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PIP₂: choreographer of actin-adaptor proteins in the HIV-1 dance

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Abstract

The actin cytoskeleton plays a key role during the replication cycle of human immunodeficiency virus-1 (HIV-1). HIV-1 infection is affected by cellular proteins that influence the clustering of viral receptors or the subcortical actin cytoskeleton. Several of these actin-adaptor proteins are controlled by the second messenger phosphatidylinositol 4,5-bisphosphate (PIP₂), an important regulator of actin organization. PIP₂ production is induced by HIV-1 attachment and facilitates viral infection. However, the importance of PIP₂ in regulating cytoskeletal proteins and thus HIV-1 infection has been overlooked. This review examines recent reports describing the roles played by actin-adaptor proteins during HIV-1 infection of CD4⁺ T cells, highlighting the influence of the signaling lipid PIP₂ in this process.

Keywords

PIP₂; actin-binding proteins; HIV-1

Introduction

The central role of actin polymerization (see **Glossary**) during infection by human immunodeficiency virus-1 (HIV-1) has been known for several decades. However, recent reports suggest a more sophisticated involvement of the actin cytoskeleton throughout the viral replication cycle. Cortical actin determines T cell susceptibility to HIV-1 infection, and viral internalization is proportional to actin polymerization in the target cell [1]. In contrast, other studies demonstrate that efficient viral entry after membrane fusion requires local actin depolymerization [2, 3]. These evidences suggest that HIV-1 entry into the host cell requires a dynamic reorganization of the actin cytoskeleton.

Infection is modulated at early stages by cellular proteins that affect viral receptor clustering, such as EWI-2, moesin and filamin-A [4-6], or the subcortical actin cytoskeleton,

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such as drebrin, syntenin-1, α -actinin, gelsolin, talin, vinculin, cofilin, profilin, WASP, WAVE-2, and Arp2/3 [2, 3, 5, 7-12] (Table 1). Some of these proteins are actin-adaptor proteins and directly bind to actin microfilaments, whereas others bind to F-actin through linker proteins such as ERM (ezrin-radixin-moesin family). While receptor clustering requires actin polymerization, subsequent internalization of the viral core requires local actin depolymerization (Table 2). Actin filaments then accompany the viral components through the cytoplasm towards the nucleus [13-15] and might ultimately regulate HIV-1 assembly and release. The virus thus controls actin dynamics throughout the cycle for its own profit, promoting local actin polymerization or depolymerization as required at each step.

PIP₂ metabolism

Several of the above-mentioned proteins bind to and are regulated by the second messenger phosphatidylinositol 4,5-bisphosphate (PIP₂), a phosphoinositide (PPI) mainly located at the plasma membrane [16]. PPIs are phosphorylated derivatives of phosphatidylinositol (**see Glossary**) that function as second messengers, docking sites for specific proteins, and allosteric regulators. Each PPI is predominantly positioned at specific cellular membranes and performs its roles locally [16]. PPI production and degradation are highly controlled. PIP₂ is produced mainly by the action of two kinases (Box 1), PIP5K and PIP4K, which respectively phosphorylate PI(4)P (phosphatidylinositol 4-phosphate) and PI(5)P (phosphatidylinositol 5-phosphate) to generate PIP₂ [17]. PI(4)P is much more abundant than PI(5)P, so the major pathway for PIP₂ production is PI(4)P phosphorylation by PIP5K. PIP5K is recruited to the plasma membrane by RhoA, Rac1 and Arf6 (ADP ribosylation factor 6) [17]. Regulation by Arf6 is especially important because it both activates PIP5K activity and recruits phospholipase D (PLD) (Box 1). PLD activity further activates PIP5K through the generation of phosphatidic acid, a PIP5K activator (Box 1). Moreover, PLD is itself activated by PIP₂, generating a positive feedback loop that amplifies the initial Arf6 signal (Box 1). Prolonged expression of a constitutively active Arf6 mutant stimulates PIP₂ synthesis and induces, as a consequence, plasma membrane internalization and PIP₂ accumulation at intracellular vesicles [18]. Some PIP₂-regulated proteins modulate PIP₂ dynamics; for example, profilin and gelsolin stimulate phosphoinositide 3-kinase (PI3K) activity, which converts PIP₂ into phosphatidylinositol 3,4,5-trisphosphate (PIP₃) [19], while cofilin acts as a PIP₂ sensor [20]. Several enzymes catalyze PIP₂ degradation (Box 1): phospholipase C (PLC) hydrolyzes PIP₂ to produce the second messengers inositol (1,4,5)-trisphosphate (IP₃) and diacylglycerol (DAG); PI3K phosphorylates PIP₂ to generate PIP₃; and PPI-phosphatases dephosphorylate PIP₂, generating PI(4)P and PI(5)P [17]. PIP₂ homeostasis is thus highly regulated by a complex balance of synthesis and hydrolysis (Box 1).

PIP₂ is involved in many processes related to the actin cytoskeleton. In general, PIP₂ synthesis initiates actin assembly, whereas PIP₂ depletion triggers actin depolymerization [17]. HIV-1 viral envelope glycoproteins (Env) trigger PIP5K α activity and PIP₂ enrichment in the vicinity of the viral attachment site, facilitating viral entry [9, 21, 22]. In this review, we propose that PIP₂ coordinates actin cytoskeletal function through multiple steps of HIV-1 infection.

The actin cytoskeleton and PIP₂ throughout the HIV-1 cycle

The following sections summarize the HIV-1-induced local changes in the actin cytoskeleton during different steps of the viral cycle and how PIP₂ affects this dynamic process.

HIV entry and membrane fusion: the cortical actin network

Receptor clustering and the formation of the fusion pore

Attachment of HIV-1 to target cells is modulated by the surface organization of the HIV receptor CD4 and co-receptors CXCR4 and CCR5, which are partially clustered in resting conditions [23]. Since most studies on the role of the actin cytoskeleton during HIV-1 infection were performed with X4-tropic viruses, we will focus on HIV-1 that uses CXCR4 co-receptor to infect CD4⁺ T cells. HIV-1 entry is a cooperative process that requires the engagement of several CD4–CXCR4 complexes to efficiently trigger viral–cellular membrane fusion [24, 25]. Numerous transmembrane receptors are distributed in specialized domains called tetraspanin-enriched microdomains (TEMs) (**see Glossary**) and membrane rafts (**see Glossary**) that are differently linked to the actin cytoskeleton [26]. CD4 and CXCR4 localization and mobility within these specialized membrane domains modulate HIV-1 infection [27, 28]. Tetraspanin (**see Glossary**) CD63 negatively regulates HIV-1 infection, by modulating CXCR4 trafficking to the plasma membrane [29]. Tetraspanin CD81, which is highly expressed in CD4⁺ T cells, and its partner EWI-2 also negatively regulate HIV-1 entry [5, 30] through several putative mechanisms. First, CD81 directly associates with CD4 and integrins [26], and could be able to influence the surface organization of the HIV receptor and virus-cell adhesion. Second, both CD81 and EWI-2 bind to F-actin, CD81 through ERM1 and ERM2 and EWI-2 through ERM1, α -actinin and filamin [5, 26]. PIP₂ regulates the EWI-2–F-actin connection during T cell activation, favoring EWI-2 association with ERM1 to the detriment of association with α -actinin [5]. It is conceivable that during HIV-1 attachment, when PIP₂ production is triggered [21], α -actinin will dissociate from TEMs. In this case, TEMs would remain linked to the actin cytoskeleton through ERM1, which are activated by PIP₂. Third, in epithelial cells CD81 recruits PIP4KII to the plasma membrane [31], which could initiate a positive feedback loop generating more PIP₂ locally during HIV-1 infection. Further studies are needed to explore this pathway in CD4⁺ T cells.

Before viral contact, actin filaments (**see Glossary**) in resting CD4⁺ T cells are mainly organized in a cortical network beneath the plasma membrane, arranged in bundles and gel networks (Figure 1A). This actin web is connected to transmembrane receptors through several linker proteins, including filamin, α -actinin, syntenin and drebrin. At this stage, basal ERM phosphorylation is observed in T cells [4], and inactive ERM1 are in a closed conformation, since they are neither phosphorylated nor PIP₂-bound [4]; CD4 associates with syntenin-1, a PSD-95/Disc large/ZO-1 (PDZ)-bearing protein involved in receptor signaling and internalization [9, 32]; and CXCR4 interacts with drebrin, an actin-binding protein related to the ADF-cofilin family [10]. Once HIV-1 attaches to the target cell (Figure 1B), the viral envelope glycoproteins (Env) sequentially engage CD4 receptor and CXCR4 co-receptor, inducing their clustering [15]. The actin cytoskeleton is also involved in the

clustering [33] and stabilization [2] of CD4–CXCR4 complexes during HIV-1 attachment. HIV-dependent CD4–CXCR4 clustering activates PIP5KI- α through a CD4-derived signal [21]. HIV-1 entry into CD4⁺ T cells can be inhibited by PIP5KI- α or Arf6 silencing, which in turn block PIP₂ production [21, 22].

PIP₂ generation after HIV-1 attachment may coordinate serial changes in the actin cytoskeleton that allow and support HIV-1 infection. PIP₂ first inhibits α -actinin and filamin binding to F-actin and activates ERM-linking activity (Table 1, Figure 1B). Interestingly, α -actinin-4 negatively regulates HIV-1 infection in CD4⁺ T cells ([5], Table 1). However, in HEK cells, filamin-A is needed for HIV-induced CD4–CXCR4 clustering and actin dynamics ([6], Table 1). Additional experiments are needed to define the role of filamin-A during HIV-1 infection in lymphocytes. Concomitantly, PIP₂ binding activates ERMs, triggering a conformational change that opens its intramolecular self-inactivating interaction (Figure 1B). Fully activated ERMs are Thrphosphorylated, and HIV-1 induces ERM phosphorylation by a CD4-derived signal [4]. Blocking this signal also inhibits PIP₂ induction [4, 21], demonstrating that HIV-induced ERM activation and HIV-triggered PIP₂ production are interrelated. Moesin activation is required for CD4–CXCR4 clustering and polarized capping in response to HIV-1 Env stimulation ([4], Table 1), and silencing of moesin inhibits X4-tropic HIV-1 infection in human CD4⁺ T cells. However, there are discrepancies about the effect of each ERM member on HIV X4/R5 infection [4, 34–36], possibly due to the use of different cell lines and virus strains; further studies are therefore needed to clarify the involvement of ERMs in HIV entry. Overall, HIV-1-induced PIP₂ production would result in less crosslinked and less bundled actin filaments, allowing a new distribution in which long actin filaments would be linked to membrane receptors through ERMs (Figure 1B).

Other proteins that are recruited to the plasma membrane likely upon PIP₂ production include syntenin-1, gelsolin and cofilin, resulting in inhibition of their functions and their capacity to bind F-actin (Table 1, Figure 1B). The two tandem PDZ domains of syntenin-1 exclusively bind either PIP₂ or proteins [32]. PIP₂ production at the viral attachment area could therefore partially release syntenin-1 from CD4 [9], allowing CD4 to connect with F-actin through the interaction with ERMs. Further studies are needed to demonstrate this dissociation, which could underlie the ability of syntenin-1 to impede HIV-1-induced syncytia formation and viral infection ([9], Table 1). PIP₂-mediated inhibition of gelsolin and cofilin would allow local actin polymerization and stabilization, which is triggered after HIV-1 attachment. This possibility is supported by overexpression of gelsolin in CD4⁺ T cells, which reduces F-actin content and impairs Env-induced CD4–CXCR4 clustering, HIV-1 entry, and infection ([8], Table 1). Cofilin is inactivated by phosphorylation or PIP₂ binding, and cofilin–PIP₂ binding is the main inactivation mechanism at the plasma membrane [37]. Cofilin can also be inactivated in a PAK2-dependent manner by the HIV accessory protein Nef [38], which binds to actin [15]. Soon after Env engagement (30 sec), cofilin is phosphorylated in a Rho-dependent manner [6], and this may act together with PIP₂ binding to inactivate cofilin. The accumulated evidence thus shows that PIP₂ production at the viral attachment site corresponds with changes in the actin cytoskeleton from cortical bundles to more elongated structures (Figure 1B-C, Table 2).

Membrane fusion and viral core internalization

Actin polymerization is needed for HIV-induced membrane fusion, since inhibition of various proteins involved in actin elongation arrests HIV infection at the fusion pore [10-12, 39]. Inhibition of the Arp2/3 actin nucleator complex (**see Glossary**), which is responsible for actin-branched polymerization, reduces HIV-1 infection [39]. Pseudotyping the virus with the envelope G glycoprotein of the vesicular stomatitis virus (VSV-G) overcomes this reduction, suggesting that the Arp2/3-dependent step is related to HIV-1 Env-induced membrane fusion [39]. Moreover, HIV-1 Nef also inhibits the Arp2/3 nucleation promoter N-WASP [40]. Several groups have reported that Env activates the Rho GTPase Rac ([38], Table 1), which can also be activated by Nef, together with P21-activated kinase 2 (PAK2) and the Rho GTPase exchange factor Vav [38]. Rac triggers the WAVE-2 complex, another promoter of Arp2/3 nucleation capacity [11], and HIV-1 entry is impaired by silencing proteins involved in the Rac–Arp2/3 cascade (Tiam-1, Abl kinase and WAVE2) [11]. PIP₂ activates N-WASP, and consequently Arp2/3 actin nucleation ([16, 41]; Table 1). Rho GTPases and PIP₂ are reciprocally regulated, and Rac1 promotes PIP₂ synthesis by activating PIP5K ([17], Table 1). In contrast, in fibroblasts PIP₂ inhibits Abl kinase ([42]; Table 1); but further research is needed to ascertain whether this also occurs in lymphocytes. Activation of the Arp2/3 nucleation complex would trigger a second change in actin organization: from elongated non-parallel structures to highly polymerized branched actin (Figure 1B-C). The actin-severing protein profilin is also regulated by PIP₂ (Table 1). Profilin recruitment to the plasma membrane is needed for efficient HIV-1 infection of macrophages [12], and in CD4⁺ T cells profilin recruitment to the viral attachment sites is promoted by silencing drebrin, a negative regulator of HIV-1 infection [10].

After pore formation, the local highly polymerized actin structure generated could present a physical barrier to the release of the viral core into the cytosol (Figure 1C-D). Recent reports support the notion that HIV-1 membrane fusion induces local actin depolymerization to overcome this [3, 8, 43]. As early as one hour after HIV-1 contact, a CXCR4-derived signal dephosphorylates cofilin, inducing its actin-severing activity and actin depolymerization ([3], Table 1). By this stage, PIP₂ has already dissipated [21], allowing cofilin activation. Thus, in a further adjustment of actin distribution, the highly polymerized branched structure is locally cleared by cofilin activation (Figure 1D, Table 2).

HIV-1 can additionally enter CD4⁺ T cells by clathrin-dependent endocytosis [44]. The actin cytoskeleton appears to play a negligible role in HIV-1 infection via this route. However, dynamin activity has been shown to be essential for HIV-1 endocytic entry ([44], Table 1). PIP₂ activates and recruits dynamin to the plasma membrane and is absolutely necessary for clathrin/dynamin-mediated endocytosis ([16], Table 1), suggesting that PIP₂ might also regulate this alternative entry pathway.

To recapitulate, during HIV-1 entry the virus first induces local PIP₂ production and actin polymerization, and thereafter it triggers actin depolymerization disrupting the cortical F-actin barrier to allow proper viral entrance.

Post-entry steps: interactions between virion components and the cytoskeleton meshwork

HIV-1 replication requires viral uncoating, reverse transcription (RT) and viral DNA insertion into the host cell genome. Although the accurate timing of these steps is not yet well defined, it is clear that the host cell cytoskeleton is involved in the active transport of viral components into the nucleus.

The HIV core contains the viral capsid, the genomic RNA, the accessory proteins Vif, Nef and Vpr, and viral enzymes, as well as several host cell proteins [45]. Some of these viral proteins, including Gag, reverse transcriptase, integrase and Nef, directly interact with actin [15], indicating a possible role for the actin cytoskeleton during uncoating. Host cell proteins packaged into viral particles, such as actin, actin-binding proteins and membrane proteins [45, 46], affect HIV-1 post-entry steps. For example, tetraspanins CD63, CD9, CD81 and CD82 incorporated into new virions attenuate subsequent viral infection [47], and CD63 positively regulates HIV-1 RT without any effect on virus attachment [48]. PIP₂ might modulate the role played by tetraspanins during HIV-1 replication, as it stimulates CD81 interaction with ERMs [5], and its subcellular localization in prostate cancer cells is regulated by CD82 [49].

The efficiency of RT in synthesizing double-stranded viral DNA depends on actin polymerization [13], and viral proteins that are integral components of the RT complex (**see Glossary**) interact with actin [15], suggesting that the RT complex is anchored to the cortical actin. RT is triggered by Env–CXCR4 binding [2, 3], and is regulated by cofilin and LIM domain kinase 1 (LIMK1) [2, 3]. LIMK1 phosphorylates cofilin, inactivating its severing activity. Cofilin and LIMK1 phosphorylation can be induced by HIV-1 Nef [50]. Cofilin activity can also be inactivated by PIP₂ binding [15]. Cofilin knockdown increases the cortical actin density and favors viral DNA synthesis ([3], Table 1), whereas LIMK1 downregulation decreases actin density and impairs both HIV DNA synthesis and viral nuclear migration, without significant differences in viral entry [2]. These data indicate that RT is driven by actin polymerization (Figure 2). Other cytoskeletal PIP₂-modulated proteins with roles in RT include talin-1 and vinculin, whose downregulation promotes paxillin tyrosine phosphorylation and specifically enhances viral DNA synthesis, while paxillin knockdown decreases HIV-1 infection ([7], Table 1). Since this study was performed with VSV-G pseudotyped HIV-1, an altered viral entry could not account for the observed effects in silenced cells. PIP₂ production would therefore be predicted to stimulate actin polymerization and promote viral RT (Figure 2, Table 2).

The translocation of the viral preintegration complex (PIC) (**see Glossary**) to the nucleus in adherent cell lines requires active actin and microtubule transport [13, 14], but in resting CD4⁺ T cells microtubule integrity seems not to be necessary [51]. Since T cells possess a thin cytoplasm, transport along actin filaments may be sufficient for PIC nuclear transport [3]. This process is regulated by increased actin treadmilling (**see Glossary**) induced by cofilin activation ([3], Figure 2). Since PIP₂ inhibits cofilin–actin binding, these data suggest that low PIP₂ levels are necessary for proper HIV-1 nuclear migration (Figure 2, Table 2).

Actin-dependent viral nuclear localization and DNA integration also occurs through chemokine-mediated cofilin activation [52]. Very recently, the myxovirus resistance protein (MxB), an interferon (IFN)-induced dynamin-like GTPase involved in nucleo-cytoplasmic transport, was shown to inhibit HIV-1 nuclear translocation and DNA integration [53]. The MxB-related protein dynamin is activated by PIP₂ (Table 1). Nuclear uptake of virus and DNA integration, but not viral entry, are also enhanced by knocking down the adapter-related protein complex 2 α (AP-2 α) ([54], Figure 2), an endocytic clathrin-adaptor complex that directly interacts with and activates PIP5K γ , modulating PIP₂ production ([55], Table 1). In this way, the PIP₂ pool might also be important for HIV-1 nuclear internalization and DNA integration (Table 2).

Interestingly, actin, actin-binding proteins and PIP₂ can be found in the nucleus [56], implying their possible role in influencing actin dynamics at this location, and as a consequence in modulating HIV DNA integration, viral gene transcription and nuclear export. In this sense, nuclear actin is important for efficient nuclear export of unspliced viral RNA, a process mediated by the HIV accessory protein Rev [57]. Another viral accessory protein, Tat, which regulates HIV-1 transcription [58], co-immunoprecipitates with β -actin [59] and modulates actin remodeling [60]. Tat directly interacts with PIP₂, and associates with protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) [58]. These phosphatases, which positively regulate HIV-1 transcription ([58], Table 1), modulate PIP5K-I β activity and PIP₂ production [61], suggesting that Tat could modulate PIP₂ production. However, the potential role of the actin cytoskeleton and PIP₂ for HIV DNA integration, viral gene transcription and nuclear export remains poorly understood.

Contribution of the cytoskeleton to viral assembly and release

To allow viral particle formation, transcribed viral proteins are targeted to the plasma membrane, where Gag multimerizes, the viral genomic RNA is encapsidated, and Env proteins are incorporated to the nascent virions. The new viral progeny then buds and is released. Although the exact temporal order of these events remains to be determined, HIV-1 Gag is involved in each step [45, 62], and actin-disrupting drugs reduce viral production. However, the specific role of the actin cytoskeleton during the late phase of infection remains undetermined [38].

HIV-1 assembly and budding at the plasma membrane preferentially takes place at TEMs and membrane rafts [62]. Tetraspanin CD81 co-immunoprecipitates with Gag, and several tetraspanins colocalize with Gag and Env in virus-producer cells [62]. In macrophages, assembly occurs in deeply invaginated plasma membranes enriched in PIP₂, tetraspanins, integrins and the actin-linker proteins talin, vinculin and paxillin [63, 64]. Actin-dependent organization of these compartments is important for controlled viral particle release [64]. However, the importance of tetraspanins for HIV-1 release remains a controversial issue, despite their presence at both viral exit sites and budding virions. Indeed, although some groups have suggested that tetraspanins are positive viral release factors, other groups have not observed any effect when knocking-down tetraspanins in virus-producer cells [62]. Nevertheless, since virion-associated tetraspanins impair Env-dependent fusion, HIV-1 assembly within TEMs would prevent syncytia formation, avoiding fusion between infected

and uninfected cells [65, 66]. In this regard, Env accumulation at assembly sites represses its fusion capacity, allowing efficient viral transmission [67]. Recent evidence supports the view that Gag triggers the clustering of specialized microdomains, rafts and TEMs in a stepwise assembly process ([68, 69], Figure 2). During microdomain coalescence, either acylation of Gag or its binding to PIP₂ is sufficient to allow Gag binding to the membrane and subsequent viral assembly [68].

HIV-1 Gag directly associates with PIP₂, and the Gag residues responsible for this interaction have been characterized [70, 71]. Gag is specifically targeted to PIP₂-enriched membranes and PIP₂ is essential for efficient Gag binding to the plasma membrane ([70-72], Figure 2). This targeting is regulated by Gag association with RNA, which prevents Gag binding to non-PIP₂-enriched membranes [71, 73]. Moreover, virions are enriched in membrane-associated PIP₂ in a Gag-dependent manner [71]. Gag is also connected to cytoskeleton proteins. During assembly, HIV-1 triggers the formation of filamentous actin structures that disappear after virus release ([74], Figure 2). Gag association with filamin-A is essential for its proper localization at the plasma membrane, as well as for particle assembly and viral release ([75], Table 1, Figure 2). During these processes, PIP₂ production and hydrolysis are both required (Table 2). Gag interacts with Tsg101 and Alix, components of the ESCRT (endosomal sorting complex required for transport) pathway that are essential for virion fission during the budding process ([45], Table 1, Figure 2). Tsg101 and Sprouty2 regulate PIP₂ hydrolysis by PLC γ , which is essential for HIV-1 production ([76, 77], Table 1, Figure 2). PIP₂ turnover is thus a critical event during HIV-1 assembly and release.

Actin dynamics during cell-to-cell virus transfer

HIV-infected cells can induce numerous types of cell-cell contacts with uninfected target cells- filopodial bridges, tunnels, membrane nanotubes and virological synapses (VS)- increasing the efficiency of viral dissemination [78]. HIV-induced cell-cell contacts are characterized by Env-dependent recruitment of membrane receptors [79, 80]. Several cellular molecules accumulate at HIV-induced cell-cell contacts: adhesion molecules and integrins, markers of TEMs and membrane rafts, signaling molecules and the cytoskeleton proteins actin, talin, α -actinin, ERMs, syntenin-1 and drebrin [4, 5, 9, 10, 66, 80]. Membrane microdomains are important for VS formation, and membrane proteins included in TEMs are important for cell-cell viral transmission [81]. A correct balance of TEM components seems to be necessary for efficient viral spreading through VS. PIP₂, a possible modulator of interactions within TEMs, could play a role in this process.

Actin remodeling is essential for VS formation [79, 80]. CD4 and coreceptor clustering at the VS is dependent on filamin-A expression [6] and on myosin-driven transport [80]. In addition, cell-cell viral dissemination and uropod formation require myosin light chain kinase (MLCK) ([82], Table 1), which phosphorylates myosin proteins, triggering actin polymerization [83]. Actin clearance at the VS [43] depends on low levels of PIP₂ enrichment at the contact, in a process affected by syntenin-1 [9]. Since PIP₂-regulated actin-binding proteins accumulate at the VS and modulate viral dissemination, PIP₂ is a candidate coordinator ensuring the correct signals that will trigger VS formation and efficient HIV cell-cell dissemination.

Concluding remarks

Accumulating data establish that HIV-1 controls actin dynamics during the various steps of its replication cycle (Table 2) through the stimulation or repression of several actin-binding proteins. Recent evidence highlights the importance of PIP₂ in regulating the activity of several of these actin-related proteins, and its involvement in actin cytoskeleton turnover. However, so far few reports have demonstrated a direct role for PIP₂ during HIV-1 infection, demonstrating that HIV-1 contact induces PIP₂ generation during viral entry, and that PIP₂ production and hydrolysis are essential for viral assembly and release (Table 2). We propose that this PPI plays crucial roles at each step of the HIV-1 infection cycle. Further investigation into PIP₂-dependent regulation of actin turnover is needed to provide better understanding of the replication cycle (Box 2), an essential step toward identifying new anti-HIV targets and therapies.

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Glossary

Actin filaments	monomers (G-actin) are added to actin (+) ends and dissociated from actin (−) ends.
Actin polymerization	actin monomers are added to the actin (+) ends and stabilized at the (−) ends, leading to the extension of the filamentous actin (F-actin).
Actin treadmilling	process that occurs when the same filament is simultaneously extended at the (+) end and depolymerized at the (−) end.
Arp2/3 actin nucleator complex	triggers the formation of new actin nucleation cores, thereby stimulating actin polymerization. Arp2/3 nucleation activity is stimulated by WASP, N-WASP and WAVE-2.
Membrane rafts	are small liquid-ordered membrane microdomains, enriched in saturated lipids and specific glycosylphosphatidylinositol (GPI)-linked proteins.
Phosphatidylinositol	can be reversibly phosphorylated to generate different phosphoinositides.
Preintegration complex (PIC)	is an integration-competent HIV-1 complex formed upon viral DNA synthesis in the cytoplasm that is able to efficiently integrate the viral genome into the target cell genome.
Reverse transcription (RT) complex	is originated from the viral core and is responsible for reverse transcription of the viral genome, converting viral single-stranded positive RNA into double-stranded DNA.

Tetraspanins

are four-span transmembrane proteins containing a highly conserved CCG motif in the larger of the two extracellular domains.

Tetraspanin-enriched microdomains (TEMs)

are platforms organized by tetraspanin proteins, and include partner proteins such as membrane proteins, signaling molecules and cytoskeletal proteins.

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Box 1**PIP₂ metabolism**

PIP₂ (phosphatidylinositol-4,5-bisphosphate) is generated by PIP5KI or PIP4KII, which respectively phosphorylate PI(4)P (phosphatidylinositol 4-phosphate) and PI(5)P (phosphatidylinositol 5-phosphate). Arf6 (ADP ribosylation factor 6) and PLD (phospholipase D) activate PIP5KI. PIP₂ is catalyzed by PLC (phospholipase C), producing IP₃ (inositol-1,4,5-trisphosphate) and DAG (diacylglycerol), and PIP₂ can be converted into PIP₃ (phosphatidylinositol-3,4,5-trisphosphate) by phosphorylation by PI3K. PIP₂ can also be dephosphorylated by different phosphatases.

Box 2**Outstanding Questions**

- Does HIV-1 directly causes PIP₂ depletion after membrane fusion or PIP₂ depletion is a consequence of viral-induced actin cytoskeleton changes?
- What are the dynamics and the timing of PIP₂-induced modulation of actin-binding proteins during HIV-1 infection?
- How is the PIP₂ pool tuned throughout HIV-1 infection?
- Is PIP₂ mainly acting as a recruiter of cellular and viral proteins to the plasma membrane?
- Is nuclear PIP₂ playing a role in HIV-1 nuclear migration and DNA integration?

Figure 1. Actin cytoskeleton turnover during HIV attachment and entry steps.

(A) Initial situation. The actin cortical cytoskeleton is arranged in bundles, and transmembrane receptors are organized in TEMs. (B) HIV-1-induced receptor clustering activates PIP5K, which generates PIP₂. PIP₂ binding inhibits the interactions of several proteins (syntenin, α-actinin, and cofilin) in favor of other proteins that become activated (ERMs). The actin cytoskeleton and the clustering of HIV-1 receptors cooperate during viral attachment. (C) Signaling derived from CD4 and CXCR4 triggers Rac-1. Rac-1 associates with WAVE-2 complex, which activates the Arp2/3 actin nucleator. The actin cytoskeleton

is locally remodeled into a branched and highly polymerized web. **(D)** Rac-1-derived signals activate cofilin. Cofilin, profilin and gelsolin depolymerize F-actin, allowing the release of the viral core into the cell. In the illustration, virus particles and molecules are not to scale.

Figure 2. HIV-1 replication is regulated by actin-binding and PIP₂-modulated proteins. After HIV-1 fusion with the plasma membrane, actin polymerization facilitates RT. During this step, cofilin is inactivated by LIMK1-dependent phosphorylation. Talin and vinculin are inactive, while phosphorylated paxillin promotes viral DNA synthesis. PIC nuclear uptake depends on cofilin activation, thereby inducing actin depolymerization. HIV nuclear transport and DNA integration are also regulated by AP-2. After transcription of viral genes, virion assembly takes place at coalesced TEMs and membrane rafts at the plasma membrane, where Gag associates with PIP₂ and filamin-A. Filamentous actin structures are formed during assembly. ESCRT proteins (Tsg101 and Alix) drive viral fission. During the final step of the viral cycle, Sprouty2 regulates PLC-dependent PIP₂ hydrolysis. HIV-1 virions bud from the cell, generating the new progeny. Cellular filamentous actin structures dissipate after virus release. In the illustration, virus particles and molecules are not to scale.

Table 1
PIP₂ regulation of actin-related proteins and their role during the HIV-1 cycle

Protein	Protein function	PIP ₂ regulation	Refs	Effect on HIV cycle	Refs
α-Actinin	Organizes actin filaments in parallel bundles	Inhibited by PIP ₂	[16]	α-Actinin restricts HIV-1 entry	[5]
Abl kinase	Phosphorylates WAVE2 and activates Arp2/3	Inhibited by PIP ₂	[42]	Abl kinase activity favors HIV-1 entry	[11]
AP-2	Clathrin-adaptor complex	AP-2 interacts with PIP5K1γ, regulating PIP ₂ production	[55]	AP-2 negatively regulates nuclear translocation and viral DNA integration	[54]
Cofilin	Depolymerizes F-actin at the (-) ends, generates actin nuclei (Arp2/3 activation)	Severing function is inhibited by PIP ₂	[20]	Cofilin promotes HIV-1 latent infection of resting CD4 T cells, facilitating HIV-1 migration to the nucleus and viral DNA synthesis	[3]
Dynamitin	Clathrin-mediated endocytosis	Recruited and activated (GTPase activity) by PIP ₂	[16]	Dynamitin activation is needed for the HIV-1 endocytic entry pathway	[44]
ERMs	Links F-actin and transmembrane receptors, organizes actin into stress fibers	Activated by PIP ₂ (open conformation)	[16]	ERMs facilitate Env-induced CD4-CXCR4 clustering and actin redistribution during HIV-1 entry	[4, 35]
Filamin	Cross-links F-actin, organizing the cytoskeleton in a gel network	Inhibited by PIP ₂ (actin binding capacity)	[84]	Filamin favors HIV-1-induced CD4-CXCR4 clustering and contributes to particle assembly by interacting with Gag	[6] [75]
Gelsolin	Sever filament (+) ends, promote actin polymerization	Dissociated from F-actin by PIP ₂	[16]	Gelsolin silencing or overexpression restricts HIV-1-infection	[8]
Myosin light chain kinase (MLCK)	Myosin phosphorylation	Activated by PIP ₂ hydrolysis	[83]	MLCK induces CD4 or CXCR4 Env-dependent clustering at the VS, favoring viral dissemination	[80, 82]
Paxillin	Adaptor protein at focal adhesions	Its tyrosine phosphorylation correlates with PIP ₂ synthesis	[85]	Paxillin favors HIV-1 infection	[7]
PP1/PP2A	Serine/threonine phosphatases	PP1/PP2A inhibitor impairs PI4P5K-Iβ PIP ₂ synthesis	[61]	PP1/PP2A favors HIV-1 transcription	[58]
Profilin	Promotes actin assembly at (+) ends, enhances F-actin growth	Inhibited by PIP ₂ (binding capacity to actin and Poly-Pro bearing proteins)	[16]	Profilin supports virion production Local profilin increase makes cells more permissive to infection	[12] [10]
Rac	Promotes branched-actin polymerization	Rac activates PIP5K, and is activated by PIP ₃	[16]	Rac is needed for HIV-1-induced membrane fusion	[38]
Sprouty2	Inhibits receptor tyrosine kinases	Inhibits PIP ₂ hydrolysis	[76]	Sprouty2 impairs HIV-1 release	[76]
Syntenin-1	Scaffold protein	PIP ₂ inhibits PDZ protein-protein association	[32]	Syntenin-1 inhibits HIV-1 entry	[9]
Talin	Associates with and activates β1/3 integrins	PIP ₂ promotes talin activation	[16]	Talin blocks retroviral infection by inhibiting paxillin phosphorylation	[7]
Tsg101	Controls endosomal cargo sorting and trafficking	Facilitates PIP ₂ hydrolysis	[77]	Tsg101 is essential for HIV budding	[77]

Protein	Protein function	PIP ₂ regulation	Refs	Effect on HIV cycle	Refs
Vinculin	Scaffold protein involved in focal adhesion	Increases its binding to WASP and talin, dissociation from F-actin	[16]	Vinculin blocks retroviral infection by inhibiting paxillin phosphorylation	[7]
WASP	Activates Arp2/3, inducing actin branching	Activation by PIP ₂	[41]	Inhibition of Arp2/3 blocks HIV-1 replication	[39]

Table 2
Summary of PIP₂ and actin turnover at the different steps of HIV-1 infection in CD4⁺ T cells

HIV-1 cycle	PIP ₂ ^a	Actin cytoskeleton ^a
Attachment	Production	Polymerization
Core internalization	Degradation	Depolymerization
RT	Production ^b (N.D.)	Polymerization
Nuclear migration and DNA integration	Degradation ^c (N.D.)	Depolymerization
Assembly	Production	Polymerization ^d (N.D.)
Budding	Degradation	Depolymerization ^e (N.D.)

^a Abbreviation: N.D., not demonstrated

^b Cofilin inactivation favors viral DNA synthesis, and PIP₂ inhibits cofilin actin-severing activity.

^c Actin treadmilling is induced by an increase in cofilin activity.

^d HIV-1 Gag associates with filamin-A during assembly. This actin cross-linking protein organizes the actin cytoskeleton in gel networks. During the late steps of HIV-1 infection, virus-induced filamentous actin structures disappear after virus release.

^e During the late steps of HIV-1 infection, virus-induced filamentous actin structures disappear after virus release.