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Cleavage of the Murine Leukemia Virus Transmembrane Env Protein by Human Immunodeficiency Virus Type 1 Protease: Transdominant Inhibition by Matrix Mutations

ROSEMARY E. KIERNAN† AND ERIC O. FREED*

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-0460

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We have identified mutations in the human immunodeficiency virus type 1 (HIV-1) matrix protein (MA) which block infectivity of virions pseudotyped with murine leukemia virus (MuLV) envelope (Env) glycoproteins without affecting infectivity conferred by HIV-1 Env or vesicular stomatitis virus G glycoproteins. This inhibition is very potent and displays a strong transdominant effect; infectivity is reduced more than 100-fold when wild-type and mutant molecular clones are cotransfected at a 1:1 ratio. This phenomenon is observed with both ecotropic and amphotropic MuLV Env. The MA mutations do not affect the incorporation of MuLV Env into virions. We demonstrate that in HIV-1 virions pseudotyped with MuLV Env, the HIV-1 protease (PR) efficiently catalyzes the cleavage of the p15(E) transmembrane (TM) protein to p12(E). Immunoprecipitation analysis of pseudotyped virions reveals that the mutant MA blocks this HIV-1 PR-mediated cleavage of MuLV TM. Furthermore, the transdominant inhibition exerted by the mutant MA on wild-type infectivity correlates with the relative level of p15(E) cleavage. Consistent with the hypothesis that abrogation of infectivity imposed by the mutant MA is due to inhibition of p15(E) cleavage, mutant virions are significantly more infectious when pseudotyped with a truncated p12(E) form of MuLV Env. These results indicate that HIV-1 Gag sequences can influence the viral PR-mediated processing of the MuLV TM Env protein p15(E). These findings have implications for the development of HIV-1-based retroviral vectors pseudotyped with MuLV Env, since p15(E) cleavage is essential for activating membrane fusion and virus infectivity.

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The human immunodeficiency virus type 1 (HIV-1) matrix protein (MA) performs several important roles in the virus life cycle (for a review, see reference 13). MA is critical to the targeting of the Gag precursor to the plasma membrane. Mutation of the N-terminal glycine, which serves as a myristic acid acceptor site, generally abolishes virus assembly (3, 21, 24, 47), and substitutions and deletions within a highly basic domain near the MA N terminus disrupt proper Gag targeting and virus assembly (15, 69, 70). Consistent with a role for MA in Gag trafficking and plasma membrane targeting, single amino acid changes between MA residues 84 and 88 redirect virus assembly to a cytoplasmic compartment (21). Large deletions in MA also retarget significant amounts of assembly to the cytoplasm (12, 22, 53). MA is also required for efficient incorporation of full-length HIV-1 envelope (Env) glycoproteins into virions. Deletions and multiple amino acid substitutions throughout MA impair Env incorporation (9, 68), and single amino acid substitutions near the amino terminus of MA can abrogate Env incorporation (16, 19, 45). Several reports have also implicated HIV-1 MA in an early step in the virus life cycle prior to the completion of reverse transcription (4, 52, 67). We recently reported that mutation of the highly conserved Leu at residue 20 impairs an early step in virus infection, potentially by destabilizing the viral core complex early postentry (36).

Env glycoproteins are incorporated into virions during the budding of virus particles from the plasma membrane of infected cells. Due to their unusually long cytoplasmic domains, HIV-1 Env glycoproteins are generally not incorporated into nonlentiviral particles. In contrast, heterologous Env glycoproteins can be readily incorporated into HIV-1 virions. For example, the Env glycoproteins of murine leukemia virus (MuLV) and human T-cell leukemia virus type 1, the vesicular stomatitis virus G glycoprotein (VSV-G), and the gD glycoprotein of pseudorabies virus can be incorporated into HIV-1 particles (23, 38, 40, 60); in many cases, the pseudotyped virions are infectious in biological assays. The pseudotyping of HIV-1 virions with heterologous Env glycoproteins has been exploited extensively in the development of HIV-1-based retroviral vectors (7, 27, 38, 46, 55).

Retroviral Env complexes are composed of a surface glycoprotein (SU), which is responsible for receptor binding, and a transmembrane protein (TM), which is involved in membrane fusion (for a review, see reference 18). In several retroviral systems, the cytoplasmic domain of the TM Env glycoprotein is cleaved during or after virus budding by the viral protease (PR). This late cleavage event, which occurs with MuLV (26, 28, 33, 61), equine infectious anemia virus (56), and Mason-Pfizer monkey virus (M-PMV) (1, 2, 59), has been reported to activate the fusion potential of the Env glycoprotein complex. Mutations which remove the TM cytoplasmic tail C terminal to the cleavage site can lead to enhanced fusogenicity, resulting in increased cell-to-cell fusion in Env-expressing cells (1, 50, 54, 59). In the case of MuLV, in which this phenomenon has been the most extensively studied, the 16 C-terminal residues of the TM are removed, converting the 15-kDa TM protein [p15(E)] to a 12-kDa protein [p12(E)] and a 16-residue peptide [p2(E) or R]. Mutations which prevent the removal of the MuLV R peptide severely attenuate virus infectivity (50, 54). It has been proposed that the C termini of these retroviral TM proteins suppress membrane fusion until virus release has been com-

* Corresponding author. Mailing address: Bldg. 4, Rm. 307, NIAID, NIH, Bethesda, MD 20892. Phone: (301) 402-3215. Fax: (301) 402-0226. E-mail: EFreed@nih.gov.

†Present address: IGH, CNRS-UPR 1142, Montpellier, France.

pleted, thereby limiting Env-induced cytotoxicity (54, 65). Interestingly, mutations which shorten the cytoplasmic tail of lentiviral TM Env glycoproteins have also been reported to increase fusogenicity (10, 11, 16, 32, 44, 56, 57, 63, 71).

Single amino acid substitutions in HIV-1 MA can block HIV-1 Env incorporation (16, 19, 45). Therefore, in our ongoing characterization of HIV-1 MA function, we have frequently used MuLV Env pseudotypes to examine the phenotypes of MA mutations. In this report, we describe an HIV-1 MA mutant, 20LK/73EK/82AT, which cannot be effectively pseudotyped with MuLV Env. The virus was initially obtained as a cell culture revertant (35) of a previously described mutant, 20LK (36), and replicated with near-wild-type (wt) kinetics in H9 cells. In single-cycle assays, 20LK/73EK/82AT displayed near-wt infectivity when pseudotyped with either HIV-1 Env or VSV-G. In contrast, the mutant virus was almost completely noninfectious when pseudotyped with MuLV Env. In addition, the mutant MA transdominantly inhibited wt infectivity in coexpression experiments. We determined that the mutations did not affect incorporation of MuLV Env into virions but almost completely abolished cleavage of the MuLV TM protein from p15(E) to p12(E). Furthermore, transdominant inhibition of infectivity could be correlated with levels of p15(E) cleavage. Infectivity of the mutant MA virus was largely rescued by pseudotyping with a truncated p12(E) form of MuLV Env.

MATERIALS AND METHODS

Plasmids, cells, and transfections. HIV-1 MA mutations were introduced into an *env*-negative derivative of pNL4-3, pNL4-3KFS (14, 19), or a luciferase-expressing, *env*-negative pNL4-3 derivative, pNLuc (36). The *env*-negative clones were pseudotyped as indicated with HIV-1 Env, VSV-G, amphotropic MuLV (ampho-MuLV) or ecotropic MuLV (eco-MuLV) Env, or a truncated version of eco-MuLV Env. The following Env expression vectors were used: for HIV-1 Env, pHenv (20); for ampho-MuLV Env, pSVAMLVenv (38) and pCAE (50); for eco-MuLV Env, pCEE (50); for truncated [p12(E) form] eco-MuLV Env, pCEETR (50); and for VSV-G, pHCMV-G (66). The HIV-1 PR-defective derivative of pNL4-3, which contains a mutation in the PR active site, has been described previously (30). Virus stocks were prepared by cotransfection of HeLa or 293T cells (17, 36) with pNL4-3 derivatives and Env expression vectors as indicated.

Single-cycle infectivity assays and PCR analysis. Relative infectivity of pseudotyped virions was measured by luciferase and MAGI assays. For luciferase assays, pseudotyped virions were produced from 293T cells by cotransfecting wt (pNLuc) or mutant (pNLuc/20LK/73EK/82AT) luciferase-expressing clones with Env-expressing vectors. The virus stocks were normalized for reverse transcriptase (RT) activity (17) and were used to infect H9 cells or NIH 3T3 cells. Relative infectivity was measured by luciferase assay as described previously (36). Relative infectivities of pNLuc pseudotyped with the different Env glycoproteins (measured in arbitrary light units) were as follows: HIV-1 Env in H9 cells, 1; ampho-MuLV Env in H9 cells, approximately 200; eco-MuLV Env in NIH 3T3 cells, approximately 120; truncated eco-MuLV Env in NIH 3T3 cells, approximately 100; and VSV-G in H9 cells, approximately 1,200. For analysis by MAGI assay (37), pseudotyped virions were produced from HeLa cells by cotransfecting wt (pNL4-3KFS) or mutant (pNL4-3KFS/20LK/73EK/82AT) *env*-negative molecular clones with pSVAMLVenv. For PCR analysis, HeLa cells were cotransfected with wt (pNL4-3KFS) or mutant (pNL4-3KFS/20LK/73EK/82AT) *env*-negative molecular clones and Env-expressing vectors as indicated. Virus stocks were normalized for RT activity and were used to infect H9 or NIH 3T3 cells. At 18 h postinfection, cells were lysed and analyzed by PCR using primers specific for HIV-1 long terminal repeat (LTR) DNA (36) or for human, mouse, and rat α-tubulin DNA (Clontech). The amplified DNA was electrophoresed on agarose gels and subjected to Southern blotting with HIV-1 LTR or α-tubulin-specific probes. PCR and Southern blotting were performed as described previously (36). For detection of α-tubulin DNA, the PCR product amplified from the positive control provided (Clontech) was ^{32}P labeled by random priming and used as a probe.

To assess the effect of mutant MA on infectivity in *trans*, wt and mutant molecular clones were cotransfected into 293T cells along with pSVAMLVenv DNA at various DNA ratios. The amount of molecular clone DNA was held constant at 5 μg. Virus stocks were harvested, normalized for RT activity, and used to infect H9 cells.

Immunoprecipitations. Methods used for metabolically labeling transfected HeLa cells, pelleting virions in an ultracentrifuge, preparing cell and virion lysates, and immunoprecipitating viral proteins have been described previously (17, 64). Virus-specific proteins were immunoprecipitated with either a mixture of AIDS patient sera (human HIV immunoglobulin; obtained from the NIH AIDS Research and Reference Reagent Program) and anti-Rauscher MuLV gp70 antiserum (Quality Biotech, Inc.) or anti-Rauscher MuLV gp70 antiserum alone, as indicated. Because gp70 and p15(E) are covalently linked by disulfide bonds, p15(E) can be detected readily with anti-gp70 antiserum (48, 49).

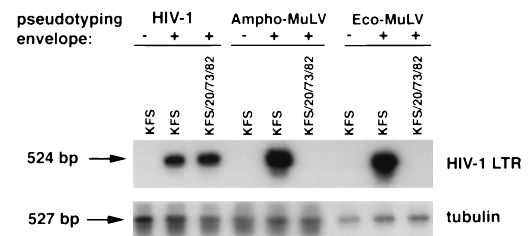

FIG. 1. PCR amplification of viral DNA following infection by wt and MA mutant pseudotypes. The *env*-negative HIV-1 molecular clone pNL4-3KFS (KFS) and the 20LK/73EK/82AT MA mutant derivative (KFS/20/73/82) were pseudotyped with vectors expressing the indicated Env glycoproteins. Eighteen hours after infection, H9 (for HIV-1 and ampho-MuLV Env) or NIH 3T3 (for eco-MuLV Env) cells were lysed and viral DNA was amplified by PCR (Materials and Methods). As a PCR control, cell lysates were also amplified with primers specific for α-tubulin. Nonpseudotyped KFS served as a negative control. The amplified DNA was electrophoresed and subjected to Southern blotting with HIV-1 LTR- or α-tubulin-specific probes.

RESULTS

Identification of an HIV-1 MA mutant which cannot be effectively pseudotyped by MuLV Env. The HIV-1 MA mutant 20LK/73EK/82AT was initially obtained as a viral revertant of a previously reported MA mutant, 20LK (36), and grows with near-wt replication kinetics in H9 cells (35). In characterizing the mechanism of reversion of this virus, we performed single-cycle infectivity assays by pseudotyping an *env*-negative, luciferase-expressing molecular clone (pNLuc) with different viral Env proteins. When pseudotyped with either HIV-1 Env or VSV-G, 20LK/73EK/82AT showed 75% ± 7% or 64% ± 7%, respectively, of wt infectivity. In striking contrast, when pseudotyped with either ampho-MuLV or eco-MuLV Env, 20LK/73EK/82AT was almost completely noninfectious (0.03% ± 0.01% or 0.05% ± 0.04% of wt activity). The same result was obtained when a different *env*-negative clone (pNL4-3KFS/20LK/73EK/82AT) was pseudotyped with ampho-MuLV Env and assayed by the MAGI infectivity assay (data not shown).

Results obtained by single-cycle infectivity assays were confirmed by PCR analysis of H9 or NIH 3T3 cells infected with HIV-1 or MuLV Env pseudotypes (Fig. 1). Consistent with the near-wt infectivity of 20LK/73EK/82AT in both single-cycle assays and spreading infections, a comparable amount of viral DNA was detected in H9 cells infected with wt and 20LK/73EK/82AT HIV-1 Env pseudotypes. In contrast, no HIV-1-specific DNA could be detected in H9 or NIH 3T3 cells infected with 20LK/73EK/82AT pseudotyped by ampho-MuLV or eco-MuLV Env, respectively.

The 20LK/73EK/82AT HIV-1 MA mutant potently and transdominantly inhibits infectivity conferred by MuLV Env. We next examined whether the 20LK/73EK/82AT mutant would transdominantly interfere with the ability of wt HIV-1 molecular clones to be effectively pseudotyped by MuLV Env. Ampho-MuLV pseudotypes were obtained by cotransfecting wt and 20LK/73EK/82AT MA mutant molecular clones at various ratios of input DNA (10:1, 5:1, 2:1, 1:1, 1:2, 1:5, and 1:10). The resulting virus stocks were normalized for RT activity, and infectivities were analyzed by luciferase assay (Table 1). The

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TABLE 1. Effect of varying the ratio of wt to mutant DNA on the infectivity of ampho-MuLV Env-pseudotyped virions[a]

Molecular clone	wt:mutant DNA	Relative infectivity
pNLuc		
Alone		100
+pNLuc/20LK/73EK/82AT	10:1	33 ± 5
	5:1	19 ± 4
	2:1	4 ± 1
	1:1	0.6 ± 0.1
	1:2	0.2 ± 0.04
	1:5	0.05 ± 0.01
	1:10	0.04 ± 0.01
pNLuc/20LK/73EK/82AT		0.03 ± 0.01

[a] pNLuc and/or pNLuc/20LK/73EK/82AT were cotransfected into 293T cells with the ampho-MuLV Env expression vector pSVAMLVenv. Transfections were performed with a total of 5 μg of molecular clone DNA at the indicated ratios, with 5 μg of pSVAMLVenv. Virus stocks were harvested and normalized for RT activity, and infectivity was quantitated by luciferase assay after infection of H9 cells. Data are averages from at least six independent assays ± standard error.

results demonstrated that the 20LK/73EK/82AT MA mutant exerted a potent transdominant inhibition on wt infectivity when the two were coexpressed. Even at a 10-fold excess of wt DNA, viral infectivity was significantly reduced. As the ratio of wt to mutant DNA decreased, viral infectivity was progressively reduced.

The 20LK/73EK/82AT MA mutant does not affect MuLV Env incorporation into HIV-1 virions. To elucidate the mechanism by which the 20LK/73EK/82AT MA mutant inhibits infectivity in the context of MuLV Env pseudotypes, we first analyzed whether the MA mutations affected incorporation of MuLV Env glycoproteins into virions. HeLa cells, cotransfected with wt pNL4-3KFS or KFS/20LK/73EK/82AT and pSVAMLVenv, were labeled overnight with [^{35}S]Cys. Virions were pelleted in an ultracentrifuge, and cell- and virion-associated proteins were immunoprecipitated with a mixture of AIDS patient serum and anti-MuLV Env antibody. As shown in Fig. 2, the 20LK/73EK/82AT MA mutant did not affect incorporation of the MuLV Env glycoprotein complex into virions (compare the levels of virion-associated gp70 in KFS and KFS/20/73/82 lanes). Cells transfected with the 20LK/73EK/82AT mutant showed reduced levels of Pr55^{Gag} relative to wt-transfected cells, due to an increased rate of Pr55^{Gag} processing induced by the 20LK mutation (35, 36).

HIV-1 PR cleaves MuLV p15(E); this cleavage is blocked in 20LK/73EK/82AT MA mutant virions. Studies conducted in several laboratories have demonstrated that the fusogenic potential of the MuLV Env glycoprotein complex is activated by removal of the 16 C-terminal residues of the TM protein (50, 54). This cleavage, which converts the p15(E) TM protein to the p12(E) form, is performed by MuLV PR (8, 34, 58). We first determined whether the HIV-1 PR could mediate this cleavage reaction in HIV-1 virions pseudotyped with MuLV Env. pNL4-3KFS virions pseudotyped with MuLV Env were compared to those containing a mutation in the HIV-1 PR active site (30). In pNL4-3KFS pseudotypes (Fig. 3, KFS lane), the p12(E) form of the MuLV TM was the predominant species detected. In PR$^-$ virions, however, only the uncleaved p15(E) form of the MuLV TM was observed (Fig. 3, PR$^-$/KFS lane). These results demonstrate that in HIV-1 virions pseudotyped with MuLV Env, the HIV-1 PR efficiently cleaves MuLV TM from p15(E) to p12(E). In contrast to what we

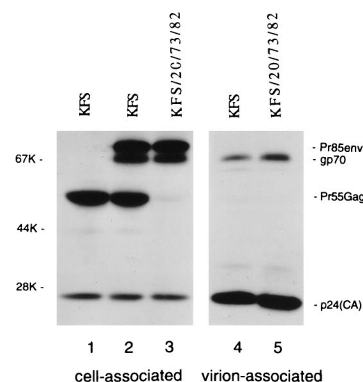

FIG. 2. Incorporation of MuLV Env into wt and MA mutant virions. HeLa cells were cotransfected with the *env*-negative HIV-1 molecular clone pNL4-3KFS (KFS; lanes 1, 2, and 4) or the HIV-1 MA mutant derivative pNL4-3KFS/20LK/73EK/82AT (KFS/20/73/82; lanes 3 and 5) and the ampho-MuLV Env expression vector pSVAMLVenv. Transfected cells were metabolically labeled with [^{35}S]Met; cell- and virion-associated material was then prepared and immunoprecipitated with AIDS patient serum (lane 1) or a mix of AIDS patient serum and anti-MuLV Env antiserum (lanes 2 to 5) (Materials and Methods). Immunoprecipitates were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and proteins were detected by fluorography. Positions of molecular weight markers are shown on the left in kilodaltons; positions of the MuLV Env precursor Pr85env and MuLV SU glycoprotein gp70 and of the HIV-1 Gag precursor Pr55^{Gag} and p24(CA) are indicated on the right.

observed in wt HIV-1 virions pseudotyped with MuLV Env, 20LK/73EK/82AT MA mutant pseudotypes contained almost exclusively the uncleaved p15(E) form of MuLV Env (Fig. 3, KFS/20/73/82 lane). These results indicate that cleavage of p15(E) to p12(E) is markedly impaired by the 20LK/73EK/82AT MA mutations.

As shown in Table 1, the 20LK/73EK/82AT MA mutant inhibited wt infectivity in a markedly transdominant manner. We therefore sought to determine whether this transdominant inhibition of viral infectivity could be correlated with the efficiency of p15(E) cleavage in the pseudotyped virions (Fig. 4). pNL4-3KFS and pNL4-3KFS/20LK/73EK/82AT molecular clones were cotransfected at various DNA ratios (5:1, 2:1, and 1:1) with a fixed amount of MuLV Env expression plasmid. Transfected cells were metabolically labeled with [^{35}S]Met, and virion-associated proteins were immunoprecipitated with an anti-MuLV Env antiserum (Materials and Methods). Consistent with the data presented in Fig. 3, in wt MA pseudotypes, the cleaved p12(E) form of MuLV Env predominated (Fig. 4,

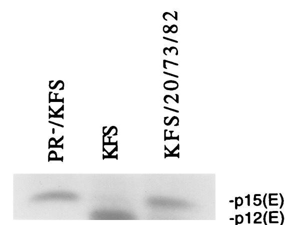

FIG. 3. Immunoprecipitation of MuLV Env from wt, PR$^-$, and MA mutant virions. HeLa cells were cotransfected with the *env*-negative HIV-1 molecular clone pNL4-3KFS (KFS), the HIV-1 MA mutant derivative pNL4-3KFS/20LK/73EK/82AT (KFS/20/73/82), or a derivative of pNL4-3KFS containing a mutation in the HIV-1 PR active site (PR$^-$/KFS) and the ampho-MuLV Env expression vector pSVAMLVenv. Transfected cells were metabolically labeled with [^{35}S]Met, and virion-associated material was prepared and immunoprecipitated with anti-MuLV Env antiserum. Positions of the uncleaved p15(E) and cleaved p12(E) forms of the MuLV TM protein are indicated.

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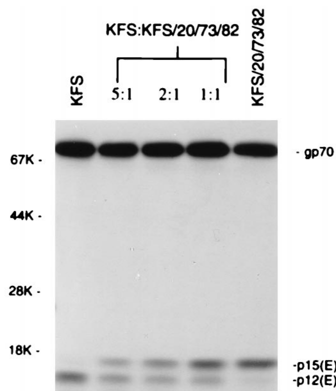

FIG. 4. Immunoprecipitation of MuLV Env from wt and MA mutant virions. HeLa cells were cotransfected with the *env*-negative HIV-1 molecular clone pNL4-3KFS (KFS), the HIV-1 MA mutant derivative pNL4-3KFS/20LK/73EK/82AT (KFS/20/73/82), or various ratios of wt and mutant DNAs (KFS:KFS/20/73/82 ratio indicated) together with the ampho-MuLV Env expression vector pSVAMLenv. Transfected cells were metabolically labeled with [^{35}S]Met, and virion-associated material was prepared and immunoprecipitated with anti-MuLV Env antiserum. Positions of molecular weight markers are shown on the left in kilodaltons; positions of the MuLV SU glycoprotein gp70 and of the uncleaved p15(E) and cleaved p12(E) forms of the MuLV TM protein are indicated on the right.

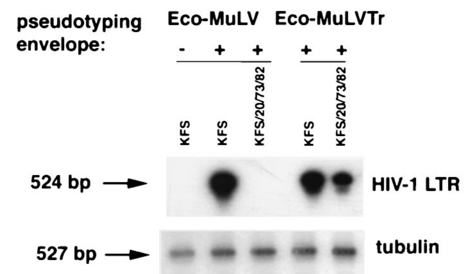

FIG. 5. PCR amplification of viral DNA following infection by wt and MA mutant pseudotypes. The *env*-negative HIV-1 molecular clone pNL4-3KFS (KFS) and the 20LK/73EK/82AT MA mutant derivative (KFS/20/73/82) were pseudotyped with a vector (pCEE) expressing the full-length eco-MuLV Env (Eco-MuLV) or a vector (CEETR) expressing the p12(E) truncated form of MuLV Env (Eco-MuLVTr). Eighteen hours after infection of NIH 3T3 cells, lysates were prepared and viral DNA was amplified by PCR (Materials and Methods). As a PCR control, cell lysates were also amplified with primers specific for α-tubulin. Nonpseudotyped KFS served as a negative control. The amplified DNA was electrophoresed and subjected to Southern blotting with HIV-1 LTR- or α-tubulin-specific probes.

KFS lane). In the 20LK/73EK/82AT MA mutant pseudotypes, p15(E) cleavage was largely blocked (Fig. 4; KFS/20/73/82 lane). When wt and mutant DNAs were coexpressed, the efficiency of p15(E) cleavage decreased as the ratio of wt to mutant DNA was reduced (Fig. 4; KFS:KFS/20/73/82 5:1, 2:1, and 1:1 lanes).

Infectivity defect exerted by the 20LK/73EK/82AT MA mutant can be rescued by truncation of p15(E). The results presented above and in Fig. 3 suggest that the 20LK/73EK/82AT MA mutant inhibited infectivity of MuLV Env by blocking cleavage of the MuLV TM p15(E). A prediction of this hypothesis is that infectivity of mutant MA virions might be rescued when pseudotyped by the truncated p12(E) form of MuLV Env. To test this hypothesis, we cotransfected pNL4-3KFS or the 20LK/73EK/82AT mutant derivative with a vector, pCEETR (50), which expresses an eco-MuLV Env mutant containing a stop codon immediately after the p15(E) cleavage site. This vector thus synthesizes only the p12(E) form of MuLV TM (50), a point that was confirmed by immunoprecipitation analysis (data not shown). Infectivity of the pseudotyped virions was measured in NIH 3T3 cells. The infectivity of the 20LK/73EK/82AT mutant in NIH 3T3 cells was increased 400-fold when pseudotyped by the truncated form of eco-MuLV TM compared with pseudotypes bearing full-length TM. This result was confirmed by PCR analysis of NIH 3T3 cells infected with wt or mutant MA pseudotyped by full-length or truncated Env. Consistent with the data presented in Fig. 1, no viral DNA could be detected in cells infected with the 20LK/73EK/82AT MA mutant pseudotyped by full-length eco-MuLV Env (Fig. 5, Eco-MuLV). In contrast, viral DNA synthesis was readily detectable in cells infected with the 20LK/73EK/82AT MA mutant pseudotyped by truncated eco-MuLV Env (Fig. 5, Eco-MuLVTr).

Evaluation of other MA mutants for effects on p15(E) cleavage. Since the mutant MA described in this report contains changes at residues 20, 73, and 82, we sought to determine which of the individual changes contributed to the phenotype observed. Ampho-MuLV Env pseudotypes were evaluated by luciferase-based infectivity assays and/or immunoprecipitation analysis. Infectivity assays revealed that of the three individual changes, only 82AT contributed significantly to the observed phenotype; this mutant displayed approximately 1% relative infectivity when pseudotyped by MuLV Env despite the finding that pNL4-3/82AT replicated with near-wt kinetics in H9 cells (35). 73EK was not further assessed by immunoprecipitation of pseudotyped virions, as this mutation causes an assembly and release defect resulting in an approximately 10-fold reduction in virion production (35). The results obtained by infectivity assay for 20LK and 82AT were confirmed biochemically by immunoprecipitation of pseudotyped virions. In 20LK as in wt pseudotypes, the p12(E) form of MuLV TM predominated, whereas 82AT pseudotypes showed an approximately 1:1 ratio of p15(E) to p12(E) (data not shown). Thus, although the 82AT single mutant caused a significant inhibition of p15(E) cleavage, the triple mutant (20LK/73EK/82AT) displayed a more pronounced phenotype.

Additional HIV-1 MA mutants were assessed biochemically for effects on p15(E) cleavage. The 12LE and 30LE MA mutants were previously demonstrated to abolish incorporation of HIV-1 Env but to be readily pseudotyped by MuLV Env (19). Both mutants displayed wt levels of p15(E) cleavage when pseudotyped by MuLV Env (data not shown).

DISCUSSION

In this report, we demonstrate that HIV-1 PR efficiently catalyzes the cleavage of the MuLV TM Env protein from p15(E) to p12(E) in HIV-1 virions pseudotyped with MuLV Env. The cleavage reaction can be inhibited by specific mutations in HIV-1 MA in a potent and transdominant manner, thereby blocking virus infectivity. This inhibition of infectivity can be largely reversed by pseudotyping with the truncated p12(E) form of MuLV Env, confirming the requirement for this cleavage in the activation of Env fusogenicity. MuLV p15(E) processing by HIV-1 PR appears to be quite efficient relative to processing by MuLV PR; in MuLV virions, significant amounts of unprocessed p15(E) are detected (24, 30), whereas we observed almost complete processing to p12(E) in virions containing wt HIV-1 PR and MA. Despite the highly efficient cleavage of p15(E) to p12(E) in pseudotyped virions, we consistently observed no cell-associated p12(E) (data not

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shown), indicating that significant p15(E) cleavage by HIV-1 PR occurs only during or after virus release.

Our results indicate that even a modest defect in p15(E) cleavage dramatically reduces virus infectivity (Fig. 4 and Table 1). For example, at a 2:1 ratio of wt to mutant Gag DNA, the ratio of p15(E) to p12(E) was approximately 1:1 (as determined by phosphorimage analysis), yet infectivity was reduced 25-fold. At a p15(E):p12(E) ratio of approximately 2:1 (Fig. 4, 1:1 lane) infectivity was reduced more than 100-fold. In previous experiments in which full-length and R-peptide-truncated MuLV Env glycoproteins were coexpressed, it was observed that the presence of full-length Env protein did not transdominantly suppress the ability of the truncated Env to induce cell-to-cell fusion (65). This discrepancy may reflect differences in the mechanism of membrane fusion in the context of syncytium formation versus fusion between the viral envelope and host cell plasma membrane during infection.

The data presented in this report are reminiscent of those from a previous study in which mutations in M-PMV MA were shown to block cleavage of the M-PMV TM protein catalyzed by the M-PMV PR (2). The present study extends these results by demonstrating that inhibition of HIV-1 PR-mediated TM cleavage can occur with MuLV. Furthermore, the data presented here indicate that PR-mediated TM cleavage can be inhibited by mutations in a heterologous (HIV-1) MA protein. We predict based on our results that MuLV MA mutations might also inhibit p15(E) cleavage, although to our knowledge no such mutation has been described. It remains to be determined whether the 82AT and 20LK/73EK/82AT HIV-1 MA mutants are unique in their ability to block p15(E) cleavage or whether other HIV-1 MA mutants display this phenotype.

Several models could explain the ability of HIV-1 MA mutations to interfere with HIV-1 PR-mediated cleavage of the MuLV TM. (i) An interaction between MA and the TM might be required to facilitate cleavage by PR, and this interaction could be disrupted by the 20LK/73EK/82AT changes. This model is somewhat unlikely in light of a previous report in which an HIV-1 mutant lacking a large portion of MA could be rendered infectious with MuLV Env (62). Although the extent of TM cleavage was not determined in that study, our data suggest that TM cleavage would have been required to achieve the observed levels of infectivity. (ii) HIV-1 MA mutations could induce an interaction between MA and the MuLV TM which obscures or alters the conformation of the sequence recognized by PR. (iii) Both wt and mutant HIV-1 MA might interact with MuLV TM, but the nature of the interaction may be altered by MA mutations, thereby preventing PR-mediated processing.

The results presented here raise the possibility that heterologous retroviral Env and MA proteins interact, suggesting the presence of highly conserved structural motifs in these proteins. We have previously demonstrated that single amino acid changes in HIV-1 MA can block the incorporation of HIV-1 Env into virions without affecting the incorporation of MuLV Env (19). Other groups have described more drastic MA mutations which perturb HIV-1 Env incorporation without affecting levels of MuLV Env in virions (41, 62). Taken together, these studies suggest that the incorporation of full-length HIV-1 Env glycoproteins into virions requires a specific interaction with, or at least accommodation by, the HIV-1 MA. In contrast, the incorporation of Env proteins with short cytoplasmic tails is relatively nonspecific and does not require an interaction with MA. Thus, any interaction that might occur between MuLV Env and HIV-1 MA is clearly not required for incorporation of the MuLV Env protein into HIV-1 virions. Consistent with the hypothesis that heterologous retroviral Env and Gag proteins may interact at some stage during virus assembly and release is the recent observation that MuLV Env directs basolateral budding of HIV-1 Gag proteins in polarized epithelial cells (39). In the latter study, polarized budding was dependent upon a Tyr-X-X-Leu motif (where X is any amino acid) in the cytoplasmic domain of MuLV p15(E). It is interesting that nuclear magnetic resonance spectroscopy and X-ray crystallography data are now available for a number of retroviral MA proteins; although these proteins display little or no amino acid sequence homology, their overall structures are remarkably well conserved (5, 6, 29, 42, 43, 51).

Because of its relatively promiscuous incorporation into virions, stable SU-TM interaction, and ability to confer high levels of infectivity, the MuLV Env glycoprotein has been used extensively in HIV research and in the development of HIV-based vectors (7, 19, 27, 36, 38, 40, 46, 55, 62). The data presented here demonstrate that HIV-1 MA substitutions can abolish infectivity of MuLV Env pseudotypes by abrogating HIV-1 PR-mediated p15(E) cleavage. These results indicate that in developing HIV-based vectors, Gag sequences which optimize MuLV TM cleavage should be selected.

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