Rescue of Human Immunodeficiency Virus Type 1 Matrix Protein Mutants by Envelope Glycoproteins with Short Cytoplasmic Domains

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The matrix (MA) protein of human immunodeficiency virus type 1 (HIV-1) forms the outer protein shell directly underneath the lipid envelope of the virion. The MA protein has a key role in different aspects of virus assembly, including the incorporation of the HIV-1 Env protein complex, which contains a transmembrane glycoprotein with an unusually long cytoplasmic tail. In this study, we compared the abilities of HIV-1 MA mutants to incorporate Env protein complexes with long and short cytoplasmic tails. While the mutant particles failed to incorporate the authentic HIV-1 Env protein complex, they retained the ability to efficiently and functionally incorporate the amphotropic murine leukemia virus Env protein complex, which has a short cytoplasmic tail. Moreover, incorporation of the autologous Env protein complex could be restored by a second-site mutation that resulted in the truncation of the cytoplasmic tail of the HIV-1 transmembrane glycoprotein. Remarkably, the second-site mutation also restored the ability of MA mutants to replicate in MT-4 cells. These results imply that the long cytoplasmic tail of the transmembrane glycoprotein is responsible for the exclusion of the HIV-1 Env protein complex from MA mutant particles.

The formation of an infectious human immunodeficiency virus type 1 (HIV-1) virion requires the coordinated assembly of the viral internal structural proteins, enzymes, surface glycoproteins, and genomic RNA. The internal structural proteins of the virion are synthesized on free ribosomes as part of the Gag polyprotein precursor (Pr55^{gag}) (20, 44). During translation of the *gag* gene, occasional ribosomal frameshifting into the overlapping *pol* frame ensures the synthesis of the viral enzymes as components of the Pr160^{gag-pol} polyprotein (20). The Pr55^{gag} and Pr160^{gag-pol} polyproteins are transported to the plasma membrane, where particle assembly and membrane extrusion occur simultaneously (14). Particle formation is thought to be driven mainly by the self-association of Pr55^{gag} molecules underneath the cell membrane, since other viral gene products are dispensable (20). After assembly is completed, part of the electron-dense outer shell of the nascent particle condenses into a cone-shaped core structure that is typical for mature HIV-1 virions (14). Virion maturation requires cleavage of Pr55^{gag} by the viral protease (17), which is brought into the virion as a component of Pr160^{gag-pol}. The proteolytic cleavage sites define different domains of Pr55^{gag}, which yield the internal structural proteins of the mature virion. These include the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins, which are common to all retroviruses, and a peptide derived from the C terminus of Pr55^{gag} (p6^{gag}), which is found only in primate immunodeficiency viruses (19, 29).

The prominent cone-shaped core of the mature virion is formed by the CA protein, which represents the largest of the cleavage products of Pr55^{gag} (14). Mutagenesis of the CA pro-

tein has indicated that it contains distinct functional domains which are crucial for different steps of the virus life cycle (8, 33, 41). Recently, it was shown that sequences within the CA domain of Pr55^{gag} bind to the cellular prolyl-isomerase cyclophilin A and mediate its specific incorporation into the virion (12, 39). The NC domain of Pr55^{gag}, which resides C terminal to the CA domain, is involved in the selective packaging of the viral genomic RNA dimer (20). The C-terminal p6 domain mediates the virion association of the accessory viral protein Vpr and facilitates the final release of budded particles from the cell surface (16, 24, 27, 31).

The MA protein, which is derived from the N terminus of Pr55^{gag}, forms a protein shell directly underneath the lipid envelope of the virion (14). The MA protein contains a stretch of basic residues which contributes to the ability of HIV-1 to replicate within growth-arrested cells (3, 40). Additional determinants which are crucial during the early steps of the virus life cycle were identified near the C terminus of the MA domain (46). During translation of the *gag* gene, the MA domain is modified by the N-terminal attachment of myristic acid, which stabilizes the interaction of Pr55^{gag} with the cell membrane and is required for particle assembly (2, 17). Sequences within the MA domain other than those required for myristylation are also critical to target Pr55^{gag} to the cell membrane (36, 49, 50). It has been reported that deletions as well as single amino acid substitutions in the HIV-1 MA domain can cause a redirection of particle assembly to intracellular sites (11, 13, 36). Conversely, a point mutation in the MA domain of Mason-Pfizer monkey virus caused particle assembly to occur at the cell membrane rather than in the cytoplasm, as is usual for type D retroviruses (34). These results suggest that the determinants which target retroviral Gag polyproteins to the viral assembly site are primarily located within the MA domains.

Another aspect of HIV-1 morphogenesis in which the MA domain has an essential role is the incorporation of the Env protein spikes into the budding virion (9, 47). The HIV-1 Env

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protein spikes consist of an oligomeric complex formed by the surface glycoprotein, gp120, and the transmembrane glycoprotein, gp41 (21). The gp120 and gp41 glycoproteins are derived from a common precursor (gp160), which is cleaved by a cellular protease during its transport through the secretory pathway (21). Although viral surface glycoproteins are not required for retroviral particle formation, their incorporation is essential for the formation of infectious virions (21). While the viral glycoproteins appear to be preferentially incorporated, the mechanism that directs them into the nascent particle remains poorly understood.

In the case of HIV-1, we and others have shown that the association of the Env protein complex with viral particles is remarkably sensitive to alterations in the MA domain of Pr55^{gag}. We previously reported that 10 of 12 small in-frame deletion or substitution mutations in the HIV-1 MA domain prevented Env protein incorporation (9), a finding consistent with the results of Yu et al. (47). In another study, the deletion of about 80% of the HIV-1 MA coding region had only about a twofold effect on the virion association of HIV-1 Env proteins (42), suggesting that smaller mutations which alter rather than essentially remove the MA domain may be more disruptive. The large deletion also had little effect on the incorporation of the Env proteins of amphotropic murine leukemia virus (A-MLV); however, the infectivity of the MA mutant particles was dramatically increased upon pseudotyping with A-MLV Env protein (42).

In this report, we show that the effects of alterations in the HIV-1 MA domain on Env protein incorporation are related to the length of the cytoplasmic tail of the transmembrane glycoprotein. Alterations in the HIV-1 MA domain that prevented the incorporation of the HIV-1 Env protein complex still allowed the efficient incorporation of the A-MLV Env protein complex, which has a much shorter cytoplasmic domain. Moreover, the incorporation of HIV-1 Env protein and infectivity of MA mutant particles could be restored by a second-site mutation that resulted in the truncation of the cytoplasmic tail of gp41. These results show that sequences within the long cytoplasmic tail of the HIV-1 transmembrane protein were responsible for the exclusion of the wild-type HIV-1 Env protein complex from MA mutant particles.

MATERIALS AND METHODS

Proviral DNA constructs. The parental proviral construct used in this study, HXBH10, is a *vpu*⁺ variant of the infectious HXBc2 proviral clone of HIV-1 (38). HXBH10-*gag*⁻ is a variant of HXBH10 that is unable to express Pr55^{gag} because of a premature termination codon in place of codon 8 of the *gag* gene and an additional frameshift mutation in the CA coding region (9). The envelope-deficient provirus HXBH10-*env*⁻fs has the *env* initiation codon replaced by ACG and also harbors a frameshift mutation at a *Kpn*I site (nucleotide [nt] 6346) in the 5' portion of the *env* gene (9). The MA mutant proviruses LS8,9→SR, Δ16-18, WA36,37→SR, Δ41-43, and Δ77-80 are isogenic to HXBH10, except for the presence of the indicated missense or in-frame deletion mutations in the MA coding region (9). In each of these constructs, a *Sal*I-*Nhe*I fragment (nt 5785 to 7264) was replaced by the corresponding fragment from HXBH10-*env*⁻fs to obtain the envelope-deficient MA mutants LS8,9→SR/*env*⁻fs, Δ16-18/*env*⁻fs, WA36,37→SR/*env*⁻fs, Δ41-43/*env*⁻fs, and Δ77-80/*env*⁻fs.

The HXBH10ΔenvCAT proviral construct is a derivative of HXBH10 with a deletion in the *env* gene and a bacterial chloramphenicol acetyltransferase (CAT) gene in place of the *nef* gene (39). The pHXBH10ΔenvCAT construct was obtained by replacing nt 5372 to 5934 of the HIV-1_{HXB2}-derived sequence in pHXBΔenvCAT (18) with the corresponding sequence from HIV-1_{BH10}, thereby introducing a functional *vpu* gene. The LS8,9→SR/ΔenvCAT and Δ16-18/ΔenvCAT constructs are identical to HXBH10ΔenvCAT except for the presence of additional mutations in the MA coding region. These constructs were obtained by replacing the segment of HXBH10ΔenvCAT between the unique *Bss*HI and *Apa*I sites (nt 710 to 2009) with the corresponding segments from the MA mutants LS8,9→SR and Δ16-18.

The HXBH10ΔCT mutant has codon 713 of the *env* gene (TCA) replaced by a premature termination codon (TAA). The premature termination codon was

generated by site-directed mutagenesis using single-stranded DNA from pKS⁺EX3.1 as a template as described previously (15). An *Nhe*I-*Bam*HI fragment (nt 7259 to 8479) covering the mutated region was then reintroduced into the parental HXBH10 construct and into the mutant proviruses LS8,9→SR and Δ16-18, generating HXBH10ΔCT, LS8,9→SR/ΔCT, and Δ16-18/ΔCT.

Cell culture, transfection, and virus transmission. HeLa cells were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum. The human T-lymphoid cell lines C8166 and MT-4 were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. HeLa cells (10⁶) were seeded into 80-cm² tissue culture flasks 24 h prior to transfection. The cells were transfected by a calcium phosphate precipitation technique (5). To measure the infectivity of pseudotyped particles, equivalent reverse transcriptase units (2 × 10⁴ cpm) of filtered supernatants derived from HeLa cells cotransfected with envelope-deficient proviral plasmids and the A-MLV *env* expression vector SV-A-MLV-*env* (26) were added to C8166 cells (10⁶ cells in 4 ml of medium). The cells were incubated for 72 h, lysed, and assayed for CAT activity as previously described (18). To determine the ability of virions to initiate a productive infection, 10⁶ MT-4 cells were exposed for 12 h to supernatants from HeLa cells transfected with proviral constructs. The supernatants contained 2 × 10⁴ cpm of reverse transcriptase activity. Viral replication was monitored daily by measuring particle-associated reverse transcriptase activity in culture supernatants as described previously (6).

Viral protein analysis. Starting 48 h posttransfection, HeLa cell cultures were metabolically labeled for 12 h with [³⁵S]cysteine (50 μCi/ml). Labeled cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (140 mM NaCl, 8 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.05% sodium dodecyl sulfate [SDS]). Virions released into the supernatant were pelleted through a 20% sucrose cushion (in phosphate-buffered saline) for 90 min at 4°C and 27,000 rpm in a Beckman SW41 rotor. Pelleted virions were lysed in RIPA buffer, and viral proteins were analyzed either directly by electrophoresis through SDS-12% polyacrylamide gels or by immunoprecipitation prior to electrophoresis. HIV-1-encoded proteins were immunoprecipitated with serum from an individual infected with HIV-1. A-MLV Env protein was immunoprecipitated with a goat anti-MLV serum (Quality Biotech, Camden, N.J.).

RESULTS

Efficient incorporation of heterologous Env proteins into HIV-1 MA mutants defective for incorporation of autologous Env proteins. We reported previously that the HIV-1 capsid-Env glycoprotein interaction is very sensitive to alterations in the MA domain of the Gag polyprotein (9). An analysis of 12 HIV-1 mutants with small deletions or missense mutations throughout the MA coding region revealed that all but two alterations near the C terminus of the MA domain prevented the virion association of the viral Env proteins (9). Since HIV-1 accepts the Env proteins of widely divergent retroviruses (23, 26, 28, 37), we examined whether the integrity of the HIV-1 MA domain is also important for pseudotyping of HIV-1 particles with heterologous Env proteins. To this end, we used variants of the replication-competent HIV-1 proviral clone HXBH10 that encode either wild-type or mutant MA proteins and that contain *env* gene mutations that preclude the synthesis of the HIV-1 Env protein precursor.

The *env*-deficient HIV-1 proviral clones were transfected into HeLa cells together with an expressor construct for the Env proteins of the widely divergent A-MLV. To examine whether A-MLV Env proteins expressed in *trans* were incorporated into HIV-1 particles, [³⁵S]cysteine-labeled particulate material released from the transfected HeLa cells was pelleted through 20% sucrose and solubilized in RIPA buffer. Particle-associated viral proteins were analyzed both directly by SDS-polyacrylamide gel electrophoresis (PAGE) and by immunoprecipitation prior to electrophoresis, using a goat serum that recognizes the A-MLV surface glycoprotein gp70.

Significant amounts of A-MLV gp70 were detected in wild-type HIV-1 particle preparations (Fig. 1, lane 3), in agreement with previous reports demonstrating that HIV-1 can be pseudotyped by the A-MLV Env proteins. The appearance of the gp70 band was dependent on the coexpression of HIV-1 Gag protein (Fig. 1, lane 2), confirming that the pelletable gp70 was particle associated. Remarkably, alterations in the HIV-1 MA domain that prevented the incorporation of the

FIG. 1. Incorporation of A-MLV Env protein into wild-type (WT) and mutant HIV-1 particles. HeLa cells were transfected with HXBH10-*env*⁻fs (lane 1) or cotransfected with the A-MLV Env expressor SV-A-MLV-*env* (26) and either HXBH10-*gag*⁻ (lane 2), HXBH10-*env*⁻fs (lane 3), LS8,9→SR/*env*⁻fs (lane 4), Δ16-18/*env*⁻fs (lane 5), WA36,37→SR/*env*⁻fs (lane 6), Δ41-43/*env*⁻fs (lane 7), or Δ77-80/*env*⁻fs (lane 8). [³⁵S]cysteine-labeled particulate material released into the supernatants was pelleted through 20% sucrose and disrupted in RIPA buffer. Aliquots were either analyzed directly by SDS-PAGE (top) or immunoprecipitated with goat anti-MLV serum prior to SDS-PAGE (bottom). IN, integrase.

autologous Env protein complex (9) did not reduce the incorporation of A-MLV Env proteins (Fig. 1, lanes 4 to 8). On the contrary, the ability of HIV-1 particles to incorporate A-MLV Env proteins appeared to increase as a consequence of the alterations in the MA domain, as evident from an increase in the ratio between gp70 and HIV-1 Gag products in purified virion preparations. Thus, the requirements for the incorporation of autologous and heterologous Env proteins into HIV-1 particles differ considerably.

Replication-defective HIV-1 MA mutants are infectious when pseudotyped with A-MLV Env proteins. The results described above demonstrated that A-MLV Env proteins were efficiently incorporated into HIV-1 MA mutant particles, which, as shown previously, are unable to incorporate HIV-1 Env proteins (9). To determine whether the A-MLV Env proteins were incorporated in a functional manner, the infectivities of wild-type and MA mutant HIV-1 particles pseudotyped with A-MLV Env proteins were compared by using a previously described *trans*-complementation assay (18). To this end, the LS8,9→SR and Δ16-18 mutations were introduced into the HXBH10Δ*env*CAT proviral construct, an *env*-deficient variant of HXBH10 that carries a bacterial CAT gene in place of the *nef* gene. The proviral constructs were cotransfected into HeLa cells together with the A-MLV Env expressor construct. Reverse transcriptase activity in the culture supernatants was de-

FIG. 2. Infectivities of wild-type (WT) and mutant HIV-1 particles pseudotyped with A-MLV Env proteins. HeLa cells were transfected with HXBH10Δ*env*CAT (lane 1) or cotransfected with SV-A-MLV-*env* and either HXBH10Δ*env*CAT (lane 2), LS8,9→SR/Δ*env*CAT (lane 3), or Δ16-18/Δ*env*CAT (lane 4). Supernatants from the transfected cells were normalized to equivalent reverse transcriptase units and incubated with C8166 T-lymphoid cells, and CAT activities in the target cells were compared. c, [¹⁴C]-labeled chloramphenicol; ac, acetylated derivatives.

termined 60 h posttransfection, and equivalent reverse transcriptase units were added to C8166 target cells. To determine the ability of the pseudotyped viral particles to proceed through a single round of virus transmission, the CAT activity in the target cells was measured after a 72-h incubation period.

As shown in Fig. 2, the parental HXBH10Δ*env*CAT construct gave a strong positive signal that depended on the co-expression of the A-MLV Env proteins. For both MA mutant constructs, the CAT activity in the target cells was only slightly lower than that obtained with the parental construct. These results indicate that the previously observed inability of full-length HIV-1 proviruses that carry the LS8,9→SR or Δ16-18 mutation to initiate a productive infection (9) was largely due to the effect of the mutations on HIV-1 Env protein incorporation.

Truncation of the cytoplasmic tail of gp41 restores incorporation of HIV-1 Env proteins into MA mutant particles. The data presented above demonstrated that the A-MLV Env proteins are efficiently and functionally incorporated into the LS8,9→SR and Δ16-18 MA mutant particles, which fail to incorporate the HIV-1 Env proteins (9). These observations raised the possibility that the LS8,9→SR and Δ16-18 MA mutants were not defective for Env protein incorporation per se but were unable to accommodate the long cytoplasmic tail of the HIV-1 transmembrane glycoprotein. Lentiviruses typically have transmembrane proteins with long cytoplasmic tails of up to 220 amino acids. In the case of HIV-1, the cytoplasmic tail of the transmembrane protein comprises about 150 amino acids. By comparison, the transmembrane proteins of oncoviruses such as A-MLV have much shorter cytoplasmic tails, which do not exceed 50 amino acids (21).

To explore whether the long cytoplasmic tail of the HIV-1 transmembrane protein restricts its ability to fit into the altered MA structures, we examined whether the incorporation defect could be corrected by a truncation that largely removed the cytoplasmic tail. A premature termination codon was introduced into the parental HXBH10 proviral clone in place of codon 713 of the *env* gene, yielding the HXBH10ΔCT mutant.

FIG. 3. Cytoplasmic tail truncation restores HIV-1 Env protein incorporation into MA mutant particles. HeLa cells were transfected with the parental HXBH10 proviral DNA (wild type [wt]; lane 1) or the mutant proviral constructs HXBH10 Δ CT (lane 2), LS8,9 $\rightarrow$ SR (lane 3), LS8,9 $\rightarrow$ SR/ Δ CT (lane 4), Δ 16-18 (lane 5), Δ 16-18/ Δ CT (lane 6), and HXBH10-*gag*⁻ (lane 7). [³⁵S]cysteine-labeled particulate material released into the supernatants was pelleted through 20% sucrose and disrupted in RIPA buffer. Viral proteins were immunoprecipitated with serum from an individual infected with HIV-1 and separated by SDS-PAGE. IN, integrase; RT, reverse transcriptase.

The mutation was expected to result in an Env protein with a truncated cytoplasmic tail consisting of only seven amino acids extending from the predicted inner membrane boundary. The Δ CT mutation was also introduced into proviral clones that carry the LS8,9 $\rightarrow$ SR or Δ 16-18 mutation but are otherwise identical to HXBH10. It was shown previously that the presence of the Δ CT mutation in a replication-competent molecular clone of HIV-1 results in only a marginal delay in virus replication in MT-4 cells (43), demonstrating that the truncated Env proteins can be functionally incorporated into virions.

Transfection of the HXBH10, LS8,9 $\rightarrow$ SR, and Δ 16-18 proviral constructs, or of the corresponding Δ CT mutants, into HeLa cells and immunoprecipitation of metabolically labeled lysates showed that the full-length and truncated Env products were expressed with comparable efficiencies (data not shown). The abilities of the full-length and truncated Env proteins to associate with wild-type and MA mutant particles are compared in Fig. 3. Viral particles released from the transfected HeLa cells were partially purified by ultracentrifugation through 20% sucrose, and pelleted material was lysed in RIPA buffer and analyzed by immunoprecipitation with HIV-positive patient serum. The presence of the Δ CT mutation in the parental HXBH10 construct did not alter the ratio between surface glycoprotein gp120 and Gag proteins in virion preparations (Fig. 3, lanes 1 and 2). Thus, the C-terminal truncation of gp41 by 144 amino acids did not significantly affect the levels of HIV-1 Env proteins that were incorporated into wild-type par-

ticles, consistent with previous results (43). However, the levels of uncleaved Env precursor in virion preparations were somewhat elevated as a consequence of the truncation.

As reported previously (9), particle production was efficient in the presence of the LS8,9 $\rightarrow$ SR mutation and even elevated by the Δ 16-18 mutation, yet hardly any gp120 could be detected in the mutant particle preparations when a wild-type *env* gene was expressed (Fig. 3, lanes 3 and 5). In striking contrast, large amounts of gp120 were found associated with particles produced by the constructs that contained the LS8,9 $\rightarrow$ SR or Δ 16-18 mutation in combination with the Δ CT mutation (Fig. 3, lanes 4 and 6). Remarkably, in the latter case, the amounts of gp120 relative to those of CA protein associated with the mutant particles exceeded even the amount found in wild-type HIV-1 virions. Thus, the defect of the MA mutants in Env protein incorporation could be fully compensated for by the truncation of gp41.

Truncation of gp41 in MA mutants restores virus replication in MT-4 cells. Since the Δ CT mutation suppressed the defect in Env protein incorporation caused by the LS8,9 $\rightarrow$ SR and Δ 16-18 mutations, we explored the possibility that the replication defect caused by the MA mutations was also suppressed. MT-4 cells were exposed to equivalent amounts of virions produced in HeLa cells, and virus replication was monitored daily by measuring particle-associated reverse transcriptase activity in the culture supernatants. As reported previously (43), virus replication in MT-4 cells was only moderately affected by the removal of the 144 C-terminal amino acids of gp41 (Fig. 4). Virus replication could not be detected during a 36-day observation period after exposure of MT-4 cells to virions produced by the LS8,9 $\rightarrow$ SR and Δ 16-18 mutants (Fig. 4 and data not shown). In striking contrast, the LS8,9 $\rightarrow$ SR and Δ 16-18 mutations had no significant effect on virus replication when present in the context of the Δ CT mutant (Fig. 4). Thus, the lethal effect of the mutations in the MA domain could be compensated for by a secondary mutation in the *env* gene that restored Env protein incorporation.

DISCUSSION

This study documents the differential requirements for the incorporation of Env glycoproteins with long and short cytoplasmic tails into HIV-1 virions. We and others recently reported that the virion incorporation of the HIV-1 Env protein complex is critically dependent on the integrity of the MA domain of the HIV-1 Gag polypeptide (9, 47). Here we show that sequences within the HIV-1 MA domain, which are required for the virion association of the autologous Env proteins, are dispensable for the incorporation of the Env proteins of A-MLV, a widely divergent retrovirus. One important difference between lentiviruses such as HIV-1 and other retroviruses is the unusually long cytoplasmic tail of lentiviral transmembrane glycoproteins. Therefore, the efficient incorporation of the Env protein complex of A-MLV, but not that of HIV-1, into HIV-1 MA mutants led us to explore the possibility that the altered MA domains could accommodate only short cytoplasmic domains. As predicted by this hypothesis, HIV-1 Env proteins were efficiently incorporated into MA mutant particles following truncation of the cytoplasmic domain of the transmembrane protein.

It is noteworthy that the levels of HIV-1 Env protein incorporated into MA mutant particles upon truncation of the transmembrane protein exceeded the levels incorporated into wild-type particles, both in the presence and in the absence of cytoplasmic tail sequences. Similarly, the levels of A-MLV Env protein associated with MA mutant particles were higher than

FIG. 4. Env cytoplasmic tail truncation compensates for the replication defect of HIV-1 MA mutants. HeLa cells were transfected with the parental HXBH10 proviral DNA (wild type [wt]; open circles) or the mutant proviral constructs HXBH10ΔCT (filled circles), Δ16-18 (open triangles), Δ16-18/ΔCT (filled triangles), LS8,9→SR (open squares), and LS8,9→SR/ΔCT (filled squares). MT-4 T-lymphoid cells were then exposed to supernatants from the transfected HeLa cultures normalized to equivalent reverse transcriptase (RT) units. Viral replication in the target cells was monitored by measuring particle-associated reverse transcriptase activity in culture supernatants.

those associated with wild-type HIV-1 particles. It is possible that the alterations in the MA domain affected determinants that control the normal arrangement of Env protein spikes on the surface of the virion. However, since MA mutants pseudotyped with A-MLV or C-terminally truncated HIV-1 Env protein were infectious, at least a fraction of the Env protein must have been incorporated in a functional manner.

Some studies have suggested that alterations in the cytoplasmic domain of the HIV-1 transmembrane protein impair glycoprotein incorporation (10, 48). Combined with the observation that alterations in the HIV-1 MA domain can completely prevent the virion association of the authentic HIV-1 Env protein complex (9, 47), these results appeared to support a model of Env protein incorporation that relies on a specific interaction with a viral structural component. Evidence for a specific interaction between HIV-1 Gag and Env proteins was also provided by the finding that the Env protein determines the site of virion assembly and release in polarized epithelial cells (30). On the other hand, it was reported that the cytoplasmic domain of the HIV-1 transmembrane protein, the portion of the Env protein complex most likely to interact with a structural component, can be deleted without significant effects on Env protein incorporation (35, 43). Similarly, the cytoplasmic tails of the transmembrane proteins of simian immunodeficiency virus SIV_{mac} and Rous sarcoma virus were shown to be dispensable for glycoprotein incorporation (22, 32). The results

of the present study confirm that the cytoplasmic tail of the HIV-1 transmembrane protein is also dispensable for glycoprotein incorporation. Discrepancies between different studies may be attributable to differences in the exact changes made. Our results indicate that the HIV-1 MA domain has evolved to accommodate the long cytoplasmic tail of the HIV-1 transmembrane protein. Therefore, it is conceivable that the presence of a modified cytoplasmic tail can interfere with glycoprotein incorporation, although the removal of the cytoplasmic tail does not.

The lack of a requirement for a specific recognition of cytoplasmic tail sequences is also consistent with the well-documented ability of retroviruses to incorporate heterologous, apparently unrelated viral Env proteins (4, 7, 23, 25, 26, 28, 37, 45). In the case of HIV-1, it was demonstrated that the Env proteins of retroviruses as divergent as human T-cell leukemia virus and MLVs can be functionally incorporated, resulting in the formation of infectious pseudotype viruses (23, 26, 28, 37). It was also reported that HIV-1 virions can incorporate non-retroviral Env proteins as well as some cellular glycoproteins (1, 51). It is possible that HIV-1 can accept other Env proteins and cellular membrane proteins with relative ease because its MA domain has evolved to accommodate an unusually large Env protein complex. Interestingly, although HIV-1 can be pseudotyped with the Env proteins of widely divergent viruses, our results suggest that the reciprocal incorporation of HIV-1 Env protein into other retroviral particles may be less efficient. We previously reported that HIV-1 Env protein is not incorporated into visna virus particles (9). However, efficient incorporation could be achieved by replacing the MA domain of the visna virus Gag polyprotein by the MA domain of HIV-1 (9). It is possible that the long cytoplasmic tail of the HIV-1 Env protein complex cannot be accommodated even by the MA domain of a lentivirus that encodes a cytoplasmic tail of similar size. An incompatibility of the Env cytoplasmic tail of SIV_{mac} and the MA domain of HIV-1 also may be the basis for the observation that a cytoplasmic tail truncation significantly enhanced the incorporation of SIV_{mac} Env proteins into HIV-1 particles (52).

In an attempt to explain the preferential incorporation of viral glycoproteins in the absence of a requirement for cytoplasmic tail sequences, an active exclusion/passive inclusion mechanism was recently suggested (20). This model postulates that cellular glycoproteins are excluded from the site of virus assembly as a consequence of interactions with cytoplasmic components, while viral glycoproteins which lack such interactions can freely diffuse into the assembly site. To reconcile this model with the apparent requirement for a specific interaction between Env and Gag components in the case of HIV-1, it was proposed that some viral glycoproteins may be excluded similarly to host cell proteins, unless an interaction with the Gag precursor occurs (20). The model could be adapted to explain the differential effects of alterations in the HIV-1 MA domain on the incorporation of Env proteins with long versus short cytoplasmic domains. It may be that the long cytoplasmic domain of HIV-1, in contrast to the short cytoplasmic domain of A-MLV, contains sequences that interact with cellular factors. In the absence of a competing interaction with a functional HIV-1 MA domain, this could lead to the exclusion of full-length HIV-1 Env protein from the nascent virion. It might then be predicted that the removal of the cytoplasmic tail restores glycoprotein incorporation into MA mutant particles, since it would eliminate the need for an interaction with the MA domain. However, it is also possible that the selective exclusion of the full-length HIV-1 Env protein is due to an inability of the altered MA domains to sterically accommodate

the long cytoplasmic domain. One way to distinguish between these possibilities may be the analysis of second-site revertants, if they could be obtained.

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