Atomic view of the HIV-1 matrix lattice; implications on virus assembly and envelope incorporation

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During the late phase of HIV type 1 (HIV-1) infection cycle, the virally encoded Gag polyproteins are targeted to the inner leaflet of the plasma membrane (PM) for assembly, formation of immature particles, and virus release. Gag binding to the PM is mediated by interactions of the N-terminally myristoylated matrix (myrMA) domain with phosphatidylinositol 4,5-bisphosphate. Formation of a myrMA lattice on the PM is an obligatory step for the assembly of immature HIV-1 particles and envelope (Env) incorporation. Atomic details of the myrMA lattice and how it mediates Env incorporation are lacking. Herein, we present the X-ray structure of myrMA at 2.15 Å. The myrMA lattice is arranged as a hexamer of trimers with a central hole, thought to accommodate the C-terminal tail of Env to promote incorporation into virions. The trimer–trimer interactions in the lattice are mediated by the N-terminal loop of one myrMA molecule and α-helices I–II, as well as the 310 helix of a myrMA molecule from an adjacent trimer. We provide evidence that substitution of MA residues Leu13 and Leu31, previously shown to have adverse effects on Env incorporation, induced a conformational change in myrMA, which may destabilize the trimer–trimer interactions within the lattice. We also show that PI(4,5)P₂ is capable of binding to alternating sites on MA, consistent with an MA–membrane binding mechanism during assembly of the immature particle and upon maturation. Altogether, these findings advance our understanding of a key mechanism in HIV-1 particle assembly.

Significance

The assembly of immature HIV-1 particles is initiated by targeting of the Gag polyproteins to the plasma membrane (PM). Gag binding to the PM is mediated by the N-terminally myristoylated matrix (myrMA) domain. Formation of a Gag lattice on the PM is obligatory for the assembly of immature HIV-1 and envelope (Env) incorporation. The structure of the myrMA lattice presented here provided insights on the molecular factors that stabilize the lattice and hence favor Env incorporation. Our data support a mechanism for Gag binding to the PM during the assembly of immature particles and upon maturation. These findings advance our understanding of a critical step in HIV-1 assembly.

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The authors declare no competing interest.

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(Env) incorporation into virus particles (26–29). Genetic and biochemical studies suggested that incorporation of the Env protein into virus particles is mediated by interactions between the myrMA domain of Gag and the cytoplasmic tail of Env (gp41CT) (30–34). How the gp41CT domain of Env interacts with the Gag lattice is still unknown. It is strongly suggested that myrMA trimerization is considered an obligatory step in HIV-1 Env incorporation (28), and that myrMA–gp41(CT) interactions and Env incorporation are mediated by formation of higher-order organization (hexamers of trimers) of myrMA on the membrane (25, 26, 28, 35).

Atomic details of myrMA arrangement in the lattice, how PI(4,5)P₂ mediates myrMA binding to the PM during assembly and maturation, and how single residues in the MA can control Env incorporation are still lacking. A recent cryoelectron tomography study revealed that the myrMA protein undergoes dramatic structural changes to allow the formation of distinct hexameric lattices in immature and mature particles (36). These studies indicated that while PI(4,5)P₂ was bound in the pocket in the mature form, it was absent in the immature form. These results suggested that myrMA also undergoes a structural transformation in the immature/mature transition, perhaps priming it for virus entry and uncoating. Based on these findings, a model was proposed in which HIV-1 not only achieves assembly of the CA core surrounding the RNA genome, but it also extends to repurpose the myrMA lattice for an entry or postentry function (36).

Here, we report the X-ray structure of the HIV-1 myrMA protein at 2.15 Å. In the crystal lattice, the myrMA protein is arranged as a hexamer of trimers with a threefold rotation axis at the central hole, which is thought to accommodate Env gp41CT to promote incorporation into virions. The trimer–trimer interactions in the lattice are mediated by the N-terminal residues, indicating that this motif plays a role not only in regulating the myr switch mechanism but also in stabilizing the myrMA lattice. We show that MA mutants defective of Env incorporation induced a conformational change in myrMA, which appear to disrupt formation of the lattice. We also provide evidence that support an alternating PI(4,5)P₂ binding mechanism during assembly and maturation. These findings advance our understanding of a key mechanism in HIV-1 particle assembly.

Results
Structure Determination of the HIV-1 myrMA Trimer. NMR studies have shown that the HIV-1 myrMA protein is predominantly globular and composed of five α-helical domains, a short 3₁₀ helix, and a β-hairpin (19, 21, 22). The C-terminal α-helix of the MA extends away from the globular region, progressively transitioning into a random coil, which serves as the linker between the MA and CA domains in the immature Gag polyprotein. Due to technical challenges, determination of the structure of the HIV-1 myrMA trimer has been elusive. Herein, to facilitate crystal screening conditions, we generated an MA construct lacking the unstructured amino acids 113 to 132 (myrMA₁₁₂). Crystallization conditions were obtained and optimized for myrMA₁₁₂, which allowed for determination of the high-resolution structure by X-ray crystallography at 2.15 Å. Atomic coordinates and structure factors have been deposited in the Protein Data Bank (PDB ID code 7TBP). Data collection and refinement statistics are shown in SI Appendix; Table S1.

The asymmetric unit of the crystal structure contained three independent copies of myrMA₁₁₂ (chains a, b, and c). Like the WT myrMA protein (21, 22), the myrMA₁₁₂ structure consists of five α-helices, a 3₁₀ helix, and a β-hairpin. Residues 109 to 112 (chain a), 110 to 112 (chain b), and 108 to 112 (chain c) lacked electron density consistent with a disordered conformation, as previously shown by the NMR structural studies of the WT myrMA protein (21, 22). Interestingly, the complete N terminus, including the myr group and linkage to the N-terminal glycine, were well-defined in the electron density map encompassing chain b (SI Appendix, Fig. S1). For chains a and c, the electron density for the myr group as well as the N-terminal 2–5 (chain a) and 2–8 (chain c) residues was not observed presumably due to disorder. Each chain (a, b, and c) forms an independent trimer through crystallographic symmetry (Fig. 1). The trimeric arrangement of the three chains is very similar to each other, and to that observed for myr(–)MA, with only slight orientation differences of monomers in the trimeric assembly (SI Appendix, Fig. S2) (19). In summary, we report a structural snapshot of the myrMA and trimeric assemblies at high-resolution, which allowed for observation of the attached myr group.

Myristoyl Swapping. The X-ray structure of myrMA₁₁₂ revealed that for chain b, in addition to trimeric associations, each myrMA within the trimer has a dimeric interaction with an adjacent copy of myrMA from another trimer. This interaction is symmetrical, with a twofold rotational symmetry, and shows the myr group of one subunit in the hydrophobic cavity of the subunit across the twofold axis (Fig. 2A), introducing a myristoyl swap. Although it is possible that “myristoyl swapping” between the neighboring asymmetric units is a consequence of crystal packing, a few important structural details have emerged. First, chain b presents the myr group in an exposed conformation as related to self-association observed for WT myrMA in solution (21, 22) (SI Appendix, Fig. S3).
Second, the myr group of myrMA\textsubscript{112} adopts almost an identical conformation to that observed for the self-associated and myr-sequestered WT myrMA (21, 22), and is inserted into a cavity formed by the same exact residues of the adjacent molecule (Fig. 2\textsuperscript{B}). These conformations reveal myrMA in myr-exposed conformation (no longer self-associated) and myr-sequestered conformation (to build a broad lattice). In the trimeric structure, the exposed myr groups are quite distant from each other (51 Å) (\textit{SI} Appendix, Fig. S3). Third, the structure of the myrMA\textsubscript{112} trimer is virtually identical to that observed for the myr(\textendash)MA trimer (\textit{SI} Appendix, Fig. S2) (19), demonstrating that although the myr group was found to promote MA self-association in solution (22, 37, 38), the trimer arrangement appears to be an intrinsic property of the protein.

\textbf{Lattice Formation: Hexamer of Trimers.} A continuous 2D lattice of myrMA is observed in the crystal, building from monomer to trimer and spatially arranged trimers to yield higher level hexameric arrangements. Individual spatial arrangements of trimers formed by chain c are observed in the lattice with a threefold rotation symmetry. Layered within the crystal lattice, three sets of trimers of chain b and three sets of trimers of chain c yielded a tightly packed hexamer of trimers (lattice b/c) with a threefold rotation axis (Fig. 3\textsuperscript{A} and \textit{SI} Appendix, Fig. S4). In this structure, all six sets of trimers are aligned in an equivalent top-to-bottom orientation and the myr groups of trimers b are present in the myr-exposed conformation. The structure of the myrMA\textsubscript{112} lattice (b/c) revealed several unique features. First, the trimer\textendash trimer interactions are mediated by the N-terminal loop of one myrMA molecule and \(\alpha\)-helices I–II, as well as the \(3_{10}\) helix of a molecule from an adjacent trimer (Fig. 3\textsuperscript{B}). Several N-terminal residues—including Ala5, Leu8, and Ser9—of one molecule are in close proximity and interact with residues Leu31, Ile34, Val35, Ser38, and Gly49 of an adjacent molecule (Fig. 3\textsuperscript{C}). Second, trimer\textendash trimer packing places the myr groups in juxtaposition (Fig. 3\textsuperscript{B}). Third, MA residues Leu13, Glu17, Leu31, and Val35 implicated in Env incorporation are not exposed toward

**Fig. 2.** Myristoyl swap. (A) Cartoon representation of myrMA\textsubscript{112} chain b showing the dimeric interface formed by two trimers through a myr swapping mode (cyan and magenta). The two trimers are aligned in an antiparallel mode. (B) Cartoon representation showing two myrMA\textsubscript{112} molecules (cyan and magenta) in which the myr group (red) is sequestered in a preexisting hydrophobic cavity of an adjacent molecule. For comparison, the NMR structure of the monomeric myrMA protein (gray; PDB ID code 2H3I) is shown with the myr group buried in an identical hydrophobic cavity formed by the side chains of residues Trp16, Ile34, Leu51, Leu85, and Val88.

**Fig. 3.** Hexamer of trimer lattice formation. (A) Hexamer of trimer is formed by trimers b and c. In this lattice, the myr group (red) is extruded and projecting out-of-plane. All six trimers are in a parallel mode with a threefold rotation axis. Residues implicated in Env incorporation (Glu17, Leu13, Leu31, and Val35; magenta) are located in the trimer-trimer interface. Glu99, which was also shown to impact Env incorporation, is located in the interior of the central hole of the hexamer lattice. Other residues that have been shown to impact Env incorporation by stabilizing the myrMA trimer are shown as yellow spheres. (B) Trimer-trimer interactions are mediated by the N-terminal residues of one molecule (orange) and \(\alpha\)-helices I–II and \(3_{10}\) helix of an adjacent molecule (green). The myr group in chain c, which is not observed due to its dynamic nature, is drawn as a dotted line. (C) A close-up view of the residues involved in the trimer-trimer interface. (D) Electrostatic surface potential maps of the myrMA\textsubscript{112} lattice. The blue (+5 kT/e) (e, electron charge; k, Boltzmann constant; T, temperature) and red (−5 kT/e) colors indicate positively and negatively charged electric potentials, respectively.
Impact of Env-Defective MA Mutations on the Lattice Structure. 
As discussed in the Introduction, the hexamer of trimers lattice of myrMA is used as a model to explain the mechanism of Env incorporation into virus particles (26–29). Point mutations of MA residues Leu13, Glu17, Leu31, Val35, and Glu99 were previously found to impair Env incorporation without affecting virus particle formation (30–33). However, it is not known whether these residues interact directly with gp41CT by accommodation in the central hole or if they play an indirect role in MA-mediated Env incorporation. The structure of the myrMA_{112} lattice revealed that except for Glu99, these residues are not projecting toward the central hole but rather reside in the MA intra- and intertrimer interfaces (Fig. 3A). Herein, we employed an NMR chemical shift perturbation (CSP) assay to assess the effect of the L13E and L31E mutations on the structure of myrMA. Typically, only a few signals corresponding to amino acids in the vicinity of the mutation site exhibit major CSPs in the $^{1}H$–$^{15}N$ HSQC spectrum. Our NMR data show that, compared with the spectrum of the WT myrMA protein, numerous residues exhibited substantial CSPs for both the L13E and L31E mutants (Fig. 4A). NMR signals corresponding to residues 2 to 40 were significantly shifted or severely broadened, indicating that L13E and L31E mutations induced structural/conformational changes in the myrMA protein. Mapping the CSPs on the structure of the myrMA_{112} lattice revealed that the changes are localized to the N-terminal region and $\alpha$-helices I–II (Fig. 4B). These are the same motifs involved in the trimer–trimer interactions and appear to stabilize the lattice (Fig. 3). Surprisingly, the 2D $^{1}H$–$^{15}N$ HSQC spectra of myrMA L13E and L31E mutants are nearly identical (SI Appendix, Fig. S5), indicating that substitution of either of these two leucine residues causes a similar conformational/structural change in the protein.

Next, we examined whether L13E and L31E mutations had any effect on the position of the myr group. Previous NMR studies of the WT myrMA protein have shown that a subset of $^{1}H$ and $^{15}N$ resonances for amino acid residues 3–18, Val35, Trp36, Arg39, Gly49, Glu52, His89, and Gln90 are sensitive to the position of the myr group and shift progressively toward the corresponding frequencies observed for myr(–)MA upon increasing protein concentration, indicating exposure of the myr group and a concomitant shift in the monomer–trimer equilibrium toward the trimeric species (21). Similar CSPs were observed

Fig. 4. Structural analysis of myrMA mutants that impair Env incorporation. (A) Overlay of 2D $^{1}H$–$^{15}N$ HSQC spectra for WT (black) and mutant L13E (Top) and L31E (Bottom) myrMA proteins collected at 120 $\mu$M (red). Substantial CSPs or severe broadening of signals corresponding to residues 2 to 40 are observed in the NMR spectra of myrMA L13E and L31E mutants when compared with the spectrum of the WT myrMA protein. (B) CSPs induced by the L13E and L31E mutants are mapped to the N-terminal loop and $\alpha$-helices I–II of myrMA (slate). These CSPs are suggestive of a conformational change in the packing of $\alpha$-helices, which also altered the position of the myr group (red) and ultimately lattice formation.
upon changing the protonation state of His89 by changing the solution pH to 5, which stabilized the salt bridge formed between the side chains of His89 and Glu12, leading to exposure of the myr group and a shift in the equilibrium from monomer to trimer (37). As shown in the 2D 1H–15N HSQC data for the L13E and L31E mutants (Fig. 4 and SI Appendix, Fig. S6), signals corresponding to residues 3–18, Val35, Trp36, Arg39, Gly49, Glu52, His89, and Gln90 shift toward the corresponding frequencies observed for myr(−)MA, suggesting that L13E and L31E mutations caused a perturbation in the position of the myr group and a shift in equilibrium to the myr-exposed state.

A possible explanation of the CSPs observed for signals of residues 2 to 40 is that these changes are caused by changes in the conformation and position of the myr group only. To test this hypothesis, we collected 2D 1H–15N HSQC data for the myr(−)MA L13E and L31E proteins (SI Appendix, Fig. S7). Similar to the corresponding myrMA proteins, numerous signals corresponding to residues in the N-terminal loop and α-helices I–II exhibited significant CSPs or severe broadening, indicating a conformational change in these motifs (SI Appendix, Fig. S7). Taken together, our NMR data indicated that substitution of Leu13 or Leu31 to glutamate induced a conformational change in the N-terminal loop and α-helices I–II, which likely destabilized the trimer–trimer interface and therefore the hexamer lattice.

Alternate Binding of PI(4,5)P₂ to myrMA. Previous NMR-based structural studies revealed that binding of tr-PI(4,5)P₂ to HIV-1 myrMA induced a conformational change that triggered myristate exposure, and that tr-PI(4,5)P₂ adopted an “extended lipid” conformation in which the inositol head group and 2′-acyl acid chain bind to a hydrophobic cleft, and the 1′-acyl and exposed myr group bracket the HBR implicated in membrane binding (22). NMR-based liposome binding studies with native lipids, however, suggested that the acyl chains may not be involved in myrMA–PI(4,5)P₂ interaction, and that interaction is mediated predominantly by electrostatic interactions between the HBR and the headgroup of PI(4,5)P₂ (39). Yet, recent cryotomography data suggested that PI(4,5)P₂ may bind to myrMA differently in the immature and mature HIV-1 particles (36). The lack of electron density corresponding to PI(4,5)P₂ in the immature particle reconstruction suggested that interactions may be limited to the headgroup of PI(4,5)P₂ and the HBR of myrMA (36). However, electron density attributed to the PI(4,5)P₂ acyl chain inserted in a cleft in myrMA was observed in the mature particle (36), consistent with the NMR structural studies (22). How PI(4,5)P₂ binds to MA in the immature particle is not clear.

Here, we assessed how myrMA may utilize an alternating PI(4,5)P₂ binding mechanism during assembly and maturation. We obtained 2D 1H–15N HSQC NMR data on the WT myrMA protein as titrated with inositol 1,4,5-trisphosphate (IP₃), the headgroup of PI(4,5)P₂. As shown in SI Appendix, Fig. S8, a subset of signals corresponding to residues Lys27, Gln28, Lys30, and Lys32 (group 1) exhibited significant CSPs upon addition of IP₃. Interestingly, these residues are different from those involved in binding of tr-PI(4,5)P₂ (22). Mapping the CSPs on the structure of the myrMA₁₋₁₂ protein revealed that these interacting residues are located in the HBR and are not part of the hydrophobic cleft (Fig. 5). Of note, the 1H–15N resonances for Arg22, Trp36, Arg76, Thr81, and Ser77 were unaffected by IP₃ titration; the latter four residues were previously shown to interact with the 2′-acyl chain of tr-PI(4,5)P₂ (22). These results indicated that the headgroup of PI(4,5)P₂ binds to myrMA via a different site (Fig. 5 A–D). Additionally, a second group of signals corresponding to residues 2–18, Arg39, Leu75, and His89 (group 2) that are well removed from group 1 residues in the myrMA structure also exhibited IP₃-dependent CSPs and shifted progressively toward values observed in the myr(−)MA spectrum (SI Appendix, Fig. S8). Similar changes were observed previously upon binding of tr-PI(4,5)P₂ to myrMA, indicative of a shift in the myr switch equilibrium from a predominantly myr-sequestered to myr-exposed state. These results indicated that binding of the headgroup of PI(4,5)P₂ is sufficient for triggering myr exposure. Altogether, these findings indicate that PI(4,5)P₂ is capable of binding to myrMA via an alternate mechanism during assembly and maturation.

Discussion

Structural and biophysical studies of retroviral MA proteins and their interactions with phospholipids and membrane mimetics (biscelles, micelles, liposomes, and lipid nanodiscs) provided invaluable insights on key molecular determinants of MA-mediated Gag assembly on the PM (2, 22, 39–52). In this
report, we provide high-resolution structural details of the myrMA trimer and hexamer lattice, the impact of myrMA mutations defective of Env incorporation on the myrMA structure and lattice formation, and provide evidence to support an alternating PI(4,5)P₂ binding mechanism during assembly and maturation. We provide evidence that myrMA is capable of forming a well-ordered hexamer of trimers lattice even in the absence of PI(4,5)P₂ or membrane. Previous cryoelectron tomography data revealed that the HIV-1 Gag lattice manifests as a cage of interconnected hexamers that cover most of the inner surface of the viral envelope (53, 54). Furthermore, cryo-electron diffraction studies obtained from 2D crystals of myrMA grown on a lipid monolayer containing PI(4,5)P₂ suggested that myrMA organizes as hexamers of trimers (23). The hexamer of trimers lattice is increasingly used as a model to explain the mechanism of Env incorporation into virus particles (26–29). These findings led us to hypothesize that forces stabilizing the myrMA lattice are intrinsic to the protein and that binding to lipids and membranes may play a role in altering the orientation or conformation of the lattice.

Recent cryotomography data revealed distinct trimer–trimer packing in the immature and mature HIV-1 particles (Fig. 6) (36). Subtomogram averaging analysis of the immature particle revealed a hexamer of myrMA trimer lattice with large holes at the sixfold axis (Fig. 7A). Similar analysis of the mature particle revealed large patches of a hexagonal lattice of myrMA trimers also with a sixfold rotation axis, but with a significantly smaller central hole (Fig. 7B). Because of the low resolution of the cryotomography data, atomic details and the type of residues involved in the trimer–trimer interface were not clearly identified. In the immature particle, the reconstruction model suggested that trimer–trimer contacts are mediated by the N-terminal residues, α-helix I, and the 3₁₀ helix (Fig. 6) (36). In the mature particle, the HBR loop faces acidic residues in the N terminus of α-helix IV and the 3₁₀ helix of an adjacent myrMA monomer, to form a dimeric interface that links trimers together (Fig. 6). The PI(4,5)P₂ binding cleft adjacent to the HBR is positioned at the center of the dimer interface (Fig. 7A) (36). Although it offers a different arrangement (threefold vs. sixfold symmetry), the X-ray structure has several features similar to that of the immature lattice in which the N-terminal residues of myrMA are directly involved in trimer–trimer contact, placing the myr groups of MA subunits in juxtaposition (Figs. 6 and 7). These findings indicate that the N-terminal loop (residues 2 to 10) of myrMA is a key motif not only in regulating the myr switch mechanism but also in stabilizing the MA lattice.

Comparison of our X-ray structure with the MA lattice models constructed from the low-resolution reconstruction data (7 to 22 Å) (23, 36) revealed a few intriguing differences. While models of the immature and mature particles (Fig. 7A), as well as that of myrMA assembled on a membrane monolayer containing PI(4,5)P₂ (Fig. 7C), adopt a hexameric arrangement with C₆ rotation axis, they differ in the arrangement of the trimeric units, trimer–trimer contact points, and size of the central hole (Fig. 7). Our X-ray structure of the myrMA lattice offers yet a different arrangement with a threefold rotation axis but relatively similar trimer–trimer contact points to the model of the immature particle (Figs. 6 and 7). These findings lead us to propose a "structural plasticity” feature of the myrMA lattice in which the trimer units are capable of adopting different orientations, while maintaining a relatively unchanged myrMA structure.

Despite the accumulating evidence for an interplay between Gag assembly on the PM and the incorporation of Env into virus particles, this mechanism is not fully understood. There is
mounting evidence that Env incorporation may require formation of a Gag lattice (hexamers of trimers) on the membrane (25, 26, 28, 35). The hypothesis of an interplay between myrMA and gp41CT is supported by the finding that point mutations in the MA—such as L13E, E17K, L31E, V35E, and E99V—had adverse effects on Env incorporation without affecting virus particle formation (30–34). Likewise, nonconservative mutations in the trimer interface of the MA (A45E, T70R, and L75G) (Fig. 3) were found to impair Env incorporation and infectivity, indicating that MA trimerization is an obligatory step for Env incorporation (28). The trimer interface was characterized by hydrogen–deuterium exchange mass spectrometry and structural studies of a compensatory mutant that rescues Env incorporation (51, 55). Nanoscale single-particle tracking of Env on the PM has demonstrated that Env immobilization at sites of Gag assembly is regulated by gp41CT and specific MA residues such as Leu13, and that Env is restricted in subviral regions within the Gag lattice (56). Collectively, these studies led to a model for Env incorporation in which the gp41CT domain is accommodated in the central aperture formed by the hexamer of trimers (27, 28). Our structural data show that Leu13, Glu17, Leu31, and Val35 reside in the myrMA intra- and intertrimer interfaces, whereas Glu99 is projecting toward the central hole (Fig. 3).

Furthermore, we provide evidence that substitution of Leu13 or Leu31 to glutamate induced a conformational change in the N-terminal loop and α-helices I–II, leading to perturbation of the myr group position, destabilization of the trimer–trimer interface, and therefore the lattice. Most importantly, NMR data show that both mutations induced a similar structural change in the myrMA protein, suggesting that the defect in Env incorporation caused by these mutations is in consequence of a structural change in the MA and destabilization of the lattice. It has yet to be determined whether these mutations have any effect on MA–membrane binding. Previous studies have shown that substitution of Val35 to Ile compensated for the effects of L13E mutation and rescued Env incorporation defect (29, 31). As shown in Fig. 3C, Val35 is located in a relatively hydrophobic nest (Ala5, Leu8, Leu31), which stabilizes the trimer–trimer interface and consequently the lattice. We hypothesize that although this mutation is conservative (Val to Ile), the more branched hydrophobic side chain of Ile may stabilize the lattice by making intermolecular contacts with hydrophobic residues on the adjacent molecule.

We also provide evidence that support a molecular switch mechanism for MA–membrane binding during virus assembly and maturation. The HBR in MA (Fig. 5) is highly conserved in almost all retroviral MA proteins and serves as the binding site for acidic phospholipids in the inner leaflet of the PM (1, 57). A few models of the HIV-1 myrMA trimer bound to membranes have been proposed based on structural, biochemical, biophysical, and functional studies (2, 22, 39). Our data indicate that, in addition to the previously described mechanism of PI(4,5)P₂ binding to myrMA (22), the protein is capable of binding to the headgroup of PI(4,5)P₂ via a second site in the HBR, also promoting myr exposure. These findings support an alternating PI(4,5)P₂ binding mechanism in the immature and mature particles, as described recently (Fig. 5) (36). Interestingly the absence of electron density for PI(4,5)P₂ in the cryotomography reconstruction of the immature myrMA lattice suggested that interactions of myrMA with PI(4,5)P₂ are mediated by interactions of the HBR and the headgroup of PI(4,5)P₂ (36). We have shown that the headgroup of PI(4,5)P₂ can interact directly with Lys27, Lys30, and Lys32, which are not directly involved in binding of n-PI(4,5)P₂ (Fig. 5) (22) but are considered key residues for Gag binding to the PM, virus assembly, and particle release (39, 58, 59). On the other hand, the observation of electron density for an extended acyl chain attributed to the PI(4,5)P₂ ligand in the subtomogram reconstruction of the mature particle led to the suggestion that maturation of Gag results in the partial removal of up to ∼2,500 of the ∼150,000 lipids in the inner leaflet of the viral membrane (36). Interestingly, in a recent X-ray structure of the HIV-1 myr(–)MA protein in complex with inositol hexakisphosphate (IP₆), the asymmetric unit contained three types of structures in which the IP₆ ligand interacted with various residues in the HBR (60). One of the three structures is almost identical to that described for myrMA bound to IP₃ (Fig. 5 A and C), consistent with the hypothesis that MA contains alternate PI(4,5)P₂ binding sites and that the conformational switch of myrMA during maturation may also involve lipid/membrane reorganization.

In conclusion, we provide an atomic view for the HIV-1 myrMA lattice, which reveals invaluable structural insights on the arrangement of the myrMA subunits, trimers, trimers–trimer interfaces, myr swapping, impact of MA mutations defective of Env incorporation on the structure of myrMA, and consequently lattice formation. Our data also support an alternating MA–PI(4,5)P₂ binding mechanism during virus assembly and maturation. These findings fill a major gap in our understanding of the mechanisms of Gag assembly on the PM and Env incorporation into virus particles. In a very recent study by Modena and the NIH, it was shown that messenger RNA vaccine coexpressing membrane-anchored HIV-1 Env and simian immunodeficiency virus Gag proteins to generate virus-like particles induced antibodies capable of broad neutralization and reduces the risk of infection in rhesus macaques (61). The findings described here may facilitate the development of new therapeutic agents that inhibit Gag assembly, Env incorporation, and ultimately virus production.

Materials and Methods

Plasmid Construction. A plasmid encoding for HIV-1 MA (132 amino acid residues; pNL4-3 strain) and yeast N-terminal myristoyltransferase was provided by Michael Summers, Howard Hughes Medical Institute, University of Maryland Baltimore County, Baltimore, MD. Using a QuickChange Lightning site-directed mutagenesis kit (Agilent Technologies), the MA gene was modified by inserting a stop codon after amino acid residue 112 codon, yielding an MA gene encoding for untagged MA112. (Note: The N-terminal Met, which is absent in the myrMA protein, is designated as residue 1.) The L13E and L31E MA mutant constructs were generated using a QuickChange Lightning site-directed mutagenesis kit. Forward and reverse primers (Integrated DNA Technologies) extended 15 base pairs on either side of the mutation codon. DNA sequences were verified by plasmid sequencing at the Hefflin Genomics Core at the University of Alabama at Birmingham.

Protein Expression and Purification. The myrMA112 protein was overexpressed in Escherichia coli BL21CodonPlus (DE3) RIL cells (Agilent Technologies). Cells were grown at 37 °C in Luria–Bertani broth medium containing 100 mg/L ampicillin. Media were supplemented with 60 μM myristic acid (Sigma Aldrich) when A₆₀₀ reached ∼0.2. At A₆₀₀ 0.7 to 0.8, cells were induced with 0.5 mM isopropyl β-D-thiogalactopyranoside (Gold Biotechnology). Cells were then grown at 37 °C for 4 h, spun down, and stored at −80 °C. The cell pellet was resuspended in lysis buffer containing 20 mM phosphates (pH 8.0), 1 M ammonium sulfate, 2 mM DTT, and 1× inhibitor mixture (EMD Millipore). Cells were then sonicated, and lysate was separated from pellet by spinning down at 35,000×g for 30 min at 4 °C. The supernatant was loaded into a HiTrap Butyl FF column (Cytiva) equilibrated in lysis buffer (excluding protease inhibitors). Resin was then washed with lysis buffer (excluding protease inhibitors). The myrMA112 protein was eluted via a gradient using a buffer containing 20 mM sodium phosphates (pH 8) and 2 mM DTT. Protein purity was verified by SDS/PAGE. Fractions were pooled and buffer exchanged to 50 mM sodium phosphate (pH 7.5) and 0.5 M NaCl. The myrMA112 protein stock was equilibrated in lysis buffer (excluding protease inhibitors). The myrMA112 protein stock was equilibrated in lysis buffer (excluding protease inhibitors).
containing the protein were pooled and dialyzed in a buffer containing 20 mM phosphates (pH 8.0), 1 M ammonium sulfate, and 2 mM DTT. Purification steps described above were repeated to remove minor contaminants. The protein was then run on a size-exclusion column (Hiload 16/60 Superdex 75, Cytiva) in a buffer containing 20 mM Hepes (pH 8) and 100 mM NaCl. 15N-labeled WT myrMA, WT myr-MJM, myrMA L13E, myrMA L31E, and myr-MJM L31E samples were prepared as described previously (21, 22, 40).

X-Ray Crystallography and Structure Determination. The HIV-1 myrMA(12) protein (20 mg/mL) in 20 mM Hepes (pH 8) and 100 mM NaCl was subjected to crystallization trials. Diffraction quality crystals were obtained using hanging-drop vapor diffusion in a solution of 40% PEG 400 (Hampton Research) and 0.1 M Bis-Tris-propane buffer (pH 4.7). Crystals were cryocooled in the same conditions. X-ray diffraction data were collected at the Advanced Photon Source, Southeast Regional Collaborative Access Team (SER-CAT) beamline 22-ID. Raw intensity data were processed with the HKL2000 software package (62). The initial electron density map was generated via molecular replacement with PHASER (63) using the previously solved structure of the WT myr-MJM protein (PDB ID code 1HIW) (19). The structure was then iteratively refined with PHENIX (64) and with manual manipulation in Coot (65). Visualization of structures was performed using PYMOL (66).

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NMR Spectroscopy. NMR data were collected at 35 °C on a Bruker Avance III (600 MHz 1H) equipped with a cryogenic triple-resonance probe, processed with NMRpipe (67) and analyzed with NMRView (68). The 15N-labeled protein samples were prepared at 120 μM in 50 mM sodium phosphates (pH 5.5), 100 mM NaCl, 1 mM EDTA, and 2 mM TCEP.

Lipid NMR Titrations. 1H–13N HSQC NMR titrations with IP3 (Echelon Biosciences) were conducted with a 100-μM sample of 15N-labeled myrMA in 50 mM sodium phosphates (pH 5.5) and 2 mM TCEP. A stock solution of IP3 was prepared in deionized water at 20 mM.

Data Availability. The atomic coordinates of myrMA(12) have been deposited in the Protein Data Bank, PDB ID code 7TBp.

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