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TM Domain Swapping of Murine Leukemia Virus and Human T-Cell Leukemia Virus Envelopes Confers Different Infectious Abilities despite Similar Incorporation into Virions

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We investigated the influence of transmembrane protein (TM) domains on incorporation of retroviral envelopes into virions and on infectivity. We introduced complete, truncated, or chimeric Friend murine leukemia virus (F-MuLV) and human T-cell leukemia virus type 1 (HTLV-1) envelopes into an MuLV particle-producing complementation cell line. As shown previously for HTLV-1 envelopes containing extracellular domains of F-MuLV TM (C. Denesvre, P. Sonigo, A. Corbin, H. Ellerbrok, and M. Sitbon, J. Virol. 69:4149–4157, 1995), reverse chimeric F-MuLV envelopes containing the extracellular domain of HTLV-1 TM were not processed. In contrast, a chimeric MuLV envelope containing the entire HTLV membrane-spanning and cytoplasmic domains (FHTMi) was efficiently processed, fusogenic as tested in a cell-to-cell assay, and efficiently incorporated into MuLV particles. However, these MuLV particles bearing FHTMi envelope proteins could not infect mouse or rat cells which are susceptible to wild-type F-MuLV. Therefore, envelopes which are readily fusogenic in cell-to-cell assays and also efficiently incorporated into virions may not necessarily confer virus-to-cell fusogenicity. HTLV envelopes, whether parental, chimeric (containing the MuLV cytoplasmic tail) or with a truncated cytoplasmic domain, were incorporated into MuLV particles with equal efficiencies, indicating that the cytoplasmic tails of these envelopes did not determine their incorporation into virions. In contrast to FHTMi envelope, HTLV-1 envelopes with F-MuLV membrane-spanning and cytoplasmic domains, as well as wild-type HTLV-1 envelopes, conferred virion infectivity. These results help to define requirements for envelope incorporation into retroviral particles and their cell-free infectivity.

The envelope (Env) of replication-competent retroviruses is produced from a glycoprotein precursor which is processed into an entirely extracellular surface subunit (SU) and a transmembrane subunit (TM). This glycoprotein complex is efficiently incorporated into virions, whereas most of the surface host proteins are excluded (19). By analogy with other enveloped viruses such as alphaviruses (29), it has been proposed that selective Env incorporation into virions is due to specific interactions between Env glycoproteins and internal viral proteins. The TM of the avian Rous sarcoma virus (RSV) can be chemically cross-linked in virions to the matrix protein (MA), suggesting that these two proteins are indeed in close contact and that their interaction may be necessary for Env incorporation into virions. Mutagenesis of the human immunodeficiency virus type 1 (HIV-1) MA is usually associated with a defect in Env incorporation, lending support to this hypothesis (9, 35). The retroviral TM subunit contains three major domains: (i) the external domain located at the extracellular side of the lipid bilayer, (ii) the membrane-spanning domain allowing anchorage of Env into the lipid bilayer, and (iii) the cytoplasmic domain of variable length, for which no clear function has been established. As the MA protein is anchored at the cytoplasmic face of the lipid bilayer, the region of the TM most likely to interact with the MA is its cytoplasmic domain. However, some observations suggest that the cytoplasmic domain is not essential in envelope incorporation. Indeed, an RSV envelope lacking its cytoplasmic domain is efficiently incorporated into virions (25). In addition, TM-specific sequences are apparently not required for envelope incorporation into RSV particles, since the divergent influenza virus hemagglutinin (HA) glycoprotein complex is efficiently incorporated into RSV particles (8). Moreover, substitution of the RSV Env cytoplasmic domain for that of HA did not increase HA incorporation into RSV virions (8). However, a negative effect of the Env cytoplasmic tail, which seems to depend on interaction with the MA, has been observed. For instance, certain mutations in the HIV-1 MA block the incorporation of wild-type HIV-1 Env but not that of either a mutant HIV-1 Env with truncated cytoplasmic domain or an amphotropic murine leukemia virus (MuLV) Env (12, 24).

The human T-cell leukemia virus type 1 (HTLV-1) Env can pseudotype viral cores constituted of HIV-1 or Moloney MuLV Gag and Pol proteins, although yielding poorly infectious MuLV particles (23, 32). Whether this low infectivity is due to a low efficiency of HTLV-1 Env incorporation or to alteration of another function remains unclear. We chose here to address the mechanisms for the preferential incorporation of retroviral Env into virions and their subsequent infectivity and more particularly to monitor the influence of divergent cytoplasmic domains in this process. We used wild-type, de-

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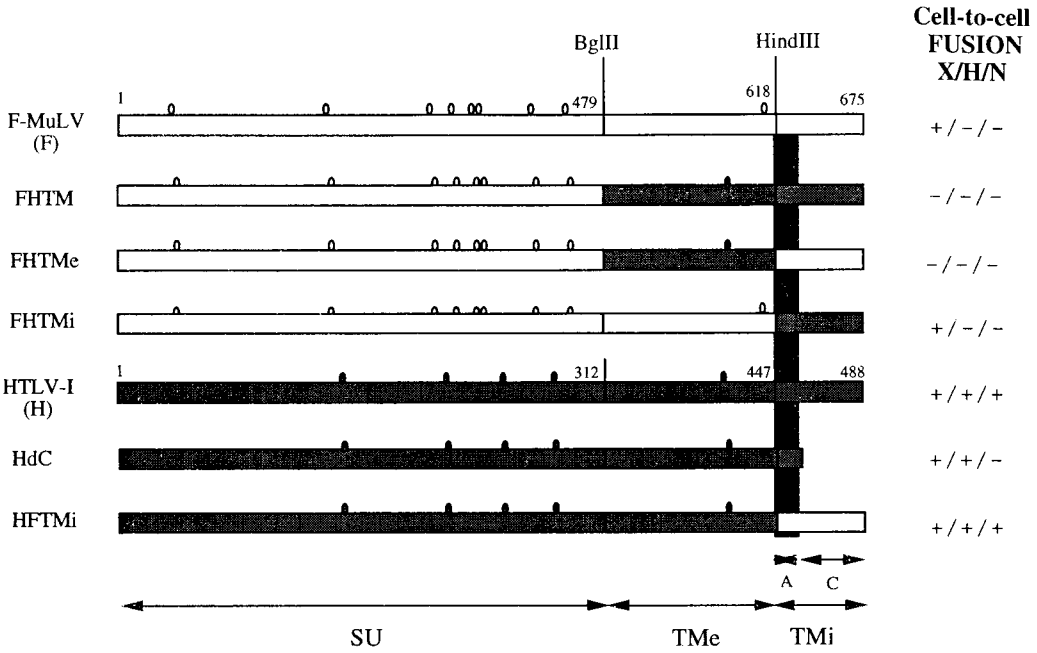

FIG. 1. Structures and summary of the fusogenic properties of parental, chimeric, and truncated envelopes. F-MuLV (open boxes) and HTLV-I (shaded boxes) Env are represented as uncleaved protein precursors numbered from the AUG initiation codons and anchored in the plasma membrane, symbolized by the vertical solid box. Locations of the SU and TM components are indicated at the bottom, with the TM comprising the external (TMe) and intracellular (TMi) domains. The TMi domain is further divided into membrane-spanning anchor (A) and cytoplasmic (C) domains. Note that contrary to what was previously and erroneously indicated (6), all F-MuLV cytoplasmic domains, including those in HFTMi, comprise the entire carboxy-terminal R peptide. Potential N-linked glycosylation sites are indicated by small circles. Fusogenicities of previously reported HTLV Env (6) are also shown for comparison. Fusion indicator cell lines stably expressing HIV-1 Tat protein were rat XC-Tat (X), human HeLa-Tat (H), and mouse NIH 3T3-Tat (N). The envelope was considered fusogenic (+) when more than 10 fusion events, identified as blue foci, were observed; nonfusogenic combinations are indicated by −.

leted, and chimeric Env proteins derived from HTLV-1 and a divergent murine retrovirus, Friend MuLV (F-MuLV). We observed that the origin of the anchorage domain and the presence or origin of the cytoplasmic domain did not detectably influence Env incorporation into MuLV particles. However, a chimeric F-MuLV Env containing the cytoplasmic tail of HTLV-1, although efficiently fusogenic in a cell-to-cell assay and efficiently incorporated into MuLV cores, did not confer infectivity to virions. In contrast, chimeric HTLV-1 Env containing the F-MuLV cytoplasmic tail yielded infectious virions.

(This work is part of C.D.'s Ph.D. thesis requirements.)

MATERIALS AND METHODS

DNA, cells, and antibodies. Plasmids pBEH/H and pBSE/F contain the coding sequences of HTLV-1 strain MT2 *env* and F-MuLV strain 57 *env*, designated H and F, respectively. Three allelic restriction sites, located at the cleavage site between SU and TM, at the 5′ end of the membrane-spanning region, and after the *env* stop codon, were introduced in the two constructs to allow domain swapping between these two envelopes (6). It is important to note that contrary to the erroneous representation of HFTMi (the nomenclature used is described below) in Fig. 2B of our previous report (6), this chimeric Env does contain all of the F-MuLV cytoplasmic tail, including the R peptide. The Env expression vectors (pCEL series) containing the envelopes derived from HTLV-1 (H, HdC, and HFTMi) or the parental F-MuLV Env (F) were previously described (6). When indicated, the pFB3 expression vector (16) was also used with envelopes derived from F-MuLV. Plasmid FBASALF contains the amphotropic MuLV *env* under the control of F-MuLV FB29 promoter (4). All cell lines were maintained at 37°C in a 5% CO_2 atmosphere. Cos cells stably expressing the *lacZ* gene under the control of the HIV-1 long terminal repeat (CosLTRlacZ cells) and the Tat-expressing cell lines were obtained and grown in conditions previously described (6). TELCeB6 cells are human rhabdomyosarcoma TE671 cells, which stably express Gag and Pol Moloney MuLV and contain a *lacZ* retroviral vector (5). TELCeB6 and human rhabdomyosarcoma RD cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, glutamine, penicillin, and streptomycin (complete growth medium). Two anti-HTLV-1 TM monoclonal antibodies, 5a and 39b, were used (1). Different antibodies against MuLV proteins generously provided by A. Rein were used: (i) a goat anti-MuLV SU gp70 (31), (ii) a rabbit anti-MuLV TM p15E (28), (iii) and a goat anti-MuLV CA p30 (31).

Construction and nomenclature of chimeric envelope genes. Chimeric FH Env proteins containing HTLV-1 sequences were designated FHTM, FHTMe, and FHTMi. In this nomenclature, the first and second letters indicated the origin of the background sequence and that of the substituted heterologous sequence, respectively, with F or H standing for F-MuLV or HTLV-1. The last letters indicated the nature of the heterologous substituted domain, with TM denoting the complete TM subunit substitution, TMe denoting a substitution that included the extracellular portion of the TM comprised between the *Bgl*II and *Hin*dIII restriction sites, and TMi denoting a substitution of the *Hin*dIII-*Mlu*I fragment encompassing most of the membrane-spanning anchor and the whole cytoplasmic domain (Fig. 1). All substitutions were verified by DNA sequencing of the junctions.

Transfection and expression systems. Pulse-chase and cell-to-cell fusion analyses were performed after transfection of either 5×10^5 CosLTRlacZ cells with 10 μg of DNA in 10-cm-diameter plates or 0.5×10^5 to 1×10^5 CosLTRlacZ cells with 1 μg of DNA in six-well plates, using the DEAE-dextran—chloroquine and dimethyl sulfoxide technique (26). Determination of glycoprotein incorporation into virions and infectivity assays were performed after transfection of either 10^6 TELCeB6 cells with 15 μg of DNA in 10-cm-diameter plates or 1×10^5 to 2×10^5 TELCeB6 cells with 3 to 5 μg in six-well plates, using the calcium phosphate technique (the number of cells indicated corresponds to the number of cells seeded 24 h before transfection).

Metabolic labeling, immunoprecipitation, and incorporation of viral glycoproteins into virions. Forty-eight hours after transfection, CosLTRlacZ cells were starved in cysteine- and methionine-free culture medium for 1 h and pulse-labeled at 37°C for 30 min in 3 ml of cysteine- and methionine-free RPMI containing 50 μCi of [^{35}S]cysteine and [^{35}S] methionine (ICN) per ml and 2% dialyzed fetal calf serum. For pulse-chase labeling, labeled cells were chased in complete growth medium supplemented with methionine (1 mM) and cysteine (2.5 mM) for 2 to 6 h. Cells were lysed as previously described (6). Lysates were precleared with normal rabbit serum and immunoprecipitated with a rabbit anti-MuLV TM p15E serum and goat anti-MuLV SU gp70 serum. Immunoprecipitates were electrophoresed in sodium dodecyl sulfate (SDS)–12% polyacrylamide gels under reducing conditions in parallel with prestained molecular weight markers (Low Range Protein Standards; Bio-Rad). For analyses of glycoprotein incorporation into virions, transfected TELCeB6 cells were labeled overnight in 6.5 ml of cysteine- and methionine-free RPMI containing 75 μCi of

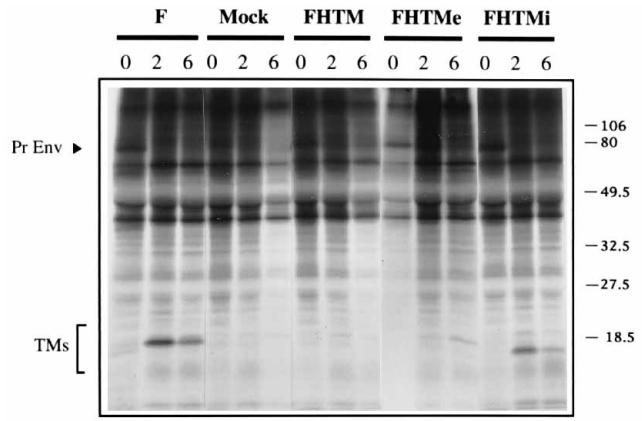

FIG. 2. Pulse-chase assays of parental and chimeric envelopes derived from F-MuLV. Forty-eight hours after transfection, CosLTRlacZ cells were pulse-labeled for 30 min with [^{35}S]cysteine and [^{35}S]methionine (lanes 0) and chased for 2 (lanes 2) and 6 (lanes 6) h. Cell lysates were subjected to immunoprecipitation with a MuLV TM p15E antiserum mixed with a MuLV SU gp70 antiserum without preenrichment of glycoproteins on lentil lectin. Positions of migration of the uncleaved SU/TM precursors (Pr Env) and of the TM envelope products are indicated. Sizes are indicated in kilodaltons.

[^{35}S]cysteine and [^{35}S]methionine (ICN) per ml and 2% dialyzed fetal calf serum. Labeling was performed 8 h after transfection for HTLV-1 envelopes expressed by the pCEL vector and 54 h after transfection for F-MuLV envelopes expressed by the pFB3 vector. Supernatants were harvested and filtered through a 0.45-μm-pore-size filter. For HTLV-1 envelopes, the supernatants of two 10-cm-diameter plates transfected with the same DNA preparation were pooled, whereas for F-MuLV, the supernatant of a single plate was used. Supernatants were ultracentrifuged on a 2-ml 20% sucrose cushion for 3 h at 24,000 rpm in an SW41 rotor (Beckman). The pellets were lysed by adding 150 μl of lysis buffer (50 mM Tris HCl [pH 7.5], 15 mM NaCl, 5 mM $MgCl_2$, 5 mM KCl, 1% Triton X-100, 0.5% sodium deoxycholate). One-fifth of the lysate was preserved and electrophoresed as such for a crude analysis of the virus protein content, whereas the remaining lysate was submitted to immunoprecipitation with anti-Env antibodies followed by anti-MuLV CA immunoprecipitation of the corresponding supernatants. After labeling, whole cells were lysed as described above for the CosLTRlacZ cells and immunoprecipitated with the same panel of antibodies as used for virions. Lysates of cells transfected with envelopes derived from HTLV-1 and F-MuLV were enriched in glycoprotein content with lentil lectin before immunoprecipitation (6).

Cell-to-Cell fusion assay with F-MuLV-derived envelopes. The cell-to-cell fusion assay was performed as described previously (6). Briefly, 48 h after transfection, CosLTRlacZ cells were cocultivated with Tat-expressing cells and stained in situ to detect β-galactosidase expression. Each blue focus corresponded to at least one cell-to-cell fusion event. All blue foci visible under a stereomicroscope at a magnification of ×40 were counted, regardless of size and morphology.

Analyses of viral infectivity. Culture medium (2 ml) of the TELCeB6 cells in six-well plates was changed at different times after transfection, harvested 16 h later, and filtered (through a 0.45-μm-pore-size filter). Five hundred microliters of the fresh filtered culture medium (48 h after transfection) was used to infect target cells in the presence of 8 μg of Polybrene per ml for testing virions from F- and FHTMi-transfected cells (Fig. 1). For analysis of virions harvested from cells transfected with the amphotropic and the H-series envelopes (Fig. 1), upon harvest of 16-h supernatant (24 h after transfection), fresh medium was added to the transfected TELCeB6 cells and an additional harvest was performed the following day (48 h after transfection). The 2-ml supernatants were filtered and placed on RD and XC-Tat cells without Polybrene. Two days after infection, cells were fixed with 0.5% glutaraldehyde and stained to reveal the presence of β-galactosidase activity. Infectious titers were expressed as the number of β-galactosidase-positive blue foci obtained per milliliter of medium from transfected TELCeB6 cells. All experiments were done in duplicate and repeated several times. In each experiment, F or the wild-type amphotropic Env was used as a positive control envelope.

Statistical methods. The nonparametric statistics used were the Mann-Whitney *U* test for comparison of the number of blue foci obtained for fusion of F and FHTMi with XC-Tat indicator cells. *P* values of <0.05 were considered significant.

RESULTS

Expression and cell-to-cell fusogenicity of F-MuLV envelopes containing HTLV-1 TM sequence. Expression and maturation of parental and chimeric F-MuLV Env containing the entire HTLV-1 TM sequence (FHTM) or either the extracellular (FHTMe) or the intracellular (FHTMi) region of the TM were examined in CosLTRlacZ cells. Protein precursors of all these envelopes were detected after a 30-min [^{35}S]Met and [^{35}S]Cys pulse-label (Fig. 2, lanes 0). Because most of the mature SU products were masked by background signal, SU/TM cleavage was more readily monitored by detection of TM products. After a 2-h (lanes 2) and a 6-h (lanes 6) chase, F and FHTMi Env precursors seemed entirely chased into the corresponding cleaved subunits. Contrastingly, the FHTM and FHTMe uncleaved precursors were detectable after a 2-h chase with no detectable mature TM products, suggesting an early blockade in the processing of these chimeric Env. In the absence of maturation, these precursors seemed to be degraded within the 6-h chase. Therefore, from these and previously reported results (6), we concluded that HTLV-1 or F-MuLV substitutions which included heterologous extracellular TM sequences led to chimeric Env precursors which were blocked in the early maturation process despite efficient protein expression. On the other hand, reciprocal substitutions of the TMi sequences resulted in efficiently expressed and processed envelopes.

Biological activity of the efficiently processed chimeric FHTMi was tested in a cell-to-cell fusion assay performed on rat XC-Tat cells and murine NIH 3T3-Tat cells, which both express the ecotropic MuLV receptor. FHTMi was fusogenic for the XC-Tat cells, although with a significantly lower efficiency than the homogeneous parental F Env ($P < 0.0001$) (Table 1). Like the parental F Env, FHTMi was not detectably fusogenic with the NIH 3T3-Tat cells. By comparison and as previously reported (6), the homogeneous HTLV-1 (H) and the reverse chimeric (HFTMi) Env were fusogenic with similar efficiencies on both cell lines (Table 1). Therefore, significantly different fusogenic properties associated with the TMi of F-MuLV and HTLV-1 were observed in the F-MuLV but not the HTLV-1 background.

Incorporation into MuLV virions of parental, truncated, and chimeric envelopes. The incorporation of the parental or chimeric F-MuLV SU-containing fusogenic Env (F and FHTMi) into MuLV was determined by a complementation assay with the TELCeB6 cell lines expressing MuLV Gag and Pol products. Fifty-six hours after transfection with the F-MuLV-derived Env, cells were labeled for approximately 16 h. Crude pelleted virions or immunoprecipitates with either an anti-

TABLE 1. Fusogenic properties of parental and chimeric envelopes derived from F-MuLV

Envelope(s)	Expt(s)	Mean no. of blue foci	
		XC-Tat	NIH 3T3-Tat
F	a	700, 830, 800	<10
	b	756, 657, 614	<10
	c	830, 730, 810	<10
FHTMi	a	258, 229, 208	<10
	b	254, 405, 401	<10
	c	378, 331, 364	<10
Mock	a, b, c	<10	<10
H and HFTMi[a]		>1,000	>500
HdC[a]		>1,000	40

[a] Data are from reference 6.

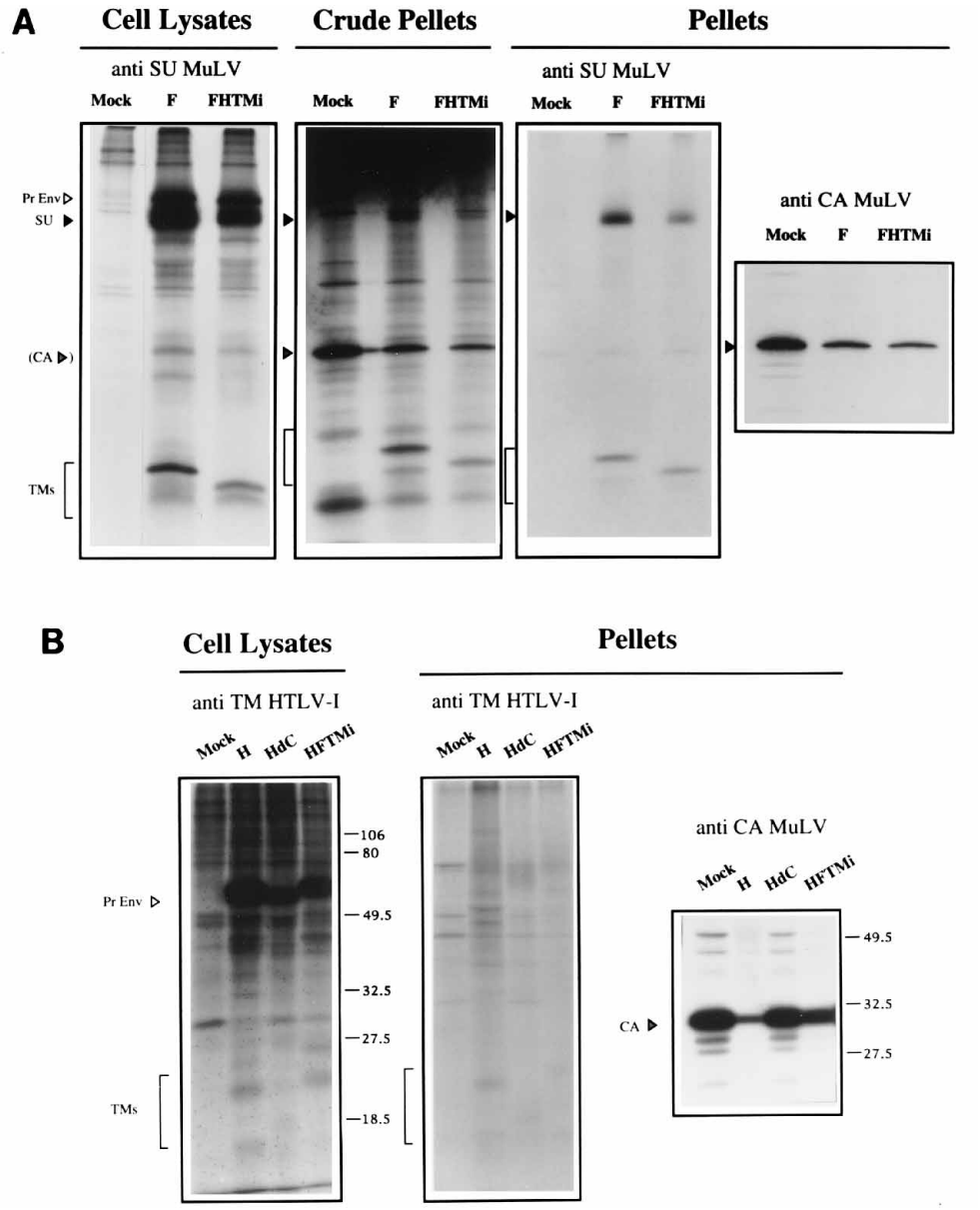

FIG. 3. Envelope incorporation into MuLV virions. TELCeB6 cells expressing different *env* genes were labeled overnight with [^{35}S]cysteine and [^{35}S]methionine. (A) Incorporation of MuLV F and FHTMi Env 70 h posttransfection. SDS-PAGE was performed in the presence of standard molecular mass markers ranging between 106 and 18.5 kDa. In the cell lysates panel, Env products associated with cells at the end of the labeling were immunoprecipitated from lysed cells by using a MuLV SU antiserum after enrichment of glycoproteins on lentil lectin. In the crude pellets panel, virus particles released into the medium were ultracentrifuged at 24,000 rpm through a 20% sucrose cushion prior to solubilization in lysis buffer. In the pellets panel, the crude pellets were further submitted to immunoprecipitation with antisera specific for either surface (anti-SU) or capsid (anti-CA) MuLV proteins. Capsid and Env products are indicated. Open, solid, and shaded arrowheads indicate precursor (Pr) Env, SU, and CA, respectively. The most abundant TM band in the F lanes migrated at the same level as the 18.5-kDa marker. (B) Incorporation of the HTLV-1 H, HdC, and HFTMi Env 24 h posttransfection. In the cell lysates panel, cell lysates were enriched in glycoprotein contents on lentil lectin and immunoprecipitated with a combination of anti-HTLV-1 TM monoclonal antibodies. In the pellets panel, virus particles released in the medium were prepared as described above and virions were immunoprecipitated with either anti-HTLV-1 TM monoclonal antibodies or a MuLV CA antiserum as indicated. For indication, the major TM product as seen in the H lanes migrated slightly above the 18.5-kDa marker.

MuLV SU gp70 serum or an anti-MuLV CA p30 serum were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) along with their corresponding cell lysates. Preparations from mock-transfected cells analyzed in parallel yielded Gag and Pol products only (Fig. 3A). Cells expressing F or FHTMi Env released virions containing similar amounts of mature SU and SU-associated TM products. Also, two SU-associated bands were observed in the TM migration area, with the lower band being relatively less abundant in the cell lysates (Fig. 3A). It is most likely that the major upper band corresponded to the uncleaved TM precursor, or pre-15E (15, 17, 28), and that the lower band corresponded to the mature TM p15E lacking the R peptide. That more cleaved TM product was observed in virions than in cell lysates is compatible with R cleavage occurring late in viral production, mostly within the virions (15, 17). We also observed a slightly faster-migrating minor band in the TM migration area of FHTMi lanes (Fig. 3A). Although the exact origin of this lower band was unclear, it was likely to

TABLE 2. Infectivities on different cell lines of MuLV virions produced in the presence of F-MuLV- and HTLV-1-derived envelopes[a]

Expt	Time (h) after transfection	Envelope(s)	Titer of supernatant (no. of blue foci/ml)		
			XC-Tat	NIH 3T3-Tat	RD
1	48	F	$(10–26) \times 10^3$	$(22–42) \times 10^3$	ND
		FHTMi	0	0	ND
2	24	A	2×10	ND	$(2–4) \times 10^2$
		H	0	ND	$(4–5) \times 10$
		HFTMi	0	ND	$(2–7) \times 10^2$
		HdC	0	ND	0
	48	A	5×10^3	ND	$(1–34) \times 10^3$
		H, HFTMi, and HdC	0	ND	0

[a] Titer ranges were determined from results of two to four experiments. Envelope designations are as in Fig. 1. Envelope A refers to amphotropic MuLV envelope produced with plasmid FBASALF (see Materials and Methods). ND, not done.

correspond to a TM cleavage product, since this band was not detected in the CosLTRlacZ cell line, which lacks the viral protease responsible for late TM cleavage (not shown). Since no uncleaved precursor product was detected in association with virions, chimeric FHTMi Env was most likely processed and incorporated into MuLV particles as efficiently as the parental F Env.

We also examined the incorporation of HTLV-1 SU-containing envelopes into MuLV particles. These experiments were limited by the fact that these envelopes exerted a much higher toxicity for TELCeB6 than for CosLTRlacZ cells. Interestingly HTLV-1 envelope cellular toxicity has been previously described for a nonrelated MuLV-packaging cell line (32). Because these envelopes were highly toxic for TELCeB6 cells, supernatants were harvested as early as 24 h posttransfection. Cells were labeled for 16 h beginning 8 h after transfection with the expression plasmids containing the homogeneous complete (H) HTLV-1 Env, the cytoplasmic tail-deleted (HdC) HTLV-1 Env, or the chimeric complete HFTMi Env. Cell lysates and pelleted virions were immunoprecipitated with a mixture of two anti-HTLV-1 TM monoclonal antibodies and then with a MuLV CA p30 antiserum (Fig. 3B).

Despite the HTLV envelope toxicity toward TELCeB6 cells and the subsequent low levels of virion recovery, H, HdC, and HFTMi Env appeared to be incorporated with similar efficiencies into MuLV pelleted particles. This finding indicated that HTLV-1 Env incorporation did not require a cytoplasmic domain. Also, the presence in HFTMi of the F-MuLV anchorage and cytoplasmic domains, including the R peptide, did not detectably enhance HTLV-1 Env incorporation into MuLV particles. Because of low corresponding TM signals in virions, we could not determine whether the R peptide in HFTMi was cleaved. Such a cleavage was not detected also in the more abundant cell-associated materials.

Lack of infectivity of MuLV virions containing fusogenic chimeric envelope. Envelope-containing virions obtained by transfection of *trans*-complementing cells were tested for infectivity for rat (XC-Tat), murine (NIH 3T3-Tat), and human (RD) cell lines through transduction of the *lacZ* gene. The murine and rat cell lines were susceptible to infection by virions containing the parental F Env (Table 2), whereas no infection was detected on either cell line with virions containing the chimeric FHTMi Env.

We also tested the abilities of chimeric envelopes containing HTLV-1 SU to confer infectivity toward the HeLa-Tat, XC-Tat, and RD cell lines, which are susceptible to HTLV-1 envelope-dependent cell-to-cell fusion. Low titers of infectious virions with complete homogeneous (H) or chimeric (HFTMi) Env were detected on RD cells, whereas no infectivity was detected for XC-Tat, 3T3-Tat, or HeLa-Tat cells (Table 2 and data not shown). Interestingly, at 24 h posttransfection, the viral titer obtained on RD cells with the chimeric HFTMi Env was slightly higher than that observed with the homogeneous H Env (2×10^2 versus 50 blue plaques per ml). No infectivity, including for RD cells, was detected for virions containing the HdC Env. Also, supernatants from H and HFTMi Env-transfected cells harvested 48 h posttransfection were no longer detectably infectious (Table 2). Since the amphotropic MuLV Env yielded infectious particles at both 24 and 48 h after transfection (Table 2), this result likely reflects the low number of remaining producing cells as a result of the toxicity of the HTLV envelopes.

DISCUSSION

To study the influence of the TM cytoplasmic tail on incorporation of retroviral Env into virions and the resulting infectivity, we analyzed the MuLV particles produced in the presence of heterologous, deleted, and chimeric envelopes derived from F-MuLV and HTLV-1. The homogeneous HTLV-1 Env (H) and its chimeric derivative containing the complete F-MuLV intracellular domains (HFTMi) conferred similar infectivities to MuLV particles. On the other hand, the reverse chimeric Env (FHTMi) did not confer any detectable infectivity, in contrast to its parental F-MuLV Env. Also, the FHTMi Env cell-to-cell fusogenicity was two- to threefold less than that of the parental F Env. We did not detect for FHTMi reduced Env precursor production, slower kinetics of maturation, detectable defects in SU/TM cleavage, or reduced incorporation into virions. Therefore, differences between cell-to-cell fusogenicities of F and FHTMi were most likely due to a weaker fusogenic activity of the latter. We have previously reported that under identical experimental conditions and in the absence of MuLV protease that might cleave the TM carboxy-terminal R peptide and increase fusogenicity (28), the chimeric Env HFTMi is as fusogenic as the HTLV-1 parental Env (6). Here, we observed that TMi domains of F and H Env influence differently cell-to-cell fusogenicity, independently of R-peptide cleavage, when placed within the F-MuLV background.

We also observed that Env precursors which include a homologous (H) or heterologous (HFTMi) cytoplasmic tail appear to accumulate intracellularly more readily than deleted HdC Env (reference 6 and Fig. 3B). Furthermore, full-length H and HFTMi Env were reproducibly more cytotoxic toward TELCeB6 cells than was HdC Env. This finding suggested that the presence of either the HTLV or the F-MuLV cytoplasmic tail was likely to reduce envelope intracellular transport and increase HTLV toxicity toward TELCeB6 cells.

As previously reported by others for Moloney MuLV (21), F-MuLV was nonfusogenic for NIH 3T3-Tat cells in the cell-to-cell fusion assay. However, incorporation of the parental F-MuLV Env into MuLV virions led to infectious ecotropic virions, whereas the chimeric FHTMi Env conferred no detectable infectivity toward XC-Tat and NIH 3T3-Tat cells. This was not due to a detectable reduction of envelope incorporation into virions or to instability of the SU-TM complex, since the SU and TM of both parental and chimeric envelopes were equally well detected after immunoprecipitation. Therefore, lack of infectivity of FHTMi was most likely due to a further downstream blockade, between receptor recognition and release of the capsid in the cytoplasm. MuLV particles harboring H Env are poorly infectious when tested in a cell-free assay on permissive cells. Low infectivity of HIV-1 particles pseudotyped with HTLV-1 Env has been previously described (23), and homogeneous HTLV-1 particles exhibit low infectivity on various cell lines (7, 11). However, HTLV-1 Env is highly fusogenic for many cell lines in cell-to-cell assays, and infection is much more efficient by cocultivation (2, 23). Altogether, our present results and these observations strongly suggest that requirements for cell-to-cell fusion and virus-to-cell fusion mediated by retroviral envelopes are distinct.

Infectious virions containing an HTLV-1 Env and MuLV Gag/Pol products could be detected only on human RD cells, in agreement with a previous report describing RD cells as one of the types of cells most sensitive to infection by recombinant HTLV-1 virions (3). Lack of infectivity for RD cells of virions containing HdC Env did not seem to be due to lack of Env incorporation but most likely resulted from a less efficient fusogenicity of this Env. Interestingly, the chimeric Env HFTMi was found to confer at least the same level of infectivity for RD cells as the parental H Env, underscoring in this case the efficient substitution of a heterologous cytoplasmic domain.

F and FHTMi Env appeared to be incorporated into virions with similar efficiencies, which suggested that Env incorporation does not require sequence-specific interaction between the virion core and the TMi domains of the envelope. However, the cytoplasmic tail might be responsible for an exclusion process (18). Indeed, whereas envelopes for several viruses whose cytoplasmic tails are partially or totally deleted are efficiently incorporated into virions (13, 20, 25, 27, 33, 36), HIV-1 Env containing a long cytoplasmic tail is not incorporated into MuLV particles. It is interesting that both the long cytoplasmic domain of HIV-1 Env and its lack of incorporation into MuLV particles remain exceptions that contrast with envelope proteins of both retroviral (HTLV-1, Gibbon ape leukemia virus, and RSV [22, 30, 34]) and nonretroviral (vesicular stomatitis virus and Semliki Forest virus [10, 30]) origins. Given these data, we suggest the simple rule that retroviral cores allow incorporation of heterologous envelopes whose cytoplasmic tails are smaller than that of the original parental envelope. The molecular basis of this rule may well be that, given the intimate MA-membrane association (14), the space left between the MA proteins and the membrane might be adapted to accommodate only its cognate cytoplasmic domain. Accordingly, in contrast to the absence of pseudotyping of MuLV with HIV-1 Env, F-MuLV or HTLV-1 Env is efficiently incorporated into HIV-1 virions. Also in agreement with this model is the fact that mutations of HIV-1 MA sequence which result in lack of incorporation of wild-type HIV-1 Env (cytoplasmic tail of approximately 150 amino acids) did not impair efficient incorporation of either amphotropic MuLV Env or truncated HIV-1 Env (both with cytoplasmic tails of 20 to 30 amino acids) (12, 24). Such MA mutations might then have resulted in a volume reduction or modification of the available space between MA and the lipid bilayer.

It remains to be elucidated whether the relative efficiency of Env protein incorporation results from a passive mechanism (i.e., random capture of Env present at the cell surface) in combination with a negative selection based on the steric criteria proposed above or from an active mechanism of incorporation (18). Since the cytoplasmic domain is not likely to be involved in the active process, as shown by deletions of the Env cytoplasmic domain of HTLV-1 (this report), RSV (25), HIV-1 (33), and simian immunodeficiency virus (20), it might be of interest to evaluate such a role for the two remaining membrane-associated domains which are common to all retroviruses, i.e., the TM membrane-spanning domain and the MA membrane-anchoring domain. In conclusion, the patterns of envelope protein incorporation into retroviral cores may thus result from hydrophobic interactions combined with a steric exclusion.

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