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Roles played by acidic lipids in HIV-1 Gag membrane binding

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Abstract

The MA domain mediates plasma membrane (PM) targeting of HIV-1 Gag, leading to particle assembly at the PM. The interaction between MA and acidic phospholipids, in addition to N-terminal myristoyl moiety, promotes Gag binding to lipid membranes. Among acidic phospholipids, PI(4,5)P₂, a PM-specific phosphoinositide, is essential for proper HIV-1 Gag localization to the PM and efficient virus particle production. Recent studies further revealed that MA-bound RNA negatively regulates HIV-1 Gag membrane binding and that PI(4,5)P₂ is necessary to overcome this RNA-imposed block. In this review, we will summarize the current understanding of Gag-membrane interactions and discuss potential roles played by acidic phospholipids.

Keywords

HIV-1 Gag; PI(4,5)P₂; membrane binding; acyl chains; virus assembly

Acidic phospholipids play an important role in recruiting cytoplasmic proteins to membrane (1–3). They act as binding partners for these proteins thereby functioning as membrane anchors (1). The mechanisms of protein interactions with acidic lipids fall mainly into two broad categories. One category involves structure-dependent interactions of binding motifs or domains with specific lipid headgroups. A well-studied example of this class is the pleckstrin homology (PH) domain of phospholipase C δ 1 (PH_{PLC δ 1}). PH_{PLC δ 1} is shown to specifically bind to both plasma-membrane-specific phosphoinositide PI(4,5)P₂ and its soluble headgroup analog D-myo-inositol 1,4,5 trisphosphate (2–6). The second category of protein interactions with acidic lipids is mediated by unstructured clusters of basic residues that mainly mediate non-specific electrostatic interactions with charged lipids in the membrane. For example, basic residues in proteins such as K-Ras, Src and MARCKS have been shown to bind acidic lipids via non-specific electrostatic interactions (2, 3).

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The structural protein of HIV-1, Gag, drives the assembly process of the virus particle (7–9). Gag is synthesized as a polyprotein Pr55^{Gag}, which contains four major structural domains, namely matrix (MA), capsid (CA), nucleocapsid (NC), and p6, as well as spacer peptides SP1 and SP2. Each of these domains performs a specific and distinct function during the assembly process. The N-terminal MA domain targets Gag to the site of assembly and mediates membrane binding. In addition, MA is important for incorporation of viral glycoprotein Env into assembling particles. Prior to or after Gag membrane binding, Gag forms multimers. This multimerization is promoted mainly by two downstream regions of Gag. The C-terminal domain of CA mediates Gag-Gag interactions through the dimerization interface, whereas NC promotes higher order multimerization via its ability to bind RNA. The NC domain has zinc finger motifs that determine the RNA specificity of NC in genomic RNA encapsidation although non-genomic RNAs can promote Gag multimerization. The p6 domain recruits the host ESCRT machinery that helps in the scission of the nascent virus particles from the membrane.

The subcellular location at which the virus assembles is an important determinant for efficient and productive virus particle production, since it affects the efficiency of accumulation of various virus components and the fate of the progeny virions after assembly. For HIV-1, assembly takes place at the plasma membrane (PM) in most cell types including a natural host, T cells. However, in another natural host, macrophages, it was initially observed that the virus assembles in structures resembling late-endosomal multivesicular bodies (MVBs) (10, 11). However, more recent studies have suggested that these compartments, termed virus containing compartments (VCCs), are actually PM invaginations that extend deep into the cytoplasm (12–14) although a population of them is not accessible to the external medium (15).

Proper subcellular localization and membrane binding of retroviral Gag proteins are mediated by the N-terminal MA domain. In this article, we review our current understanding of the mechanisms by which Gag binds the cellular membranes with a particular emphasis on the role of acidic phospholipids.

Membrane binding of Gag via bipartite signals within MA

The N terminus of the MA domain is co-translationally modified by the addition of the 14-carbon acyl chain or the myristate moiety (Fig. 1A). Amino acid substitutions of either the N-terminal glycine that serves as the site for the attachment of the myristate moiety or residues that are essential for recognition by N-myristoyl transferase result in defective membrane binding of Gag and virus release in cells (16–19). Consistent with these observations, using liposome flotation assay it was observed that myristoyl-deficient Gag proteins fail to bind membranes efficiently (20). Besides playing an essential role in membrane binding, myristate has also been implicated in oligomerization of HIV-1 MA (21).

Studies on many myristoylated proteins have shown that the myristate moiety can be either ‘sequestered’ within the protein molecule or ‘exposed’ to the environment around it (Fig. 1B). A transition between these states is termed ‘myristoyl switch’ (22–26). A study that

analyzed the membrane binding properties of the HIV-1 MA domain alone and full-length Gag found that the MA domain alone was not capable of binding to membranes whereas full-length Gag bound to membranes efficiently (18, 27–29). Based on this observation it was proposed that the MA domain alone adopts a conformation different from full-length Gag and hypothesized that a conformational change might affect the exposure of myristate. Consistent with this hypothesis, some amino acid substitutions in MA impair Gag membrane binding without affecting myristoylation itself (30, 31). Subsequently, NMR studies using myristoylated HIV-1 MA confirmed the existence of the myristoyl switch mechanism for HIV-1 MA (21). The study showed that the myristoyl moiety was sequestered within the hydrophobic cleft of the MA domain. Additionally, this study also found that MA exists as a mixture of a myristate-sequestered monomer and a myristate-exposed trimer in equilibrium, suggesting a link between myristoyl exposure and Gag-Gag association. Indeed, addition of the CA domain at the C terminus of MA shifted the equilibrium towards the myristate-exposed form, suggesting that promoting self-association between Gag molecules induces myristate exposure. Besides HIV-1, MA domains from other retroviruses such as HIV-2 and MPMV have also been shown to sequester myristate (32, 33). Interestingly, the study on HIV-1 MA showed that in addition to Gag multimerization, an interaction of MA with a soluble analogue of PI(4,5)P₂ triggers myristate exposure (discussed later) (34). In contrast, it was shown that PI(4,5)P₂ does not trigger myristate exposure of HIV-2 MA (33) and MPMV MA (32). Therefore, mechanisms regulating myristate exposure might differ between different retroviral MA domains. In addition to PI(4,5)P₂ and Gag oligomerization, changes in pH and binding of calmodulin to MA have been reported to induce myristate exposure (35, 36). Myristoyl switches have been shown to cause conformational changes in proteins such as ARF-1 (24), c-Abl (23), and recoverin (22, 25). In contrast, in the case of HIV-1, no large-scale changes in tertiary structures of the MA domain were observed regardless of whether myristate was sequestered or exposed (21). However, involvement of MA structural changes in the context of full length Gag and in the presence of lipid bilayers remains to be tested. Likewise, the requirement for various triggers remains to be examined in the presence of lipid bilayers, since spontaneous myristoyl exposure has been observed in NMR (37) and in silico (38).

Studies on other myristoylated proteins suggest that although essential, the myristate moiety alone is insufficient for stable retention of proteins on the membrane (26, 39). It is thought that myristoylated proteins bind membranes reversibly and dissociate rapidly (40, 41). The lack of stability in membrane association mediated by myristate insertion is attributed at least partly to the length of the myristate acyl chain. The short 14-carbon myristoyl chain is thought to be inadequate in providing hydrophobic interactions sufficient for stable membrane association (41). It has been suggested that a ‘secondary signal’ besides the myristate moiety is necessary to achieve efficient and stable membrane binding. This is termed the ‘two-signal hypothesis’ (42). Many cytosolic proteins use the ‘two-signal’ mechanism for achieving stable and efficient membrane binding. For example, membrane binding of Src is mediated by the myristate moiety and the secondary signal that is provided in the form of a stretch of basic residues or ‘polybasic cluster’. The polybasic cluster is known to mediate interactions with acidic phospholipids. The electrostatic interaction of the polybasic cluster with acidic lipids, along with the hydrophobic interactions provided by

myristate insertion into lipid bilayers, results in efficient membrane binding. Besides Src, many proteins such as HIV-1 Nef, MARCKS, and histophilin use the 'two-signal' mechanism to achieve stable membrane binding (43–45). Besides polybasic clusters, a variety of other factors could act as the 'second-signal'. The second signal can be in the form of additional acyl chain modifications such as palmitoylation or prenylation (46–48). These additional acyl chains augment membrane binding by increasing the hydrophobic interactions with the membrane. Protein-protein interactions that increase the clustering of several low affinity interactions with the membranes can also act as a secondary signal via increasing the avidity of the protein complex. In case of HIV-1 Gag, it was shown that a second signal is provided by a stretch of polybasic residues in MA referred to as the highly basic region (HBR). The involvement of HIV-1 MA basic residues in membrane binding via interaction with acidic phospholipids was first biochemically shown in a study by Zhou, et al. (49). Using a liposome flotation assay, this study demonstrated that the N-terminal 31 amino acid residues of MA fused to heterologous proteins promoted the HBR-dependent binding to phosphatidylserine-containing liposomes. Subsequently, structural studies showed that HBR forms a basic patch on the MA surface, which is likely to constitute an interface for acidic phospholipids (50, 51).

The inner leaflet of the PM where HIV-1 assembles is enriched in acidic phospholipids such as phosphatidylserine (PS) and phosphoinositides. As a result, the inner leaflet of the plasma membrane surface is negatively charged. Basic residues within MA domains of various retroviruses are likely to interact with these acidic lipids in cellular membranes. Consistent with this hypothesis, substitutions of the MA basic residues have been shown to affect targeting and membrane binding of many retroviral Gag proteins (52–61). Furthermore, a comparative structural analysis in combination with homology modeling suggested that myristoylated and non-myristoylated MA domains from diverse retroviruses have a basic patch or patches on the surface of MA (62). However, whether the basic patch(es) mediate interactions with specific acidic phospholipids had not been well understood until recently.

The essential role for PI(4,5)P₂ in PM targeting and membrane binding of Gag

As mentioned earlier, in most cell types HIV-1 assembly takes place specifically at the PM. However, besides PM other cellular membranes also contain acidic phospholipids. Therefore it was hypothesized that a PM-specific acidic phospholipid is responsible for Gag-PM localization. Indeed, cell-based studies suggested that this differential binding is likely facilitated by a PM-specific acidic phospholipid PI(4,5)P₂. When cellular PI(4,5)P₂ levels were perturbed either by depletion or ectopic production of PI(4,5)P₂, HIV-1 Gag failed to bind the PM and/or mislocalized to intracellular compartments, and virus release was impaired (63–66). Consistent with these observations, using an inducible PI(4,5)P₂ depletion strategy (67), we observed that rapid and acute depletion of PI(4,5)P₂ resulted in markedly decreased virus particle release (unpublished data). These observations suggest that PI(4,5)P₂ plays an important role in Gag binding to the PM and virus release. Besides HIV-1, depletion of PI(4,5)P₂ has been shown to affect PM localization and virus production of other retroviruses such as HIV-2, MLV and M-PMV (33, 55, 59, 64). However, not all

viruses are sensitive to PI(4,5)P₂ depletion. For example, PM localization and virus particle release of EIAV (54), RSV (63) and HTLV-1 (68) have been observed to be unaffected by PI(4,5)P₂ depletion [although another study observed significant effects on RSV Gag localization and virus release (69)]. These studies suggest that membrane targeting of Gag of some retroviruses is regulated by mechanisms that do not depend on PI(4,5)P₂ levels.

It is likely that a direct MA-PI(4,5)P₂ interaction underlies the observed defects in HIV-1 Gag localization and membrane binding following cellular PI(4,5)P₂ perturbation. The interaction between phosphoinositides and HIV-1 Gag was first inferred from an in vitro Gag assembly study in which inositol phosphates were shown to be essential for Gag to form proper virus-like structures (70). Subsequently, protein footprinting and NMR-based studies demonstrated the direct interaction of HIV-1 Gag derivatives with short-acyl-chain forms of PI(4,5)P₂ in solution (34, 71). Furthermore, studies using liposome flotation assays showed that Gag membrane binding is significantly enhanced in the presence of PI(4,5)P₂ with native-length acyl chains in the liposomal membranes (20, 53, 72). Finally, Gag-PI(4,5)P₂ interactions in cells is supported by a lipidomic study demonstrating that the HIV-1 viral membrane is enriched in PI(4,5)P₂ relative to the plasma membrane of the virus-producing cells and that this enrichment occurs in a MA-dependent manner (64).

Whether HIV-1 Gag binding to PI(4,5)P₂ is driven solely by electrostatic interactions or by specific recognition of headgroup of lipids is under ongoing investigation. In vitro assays showed that Gag binds more efficiently to PI(4,5)P₂-containing liposomes than to liposomes containing palmitoyl-oleoyl-PS (POPS) (an abundant form of PS in cell membranes; Fig. 2A) even when the overall negative charge of liposomes was matched (53, 73, 74). Furthermore, although liposomes containing PI(3,5)P₂, a phosphoinositide with the same charge as PI(4,5)P₂, enhanced Gag binding to liposomes, the enhancement was consistently lower than that mediated by PI(4,5)P₂ (53). However, in some experimental systems, the difference between PS- and PI(4,5)P₂-containing liposomes was not observed when charge is matched (63). This difference may be partly due to difference in acyl chains of phospholipids used in the studies, since it was shown that liposomes containing dioleoyl phospholipids recruits HIV-1 Gag more efficiently than those containing palmitoyl-oleoyl lipids in the absence of PI(4,5)P₂ (72). Notably, a structural study by Saad et al has observed that MA binds specifically to PI(4,5)P₂ and PI(3,4,5)P₃ but failed to bind to other phosphoinositides such as PI(3,5)P₂ (34). Furthermore, a Gag derivative that has the same overall charge but a different HBR sequence failed to bind to PI(4,5)P₂-containing membranes (75) (discussed later). Altogether, these results suggest that Gag binding to membranes involve electrostatic and structure-dependent association between MA and the lipid headgroups but could also be modulated by the acyl chains of lipids.

The highly basic region (HBR) in MA: an interface for PI(4,5)P₂

The molecular determinants in HIV-1 Gag for interaction with PI(4,5)P₂ were first examined in the protein footprinting study by Shikrabai et al. (71). This study examined the accessibility of lysines in non-myristoylated Gag proteins in the presence of soluble analogues of PI(4,5)P₂. This study showed that Lys 29 and Lys 31 in the MA HBR were inaccessible to the solvent in the presence of soluble (short-acyl-chain form) PI(4,5)P₂,

suggesting that these lysines directly interact with PI(4,5)P₂. Subsequently, an NMR study by Saad et al determined the structure of myristoylated-MA-soluble-PI(4,5)P₂ complexes and showed that different basic residues (Arg 21 and Lys 26) in HBR form an interface for PI(4,5)P₂ (34). In liposome flotation studies, various basic amino acid substitutions in MA HBR were examined for effects on PI(4,5)P₂-dependent membrane binding (20, 75). Among them, basic-to-neutral or basic-to-acidic substitutions at residues 17, 29, and 31 showed a significant reduction in PI(4,5)P₂-dependent liposome binding (20, 53). Interestingly, as mentioned earlier a Gag mutant in which lysines and arginines were exchanged (HBR/RK switch) failed to bind to PI(4,5)P₂-containing liposomes (Fig. 2B), suggesting that not just the overall charge but specific amino acid sequence in the HBR is necessary for Gag-PI(4,5)P₂ interactions (75). Collectively, these observations suggest that basic residues within the MA HBR mediate Gag interaction with PI(4,5)P₂. Of note, a Gag derivative lacking a majority of MA except the N-terminal myristoylation signal binds membranes and produces virus particles (76–80). Therefore, it is possible that the role of HBR in mediating membrane binding is essential only in the context of the full MA domain.

As mentioned earlier, besides HIV-1, MA domains of other retroviral Gag proteins also have basic residues that are implicated in binding to acidic lipids. However, except for MLV MA for which mutations in a basic amino acid cluster inhibit binding to liposomes containing PS and PI(4,5)P₂ (55), little is known about molecular determinants in MA for acidic lipid interactions. NMR studies have identified MA basic residues that are affected by PI(4,5)P₂ titrations for HIV-2, EIAV, and MPMV (33, 59, 81). However, whether these residues are actually involved in PI(4,5)P₂-dependent membrane binding has yet to be biochemically determined. Further studies on structure-function relationships may potentially elucidate diverse modes of MA interactions with acidic phospholipids, since the organization of basic residues is rather diverse among different retroviral MA domains (62).

Modulation of Gag membrane binding by acyl chains of phospholipids

While interactions between basic residues and the headgroup of PI(4,5)P₂ is likely to play a key role, acyl chains of this lipid have also been implicated in membrane binding of HIV-1 Gag. The NMR study using myristoylated MA and soluble PI(4,5)P₂ showed that the sn-2 acyl chain of water-soluble PI(4,5)P₂ is sequestered within the hydrophobic cleft in the MA that is different from the cleft that sequesters myristate (34). This finding led to a hypothesis in which MA sequestration of the sn-2 acyl chains of plasma membrane PI(4,5)P₂, which is usually unsaturated, contributes to Gag-membrane binding as well as Gag association with membrane microdomains (discussed later). Consistent with this finding, a surface plasmon resonance study showed that water-soluble PI(4,5)P₂, which has short (8-carbon) acyl chains, had higher affinities to both full-length Gag and isolated MA than inositol phosphates such as IP₃ that corresponds to the headgroup of PI(4,5)P₂ (82). Acyl chain saturation in the context of PI(4,5)P₂ with native-length acyl chains was found to have no significant effect on interactions between isolated MA purified from bacteria and PI(4,5)P₂ in a liposome-based study (83). In contrast, in two different liposome/vesicle-based assay methods, we observed that PI(4,5)P₂ with two saturated (dipalmitoyl) acyl chains [DP-PI(4,5)P₂] is unable to support full-length Gag binding to membrane unlike PI(4,5)P₂ with at least one unsaturated acyl chain (Olety, Veatch, and Ono, submitted). Identifying the origin

of this difference might potentially help us elucidate the role of PI(4,5)P₂ acyl chains in Gag membrane binding.

As mentioned earlier, Dick et al (2012) (72) observed that acyl chains of phospholipids also modulate HIV-1 Gag binding to membranes. Liposomes consisting of PC and PS, both of which have palmitoyl and oleoyl chains (POPC+POPS), are unable to support efficient Gag binding even when the PS content is elevated to 50 mol% as previously observed (53). However, when the dioleoyl form of phospholipids were used, liposomes containing 40 mol % (or higher) of PS efficiently recruited HIV-1 Gag. Currently, however, mechanisms that explain this phenomenon remain undetermined. Recently, an NMR study reported that MA also directly interacts with the acyl chains of PS, PE, and PC lipids via a hydrophobic cavity that is different from the binding site for the sn-2 acyl chain of PI(4,5)P₂ (37). The significance of this interaction in Gag membrane binding is unknown yet. Collectively, these studies suggest that phospholipid acyl chains are important for stable Gag-membrane binding although much remains to be elucidated.

Relationships between Gag membrane binding and microdomain organization

In addition to acidic lipids such as PS and PI(4,5)P₂, cholesterol in the PM is likely to play an important role in Gag membrane binding. Cholesterol is heterogeneously distributed in the PM (84, 85) and, along with lipids with saturated acyl chains, forms the PM microdomains known as lipid rafts (86). It has been shown that cellular depletion of cholesterol, which disrupts lipid rafts, reduces membrane binding of HIV-1 Gag and impairs virus release (87, 88). Consistent with these observations, recent in vitro studies showed that membrane binding of HIV-1 Gag to liposomes is enhanced in a cholesterol-dependent manner (72, 83). Perhaps not surprisingly, viral lipidomics revealed that released HIV-1 particles are enriched in cholesterol relative to the PM (64, 89) although another study did not detect a significant enrichment of cholesterol (90). Moreover, Gag has been shown to associate with the detergent resistant membrane (DRM) fractions (87, 91), which are enriched in raft proteins [although DRMs do not exactly reflect compositions of rafts (92)]. Microscopy-based studies have shown that HIV-1 Gag colocalizes or copatches with many lipid raft markers (75, 91, 93, 94). Finally, a study using a fluorescent lipophilic dye that alters its emission spectra in response to the order of the surrounding membrane showed that the envelope membrane of released HIV-1 particles has a high degree of membrane order, a property similar to that of the tightly packed liquid-ordered membrane structure, which lipid raft microdomains are thought to adopt (95). Consistent with the highly ordered nature of these microdomains, HIV-1 Gag modified with unsaturated myristate analogues at the N terminus failed to associate with DRM and was impaired for virus release (96). These reports collectively support the possibility that cholesterol may facilitate Gag membrane binding via formation of lipid rafts or similar structures during HIV-1 assembly.

As mentioned earlier, the NMR study on the MA-PI(4,5)P₂ complex showed that soluble PI(4,5)P₂ analogues induce exposure of the HIV-1 MA myristate moiety, while their short sn-2 acyl chain is inserted into the MA globular domain (34). Based on these observations, the study suggested a possibility that MA sequesters the unsaturated sn-2 acyl chain of

PI(4,5)P₂, while it associates with the membrane via two saturated acyl chains, i.e., the sn-1 acyl chain of PI(4,5)P₂ and the myristoyl moiety. Since saturated but not unsaturated acyl chains are favored in lipid rafts, this mechanism could potentially explain association of Gag with lipid-raft-like microdomains at the PM (34). However, a study using a giant-unilamellar-vesicle(GUV)-based in vitro system showed that HIV-1 Gag derivative failed to partition into the liquid-ordered phase, which is thought to represent lipid rafts in these systems, in the presence of PI(4,5)P₂ that has saturated sn-1 and unsaturated sn-2 acyl chains (74). Hence, the proposed model in which sn-2 acyl chain sequestration of PI(4,5)P₂ regulates Gag partitioning to lipid rafts remains to be experimentally verified.

A recent co-patching study in T cells has shown that HIV-1 Gag associates with a specific subset of microdomains enriched in uropod-localizing transmembrane proteins such as CD43, CD44, and PSGL-1 (75, 97). This association depends on the basic charge of MA HBR (75). Moreover, super-resolution localization microscopy analysis showed that juxtamembrane polybasic sequences of the transmembrane proteins are also important for co-clustering between Gag and these uropod-directed proteins (Grover, Veatch, and Ono, submitted). It has been shown that proteins with basic interfaces can recruit acidic lipids that in turn can facilitate recruitment and clustering of these proteins into microdomains (98). Consistent with the presence of microdomains enriched in acidic lipids, fluorescently tagged probes that bind specifically to headgroups of acidic lipids such as PI(4,5)P₂ and PS (e.g., GFP-tagged PH_{PLC81} or LactC2 domains) have been observed to localize non-homogeneously on the membrane (2, 99–102). Therefore, it is tempting to speculate that interactions of MA HBR with acidic lipids such as PI(4,5)P₂ and PS nucleate the formation of microdomains rich in acidic lipids, which in turn attract membrane proteins that have membrane-proximal basic clusters. Indeed, this possibility is supported by a recent simulation study in which MA is capable of sequestering PI(4,5)P₂ molecules (38). Alternatively, it is plausible that Gag localizes to pre-existing microdomains that are enriched in acidic lipids and uropod-directed transmembrane proteins.

RNA-mediated regulation of acidic-phospholipid-dependent membrane binding

Besides lipids, HIV-1 MA domain interacts with RNA (71, 103–109). HIV-1 MA is dispensable for genomic RNA encapsidation (78, 80, 110) as NC plays that role. Of note, however, several studies on MA domains of RSV and BLV have proposed a variety of functions for MA-RNA interactions including regulation of viral mRNA translation and dimerization of the encapsidated viral gRNA in addition to genomic RNA encapsidation (111–114). As for the interface between HIV-1 MA and RNA, several studies have shown that basic residues within the MA HBR interact with nucleic acids (71, 106, 108, 115, 116). Furthermore, by co-immunoprecipitation of Gag derivatives lacking NC, in vitro synthesized Gag and Gag expressed in HeLa cells transfected with HIV-1 molecular clones were shown to bind RNA in a manner dependent on MA HBR (20, 117). Since MA binds both PI(4,5)P₂ and RNA, we hypothesized that RNA has a regulatory role in Gag-membrane binding. Consistent with this hypothesis, we and others have shown using liposome-based assays that MA bound to RNA is essential for ensuring specific binding to PI(4,5)P₂ (20, 83,

117). When WT and NC-deleted HIV-1 Gag proteins were treated with RNase, both Gag proteins efficiently bound to liposomes that lack PI(4,5)P₂ but contain another acidic lipid PS. When untreated, these Gag proteins did not bind to such PS-containing membranes, suggesting that RNA when bound to MA can act as a negative regulator of PI(4,5)P₂-independent membrane binding. Of note, PS is the most abundant acidic phospholipid present in several different cellular compartments other than the PM (118–120). Therefore, RNA might act as a ‘chaperone’ to target Gag to PI(4,5)P₂-containing membranes by preventing promiscuous binding to membrane containing PS and/or perhaps other acidic lipids. This mechanism is likely to take place in HIV-1-producing cells, since RNase-mediated enhancement of PI(4,5)P₂-independent liposome binding was observed not only with in vitro synthesized Gag but also with Gag in the cytosol derived from cells expressing HIV-1 molecular clones (117). RNase treatment was also observed to cause a modest increase in binding to liposomes composed solely of a neutral lipid PC, suggesting that RNA removal also facilitates myristate exposure that promotes hydrophobic interactions (20).

Since some of the HBR basic amino acids that bind PI(4,5)P₂ also bind RNA (71, 115), it is possible that PI(4,5)P₂ reverses the suppressive effect of RNA via a direct competition. Consistent with this possibility, IP6 facilitates dissociation of RNA from MA (121). Moreover, PI(4,5)P₂, but not PC or PS, efficiently outcompeted RNA for binding to MA-coated beads (115). However, these studies (115, 121) were conducted using purified MA and short acyl chain soluble lipid analogues. Therefore, it is possible that in the context of membrane binding of full-length Gag, RNA displaced by PI(4,5)P₂ may still remain bound to Gag via a secondary site in MA or NC. It is also of note that the HBR/RK switch mutant MA, which is incapable of mediating PI(4,5)P₂-dependent membrane binding (described earlier), is still susceptible to the RNA-mediated inhibition of binding to PS-containing membrane (Fig. 2B). Therefore, the overall charge of MA HBR appears to be sufficient for binding to RNA (and PS) unlike binding to PI(4,5)P₂.

Differences in mechanisms by which PI(4,5)P₂ promotes membrane binding of Gag and the PH_{PLCδ1} domain

Evidence summarized in the previous sections indicates that HIV-1 MA (and possibly other retroviral MA domains) differs from some of representative cellular PI(4,5)P₂-binding domains, such as the PH_{PLCδ1} domain, in that the specific interaction with PI(4,5)P₂ is dependent not only on the domain structure in which basic residues mediate interactions with the lipid headgroup but also on the presence of RNA that prevents non-specific interactions with other acidic lipids. Additionally, unlike HIV-1 MA, PH_{PLCδ1} interacts with PI(4,5)P₂ regardless of acyl chains of the lipid; PH_{PLCδ1}-GFP, but not HIV-1 Gag, bound to single-phase GUVs containing DP-PI(4,5)P₂, whereas both readily bound to GUVs containing PI(4,5)P₂ with unsaturated acyl chains (Olety, Veatch, and Ono, submitted). Unexpectedly, in a GUV system containing DPPS and DP-PI(4,5)P₂, which displays two (gel and liquid) phases, not only PH_{PLCδ1}-GFP but also Gag bound to the GUVs in a manner dependent on the presence of DP-PI(4,5)P₂. However, while PH_{PLCδ1}-GFP bound to the liquid phase of these two-phase GUVs, Gag bound to the gel phase. This observation suggests that both HIV-1 MA and PH_{PLCδ1} are sensitive to the environment surrounding

PI(4,5)P₂ but in different ways (Olety, Veatch, and Ono, submitted). These differences in modes of interaction with PI(4,5)P₂ between HIV-1 MA and canonical PI(4,5)P₂-binding domains represented by PH_{PLC- δ 1} suggest that the MA-PI(4,5)P₂ interaction can be viewed as an attractive therapeutic target upon further understanding of the mechanism behind it.

Conclusions

Multiple factors, including, but not limited to, N-myristoylation and the exposure/sequestration of the myristate moiety, electrostatic and structure-dependent interactions between MA basic residues and acidic phospholipid headgroups, hydrophobic interaction with phospholipid acyl chains, local domain structure of membrane bilayer, and competition between acidic lipids and RNA, determine the efficiency and specificity of membrane binding of retroviral Gag proteins. The details of molecular mechanisms by which these different factors regulate Gag membrane binding still remain to be elucidated. These include the sequence of myristate- and HBR-driven interactions, the exact roles played by acyl chains of PI(4,5)P₂ and other lipids, the impact of MA on phospholipid distribution in the bilayer, the identity of MA-bound RNA, and the fate of RNA upon MA binding to acidic lipids to list a few. Answers to these questions are likely to facilitate ongoing efforts to target MA using small chemicals (122) or RNA aptamers (109) and may lead to development of antiretrovirals that block Gag binding to membrane, the essential step in HIV-1 assembly.

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Highlights

- HIV-1 Gag binds membrane via MA N-terminal myristate and highly basic region (HBR)
- PI(4,5)P2 promotes Gag binding to the plasma membrane (PM) and virus production
- HBR binding to acidic phospholipids and RNA regulates Gag localization
- Both headgroups and acyl chains may modulate Gag membrane binding

Fig. 1.

A. Bipartite signals, the myristate moiety and the highly basic region (blue), within the matrix domain (MA) (grey) are indicated. The amino acid sequence of the MA highly basic region (HBR) of HIV-1_{NL4-3} is shown (basic residues in blue). **B.** HIV-1 Gag 'myristoyl-switch'. Gag exists either as myristate-sequestered (left) or myristate-exposed (right) forms. MA-PI(4,5)P₂ interaction or Gag multimerization induces exposure of the myristate moiety.

Fig. 2.

A. Structure of phosphatidylserine (PS) and PI(4,5)P₂. In the examples shown, saturated 16-carbon palmitoyl and unsaturated 18-carbon oleoyl chains are attached at the sn-1 and sn-2 positions, respectively. **B.** A model depicting the roles played by PS and PI(4,5)P₂ in supporting membrane binding. Binding of wild type (wt) Gag to membranes is dependent on the highly basic region (HBR) and the presence of PI(4,5)P₂ in the membrane. A Gag mutant in which lysines and arginines of HBR are switched (HBR/RK switch) retains the same overall charge but fails to bind membranes containing PI(4,5)P₂. Note that HBR/RKswitch Gag remains susceptible to RNA-mediated suppression of electrostatic interaction with PS. Amino acid sequences within the HBR of wt and HBR/RK switch Gag proteins are shown.