The Role of Envelope Glycoprotein Processing in Murine Leukemia Virus Infection

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The murine leukemia virus envelope protein is synthesized as a precursor molecule, Pr85*env*, which is proteolytically cleaved at an arginine residue to produce two mature envelope proteins, gp70 and p15(E). The results presented here indicate that mutation to lysine of the arginine found at the envelope precursor cleavage site results in a precursor which is cleaved with an efficiency at least 10-fold lower than the efficiency with which the wild-type protein is cleaved. This mutation has been used to investigate the requirement for envelope protein processing in various aspects of retroviral infection. Viruses produced by cells transfected with mutant proviral clones are approximately 10-fold less infectious than wild-type viruses. Mutant viruses are incapable of inducing XC cell syncytium formation and are 100-fold less efficient than wild-type viruses at rendering cells resistant to superinfection. Envelope glycoproteins bearing the lysine mutation are found in reduced amounts on the surface of infected cells, and as a result mutant virions contain significantly less envelope protein than do wild-type virions. The phenotypic effects of the processing mutation described here are most likely the result of this paucity of envelope glycoproteins in virions carrying the mutation.

The envelope glycoproteins of a number of viruses are synthesized as polypeptide precursors that must be proteolytically cleaved for the virus to be infectious and for virus-induced membrane fusion activity to be exhibited. Examples of such glycoproteins include hemagglutinin of influenza virus (10), F of Sendai virus (5), E2 of mouse hepatitis virus (4), gp62 of Sindbis and Semliki Forest viruses (19), and F of respiratory syncytial virus (14). This posttranslational cleavage is currently thought to be performed by trypsin or trypsinlike enzymes. Inspection of the amino acid sequences of these viruses reveals a conserved arginine residue at the cleavage site.

Retroviral envelope proteins, like those of other enveloped viruses, are synthesized as polypeptides which are proteolytically processed, after removal of a leader peptide, to the two mature envelope proteins: the larger glycosylated external receptor-binding protein and the smaller transmembrane anchor protein. The two murine leukemia virus (MuLV) envelope proteins have been termed, on the basis of apparent molecular weights, gp70 and p15(E) and are derived from the glycosylated precursor Pr85*env* (22). The amino acid sequence at the envelope precursor cleavage site, Lys/Arg-X-Lys/Arg-Arg, is highly conserved among retroviruses (24). The cleavage is made on the carboxy side of the terminal arginine by an unknown cellular enzyme; by analogy with other enveloped viruses, this enzyme may be trypsin or a trypsinlike enzyme. An additional late cleavage occurs that produces p12(E) and p2(E) from p15(E) (22). This cleavage is thought to be performed by the virally encoded *gag* protease (20). It is not known whether either cleavage is necessary for retroviral replication.

Endogenous ecotropic MuLVs are a well-characterized class of retrovirus found in the germ lines of a variety of inbred mouse strains. Two such mouse strains, AKR and BALB/c, harbor proviruses which are approximately 99.5% homologous at the nucleotide level (7). Despite this high degree of sequence conservation, these two viruses differ

phenotypically in several respects. The AKR endogenous ecotropic provirus (AKV) is 10- to 100-fold more infectious than the BALB/c virus (7), and, like most ecotropic MuLVs, AKV induces XC cell syncytium formation in the assay developed by Rowe et al. (18), whereas the BALB/c virus does not (7). Comparison of the DNA sequences of these two viruses indicates that a mutation is present in the BALB/c provirus that changes the terminal amino acid of the envelope protein cleavage sequence from Arg to Lys (7). Previous work in this laboratory indicated that this mutation may be responsible for the inability of the BALB/c endogenous virus to induce XC cell syncytia and led to the speculation that this phenotype is caused by inefficient cleavage of the envelope precursor (7).

In this report, we present evidence that the Arg-to-Lys mutation at the site of envelope precursor processing does lead to a dramatic reduction in the efficiency of envelope protein precursor processing and transport. This defect causes the mutant virus to be unable to induce XC cell fusion, to be less infectious, and to be less effective at establishing interference than the wild-type virus.

MATERIALS AND METHODS

DNA cloning and sequencing. Standard DNA cloning techniques were used in the construction of pEF38 (13). Restriction enzymes were purchased from New England BioLabs, Inc., Beverly, Mass.; digests were done as recommended by the supplier. Ligations were done with T4 DNA ligase (New England BioLabs), and the DH-5 strain of *Escherichia coli* was transformed by the method of Hanahan (2). Transformed colonies were screened by alkaline lysis and restriction enzyme analysis. M13 DNA sequencing was done as previously described (7). Details concerning the construction of pEF38 will be provided upon request.

Transfections and virus assays. NIH 3T3 cells were transfected by the calcium phosphate precipitation technique first described by Graham and Van der Eb (1) and modified by Hopkins et al. (6). Syncytium induction in the XC cell line was measured by the method of Rowe et al. (18).

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Immunofluorescence assays were done as described by Hartley et al. (3). Reverse transcriptase assays were done essentially as described by Rapp and Nowinski (15).

Cell culture and labeling. Cells were grown at 37°C in Dulbecco modified Eagle medium supplemented with 5% calf serum (HyClone, Logan, Utah) and penicillin-streptomycin (GIBCO Laboratories, Grand Island, N.Y.) in 5% CO₂. One million cells in methionine-free RPMI 1640 containing 10% dialyzed fetal bovine serum were labeled with 250 µCi of [³⁵S]methionine (>600 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Chases were done by replacing the medium with complete RPMI 1640. Labeling with sugars was done in RPMI 1640 containing 10% dialyzed fetal bovine serum and 100 µCi of tritiated sugars per million cells. The sugars used, D-(2-³H)mannose, D-(6-³H)glucosamine hydrochloride, and L-(5,6-³H)fucose, were obtained from Amersham. Endoglycosidase-H (endo-H) digests were done as recommended by the supplier (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Iodinations were done 7 h after the plating of 10⁶ virus-infected cells in 35-mm-diameter tissue culture dishes. A total of 400 µCi of ¹²⁵I and four Iodobead catalysts (Pierce Chemical Co., Rockford, Ill.) were used per plate. Labeling was allowed to proceed at room temperature for 20 min.

Preparation and immunoprecipitation of cell lysates. Labeled cells were solubilized in phospholysis buffer (1% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 0.5% deoxycholate, 10 mM NaH₂PO₄, 0.01% bovine serum albumin) containing 1% Trasylol (FBA Pharmaceuticals, New York, N.Y.), and the lysate was precleared with normal goat serum and a 10% *Staphylococcus aureus* (Cowan 1 strain) solution prepared as described by Kessler (9). For gp70 and gag protein immunoprecipitations, polyclonal antibodies were mixed with the labeled precleared cell lysates and collected with a 10% solution of *S. aureus* that had been boiled in 2-mercaptoethanol and washed in NET (0.15 M NaCl, 5 mM EDTA, 50 mM Tris) containing 0.5% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.) (17). After incubation on ice, the samples were pelleted, washed in phospholysis buffer–1% Trasylol, and solubilized by being boiled in a sample buffer containing 10% glycerol, 5% 2-mercaptoethanol, 3% SDS, and 0.5× Tris. The samples were subsequently subjected to SDS-polyacrylamide gel electrophoresis and fluorographed by using En³Hance (New England Nuclear Corp., Boston, Mass.) and X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.). To reduce the background caused by nonspecific precipitation of nonviral proteins, the following modification of the above protocol was introduced for the immunoprecipitation of [³⁵S]methionine- and ¹²⁵I-labeled cell lysates. After being washed with phospholysis buffer–1% Trasylol, the samples were suspended in phospholysis buffer–1% Trasylol, boiled, centrifuged at 10,000 × g, and reprecipitated. NCI 79S-00834 anti-gp70 and NCI 79S-450 anti-gag antisera were used. p15(E) was precipitated by first combining 2-mercaptoethanol-boiled *S. aureus* with goat anti-rat immunoglobulin G, incubating this mixture overnight at 4°C, and adding cell lysate and anti-p15(E) (Hy65; obtained from M. Cloyd, Duke University) monoclonal supernatant.

Labeling, harvesting, and immunoprecipitation of virions. Virus-producing NIH 3T3 cells were labeled for 12 h as described above, and the medium was removed and centrifuged at low speed to pellet unattached cells and cell debris. The supernatant from this centrifugation was centrifuged at 35,000 rpm in an SW70Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.), and the virus pellet was solubilized in

FIG. 1. Envelope glycoprotein processing in p623-, p7D-, and pEF38-transfected cells (panels A and B) and immunoprecipitation assay sensitivity determination (panel C). (A and B) Cells transfected in parallel with p623, p7D, or pEF38 7 days earlier were labeled for 3 h with [³⁵S]methionine and chased for 90 min in cold medium. Cell lysates were doubly immunoprecipitated with anti-gp70 antiserum. Lanes: 1, untransfected NIH 3T3 cell lysates; 2, p623-transfected cell lysates; 3, p7D-transfected cell lysates; 4, pEF38-transfected cell lysates. Panel B is a longer exposure of panel A. (C) Lysates were prepared as described above and doubly immunoprecipitated in the following mixtures. Lanes: 1, NIH 3T3 cell lysate; 2, p623-transfected cell lysate; 3, 25% p623-transfected cell lysate; 4, 10% p623-transfected cell lysate; 5, 5% p623-transfected cell lysate. K, Kilodaltons.

phospholysis buffer containing 1% Trasylol, precleared with normal goat serum and a 10% solution of *S. aureus*, and immunoprecipitated with anti-gp70 antiserum as described above.

RESULTS

Envelope glycoprotein processing in the BALB/c and AKR ecotropic MuLVs. The BALB/c ecotropic provirus bears a mutation that changes the arginine found at the site of the wild-type AKV envelope glycoprotein cleavage to lysine. To compare the envelope protein-processing characteristics of these two viruses, we transfected NIH 3T3 cells with the infectious AKV proviral clone p623 (12) and the infectious BALB/c proviral clone p7D (7). At 2 weeks posttransfection, [³⁵S]methionine-labeled cell lysates were prepared and immunoprecipitated with anti-gp70 serum. Untransfected NIH 3T3 cells were labeled as a control. Pr85env and gp70 were present in immunoprecipitates of p623-transfected cells, whereas only the precursor was detected in immunoprecipitates of p7D-transfected cells. No viral proteins were detected in immunoprecipitates of NIH 3T3 cells (Fig. 1, lanes 1 to 3). The mature envelope protein p15(E) was observed in p623-transfected but not in p7D-transfected or untransfected lysates (data not shown). These data indicated that proteolytic processing of the p7D envelope precursor to the mature envelope proteins was defective.

Transfer of the BALB/c virus processing mutation to AKV. To determine whether the amino acid change at the site of Pr85env cleavage is responsible for the envelope glycoprotein-processing defect, we constructed a plasmid, pEF38, whose proviral DNA is identical to that of p623 except for the single nucleotide change that encodes the lysine at the BALB/c virus envelope glycoprotein-processing site. Essentially, a p623 fragment spanning the processing junction was replaced with the corresponding region from p7D. This region differed between the two proviral sequences by only one nucleotide. To confirm that the desired change had been made, the region of the plasmid encoding the gp70-p15(E) junction was sequenced, and it was determined that a lysine codon was present at the site of pEF38 glycoprotein cleavage (data not shown).

TABLE 1. vEF38 versus AKV infectivity and XC cell fusion activity^a

Proviral clone	Infectivity of proviral DNA (U/μg)		Infectivity of virus (U/ml)		
	FA	XC	FA	XC	RT
p623	2.5×10^4	2.4×10^4	10^4	10^4	1
pEF38	2.5×10^3	<0.05	10^2	<10	0.3

^a 10 μg of proviral DNA was used to transfect NIH 3T3 cells, and the transfected cells were scored 6 days later for fluorescent antibody-positive foci (FA) and plaques in the UV-XC assay (XC). Medium was changed on one plate, and virus was harvested the next day. The relative units of reverse transcriptase (RT) activity in the supernatant were determined, and titers of the virus were determined on NIH 3T3 cells. The experiment was done in parallel with both plasmids three times, and each experiment gave similar results.

After its construction, pEF38 was used to transfect NIH 3T3 cells in parallel with p623. The infectivities of the two plasmids were determined by enumerating the number of fluorescent antibody-positive foci and XC plaques per microgram of proviral DNA. The infectivities of the pEF38-derived virus vEF38 and of AKV were determined with harvests from cells 7 days posttransfection. A reverse transcriptase assay was done to determine the relative amounts of virus in the AKV- and vEF38-infected cell supernatants. The data obtained from these experiments indicated that vEF38 is at least 10-fold less infectious than AKV and is unable to induce fusion of XC cells (Table 1).

vEF38- and AKV-infected cells were examined in parallel for their sensitivity to focus formation by pseudotype pools of Harvey murine sarcoma virus enveloped with amphotropic or ecotropic proteins (Table 2). These data demonstrate that vEF38 is roughly 100-fold less effective than AKV at establishing viral interference to ecotropic MuLV. vEF38 and AKV do not significantly interfere with infection by Harvey murine sarcoma virus pseudotyped with amphotropic envelopes.

Intracellular expression of EF38 envelope proteins. To test whether the single nucleotide mutation in pEF38 has an effect on envelope protein processing, we immunoprecipitated [³⁵S]methionine-labeled lysates prepared from cells 7 days after transfection with pEF38 or p623 (Fig. 1A). Both the envelope protein precursor Pr85_{env} and the mature gp70 protein were present in p623-transfected cells (lane 2), whereas only the precursor was detected in p7D-transfected cells (lane 3). In immunoprecipitates of pEF38-transfected cells (lane 4), only the precursor protein was detected. A longer exposure of this gel (Fig. 1B) indicated the presence in the EF38 lanes (lanes 4 and 5) of a protein that migrated to around 100 kilodaltons. The suspected identity of this pro-

TABLE 2. Interference properties of pEF38

Target cells	Titer of Ha-MSV pseudotypes ^a	
	4070A (amphotropic)	AKV (ecotropic)
NIH 3T3	1	1
NIH 3T3-AKV	0.5	0.00001
NIH 3T3-EF38	0.24	0.001

^a Titers of amphotropic or ecotropic pseudotypes of Harvey murine sarcoma virus (Ha-MSV) (7, 16) were determined on NIH 3T3 cells. NIH 3T3 cells confluent infected with AKV, or NIH 3T3 cells confluent infected with vEF38. Numbers are focus-forming unit titers relative to the titer of the pseudotype on NIH 3T3 cells. The percentage of cells that expressed viral antigens (i.e., were FA⁺) was 57% in the AKV-infected culture and 56% in the vEF38-infected culture.

FIG. 2. Pulse-chase and endo-H analysis of AKV and vEF38 envelope protein processing. Infected NIH 3T3 cells were labeled for 1 h with 250 μCi of [³⁵S]methionine; chased for 0, 1, 2, 3, or 5.5 h in cold medium; and doubly immunoprecipitated with anti-gp70 antiserum and subjected to SDS-polyacrylamide gel electrophoresis (lanes 1 to 5) or digested with endo-H and subjected to SDS-polyacrylamide gel electrophoresis (lanes 6 to 10). Lanes: 1 to 5, undigested (chased for 0, 1, 2, 3, and 5.5 h, respectively); 6 to 10, endo-H digested (chased for 0, 1, 2, 3, and 5.5 h, respectively). Panels: A, AKV-infected immunoprecipitates; B, vEF38-infected immunoprecipitates. K, Kilodaltons.

tein, which we refer to as Pr100_{env}, will be discussed later. The data shown in Fig. 1A and 1B clearly indicate that the Arg-to-Lys mutation inhibits the proteolytic processing of the envelope protein precursor.

To determine the limit of our ability to detect gp70 in the immunoprecipitations shown in Fig. 1, we performed the following experiment. Immunoprecipitations were done with various amounts of [³⁵S]methionine-labeled lysate from p623-transfected cells (Fig. 1C). To keep the total amount of cell protein constant, the volume of all samples was made equal by adding labeled lysate from untransfected cells. Antibody levels were maintained in excess. gp70 was detected in immunoprecipitates of mixtures containing 25% and 10% p623-transfected cell lysate but not in a mixture containing 5% p623-transfected cell lysate. Since gp70 is not detected in immunoprecipitates of vEF38- or BALB/c virus-infected cells, the vEF38 and BALB/c virus envelope precursors in these cells are processed with less than 10% the efficiency of the AKV envelope precursor.

To examine the kinetics of envelope protein processing and glycosylation, AKV- and vEF38-infected cells were labeled for 1 h with [³⁵S]methionine, chased for 0, 1, 2, 3, or 5.5 h, and immunoprecipitated with anti-gp70 antiserum (Fig. 2). One-half of the immunoprecipitated sample was digested with endo-H, an enzyme that cleaves the high-mannose oligosaccharide side chains added in the endoplasmic reticulum (8). To quantitate the amounts of viral proteins in this experiment, bands were cut from the gel and measured by liquid scintillation counting. In AKV-infected cells, a small amount of gp70 was present after the 1-h labeling period, and as the chase time increased so did the amount of gp70 relative to Pr85_{env}. In vEF38-transfected cells, a substantial amount of Pr100_{env} appeared during the labeling period and then dropped off below our level of detection within 2 h. gp70 was not detected in these cells. In both AKV- and vEF38-infected cells, the total amount of envelope protein decreased with time as it was shed from cells in virions. The apparent molecular sizes of gp70 and Pr100_{env} were not decreased by endo-H, indicating that they contained endo-H-resistant complex sugar side chains. The apparent molecular size of Pr85_{env} was decreased to 65 to 70 kilodaltons after endo-H digestion, indicating that it contained endo-H-sensitive high-mannose sugar side chains. These results suggest that since Pr100_{env} contains endo-H-

FIG. 3. Glycosylation of AKV and vEF38 envelope glycoproteins. Infected cells were labeled for 8 h with [3 H]mannose (lanes 1, 4, and 7), [3 H]glucosamine (lanes 2, 5, and 8), and [3 H]fucose (lanes 3, 6, and 9). Cell lysates were immunoprecipitated with anti-gp70 antiserum. The undefined arrow indicates the position of Pr100env. Lanes: 1 to 3, NIH 3T3 cells; 4 to 6, AKV-infected immunoprecipitates; 7 to 9, vEF38-infected immunoprecipitates. K, Kilodaltons.

resistant complex sugar side chains, this species is transported to the Golgi apparatus. The rapid appearance of Pr100env in vEF38-infected cells indicates that transport of the uncleaved precursor to the Golgi apparatus occurs efficiently relative to gp70.

To confirm the glycosylation data obtained above, cells infected with vEF38 and AKV were independently labeled with three tritiated sugars: [3 H]mannose and [3 H]glucosamine, which are known to be added in the endoplasmic reticulum, and [3 H]fucose, which is known to be added in the Golgi apparatus (8). Lysates from these sugar-labeled cells were prepared and immunoprecipitated with anti-gp70 antiserum (Fig. 3). In AKV-infected cells, both Pr85env and gp70 were labeled with [3 H]mannose and [3 H]glucosamine (lanes 4 and 5), whereas only gp70 was labeled with [3 H]fucose (lane 6). This result suggests that the AKV envelope glycoprotein precursor is proteolytically cleaved before it arrives in the compartment of the Golgi apparatus in which fucose addition occurs. In vEF38-infected cells, the precursor was labeled with [3 H]mannose and [3 H]glucosamine (lanes 7 and 8) but was not labeled with [3 H]fucose (lane 9). Pr100env was labeled with all three sugars, consistent with the notion that this species is an uncleaved envelope glycoprotein containing complex sugar side chains. gp70 was not detected in any of the vEF38 immunoprecipitates.

Display of vEF38 envelope proteins on viral envelopes and cellular membranes. To examine virion envelope glycoproteins, [35 S]methionine-labeled viruses were solubilized and immunoprecipitated with anti-gp70 and anti-gag antisera (Fig. 4). The major gag proteins detected in both vEF38 and AKV immunoprecipitates were p30 and the gag precursor Pr65. p15 and p10 contain no methionine residues and were therefore not labeled. The envelope proteins in AKV immu-

FIG. 4. Envelope proteins in AKV and vEF38 virions. [35 S]methionine-labeled viruses were solubilized and immunoprecipitated with anti-gp70 (lanes 1, 3, and 5) or anti-p15gag (lanes 2, 4, and 6) antisera. The positions of gp70, p15E, and p12E are indicated with labeled arrows; that of Pr100env is indicated by an undefined arrow. Lanes: 1 and 2, NIH 3T3 cells; 3 and 4, AKV; 5 and 6, vEF38. K, Kilodaltons.

FIG. 5. Cell surface iodination of infected cells. Infected NIH 3T3 cells were iodinated for 20 min with 400 μ Ci of 125 I. Lysates were doubly immunoprecipitated with anti-gp70 antiserum. Lanes: 1, AKV-infected immunoprecipitate; 2, vEF38-infected immunoprecipitate. K, Kilodaltons.

noprecipitates were gp70, p15(E), and p12(E). The envelope proteins detected in vEF38 immunoprecipitates were gp70 and Pr100env. Small amounts of p15(E) and p12(E) were also visible on the original fluorogram. The ratio of the amount of AKV to vEF38 used in these immunoprecipitations, as determined by the relative amount of gag proteins in lanes 4 and 6 of Fig. 4 (quantified by liquid scintillation counting), was roughly 3:1. The ratio of AKV envelope protein (lane 3) to vEF38 envelope protein (lane 5) was approximately 13:1. These data indicate that vEF38 virions contain roughly fourfold less envelope protein than do AKV virions and that vEF38 virions contain the novel 100-kilodalton envelope glycoprotein Pr100env.

To determine the relative amounts of envelope glycoprotein on cell membranes, AKV- and vEF38-infected cells were surface labeled with 125 I and immunoprecipitated with anti-gp70 antibody (Fig. 5). The levels of Pr100env and gp70 on the surfaces of vEF38-infected cells were below the limits of detection in this assay, whereas gp70 was clearly present on AKV-infected cells. These results indicate that AKV-infected cells contain significantly more cell surface envelope protein than do vEF38-infected cells.

DISCUSSION

The endogenous ecotropic virus of the BALB/c inbred strain of mouse is unable to induce the fusion of XC cells in culture and is less infectious and less effective at establishing superinfection interference than is AKV, the endogenous ecotropic virus of the AKR inbred strain of mice. A mutation is present in the BALB/c virus that converts the arginine found at the site of envelope glycoprotein cleavage to lysine. The transfer of this single nucleotide mutation to AKV allows us to examine the effects of this mutation on the biology of the virus and on the kinetics of envelope glycoprotein processing, glycosylation, and transport.

Immunoprecipitation experiments with [35 S]methionine-labeled cells infected with AKV and the lysine mutant-containing virus vEF38 indicate that the vEF38 envelope protein precursor Pr85env is cleaved to gp70 and p15(E) at least 10-fold less efficiently than is the wild-type AKV precursor. Cell surface iodination assays demonstrate that vEF38-infected cells contain significantly less envelope protein on their plasma membranes than do AKV-infected cells, and virion-labeling experiments indicate that vEF38 virions contain roughly fourfold less envelope protein than do AKV virions.

Pulse-chase labeling of vEF38-infected cells and endo-H digestion of viral envelope glycoproteins reveal a rather complex kinetics of mutant envelope protein processing and glycosylation. No gp70 is detected in vEF38-infected cells, but an uncleaved precursor containing complex sugar side chains, which we refer to as Pr100env, appears during a 1-h

labeling period and then rapidly disappears. Metabolic labeling with tritiated sugars confirms that Pr100env contains complex sugar side chains. Because of its rapid disappearance and low cell surface and virion levels, we speculate that much of the Pr100env produced in vEF38-infected cells is degraded in the Golgi apparatus or during transport from the Golgi apparatus to the cell membrane. We cannot, however, rule out the possibility that it is secreted.

XC assays demonstrate that whereas AKV is XC⁺, vEF38, like the BALB/c ecotropic virus, is XC⁻. vEF38 also resembles the BALB/c virus by being 10-fold less infectious and 100-fold less effective than AKV at rendering cells resistant to infection by MuLVs bearing ecotropic envelopes. The extent of both XC cell fusion and viral interference presumably reflects the level of envelope glycoproteins on the surfaces of infected cells. We speculate, therefore, that it is the reduction in the relative amount of envelope glycoprotein on the cell membrane that causes vEF38 to be XC⁻ and to be less effective than AKV at establishing superinfection interference. The decreased infectivity of vEF38 is most likely caused by the small amount of envelope protein in vEF38 relative to AKV virions. We do not yet know whether the Pr100env found in vEF38 virions can function in infection; the nonfunctional nature of unprocessed glycoproteins in other enveloped viruses suggests that it cannot (25). In any case, the mutant virus relies on a reduced amount of envelope glycoprotein to perform the receptor binding and membrane fusion functions that are necessary for efficient retroviral infection.

Because of the high degree of conservation of an arginine residue at the site of envelope protein proteolytic cleavage and the widespread use of similar mechanisms for the synthesis and membrane insertion of many viral envelope glycoproteins, it seems likely that the mutation described here would have similar effects on the processing and display of envelope glycoproteins in nearly all retroviruses and in a wide range of other enveloped viruses. The nature of this mutation suggests that cleavage of retroviral envelope glycoproteins does not usually involve trypsin, because this enzyme cleaves efficiently at both arginine and lysine residues (23).

In light of recent reports indicating that T-cell fusion induced by the envelope glycoprotein of the acquired immune deficiency syndrome virus (human immunodeficiency virus) may be a major contributor to the pathogenicity of this virus (11, 21), we believe that fusion-abrogating mutations such as the one reported here are of interest not only in exploring the effects of these mutations during retroviral infection and envelope protein-induced cell fusion but also in studying the role of envelope proteins in disease processes.

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