble into diverse contractile arrays such as stress fibers, muscle myofibrils, and the actomyosin cortex, where force is produced by sliding of actin filaments along bipolar myosin II filaments [1,2]. Actin filaments do not work in isolation in these processes, but their dynamics and three-dimensional organization are controlled by a large array of actin-binding proteins. These proteins can for example control actin filament nucleation (e.g. formins, Arp2/3 complex), filament elongation (e.g. VASP and heterodimeric capping protein), filament disassembly (e.g. ADF/cofilin and gelsolin), actin monomer pool (e.g. profilin, twinfilin and cyclase-associated protein), as well as cross-link actin filaments to each other (e.g. α-actinin) or to the plasma membrane (ezrin-radixin-moesin family proteins) [3].

Biochemistry of $PI(4,5)P_2$ – actin-binding protein interplay

The activities of actin-binding proteins in cells are precisely regulated through kinase-phosphatase networks, small GTPases, and membrane phospholipids. From the phospholipids, especially phosphatidylinositol phosphates, $PI(4,5)P_2$, $PI(3,4)P_2$ and $PI(3,4,5)P_3$, have been established as central regulators of the actin cytoskeleton. $PI(3,4,5)P_3$ is a signaling lipid that is typically present in the plasma membrane at very low concentrations. It controls the actin cytoskeleton mainly through activating the guanine exchange factors of Rho-family small GTPases. $PI(3,4)P_2$ is also typically present in the plasma membrane at relatively low concentrations and contributes especially to late stages of endocytosis. $PI(4,5)P_2$, however, is present in the plasma membrane at high concentrations (and may constitute up to 1-3 % of the plasma membrane lipids), and regulates the actin cytoskeleton by interacting directly with several central actin-binding proteins [4]. Moreover, $PI(4,5)P_2$ binds many signaling proteins as well as Bin/Amphiphysin/Rvs (BAR) domain scaffolding proteins, which interact with actin-binding proteins to control their activities of subcellular localization [4,5].

Several central actin-binding proteins including profilin, cofilin, twinfilin, formins, N-WASP, and ezrin/radixin/moesin (ERM) interact with $PI(4,5)P_2$. Typically, those proteins that drive actin filament disassembly or prevent actin filament assembly are inhibited by interactions with $PI(4,5)P_2$, whereas those proteins that promote actin filament nucleation/polymerization are up-regulated through interactions with $PI(4,5)P_2$ [4]. Recent work revealed that actin-binding proteins interact with membranes via similar multivalent electrostatic interactions without specific binding pockets or penetration into the lipid bilayer [6,7]. However, the membrane binding affinities and dynamics of the actin-binding proteins are drastically different. Moreover, actin-binding proteins sense different ranges of $PI(4,5)P_2$ densities, which may define their subcellular localization. Profilin and cofilin exhibit transient, low-affinity interactions with phosphoinositide-rich membranes at physiological salt concentrations. Thus, they cannot reside in the plasma membrane for longer periods of time, but are expected to mainly function at a distance from the plasma membrane to regulate actin filament disassembly and monomer recycling. In contrast, formin Dia2 and N-WASP, which promote actin filament nucleation and assembly, display relatively high affinity, stable interactions with phosphoinositide-rich membranes. Importantly, profilin, cofilin, Dia2, and N-WASP require for membrane-binding high (>5 %) 'stimulus-responsive' $PI(4,5)P_2$ density, which can be achieved via increased $PI(4,5)P_2$ synthesis or clustering [6,8]. This $PI(4,5)P_2$ density may correspond to the one present in extending lamellipodia, where Dia2 and N-WASP localize and promote actin filament

assembly. However, ezrin and moesin, which function as cross-linkers between the plasma membrane and the actin cytoskeleton, bind membranes containing much lower (1 %) $PI(4,5)P_2$ density. Moreover, ezrin and moesin bind membranes with very high affinity and low dissociation dynamics, making them suitable for stably cross-linking actin filaments to the cell cortex [6]. Thus, the $PI(4,5)P_2$ binding affinities of actin-binding proteins, as well as the phosphoinositide-densities required for their interactions with membranes, correlate precisely with their functions in cytoskeletal dynamics (**Fig. 1**).

Cellular roles of $PI(4,5)P_2$ in regulating the actin cytoskeleton

Several studies demonstrated that an increase in plasma membrane $PI(4,5)P_2$ results in elevated actin filament assembly, whereas depletion of $PI(4,5)P_2$ leads to diminished actin filament assembly and defects in actin-dependent cellular and developmental processes [4]. In the following chapters, we focus on the cellular roles of $PI(4,5)P_2$ in actin dynamics by using cell migration and clathrin-mediated endocytosis as examples. In addition to $PI(4,5)P_2$, other phosphoinositides, especially $PI(3,4)P_2$ and $PI(3,4,5)P_3$, were linked to actin dynamics in several studies [4,9–12], but are not extensively discussed below.

Cell migration

Cell migration is critical for development and physiology of multicellular animals. The two most thoroughly characterized cell migration types are *mesenchymal*, adhesion-dependent migration, and *amoeboid* migration mode that is driven by contractile actomyosin cortex of the cell [13]. From these, the role of phosphoinositides in regulating the actin cytoskeleton is better established in the mesenchymal migration mode, and therefore this will be discussed in more detail.

Mesenchymal cell migration is driven by Arp2/3-nucleated, branched actin filament networks of thin sheet-like lamellipodial protrusions at the leading edge of the cell. The coordinated polymerization of actin filaments against the membrane promotes advancement of the leading edge. Actin filament assembly at the membrane is balanced by ADF/cofilin-mediated filament disassembly at the proximal regions of the lamellipodial actin filament network [3]. In addition to the branched lamellipodial actin network, thin finger-like actin filament protrusions, called filopodia, extend from the leading edge. These structures are filled by a tightly-packed bundle of actin filaments, and they are important for the cell to sense its environment. Finally, mesenchymal cell migration depends on focal adhesions, which are complex, multi-protein structures that link the actin cytoskeleton to the extracellular matrix through integrins and actin-associated proteins such as talin, vinculin, and α-actinin [13].

$PI(4,5)P_2$ is central for cell adhesion during motility. Local synthesis of $PI(4,5)P_2$ by PIP kinase Type I γ (PIPKIγ) in focal adhesions is critical for actin-integrin force coupling and thus for integrin-mediated cell adhesion [14]. $PI(4,5)P_2$ also binds several focal adhesion proteins such as vinculin, talin, kindlin, and α-actinin [4,15]. For example, talin interacts with $PI(4,5)P_2$–rich membranes through its FERM domain and this interaction is important for releasing the auto-inhibited conformation of the protein [16,17]. Moreover, $PI(4,5)P_2$ interacts with and activates vinculin, an actin-binding protein that links the adhesion complex to actin filaments at focal adhesions [18]. These data provide an explanation why $PI(4,5)P_2$ is required for the formation of focal adhesions in migrating cells.

Studies using specific PH domains as probes revealed that $PI(4,5)P_2$ also accumulates to the actin-rich leading edge lamellipodium [19]. Acute depletion of $PI(4,5)P_2$ results in retraction of the actin-

rich leading edge, demonstrating the importance of this phosphoinositide in the generation and maintenance of lamellipodial protrusions [20]. However, PI(4,5)P_2 may have more complex and cell-type specific roles in cell migration. This is because in rapidly moving cells, such as neutrophils, PI(4,5)P_2 regulates the myosin II-driven retraction of the cell rear, and in amoeboids altering PI(4,5)P_2 levels results in changes in their migratory mode [21,22].

At lamellipodium, PI(4,5)P_2 regulates the functions of several actin-binding proteins such as Dia1 and Dia2 formins and N-WASP. These proteins shuttle between closed auto-inhibited and open active conformations. In the case of N-WASP, which is an activator of actin-nucleating Arp2/3 complex, interactions with PI(4,5)P_2 contribute to conversion of the protein to an active form at the plasma membrane [23,24]. In the case of Dia1 and Dia2 formins, which promote actin filament nucleation and polymerization, interactions with PI(4,5)P_2 appear to be important for their targeting to the PI(4,5)P_2–rich regions of the plasma membrane [25,26]. On the other hand, ADF/cofilin, and heterodimeric capping protein, which are important negative regulators of lamellipodial actin filament assembly, are inhibited through interactions with PI(4,5)P_2. This is because the membrane-binding and actin-binding surfaces overlap on the surface of these proteins [27,28].

Based on these findings, and from the recent work on dynamics of actin-binding protein PI(4,5)P_2 – interactions, we propose the following model for the role of PI(4,5)P_2 in cell migration. Elevated PI(4,5)P_2 density at the lamellipodium recruits N-WASP and certain formins to the plasma membrane, and promotes their activation. Activation of these proteins by PI(4,5)P_2 ensures that actin filament assembly predominantly occurs at the plasma membrane, and can thus be efficiently exploited to push the leading edge forward. On the other hand, elevated PI(4,5)P_2 levels prevent actin filament capping at the plasma membrane, thus further ensuring that the productive actin filament barbed ends pushing the plasma membrane are not capped by the heterodimeric capping protein. Finally, ADF/cofilins, which display only transient, low-affinity interactions with the PI(4,5)P_2 -rich membrane are not sequestered at the plasma membrane, but instead mainly function at a distance of the plasma membrane to disassemble 'aged' actin filaments. At the plasma membrane regions that do not contain high concentrations of PI(4,5)P_2, ADF/cofilins can, however, also disassemble actin filaments in the vicinity of the plasma membrane (**Fig. 2**). In reality, the situation is much more complex, because also other phosphoinositides, especially PI(3,4,5)P_3, contribute to cell migration. PI(3,4,5)P_3 activates actin filament assembly through Rho-family GTPases, and its levels oscillate at the leading edge during lamellipodial extension-retraction cycles [29,30]. Moreover, Ca^{2+} was recently demonstrated to interact with the PI(4,5)P_2 headgroups and regulate the affinities of proteins to PI(4,5)P_2 [31]. As Ca^{2+} levels oscillate at the lamellipodium, they may add another layer to the regulation of actin-binding proteins at the plasma membrane lipids [32].

Clathrin-mediated endocytosis

Clathrin-mediated endocytosis is a major route for internalization of various cargoes, including transmembrane proteins and growth factors. This process proceeds through assembly of the clathrin coat at the plasma membrane, followed by membrane bending, scission, and vesicle uncoating. Polymerization of actin filaments against the plasma membrane provides force for membrane bending and scission. Actin dynamics are essential for clathrin-mediated endocytosis in yeasts, but their importance in this process in mammals varies between different cell-types and physiological contexts [1].

Actin polymerization initiates at the onset of membrane bending and is catalyzed by the Arp2/3 complex and its activators; WASP-family proteins and type I myosins [33]. The disassembly of the endocytotic actin network coincides with the vesicle uncoating following the scission [1]. Elegant microscopy studies provided evidence that, at least in budding yeast, the rapidly growing actin filament barbed ends face towards the plasma membrane at the base of endocytic invagination. The distal parts of actin filaments are coupled to the clathrin coat through epsin and HIP1R family proteins to harness the force generated by actin polymerization for internalization of the clathrin-coated pit [34,35](Fig. 2).

Phosphoinositides, especially PI(4,5)P_2, have also a central role in clathrin-mediated endocytosis. Studies on budding yeast revealed that PI(4,5)P_2 levels increase and decline in conjunction with coat and actin assembly and disassembly, respectively [36]. Moreover, the levels of the inositol-polyphosphate 5-phosphatase, synaptojanin which dephosphorylates PI(4,5)P_2, peak at the time when coat and actin network disassembly occur, after the vesicle scission [36,37]. Functional studies on budding yeast and mammalian cells revealed that inhibition of endocytic inositol-polyphosphate 5-phosphatases such as synaptojanin and OCRL results in sustained PI(4,5)P_2 levels and actin filament accumulation on endosomes impairing their uncoating [36,38,39]. In contrast, acute depletion of PI(4,5)P_2 results in disappearance of clathrin punctae and consequent inhibition of Arp2/3-mediated actin filament assembly at the sites of endocytosis [40]. These studies established that the PI(4,5)P_2 levels should be tightly regulated for proper endocytosis.

The precise mechanisms by which PI(4,5)P_2 controls actin filament assembly and disassembly during clathrin-mediated endocytosis are still incompletely understood. PI(4,5)P_2 interacts directly with N-WASP, but this lipid can also recruit WASP-family proteins to the sites of endocytosis through various scaffolding proteins, such as the membrane curvature sensing/generating BAR and F-BAR domain proteins [5]. These proteins associate with the PI(4,5)P_2-rich endocytic neck through their banana-shaped BAR or F-BAR domains. For example, the budding yeast F-BAR domain protein Bzz1 binds yeast WASP (Las17) [41], mammalian F-BAR domain proteins Toca1 and FBP17 can stimulate actin filament assembly through N-WASP on curved membranes [42], and the mammalian endocytic BAR domain protein SNX9 directly binds N-WASP to stimulate actin filament assembly [43]. Recent studies showed that also other phosphoinositides, namely PI(3)P and PI(3,4)P_2, interact with and control the activity of SNX9 [44,45], and thus, at least in mammalian cells, the conversion of PI(4,5)P_2 to PI (3,4)P_2 and PI(3)P_3 may fine-tune the actin cytoskeleton during endocytosis [46]. In addition to WASP-family proteins, type I myosins can activate the Arp2/3 complex during endocytosis. Type I myosins bind PI(4,5)P_2, and studies on budding yeast demonstrated that this interaction can relieve the autoinhibition of myosin I and hence induce actin polymerization [47].

We propose that, similarly to the leading edge in a migrating cell, high concentrations of PI(4,5)P_2 directly recruit and anchor Arp2/3 activators, type I myosins and WASP-family proteins, to the base of an endocytic invagination. Moreover, membrane-bending BAR and F-BAR domain proteins may recruit WASP-family proteins to the neck of endocytic invagination. This leads to actin polymerization at the regions of high PI(4,5)P_2. At the same time, actin filament barbed end capping (by heterodimeric capping protein) and filament disassembly (by ADF/cofilin) are inhibited close to the PI(4,5)P_2-rich regions of the plasma membrane to further ensure accumulation of actin filaments at these sites (**Fig. 2**). After the scission, the PI(4,5)P_2-levels sharply decrease at the vesicle through action of inositol-polyphosphate 5-phosphatases. This leads to the inactivation and/or dissociation of the Arp2/3 activators from the membrane, and activation of heterodimeric capping protein and ADF/cofilin close to the membrane. Simultaneous inhibition of actin filament assembly and induction

of actin filament disassembly leads to the observed uncoating of the actin filament network form the endosome at the time of PI(4,5)P_2-hydrolysis. Such a scenario is consistent with the biochemistry of the actin-binding protein – PI(4,5)P_2 interactions [6]. Supporting this model, WASP family proteins and type I myosins accumulate to the clathrin-coated pit just prior to the actin filament assembly, whereas cofilin peaks at these sites after actin filament assembly factors and actin [1,48–50]. However, it is important to bear in mind that the clathrin-coated pits constitute of a complex network of proteins that associate with each other through multiple interactions [33]. Thus, PI(4,5)P_2 is expected to control actin filament assembly and disassembly at these sites through a large number of direct and indirect interactions.

Conclusions and future perspectives

The role of PI(4,5)P_2 in regulating actin dynamics in various cellular processes, such as cell migration and endocytosis, is now relatively well established. There is also a wealth of information on how different actin-binding proteins interact with and are regulated by PI(4,5)P_2-rich membranes in vitro. Nevertheless, the physiological importance of interactions of individual actin-binding proteins with PI(4,5)P_2-rich membranes has remained largely elusive. In the future it will be important to design mutations to various actin-binding bindings proteins that specifically inhibit their PI(4,5)P_2-binding/regulation without affecting other functions of the protein. Knockout-rescue experiments with such mutant proteins will uncover the specific contribution of lipid-binding in the sub-cellular localizations and functions of these proteins in various cellular contexts.

It is also important to elucidate how various signaling pathways regulate the synthesis and hydrolysis of PI(4,5)P_2 at the plasma membrane, and to unravel whether the levels of PI(4,5)P_2 fluctuate e.g during lamellipodial protrusion (which is driven by actin filament assembly) and retraction (which co-insides with net disassembly of actin filaments). Moreover, several proteins involved in actin-dependent processes, such as the BAR superfamily proteins, can cluster PI(4,5)P_2 and induce formation of phosphoinositide-rich membrane microdomains [51,52]. Therefore, the role of PI(4,5)P_2 clustering in regulation of actin filament assembly/disassembly in endocytosis and cell migration needs to be examined. The BAR superfamily proteins can also sense membrane curvature [53,54], and thus it will be interesting to study whether also some actin-binding proteins display curvature sensitive binding to membranes, and if their plasma membrane interactions would hence be regulated by both lipid composition and geometry of the target membrane. Finally, it is important to keep in mind that the plasma membrane is composed of an extremely complex mixture of lipids, and that in addition to phosphoinositides also other lipid-species are likely to contribute to interactions of actin-binding proteins with the membrane. This scenario is supported by recent finding demonstrating that sterol transfer from the endoplasmic reticulum to the plasma membrane facilitates actin polymerization at endocytic sites at least in budding yeast [55]. Thus, functions of PI(4,5)P_2 in regulating actin-binding proteins should also be studied in context of other lipid-species.

Defects in the actin cytoskeleton - PI(4,5)P_2 interactions are also linked to various diseases. For example, mutations in the *OCRL1* gene encoding an inositol-polyphosphate 5-phosphatases cause Lowe syndrome. In fibroblasts of Lowe syndrome patients, the elevated PI(4,5)P_2 levels result in an aberrant actin cytoskeleton because of abnormal distribution of actin-binding proteins such as α-actin and gelsolin, which also interact with PI(4,5)P_2 [56–58]. Therefore, understanding the principles by which phosphoinositides regulate the actin cytoskeleton will also be important for elucidating the molecular basis of various pathological disorders.

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FIGURES

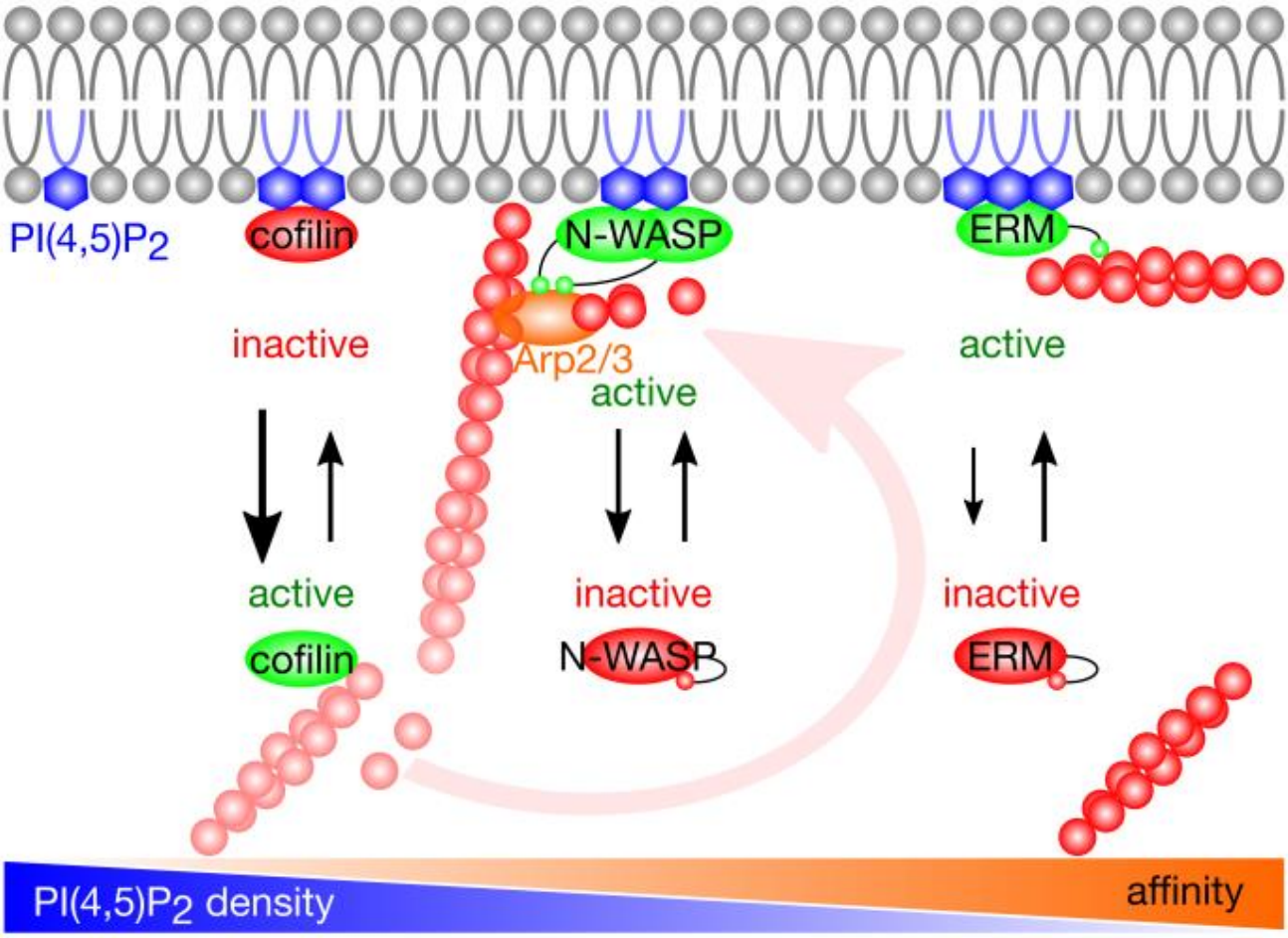

Figure 1. A working model for the regulation of actin-binding proteins by PI(4,5)P_2. Actin filament disassembly factor, cofilin, exhibits transient, low-affinity interactions with membranes under physiological salt conditions. Thus, active cofilin is not sequestered at the plasma membrane, but functions away from the membrane to promote actin filament disassembly. In contrast, the Arp2/3 activator N-WASP (and Dia2 formin; not shown in the figure) exhibits relatively high affinity, stable interactions with the membrane. The interactions of N-WASP with PI(4,5)P_2 and its other binding partners release its autoinhibitory structure, and activates N-WASP to induce Arp2/3 complex-mediated actin filament nucleation at the plasma membrane. Ezrin and moesin (ERM family) proteins, which link actin filaments to the plasma membrane, interact with membranes stably and with very high affinity. Thus, although these actin-binding proteins associate with PI(4,5)P_2 through similar multivalent electrostatic interactions, their membrane-binding affinities and dynamics are very diverse. Moreover, cofilin, N-WASP and Dia2 require a stimulus-responsive, high PI(4,5)P_2 density for interactions with the membrane, whereas ezrin and moesin can bind to the membrane at low PI(4,5)P_2 density (~1 %), which corresponds to the PI(4,5)P_2 density at the cell cortex under unstimulated conditions. The figure is modified from Senju et al., [6].

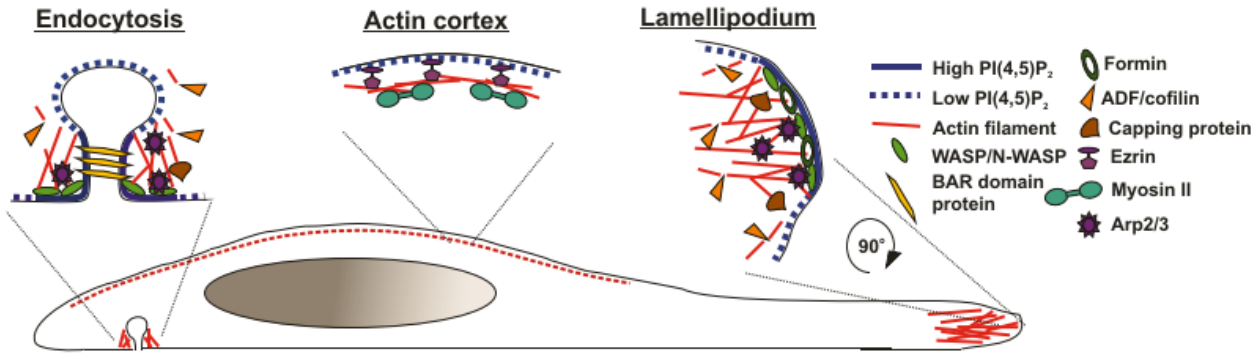

Figure 2. PI(4,5)P_2-centric view on the regulation of the actin cytoskeleton. A schematic showing a migrating cell, where the interplay between the actin cytoskeleton and PI(4,5)P_2 in three distinct processes is shown. In *clathrin-mediated endocytosis*, actin filament nucleation and polymerization are catalyzed by the Arp2/3 complex, which is activated by WASP-family proteins and type I myosins. Actin filament assembly occurs on the PI(4,5)P_2-rich areas at the base of the endocytic invagination. Moreover, curvature-sensitive BAR domain proteins at the neck of endocytic invagination may activate the Arp2/3 complex through interacting with WASP-family proteins. Actin filament disassembly by ADF/cofilins and filament barbed-end capping by heterodimeric capping protein occur away from the PI(4,5)P_2 rich membrane as well as at the membrane regions with low PI(4,5)P_2 density. Similarly, actin filament assembly at the *lamellipodium*, catalyzed by formins and WASP-family proteins, occurs at the PI(4,5)P_2-rich region of the plasma membrane, whereas ADF/cofilins and heterodimeric capping protein are active at the regions away from the PI(4,5)P_2-rich membrane. Ezrin and moesin interact, through their FERM domains, with membranes of low PI(4,5)P_2-density, and can thus cross-link the *actin cortex* also to those plasma membrane regions that do not contain high, stimulus-responsive, concentration of PI(4,5)P_2.