Maturation of the matrix and viral membrane of HIV-1

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Gag, the primary structural protein of HIV-1, is recruited to the plasma membrane for virus assembly by its matrix (MA) domain. Gag is subsequently cleaved into its component domains, causing structural rearrangement to repurpose the virion for cell entry. We determined the structure and arrangement of MA within immature and mature HIV-1 through cryo–electron tomography. We found that MA rearranges between two different hexameric lattices upon maturation. In mature HIV-1, a lipid extends into the membrane to bind with a pocket in MA. Our data suggest that proteolytic maturation of HIV-1 not only assembles the viral capsid surrounding the genome but also repurposes the membrane-bound MA lattice for an entry or postentry function and results in the partial removal of up to 2500 lipids from the viral membrane.

Assembly and budding of HIV-1 is initiated at the plasma membrane (PM) and driven primarily by a 55-kDa viral polyprotein named Gag. Gag consists of an N-terminal matrix (MA) domain, which is responsible for recruitment to the PM; the capsid (CA) domain, which induces Gag self-assembly through protein-protein interactions; the nucleocapsid (NC) domain, which recruits the viral RNA genome to the assembly site; and some small peptide domains (1, 2). Protein-protein, protein-lipid, and protein-RNA interactions lead to clustering of Gag at the assembly site, membrane bending, and subsequent release of the membrane-enveloped, immature HIV-1 particle. Concomitant with or shortly after release, the viral protease (PR) cleaves Gag at multiple positions, leading to a substantial structural rearrangement to repurpose the virus particle for entry into a target cell (3, 4). Maturation results in condensation of a ribonucleoprotein complex (RNP) from NC and RNA, which is then surrounded by a cone-shaped capsid made of CA, whereas mature MA is thought to remain associated with the viral membrane. Rearrangement of the HIV-1 envelope (Env) glycoproteins on the particle surface is presumed to render the virion fusogenic (2, 3, 5, 6).

The structure of the heterologously expressed 17-kDa PR has been determined, revealing a small, folded domain composed of five α helices and one 3_10 helix between helices 2 and 3 (7, 8). MA crystallizes as a trimer (8) and multimerizes on artificial membrane monolayers into a hexameric lattice of trimers with holes at the sixfold symmetric positions in the lattice (9). It has been suggested that these holes provide binding sites for the C-terminal tail of HIV-1 Env and promote Env incorporation into assembling virions (10, 11).

Membrane recruitment of Gag by MA is mediated by an N-terminal myristate moiety as well as a highly basic region (HBR, residues 17 to 31) (12, 13). The N-terminal myristate is thought to be in equilibrium between sequestration in a pocket within the MA domain and an exposed conformation (14). This transition is known as the “myristoyl switch.” Myristate exposure can be promoted by the trimerization of MA (14). Additionally, PI(4,5)P_2 (phosphatidylinositol 4,5-bisphosphate) in the PM is required for correct targeting of Gag (15, 16) and for stably retaining Gag at the PM (17). Early NMR (nuclear magnetic resonance) studies suggested that insertion of the 2’ acyl chain of PI(4,5)P_2 in an extended lipid conformation into a pocket on the side of MA may promote exposure of the myristate and its insertion into the lipid bilayer (18). However, this mode of PI(4,5)P_2 binding, in which the 2’ acyl chain would have to be pulled out of the bilayer, is no longer widely considered relevant for PM binding. Instead, MA-membrane interactions are believed to involve the PI(4,5)P_2 headgroup and the membrane-facing HBR (15, 17, 19, 20). MA interacts with nucleic acids in the cytosol, particularly tRNAs, through the HBR (21–23). These observations support a model in which an exchange of nucleic acid for the PI(4,5)P_2 headgroup promotes myristate exposure and stabilization of PM binding during virus assembly (21, 23, 24).

The known roles of MA are performed during virus assembly, and no function for MA during entry or postentry stages is currently established. Models for how MA functions during assembly are limited by a current lack of structural information on MA within virions. It is unclear what conformation and arrangement MA adopts in situ, and whether MA undergoes any structural changes in the process of virus maturation. In this study, we set out to determine the structure and arrangement of MA within immature and mature virus particles.

Purified mature particles were generated by transfection of a noninfectious HIV-1 proviral derivative that expresses all viral proteins except the virulence factor Nef (pcHIV plasmid that generates cHIV particles) (25). Immature cHIV particles were generated by transfection of pcHIV carrying an active site mutation in PR (pcHIV PR-). Particles were imaged without chemical fixation by using cryo–electron tomography (cryo-ET). Visual inspection of tomograms revealed the expected features: a striated CA layer in immature virus particles and conical CA cores in mature virus particles (Fig. 1, A and B). In some regions underlying the lipid envelope of mature virions, we observed regular lattice-like features (Fig. 1C).
We subjected the region underlying the viral membrane in the immature cHIV particles to reference-free subtomogram averaging. This analysis revealed a hexameric lattice of MA trimers with hexamer-hexamer spacing of 9.8 nm, as well as large holes at the sixfold axes (Fig. 2A), reminiscent of the lattices observed in membrane-associated purified MA in vitro (9). To improve the spatial resolution of the immature MA structure, we analyzed a cryo-ET dataset of fixed, immature, complete HIV-1NL4-3 particles (26). From this dataset, we obtained the same MA lattice structure at 7.2 Å resolution (Fig. 2, B to F, and fig. S1). The poorly ordered and sparse MA lattice covered only small patches of the inner membrane leaflet, whereas in other areas MA did not appear to form a lattice, suggesting heterogeneity in MA packing (Fig. 2G and fig. S2). Despite this heterogeneity, we could clearly resolve α helices (Fig. 2, C to F). The MA trimer structure, determined by crystallography [PDBID: 1HlW (9)], could be fitted into the density as a rigid body.

The MA domain of Gag is therefore present as a trimer within immature HIV-1 particles (Fig. 2D and fig. S3A).

MA helices 1 and 2 lie approximately parallel to the membrane and form a surface rich in charged and hydrophobic residues that attach MA to the inner membrane leaflet (Fig. 2, C and F). MA trimers are packed together to form a lattice by a dimeric interface where the N-terminal residues and the N terminus of helix 1 interact with themselves and the 310 helix (Fig. 2, D and E). We did not observe any density corresponding to lipids bound in the described PI(4,5)P₂ binding site on the side of MA, next to the HBR (18) (Fig. 2F). The absence of density is consistent with current models in which interactions between the headgroup of PI(4,5)P₂ and the membrane-proximal face of MA function as the major cellular determinant of efficient MA-PM targeting (17, 19). The N-terminal region of MA (residues 1 to 11), which changes conformation during the myristoyl switch (14, 27), is directly involved in trimer-trimer interactions (fig. S3B). At lower isosurface thresholds, a connection to the membrane appears in the vicinity of the N terminus of helix 1 (Fig. 2E). No notable density was observed in the myristoyl pocket (fig. S3C). The conformational equilibrium therefore shifts toward the exposed, membrane-inserted conformation in the immature virion, consistent with exposure of the myristate moiety upon MA oligomerization or membrane recruitment.

The C-terminal helix 5 of MA extends toward the center of the virus particle and is separated from the underlying CA layer by a disordered stretch containing the MA-CA cleavage site (Fig. 2C). Therefore, CA is not resolved when MA is aligned, and MA is not resolved when CA is aligned (fig. S4). The position of MA is thus constrained but not fixed relative to CA.

A number of studies have implied that holes in a hypothetical MA lattice may present a binding site for the cytoplasmic tail of Env.

Fig. 2. Immature HIV-1 matrix structure. (A and B) Slices through reconstructions of the immature HIV-1 MA lattice in cHIV PR- (A) and HIV-1NL4-3 PR- (B). The region within the yellow box in (B) is shown at a higher magnification in (D). Density is black. (C and D) Iso surface views of a cryo-ET reconstruction (gray) of an immature HIV-1NL4-3 PR- MA lattice cut perpendicular to the membrane (C) or viewed from the top toward the virus center (D). In (C), the two layers of density corresponding to the lipid headgroup layers are indicated by brackets. The structure of monomeric MA previously determined by NMR [PDBID: 2H3Q (18), colored blue to red from the N- to C-terminus, respectively], was fitted as a rigid body into the density. Helices 1, 2, and 5 are marked. (E) Same as in (C), enlarged and cut to reveal density corresponding to the N-terminal residues (black arrowhead). (F) Same as in (E) but rotated. The red arrowhead indicates the unoccupied PI(4,5)P₂ binding pocket. (G) Lattice map derived from subtomogram averaging for the immature HIV-1NL4-3 PR- MA lattice. Positions and orientations of MA trimers are illustrated by triangles colored on a scale from red (lower cross-correlation to average structure) to green (higher cross-correlation to average structure). The positions of the CA hexamers are indicated by cyan hexagons to illustrate the relationship between the MA and CA layers. (H) Immature MA lattice (gray ribbons) and residues, the mutations of which have been reported to modulate Env incorporation (colored spheres). Mutations at residues L12, L30, and L74 (red), E16 and E98 (blue), and T69 (purple) impair Env incorporation (12, 28, 30–33). Mutations in V34, F43, Q62, and S66 (green) can rescue Env incorporation defects (12, 28, 31, 43), except for those caused by mutations at T69 (purple) (28). E16 and E98 (blue) face the hole in the MA lattice in the immature virion. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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and mutations affecting MA trimerization have been shown to affect Env incorporation (9, 10, 28). We found that MA does form a lattice containing holes, although we did not observe density for the Env tail in the holes, consistent with the low Env copy number per particle (29). A number of mutations in MA have been reported to result in defects in Env incorporation (30–33) (Fig. 2H). These mutations are thus likely to modulate MA trimerization or lattice formation, and/or the myristoyl switch, but would not be presumed to directly control Env cytoplasmic tail binding within the holes. This is consistent with the model in which correct MA oligomerization is required for Env incorporation (11, 34).

We next subjected the region underlying the membrane in the mature cHIV particles to reference-free subtomogram averaging. This analysis revealed large patches of a hexagonal lattice of MA trimers with hexamer-hexamer spacing of ~8.8 nm. These dimensions are similar to the 9-nm repeating MA lattice observed in anomalous, multicolored, membranous particles (35). We resolved the structure of the lattice to a resolution of 9.5 Å (Fig. 3A). Like the immature lattice, the mature lattice is formed from MA trimers arranged in a hexagonal lattice, though it has a higher degree of regularity than the immature MA lattice (fig. S2). The arrangement of MA within the immature and mature MA lattices is, however, notably different (compare Figs. 2 and 3), as the MA lattice undergoes structural maturation to form a new, different hexagonal lattice in the mature virion.

We had previously determined the structure of the CA layer within cHIV derivatives in which either the PR cleavage site between MA and CA was blocked by mutation (HIV-1 MA-CA) or in which the cleavage site between CA and SP1 was blocked in addition (HIV-1 MA-SP1) (36–38). The CA domains and the CA lattices remain structurally immature in MA-CA and MA-SP1 particles. Upon analyzing the MA layer in these datasets, we found that the MA lattice in HIV-1, MA-CA, and MA-SP1 corresponds to that of mature particles, despite the absence of cleavage between MA and CA (Fig. 3B and fig. S5). Cleavages downstream of SP1 are therefore sufficient for MA lattice maturation, which can occur without CA lattice maturation. It is unlikely that proteolytic cleavage between CA and NC could directly trigger MA maturation through a structural signal passed through CA without inducing any detectable structural changes in CA. We therefore suggest that maturation of MA must be triggered in trans by another effector in the viroplasm or viral membrane. Further experiments will be required to elucidate the mechanism of MA maturation. Immature virions are fusion competent (3), but cleavages downstream of CA are sufficient to overcome this defect (39). These observations suggest that structural maturation of MA, rather than CA, correlates with HIV-1 becoming fusogenic, potentially by allowing Env to redistribute on the virus surface (6).

We obtained a higher-resolution 7.0-Å structure of the mature MA lattice from MA-SP1 particles. As for the immature MA lattice, the crystallographic trimer could be fitted into the density as a rigid body (Fig. 3, B to E, and fig. S1). Comparison of the immature and mature MA structures revealed important differences (Fig. 4). In the immature lattice, the HBB faces the holes at the sixfold axes, which are therefore surrounded by a basic surface (Fig. 4B). By contrast, in the mature virus MA presents a largely neutral surface toward the holes (Fig. 4B). This change could influence structural changes in the cytoplasmic tails of Env trimers that may alter their distribution and fusogenicity. In the mature virus, basic residues in the HBB loop face acidic residues in the N terminus of helix 4 (E72) and the 3i10 helix (E51) of the adjacent MA monomer, to form a dimeric interface that links trimers together (Fig. 3D, Fig. 4A, and fig. S3B). The PI(4,5)P2 binding pocket on the side of MA, adjacent to the HBB (18), is positioned at the center of the dimer interface. Unlike the immature virus, wherein the PI(4,5)P2 binding pocket is empty, it contains a density in the mature virus. Our structure does not resolve the headgroup or acyl chain of the bound lipid, but the density is consistent with the previously described extended-lipid conformation of bound PI(4,5)P2 in which the 2’ acyl chain is removed from the bilayer, whereas the 1’ acyl chain extends upward toward the bilayer (PDBID: 2H3Q and 2H3Y (18)) (Fig. 3, D and E, and fig. S6). We consider it reasonable to assign the observed density to PI(4,5)P2. The N terminus of helix 4 prominently protrudes up into the PM, indicating that, in the mature

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**Fig. 3.** Mature HIV-1 matrix structure. (A and B) Slices through reconstructions of the mature HIV-1 MA lattice in cHIV (A) and cHIV MA-SP1 (B). The region within the yellow box in (B) is shown at a higher magnification in (D). (C and D) An isosurface view of the cryo-ET reconstruction for mature cHIV MA-SP1 MA lattice fitted with the structure of monomeric MA, cut perpendicular to the membrane (C), or viewed from the top toward the virus center (D). (E and F) Same of the mature virus as in (C), enlarged and cut to reveal density corresponding to the N-terminal residues (black arrowhead), and PI(4,5)P2 (red arrowhead), and the structure of PI(4,5)P2 bound to MA as resolved by NMR [PDBID: 2H3Q (18); colored blue to red from the N- to C-terminus, respectively], was fitted as a rigid body into the density. Helices 1, 2, and 5 are marked. Density is observed in the PI(4,5)P2 binding site (red arrowhead), and the structure of PI(4,5)P2 bound to MA as resolved by NMR [PDBID: 2H3Q (18)], is shown as a stick model. (E) Same as in (C), enlarged and cut to reveal density corresponding to the N-terminal residues (black arrowhead), and PI(4,5)P2 (red arrowhead). (F) Same as in Fig. 2G, lattice map for a mature cHIV MA-SP1 MA lattice and the underlying CA lattice.
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**Fig. 4. Comparison of the immature and mature HIV-1 MA lattices.** (A) Immature and mature MA lattices are shown with one trimer aligned. In the immature lattice, contact with neighboring trimers is mediated by N-terminal regions, and the PI(4,5)P2 binding site is empty. In the mature lattice, contact with neighboring trimers is mediated by the region surrounding the occupied PI(4,5)P2 binding site. (B) Electrostatic surface potential maps of the hexameric lattice of immature and mature MA trimers. The red [−5 kT/e (k, Boltzmann constant; T, temperature; e, electron charge)] and blue (+5 kT/e) colors represent negatively and positively charged electric potentials, respectively. PI(4,5)P2 is shown in yellow. The negatively charged lipid headgroups are surrounded by positively charged f-PI(4,5)P2 with a diazirine group in the perivertebrate positions of MA and PI(4,5)P2, revealing that PR- particles, but not to MA within mature particles upon photoactivation, f-PI(4,5)P2 was efficiently crosslinked to Gag within immature PR-particles, but not to MA within mature particles (fig. S7B). This observation confirms direct interaction of the MA domain of the Gag polyprotein with PI(4,5)P2, reveals that proteolytic cleavage of Gag alters the relative positions of MA and PI(4,5)P2, and provides direct biochemical evidence for a change in MA-lipid interactions upon HIV-1 maturation.

Taken together, our results (summarized in fig. S8) revealed that MA–like CA and NC undergoes substantial structural maturation to form very different lattices in immature and mature HIV-1 (movie S1). Immature virions contain MA trimers packed together through their N termini into a poorly ordered hexagonal lattice containing basic-charged holes and disordered regions, which could accommodate the cytoplasmic tails of Env. The N-terminal myristate is predominantly membrane-inserted in the immature and mature particle. The immature MA structure provides a framework for understanding the established roles of MA in virus assembly and Env incorporation. Mature virions, by contrast, contain MA trimers packed together by means of their HBRs, resulting in a well-ordered hexagonal lattice with neutral holes. The maturation of MA is reminiscent of that of CA, which matures between two different hexameric protein lattices with two different functional roles (3). Upon maturation, both the protein arrangement and the lipid binding properties of MA change. The lipid binding pocket on the side of MA is occupied in the mature structure, containing density consistent with the previously described extended-lipid conformation of PI(4,5)P2 (fig. S6B), in which one acyl chain is removed from the membrane (18). The resolution of our density does not allow us to distinguish PI(4,5)P2 from other lipids, but its position is such that any lipid positioned within this density is pulled outward from the lipid bilayer. The virion contains approximately three PI(4,5)P2 molecules per Gag (40); we assume that the remaining PI(4,5)P2 molecules may interact with the membrane-proximal surface of MA through their headgroups, as in the immature virus. Our data therefore imply that maturation of Gag results in the partial removal of up to ~2500 of the ~150,000 lipids in the inner leaflet of the viral membrane (40). The energy barrier to the removal of acyl chains from the bilayer may be overcome by the binding of PI(4,5)P2 to MA and stabilization of the MA lattice.

In conclusion, our data suggest that the structural maturation of Gag not only condenses the RNP and achieves assembly of a viral capsid core surrounding the genome but also rearranges the membrane-bound MA layer and modulates the lipid bilayer itself. How these changes alter the properties of the virus remains to be elucidated, but we speculate that they affect not only MA and Env function but also the physical properties of the viral envelope, possibly correlating with the observed reduction in stiffness of the virus particle (41, 42). The unexpected presence of a different, well-defined MA structure in mature virions suggests that MA performs additional roles in the mature virion or after entry into the target cell. We speculate that PI(4,5)P2-stabilized MA lattices may remain associated within the membrane after virion fusion and have a postentry function, perhaps acting as signaling platforms to prepare the target cell for early HIV-1 replication.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

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