Published in final edited form as:

Trends Microbiol. 2014 July ; 22(7): 372–378. doi:10.1016/j.tim.2014.04.012.

The role of matrix in HIV-1 envelope glycoprotein incorporation

Philip R. Tedbury and Eric O. Freed

Virus-Cell Interaction Section, HIV Drug Resistance Program, Center for Cancer Research,
National Cancer Institute, Frederick, MD 21702-1201

Abstract

Incorporation of the viral envelope (Env) glycoprotein is a critical requirement for the production of infectious HIV-1 particles. It has long been appreciated that the matrix (MA) domain of the Gag polyprotein and the cytoplasmic tail of Env are central players in the process of Env incorporation, but the precise mechanisms have been elusive. A number of recent developments have thrown light on the contributions of both proteins, prompting a re-evaluation of the role of MA during Env incorporation. The two domains appear to play distinct but complementary roles, with the cytoplasmic tail of Env responsible for directing Env to the site of assembly and the matrix domain accommodating the cytoplasmic tail of Env in the Gag lattice.

Keywords

HIV-1; matrix; envelope; assembly; packaging

Combating HIV-1 and AIDS

As the main causative agent of acquired immunodeficiency syndrome (AIDS), human immunodeficiency virus type 1 (HIV-1) is one of the most significant infectious agents impacting global human health. Consequently, a great deal of research effort has been invested in understanding and combating this viral pathogen. Progress has been made on many fronts, allowing researchers to describe key processes of HIV-1 replication and subsequently develop antiretrovirals that delay or prevent the onset of AIDS and prolong the lives of infected patients [1,2]. Despite this remarkable success, neither a preventative vaccine nor a curative therapy is currently available, and resistance to available drugs is emerging. Research continues into aspects of HIV-1 replication that are not fully understood with the goal of developing novel targets for antiretroviral therapy. One such poorly understood process is the incorporation of the HIV-1 envelope (Env) glycoprotein into viral particles.

The processes associated with HIV-1 particle assembly have been studied in detail and many key features have been elucidated [3]. Gag, the protein primarily responsible for driving

Corresponding author: Freed, E.O. (efreed@nih.gov).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

assembly, is translated in the cytoplasm, then binds to the membrane via the matrix (MA) domain (Figure 1a). MA contains a binding site for phosphatidylinositol-4,5-bisphosphate (PI[4,5]P₂), which enhances targeting of Gag to the plasma membrane (PM) [4,5]. PI(4,5)P₂ binding [6], as well as Gag oligomerization [7], triggers exposure of the amino-terminal myristic acid moiety that inserts into the membrane, anchoring Gag. Gag is capable of assembling virus-like particles and budding from the membrane in the absence of any other viral protein [3].

Env is translated at the endoplasmic reticulum (ER) as the precursor polyprotein gp160, and is cotranslationally inserted into the membrane (Figure 1b) [8]. Env traffics to the PM via the Golgi apparatus and is also targeted to raft-like domains. Gp160 is glycosylated following translocation to the ER lumen, and during transport through the Golgi it is cleaved to the mature gp120 and gp41 glycoproteins, which remain non-covalently associated. Gp120 makes up the extracellular component that binds to the receptor (CD4) and co-receptors (CCR5 and CXCR4). Gp41 is a transmembrane protein; the extracellular and transmembrane regions are required for membrane fusion with target cells. The cytoplasmic tail (CT) of gp41 includes three helical regions referred to as lentiviral lytic peptides (LLP) 1–3 [9]. The LLPs have been shown to associate with the cytosolic side of the PM and may influence fusogenicity and immunogenicity of Env [10,11]. The CT also contains motifs involved in signaling, trafficking and endocytosis [12]. The active form of the Env complex is a heterotrimer (three molecules each of gp120 and gp41) [10]. Although it has long been known that MA and gp41 CT play critical roles in Env incorporation, the exact mechanism has resisted explanation. Greater understanding the functions of these protein domains is of considerable interest and could open the door to novel therapeutics. Here we discuss the current models of HIV-1 Env incorporation, in the light of recent discoveries in the field. We propose a model whereby Env traffics to sites of assembly via interactions with host cell factors, and is accommodated into the Gag lattice in a MA trimerization-dependent manner.

Four ways to incorporate an envelope

Four models have been proposed to explain the incorporation of HIV Env into virions [8,13] (Figure 2). An entirely passive mechanism would involve HIV-1 budding from the cell surface and carrying with it any Env proteins that were present at the site of assembly. A second model involves co-targeting of Gag and Env to common sites on the PM, thereby increasing the amount of Env packaged into particles. As both Gag and Env are known to traffic to lipid rafts [14–18], co-trafficking is likely to contribute to Env incorporation. The remaining two models involve specific protein–protein interactions, either the binding of Env by Gag to directly recruit Env to the particle or binding of Env and Gag to a cellular co-factor that bridges the interaction.

The evidence for these more specific models comes from two sources: (i) there have been reports, albeit limited in number, of direct interaction between lentiviral Env and Gag proteins [19–21]. (ii) Also supporting the concept of direct interactions between Gag and Env are a series of MA mutants that are unable to incorporate Env but are otherwise assembly competent; likewise, a small internal deletion in the gp41 CT leads to the formation of Env-deficient particles [22–26]. The effect of the internal CT deletion on Env

incorporation can be rescued by a mutation in MA [26], and in HeLa or 293T cells the MA mutants can be rescued by large C-terminal truncations of the CT or compensatory mutations in MA [22,24,25,27,28]. Together these data argue for an interaction between MA and the gp41 CT during particle assembly; however, they do not explain the nature of the interaction or whether it is bridged by a cellular factor. The principle argument for a cellular cofactor is the cell-type-specific requirement for the gp41 CT to achieve efficient Env incorporation. Although the Env CT is not required for receptor binding or membrane fusion, and is not required for incorporation in certain commonly used laboratory cell lines (e.g., HeLa), in most T-cell lines and in physiologically relevant cell types such as primary lymphocytes and monocyte-derived macrophages, CT-truncated Env is not efficiently incorporated into particles. These cell types are thus considered to be non-permissive for gp41 CT truncation [29,30]. The cell type-dependent requirement for the gp41 CT could be explained by a gp41 CT-interacting cellular factor that promotes Env incorporation in non-permissive cell lines.

Over the years, a number of cellular factors have been described that interact with MA and/or the gp41 CT (for review, see [8]). However, none of these has been convincingly demonstrated to be a MA–gp41 CT bridging cofactor. This raises the possibility that the cell-type-dependent requirement for the gp41 CT may be due not to the presence of a Gag–Env bridging protein but to the cell-type-specific expression of a factor that promotes Env trafficking to, or retention at, sites of particle budding. Such a factor could be non-proteinaceous in nature – for example, a specific type of lipid microdomain with which both Gag and Env associate – or it could be a protein. The Spearman laboratory recently reported that Rab11 family interacting protein 1c (FIP1c) has some of the properties of an Env incorporation co-factor [31]. This protein was shown to interact with the gp41 CT and the trafficking protein Rab14. Depletion of FIP1c in HeLa cells suppressed Env incorporation and reduced its PM localization. The CT-truncated form of Env was able to traffic to the PM with or without FIP1c; however, while full-length HIV-1 Env has been observed to colocalize with Gag at sites of virion assembly, CT-truncated Env fails to colocalize with Gag [32,33]. Consequently, the role of the gp41 CT may be to ensure trafficking of Env to a specific region of the PM; without the CT, Env still traffics to the PM but by an alternative pathway, one that is less conducive to efficient Env incorporation. The precise differences between the pathways used by full-length and CT-truncated Env are not clear and it is not known why in non-permissive cells the CT is absolutely required, while in permissive cells CT-truncated Env can be packaged, albeit less specifically. Because of the role of Rab11 family interacting proteins in recycling cargo back to the PM from an endosomal recycling compartment (ERC) [34,35], it has been suggested that Env may be directed through the ERC to reach a site on the PM that favors Env incorporation into virions [31]. This interesting hypothesis warrants further investigation.

The mechanism of Env localization to assembly sites has also been approached by using super-resolution and electron microscopy [32,33,36]. These studies revealed that full-length but not CT-truncated HIV-1 Env localizes at sites of virus assembly [32,33]. MA mutants that are unable to incorporate Env lose their colocalization with Env, suggesting that MA plays a role in either recruitment or retention of Env at sites of Gag assembly [33]. The presence of Gag greatly reduced the mobility of Env at assembly sites in a manner

dependent on Gag multimerization [32]. It is possible that Env traffics to the appropriate location at the PM, then is trapped at that site either by interaction with the MA component of the Gag lattice or due to physicochemical properties of the membrane at sites of Gag assembly. At present, it is not exactly clear what the role of MA is or what the Env seen at assembly sites represents. The amount of Env in a virion is relatively low (approximately 10 Env trimers per particle) [37], and much of the Env that is observed at sites of assembly may ultimately not be incorporated into particles. The ability of envelope proteins of a variety of different viruses to cluster at sites of HIV-1 assembly [36] argues against, but does not absolutely disprove, a requirement for direct or co-factor-mediated binding of Env to Gag. Rather, it would appear that Gag-induced properties of the PM at assembly sites favor the recruitment and retention of Env.

MA making space

The role of MA in Env incorporation appears to be distinct from that of the Env CT, despite evidence for their interdependency. Mutations in MA that block Env incorporation act specifically on the full-length Env; gp41 CT truncations rescue the incorporation defect [22,25,28]. In addition, these MA mutants are unable to package full-length Env in either permissive or non-permissive cells, demonstrating that they exert a dominant effect on incorporation even in cell types that do not require the gp41 CT for incorporation [27,38]. Identification of compensatory mutations that rescued Env-incorporation defects highlighted the importance of MA in Env packaging; mutations in either MA or the gp41 CT that blocked Env incorporation could be compensated for by mutations in MA [22–24,26,28,38]. It was also observed that a single mutation in MA, which initially arose during passage of an Env incorporation-defective mutant, was capable of rescuing a large panel of previously characterized Env-incorporation defective mutations, including the above-mentioned small deletion in the gp41 CT [24]. This substitution, glutamine 62 to arginine (62QR), is located distant from the cluster of Env incorporation defective mutations, suggesting that it does not provide rescue function simply by re-creating a disrupted MA–Env binding site. Instead, the critical feature appears to be the proximity of 62QR to the postulated MA trimer interface [24].

The structural studies of the Hill, Sundquist, and Barklis groups suggested that HIV-1 MA is trimeric and that the Env incorporation defective mutations cluster near the tips of the trimer [39,40] (Figure 3). By contrast, 62QR is located at the trimer interface and is buried away from the membrane-proximal surface of MA [24]. If the original loss of Env packaging were due to disruption of an Env-binding site at the tips of the trimer, it is difficult to envisage how 62QR would rescue Env binding. In addition, examination of residues close to 62QR on the opposing surface of the trimer interface demonstrated that the key to rescuing Env incorporation was an interaction across the trimer interface, between two MA monomers [24]. These findings demonstrate the importance of the MA trimeric structure in Env incorporation. An additional mutation was identified that blocks Env incorporation (69TR); based on the crystal structure this substitution is predicted to impair trimerization [24].

A plausible interpretation of these data is that MA trimerization is an adaptation to the large CT of HIV-1 Env. This long tail contains trafficking motifs, and has been shown to induce

nuclear factor- κ B (NF- κ B) signaling, contributing to enhanced HIV-1 gene expression [41]. It is possible that the long CT is required for functions in addition to the assembly of infectious virions; in such a scenario, lentiviruses would need to accommodate this large domain during viral assembly. The structures generated by the Barklis group showing a trimeric and non-trimeric MA indicate that MA trimerization increases the aperture at the hexameric center of the MA lattice [40,42] (Figure 3 c+d). In both trimeric and non-trimeric configurations of MA, the formation of a hexameric CA lattice drives the hexameric arrangement of Gag and therefore MA; this MA lattice structure would presumably exist in immature viral particles [43–45]. As the Env-incorporation-defective mutations also cluster around this hexameric center (made up of the tips of MA trimers), it seems reasonable to suggest that this is the site in the MA lattice into which the gp41 CT protrudes in the virion (Figure 3). Mutations that impair the ability of the MA lattice to accommodate the Env CT, either by preventing MA trimerization or by changing the properties of the aperture in the lattice, would disrupt Env incorporation. In this model, the loss of Env incorporation induced by a small deletion in the HIV-1 gp41 CT would be explained by a change in the structure or relative orientation of the CT, causing it to no longer fit into the available aperture in the MA lattice. At present, it is unknown how the compensatory mutations affect these structures.

This model of MA adopting a hexamer-of-trimers structure to accommodate the Env CT is also compatible with data from pseudotyping and competition studies. HIV-1 can package envelopes from a variety of viruses whose Env glycoproteins have short CTs. By contrast, viruses such as murine leukemia virus (MLV), whose Env has a short CT, cannot package HIV-1 Env with an intact CT [46–48]. To further elucidate potential MA Env interactions, competition analyses have been performed looking at MLV Env incorporation into HIV-1 and MLV particles. Although MLV Env can package into both HIV-1 and MLV particles, when HIV-1 and MLV Gags are co-expressed in the same cell as MLV Env, MLV Env selectively packages into MLV particles [49]. These observations suggest that the MA domains of HIV-1 and MLV Gag are able to recognize and bind their cognate Env CTs, driving selective Env packaging. However, when this hypothesis was tested by swapping MA domains of the HIV-1 and MLV Gag proteins, the original selectivity was retained, indicating that MA is not the driving force behind the selectivity for cognate Env incorporation. Rather, capsid (CA) and nucleocapsid (NC) appear to play the dominant role in directing preferential recruitment of MLV Env into MLV particles [49]. Given the short tail of the MLV Env, these data are consistent with Env clustering being due to general properties of the assembly site, rather than a direct interaction between an Env CT and its cognate Gag.

One of the difficulties in developing models for HIV-1 Env incorporation is the lack of solid structural information about the gp41 CT. The tail contains α -helical domains with strong propensities to interact with membrane bilayers. These helical motifs could also engage in intra- or inter-molecular interactions within the gp41 trimer. The lack of understanding of how the CT is oriented with respect to the membrane or the underlying Gag lattice injects a high degree of speculation into models for Env incorporation. Given the plasticity of Env structure in general it is possible that the gp41 CT could adopt multiple conformations that are regulated by the state of virion maturation or by the fusion process [50].

Future directions

If the role of MA in HIV-1 Env incorporation is one of adopting an appropriately structured lattice to accommodate the long gp41 CT then two conclusions follow: first, the hypothesis can be tested through examination of other retroviruses. Lentiviruses, most of which encode Env glycoproteins with long CTs, would be expected to share the trimeric MA configuration as an evolutionary adaptation to the long CT. Consistent with this model, the MA protein of simian immunodeficiency virus was also found to trimerize in crystallographic studies [51], however the oligomeric state of MA in virions remains to be determined for any retrovirus. By contrast, those retroviruses with short-tailed Env glycoproteins, such as MLV, should not require a trimeric MA to incorporate Env. Studies of this nature will go a long way toward explaining the relevance of MA trimers. It has also been suggested that trimerization of MA may play a role in Gag assembly [52], although it seems likely that mutations blocking MA trimerization and Gag assembly exert their phenotype via more general misfolding of the MA domain. Indeed, large deletions in MA are not incompatible with efficient Gag assembly [53]. Clearly it will be important to develop assays that can differentiate between a generally misfolded MA and one that lacks only the ability to form trimers in order to clearly determine the role of trimerization; the current methods involve direct visualization by structural methods and are extremely labor intensive.

The second consequence of functionally important MA trimerization is that MA trimers would become a potential drug target. HIV-1 therapy is in continual need of new drugs with novel mechanisms of action to combat viral variants that are resistant to existing therapies. Targeting MA trimerization may provide one such novel approach. Although efficacy would not require precise understanding of the functional relevance of MA trimers, such an understanding would undoubtedly help guide development of lead compounds to licensed therapeutics.

Concluding remarks

Recent advances have demonstrated the importance of both Env CT and MA during the incorporation of HIV-1 Env. It appears that the primary function of the HIV-1 Env CT during particle assembly is to direct the trafficking of Env to the PM, where it clusters at assembly sites via an unknown, but CT-dependent, mechanism. MA is required to permit incorporation of Env into the particle itself, potentially by forming a hexamer-of-trimers lattice that accommodates the long gp41 CT (Figure 4). Such a model would not require tight binding of MA to either the Env CT or a cellular bridging factor. Although the proposed roles of both the Env CT and MA will require further work to build on available data (Box 1), progress is being made in understanding many of the long-standing mysteries of Env incorporation.

Acknowledgments

Research in the Freed laboratory is supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research and the Intramural AIDS Targeted Antiviral Program.

References

1. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb. Perspect. Med.* 2012; 2:a007161. [PubMed: 22474613]
2. Freed, EO.; Martin, MA. HIVs and their replication . In: Knipe, DM.; Howley, PM., editors. *Field's Virology*. 6th edn. Lippincott, Williams, and Wilkins; 2013. p. 1502-1560.
3. Sundquist WI, Kräusslich H-G. HIV-1 assembly, budding, and maturation. *Cold Spring Harb. Perspect. Med.* 2012; 2:a006924. [PubMed: 22762019]
4. Ono A, et al. Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. *Proc. Natl. Acad. SciUSA.* 2004; 101:14889–14894.
5. Chukkapalli V, Ono A. Molecular determinants that regulate plasma membrane association of HIV-1 Gag. *J. Mol. Biol.* 2011; 410:512–524. [PubMed: 21762797]
6. Saad JS, et al. Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly. *Proc. Natl. Acad. SciUSA.* 2006; 103:11364–11369.
7. Tang C, et al. Entropic switch regulates myristate exposure in the HIV-1 matrix protein. *Proc. Natl. Acad. SciUSA.* 2004; 101:517–522.
8. Checkley MA, et al. HIV-1 envelope glycoprotein biosynthesis, trafficking, and incorporation. *J. Mol. Biol.* 2011; 410:582–608. [PubMed: 21762802]
9. Steckbeck JD, et al. C-terminal tail of human immunodeficiency virus gp41: functionally rich and structurally enigmatic. *J. Gen. Virol.* 2013; 94:1–19. [PubMed: 23079381]
10. Santos da Silva E, et al. The frantic play of the concealed HIV envelope cytoplasmic tail. *Retrovirology.* 2013; 10:54. [PubMed: 23705972]
11. Viard M, et al. Photoinduced reactivity of the HIV-1 envelope glycoprotein with a membrane-embedded probe reveals insertion of portions of the HIV-1 Gp41 cytoplasmic tail into the viral membrane. *Biochemistry.* 2008; 47:1977–1983. [PubMed: 18198900]
12. Postler TS, Desrosiers RC. The tale of the long tail: the cytoplasmic domain of HIV-1 gp41. *J. Virol.* 2013; 87:2–15. [PubMed: 23077317]
13. Johnson MC. Mechanisms for Env glycoprotein acquisition by retroviruses. *AIDS Res. Hum. Retroviruses.* 2011; 27:239–247. [PubMed: 21247353]
14. Nguyen DH, Hildreth JE. Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. *J. Virol.* 2000; 74:3264–3272. [PubMed: 10708443]
15. Ono A, Freed EO. Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc. Natl. Acad. SciUSA.* 2001; 98:13925–13930.
16. Yang P, et al. The cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein gp41 harbors lipid raft association determinants. *J. Virol.* 2010; 84:59–75. [PubMed: 19793805]
17. Bhattacharya J, et al. Human immunodeficiency virus type 1 envelope glycoproteins that lack cytoplasmic domain cysteines: impact on association with membrane lipid rafts and incorporation onto budding virus particles. *J. Virol.* 2004; 78:5500–5506. [PubMed: 15113929]
18. Rouso I, et al. Palmitoylation of the HIV-1 envelope glycoprotein is critical for viral infectivity. *Proc. Natl. Acad. SciUSA.* 2000; 97:13523–13525.
19. Vincent MJ, et al. Intracellular interaction of simian immunodeficiency virus Gag and Env proteins. *J. Virol.* 1999; 73:8138–8144. [PubMed: 10482563]
20. Cosson P. Direct interaction between the envelope and matrix proteins of HIV-1. *EMBO J.* 1996; 15:5783–5788. [PubMed: 8918455]
21. Manrique JM, et al. In vitro binding of simian immunodeficiency virus matrix protein to the cytoplasmic domain of the envelope glycoprotein. *Virology.* 2008; 374:273–279. [PubMed: 18255119]
22. Freed EO, et al. Single amino acid changes in the human immunodeficiency virus type 1 matrix protein block virus particle production. *J. Virol.* 1994; 68:5311–5320. [PubMed: 8035531]

23. Ono A, et al. Characterization of human immunodeficiency virus type 1 matrix revertants: effects on virus assembly, Gag processing, and Env incorporation into virions. *J. Virol.* 1997; 71:4409–4418. [PubMed: 9151831]
24. Tedbury PR, et al. Global rescue of defects in HIV-1 envelope glycoprotein incorporation: implications for matrix structure. *PLoS Pathog.* 2013; 9:e1003739. [PubMed: 24244165]
25. Brandano L, Stevenson M. A highly conserved residue in the C-terminal helix of HIV-1 matrix is required for envelope incorporation into virus particles. *J. Virol.* 2011
26. Murakami T, Freed EO. Genetic evidence for an interaction between human immunodeficiency virus type 1 matrix and alpha-helix 2 of the gp41 cytoplasmic tail. *J. Virol.* 2000; 74:3548–3554. [PubMed: 10729129]
27. Freed EO, Martin MA. Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. *J. Virol.* 1995; 69:1984–1989. [PubMed: 7853546]
28. Mammano F, et al. Rescue of human immunodeficiency virus type 1 matrix protein mutants by envelope glycoproteins with short cytoplasmic domains. *J. Virol.* 1995; 69:3824–3830. [PubMed: 7745730]
29. Murakami T, Freed EO. The long cytoplasmic tail of gp41 is required in a cell type-dependent manner for HIV-1 envelope glycoprotein incorporation into virions. *Proc. Natl. Acad. SciUSA.* 2000; 97:343–348.
30. Akari H, et al. Cell-dependent requirement of human immunodeficiency virus type 1 gp41 cytoplasmic tail for Env incorporation into virions. *J. Virol.* 2000; 74:4891–4893. [PubMed: 10775630]
31. Qi M, et al. Rab11-FIP1C and Rab14 direct plasma membrane sorting and particle incorporation of the HIV-1 envelope glycoprotein complex. *PLoS Pathog.* 2013; 9:e1003278. [PubMed: 23592992]
32. Roy NH, et al. Clustering and mobility of HIV-1 Env at viral assembly sites predict its propensity to induce cell-cell fusion. *J. Virol.* 2013; 87:7516–7525. [PubMed: 23637402]
33. Muranyi W, et al. Super-resolution microscopy reveals specific recruitment of HIV-1 envelope proteins to viral assembly sites dependent on the envelope C-Terminal tail. *PLoS Pathog.* 2013; 9:e1003198. [PubMed: 23468635]
34. Fan G-H, et al. Rab11-family interacting protein 2 and myosin Vb are required for CXCR2 recycling and receptor-mediated chemotaxis. *Mol. Biol. Cell.* 2004; 15:2456–2569. [PubMed: 15004234]
35. Nedvetsky PI, et al. A Role of myosin Vb and Rab11-FIP2 in the aquaporin-2 shuttle. *Traffic.* 2007; 8:110–123. [PubMed: 17156409]
36. Jorgenson RL, et al. Foreign glycoproteins can be actively recruited to virus assembly sites during pseudotyping. *J. Virol.* 2009; 83:4060–4067. [PubMed: 19224995]
37. Zhu P, et al. Electron tomography analysis of envelope glycoprotein trimers on HIV and simian immunodeficiency virus virions. *Proc. Natl. Acad. SciUSA.* 2003; 100:15812–15817.
38. Freed EO, Martin MA. Domains of the human immunodeficiency virus type 1 matrix and gp41 cytoplasmic tail required for envelope incorporation into virions. *J. Virol.* 1996; 70:341–351. [PubMed: 8523546]
39. Hill CP, et al. Crystal structures of the trimeric human immunodeficiency virus type 1 matrix protein: implications for membrane association and assembly. *Proc. Natl. Acad. SciUSA.* 1996; 93:3099–3104.
40. Alfadhli A, et al. HIV-1 matrix organizes as a hexamer of trimers on membranes containing phosphatidylinositol-(4,5)-bisphosphate. *Virology.* 2009; 387:466–472. [PubMed: 19327811]
41. Postler TS, Desrosiers RC. The cytoplasmic domain of the HIV-1 glycoprotein gp41 induces NF- κ B activation through TGF- β -activated kinase 1. *Cell Host Microbe.* 2012; 11:181–193. [PubMed: 22341466]
42. Alfadhli A, et al. Human immunodeficiency virus type 1 matrix protein assembles on membranes as a hexamer. *J. Virol.* 2007; 81:1472–1478. [PubMed: 17108052]
43. Wright ER, et al. Electron cryotomography of immature HIV-1 virions reveals the structure of the CA and SP1 Gag shells. *EMBO J.* 2007; 26:2218–2226. [PubMed: 17396149]

44. Huseby D, et al. Assembly of human immunodeficiency virus precursor gag proteins. *J. Biol. Chem.* 2005; 280:17664–17660. [PubMed: 15734744]
45. Briggs JAG, Kräusslich H-G. The molecular architecture of HIV. *J. Mol. Biol.* 2011; 410:491–500. [PubMed: 21762795]
46. Schnierle BS, et al. Pseudotyping of murine leukemia virus with the envelope glycoproteins of HIV generates a retroviral vector with specificity of infection for CD4-expressing cells. *Proc. Natl. Acad. SciUSA.* 1997; 94:8640–8645.
47. Wilson C, et al. Formation of infectious hybrid virions with gibbon ape leukemia virus and human T-cell leukemia virus retroviral envelope glycoproteins and the gag and pol proteins of Moloney murine leukemia virus. *J. Virol.* 1989; 63:2374–2378. [PubMed: 2784836]
48. Höhne M, et al. Truncation of the human immunodeficiency virus-type-2 envelope glycoprotein allows efficient pseudotyping of murine leukemia virus retroviral vector particles. *Virology.* 1999; 261:70–78. [PubMed: 10441556]
49. Gregory DA, et al. Multiple Gag domains contribute to selective MLV Env recruitment to MLV virions. *J. Virol.* 2012; 87:1518–1527. [PubMed: 23152533]
50. Merk A, Subramaniam S. HIV-1 envelope glycoprotein structure. *Curr. Opin. Struct. Biol.* 2013; 23:268–276. [PubMed: 23602427]
51. Rao Z, et al. Crystal structure of SIV matrix antigen and implications for virus assembly. *Nature.* 1995; 378:743–747. [PubMed: 7501025]
52. Morikawa Y, et al. Detection of a trimeric human immunodeficiency virus type 1 Gag intermediate is dependent on sequences in the matrix protein, p17. *J. Virol.* 1998; 72:7659–7663. [PubMed: 9696871]
53. Reil H, et al. Efficient HIV-1 replication can occur in the absence of the viral matrix protein. *EMBO J.* 1998; 17:2699–2708. [PubMed: 9564051]

Highlights

- Matrix and gp41 cytoplasmic tail are the key factors in HIV-1 envelope incorporation.
- Gp41 cytoplasmic tail interacts with cellular trafficking factors.
- Matrix adopts a hexamer-of-trimers structure that accommodates the gp41 cytoplasmic tail.

Box 1. Outstanding questions

- What causes diverse envelopes to cluster at sites of HIV-1 assembly, and what drives envelope selection?
- Is trimerization of MA an absolute requirement for lentiviral Env incorporation?
- Does trimerization of MA play any other role in HIV-1 assembly or replication?
- What is the structure of the HIV-1 gp41 CT?

Figure 1. Schematic of Gag and Env proteins

(A) Gag is the protein primarily responsible for driving particle assembly; it interacts with the cytosolic face of the membrane via MA and forms a lattice via CA–CA interactions. NC recruits the viral genomic RNA, which in turn contributes to Gag lattice formation. Following the formation a viral particle, late domains in p6 recruit the cellular endosomal sorting complex required for transport (ESCRT) to achieve membrane scission and complete viral budding. (B) Env is a transmembrane protein, with gp120 displayed on the extracellular side of the cell or viral membrane. Gp41 has an ectodomain that interacts with gp120, a transmembrane region and a long tail that is thought to interact with the cytoplasmic face of the PM. Lentiviral lytic peptides (LLPs) are predicted to be amphipathic, membrane-associated, alpha helices. MA, grey; gp120, blue; gp41, green; gp41 transmembrane domain, yellow.

Figure 2. Models for Env incorporation

The passive model assumes that HIV-1 buds from the membrane and simply carries with it any Env that may be present. In the cotargeting model, incorporation is enhanced by targeting both Gag and Env to raft-like regions within the PM. The direct Gag-Env interaction model proposes that the motifs within MA and gp41 that have been demonstrated to influence Env incorporation comprise interaction domains, and that this interaction (circled) is required for Env incorporation into particles. The indirect Gag-Env interaction model posits the existence of a bridging factor and explains the observation that the requirement for the gp41 CT in Env incorporation is cell type dependent. MA, grey; gp120, blue; gp41, green; gp41 transmembrane domain, yellow; lipid rafts, red; hypothetical

bridging factor, pink. Note that for simplicity the Env complex is shown as monomer (one molecule each of gp120 and gp41) rather than in its native trimeric state. This figure is adapted from Checkley *et al.* [4].

Figure 3. Trimeric arrangement of MA molecules

(A) Top down and (B) transverse views of the MA trimer structure as solved by Hill *et al.* [31]. Residues that can be mutated to block Env incorporation are shown in blue (L12, E16, L30, V34 and E98). Residues that can be mutated to restore Env incorporation are shown in green (Q62, S66); these residues are in a position to form interactions across the trimer interface to effect restoration of Env incorporation. Residue T69 (red) can be mutated to prevent Env incorporation but is not compensated by modifications of the trimer interface. Mutation of T69 has been suggested to block MA trimerization [24]. (C) and (D) show a MA monomer mapped onto non-trimeric and trimeric lattices, respectively, as described by

Alfadhli *et al.*, [40,42]. Positions of mutations that block Env incorporation are shown in blue. Trimerization enlarges the central aperture of the MA lattice (from < 3 nm to > 4 nm), which is lined by the inhibitory mutations. Together these data suggest a role for the central aperture in accommodating the gp41 CT. The model of MA monomer and trimer were generated in Pymol (www.pymol.org) based on the Protein Data Bank coordinates 1HIW [39].

Figure 4. Three steps to Env incorporation

(A) On leaving the *trans*-Golgi network, the gp41 CT interacts with FIP1c and/or other host factors, and traffics to raft-like domains of the PM. (B) At the PM, Env encounters Gag arranged into a long-range lattice. The Gag lattice traps Env in place and prevents its endocytosis. When Gag forms a virion, it carries with it some of the Env proteins. (C) Wild-type MA will accommodate the CT of gp41, causing Env to be trapped in place by the presence of Gag and incorporated into particles. (D) MA that cannot accommodate the CT of gp41 will exclude Env from the Gag lattice, leading to a random distribution in the PM and lack of Env incorporation into particles. PM, blue; lipid rafts, red; Gag lattice, grey shaded area; Env, cyan; gp41, green; gp41 transmembrane domain, yellow. Note that for

simplicity the Env complex is shown as monomer (one molecule each of gp120 and gp41) rather than in its native trimeric state.