JOURNAL OF VIROLOGY, Feb. 1995, p. 713–719 Vol. 69, No. 2
0022-538X/95/$04.00+0

Receptor-Binding Domain of Murine Leukemia Virus Envelope Glycoproteins

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Received 24 June 1994/Accepted 4 November 1994

The surface glycoprotein (SU) of murine leukemia viruses (MuLVs) comprises two domains connected by a proline-rich hinge. The interaction of MuLV particles with subgroup-specific cell surface receptors depends primarily on two variable regions (VRA and VRB) located in the amino-terminal domain. To delineate the minimal receptor-binding domains, we examined the capacity of soluble envelope fragments to compete with the entry of virus particles. Amphotropic, ecotropic, polytropic, and xenotropic truncated SUs were produced by inserting stop codons in the *env* gene of the 4070A, Friend, MCF247 and NZB MuLVs, respectively. These fragments, as well as full-length envelope glycoproteins, were stably expressed in cells bearing the corresponding receptor. Synthesis, posttranslational modifications, transport, and secretion of the *env* gene products were monitored by immunoprecipitation. Cells expressing the modified SUs or naive cells preincubated with SU-containing conditioned media were infected with different pseudotypes of a retroviral vector carrying a β-galactosidase marker gene. Reduction of cell susceptibility to infection in the presence of SU was used as a measure of receptor occupancy. The results indicated that the amphotropic and ecotropic envelope amino-terminal domains contain all of the determinants required for receptor binding. In contrast, additional sequences in the proline-rich region were needed for efficient interaction of the polytropic and xenotropic amino-terminal domains with the receptors.

Retrovirus envelope glycoproteins comprise a transmembrane (TM) molecule and a surface (SU) molecule. The entry of type C retroviruses into cells is mediated by the interaction of SU with a cell surface receptor (8, 21). Studies of natural murine leukemia virus (MuLV) recombinants have shown that receptor recognition is ensured by the amino-terminal half of the SU protein (2, 7, 10, 19, 20). Sequence comparisons of the SU amino-terminal domains from murine type C retroviruses have delineated three highly variable regions, referred to as variable region A (VRA), variable region B (VRB), and the proline-rich region (PRO), which are thought to be important for receptor recognition (1, 2, 9, 15, 16, 19). A similar organization has been recognized in feline leukemia virus and Gibbon ape leukemia virus (1). Exchanging VRA and/or VRB between envelope glycoproteins of different MuLV subgroups showed that VRA plays a predominant role in receptor choice (1, 14). Accordingly, insertion mutagenesis in VRA of Moloney MuLV SU affects the recognition of the ecotropic (E) receptor (5). Analysis of disulfide bridges in Friend E and mink cell focus-forming (MCF) MuLV SUs has indicated that VRA can form two hydrophilic loops suitable for receptor binding (11, 12). The cysteine residues involved in the formation of these potential loop structures are conserved between type C SUs. In feline leukemia virus SUs, the VRA-equivalent region (referred to as V1) has also been shown to determine receptor specificity (3, 18).

Although the participation of VRA in SU-receptor interactions has been clearly established, other determinants must be involved. For example, the host range of the 10A1 MuLV recombinant is in part determined by PRO, which is required for recognition of the 10A1 receptor (17). The receptor-binding properties of SU chimeras constructed by combining VRA, VRB, and PRO from different MuLV subgroups have indicated that efficient virus entry through the xenotropic (X) or polytropic (P) receptors requires the presence of PRO from either X or P SUs (1). However, in contrast with VRA, mutations introduced into E PRO did not alter receptor recognition (5).

In the present study, we analyzed the receptor-binding properties of native or truncated SU proteins from four MuLV subgroups. The binding of SU proteins either produced by the cell itself or exogenously added renders the viral receptor unable to mediate the entry of viral particles. Measuring the intensity of this interference therefore gives an indirect evaluation of the affinity between the SU and the receptor (6). Here, this approach was used to assay for interactions between the E, amphotropic (A), P, and X receptors and truncated envelope glycoproteins retaining the amino-terminal domain only (ST proteins), the amino-terminal domain and PRO (PRO proteins), or the entire SU moiety.

The data extend to all four MuLV SUs our original finding on the Friend E SU that the amino-terminal part of the molecule autonomously folds into an active receptor-binding structure (6). However, only A-ST and E-ST were biologically active and able to confer interference levels equivalent to those observed with full-length SUs. In contrast, addition of PRO was essential for the activity of X- and P-based SUs. These results demonstrate that the receptor-binding determinants of the E and A envelopes are all located in the amino-terminal domain of the envelope glycoprotein whereas the X and P envelopes need additional sequences located in PRO.

MATERIALS AND METHODS

Construction of truncated *env* genes (Fig. 1). All constructs were derived from the pFB.KDEL vector (6), in which *env* sequences are under the control of the FB29 Friend MuLV long terminal repeat. pA-GP was obtained by ligating the *Bgl*II-*Cla*I fragment from pCRUCA (1), which encompasses the 4070A *env* gene,

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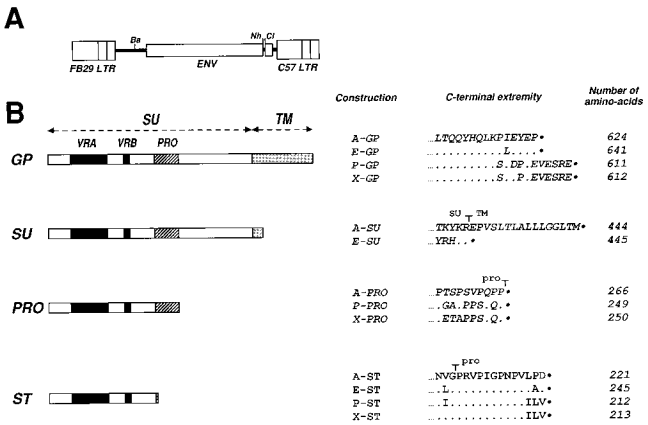

FIG. 1. Schematic representation of envelope expression vector and truncated SU fragments. (A) MuLV *env* genes were inserted between the *Bam*HI (Ba) and *Nhe*I (Nh) or *Cla*I (Cl) sites in FB-KDEL (6). (B) Schematic representation of a full-length MuLV envelope glycoprotein (GP) and of truncated envelope fragments (SU, PRO, and ST), showing VRA and VRB (black boxes), PRO (hatched boxes), and the TM anchor protein (grey boxes). The amino acid sequences shown indicate the exact termination of each protein according to the published sequences of the 4070A A (16), Friend C57 E (9), MCF 247 P (7), and NZB X (15) MuLVs. A envelope fragments were generated by inserting stop codons in the *env* gene of MuLV 4070A, giving rise to A-ST (truncation at the amino-terminal border of PRO), A-PRO (truncation at the carboxy-terminal border of PRO), and A-SU (truncation 16 amino acids downstream of the natural cleavage site between SU and TM). A-GP encodes the full-length 4070A envelope glycoprotein. E envelope fragments were generated from the Friend MuLV *env* gene. E-ST encodes the amino-terminal domain, E-SU terminates at the natural SU-TM cleavage site, and E-GP is a full-length glycoprotein. Stop codons were similarly inserted in the MuLV MCF 247 *env* gene to generate P fragments, giving rise to P-ST, P-PRO, and P-GP, and in the X MuLV NZB *env* gene to generate X-ST, X-PRO, and X-GP. LTR, long terminal repeat.

to the *Bam*HI-*Cla*I fragment of pFB.KDEL; pP-GP and pX-GP were obtained by exchanging a *Bam*HI-*Nhe*I fragment between pFB.KDEL and pCRUCM or pCRUCX (1), which contain the P MCF247 (7) and X NZB (15) *env* genes, respectively. For the X construction, the 5′ *Bam*HI restriction site was created by inserting a *Bam*HI linker in the *Acc*I restriction site (nucleotide 220). These constructs and Friend MuLV E envelope expression vector pFB3 (6) (here referred to as pE-GP) were used as starting material to obtain truncated envelope expression vectors. ST fragments were generated by inserting in-frame stop codons to terminate translation at amino acids 221, 245, 212, and 213 of the A, E, P, and X envelope proteins, respectively. PRO fragments were generated by inserting in-frame stop codons to terminate translation at amino acids 266, 249, and 250 of the A, P, and X envelope proteins, respectively. Most of the mutations were obtained by PCR synthesis. PCR fragments were sequenced. A 14-mer *Xba*I linker was inserted to generate a stop codon in pP-ST (*Bcl*I restriction site, nucleotide 1018 [7]), pX-ST (*Bcl*I restriction site, nucleotide 1015 [15]), or pA-SU (*Nco*I restriction site, nucleotide 1455 [16]).

Cells and viruses. Cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (*Mus dunni*, Rat-1, D17, CCL64, and HOS) or 10% newborn calf serum (NIH 3T3). Stocks of the helper-free E and A pseudotypes with known titers were obtained from cloned ΨCRE and ΨCRIP producers, respectively. The retroviral vector was an LXSN derivative (13) in which the *nls-lacZ* gene was inserted in the *gag* configuration. P, X, and 10A1 pseudotypes were obtained by transient expression of P-GP, X-GP, or the 10A1 *env* gene in EB8 cells (1). These cells constitutively synthesize helper-free particles that are devoid of envelope glycoproteins and contain a β-galactosidase (β-gal)-encoding retroviral genome. EB8 supernatant was harvested 64 h after transfection, filtered through 0.45-μm-pore-size filters, and used for infections. Titers ranged from 10^3 to 10^4 β-gal-positive focus-forming units (FFU)/ml.

Virus infection and interference assays. Transfection of NIH 3T3 cells and D17 cells was done by calcium phosphate coprecipitation with pSV2Neo. Stable transformants were selected with 1 mg of G418 per ml. For interference assays (6), 1 ml of viral supernatant containing approximately 200 to 300 of β-gal-positive FFU and supplemented with 8 μg of Polybrene per ml was incubated for 1 h at 37°C with 10^5 cells in 35-mm-diameter dishes and replaced with fresh medium. Cells were stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) 48 h later, and β-gal-positive FFU were scored. Data were expressed as percentages of the values scored in control cells. Conditioned media were 72-h supernatants from confluent cells. They were filtered through 0.45-μm-pore-size filters and incubated with target cells for 1 h at 37°C before virus infection. Constructions encoding envelope proteins from the same subgroup were tested in the same experiment. Each experiment was repeated at least three times.

Immunoprecipitation analysis. Confluent cells plated on 60-mm-diameter dishes were washed with phosphate-buffered saline and incubated in 1 ml of cysteine-free minimal essential medium containing 2% dialyzed newborn calf serum for 45 min at 37°C. After [^{35}S]cysteine labeling (100 μCi/ml; Amersham) for 3 h, culture medium was harvested and supplemented with 10× lysis buffer (0.5% Nonidet P-40, 150 mM NaCl, 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES], 1 mM phenylmethylsulfonyl fluoride), cells were disrupted under the same conditions, and both were clarified by centrifugation. Preclearing was performed with nonimmune goat serum and protein A-Sepharose (6MB; Pharmacia) for 16 h at 4°C. After centrifugation, supernatants were incubated with goat anti-Raucher gp70 serum (Microbiological Associates) for 2 h at 4°C and then incubated for 30 min with protein A-Sepharose. After washing in RIPA buffer (20 mM Tris [pH 7.4], 0.1% deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 0.1% Triton X-100, 150 mM NaCl), immunoprecipitates were subjected to electrophoresis on SDS–12% polyacrylamide gels followed by fluorography or digested with peptide N-glycosidase F (Biolabs) as recommended by the manufacturer prior to analysis. For pulse-chase experiments, cells were labeled for 40 min with 150 μCi of [^{35}S]cysteine and chased for the time periods indicated in the legend to Fig. 3. Cells and media were then treated as described above.

RESULTS AND DISCUSSION

Synthesis of wild-type and modified envelopes. Wild-type *env* genes of the different MuLV subgroups and genes encoding envelope fragments truncated from various portions of the carboxy-terminal domain were inserted into the FB expression vector (Fig. 1) (6). NIH 3T3 cells, which expressed the A, P, and E receptors, were stably transfected with the A, P, and E constructs, while X constructs were introduced into D17 cells, which express the X receptor. Cells were labeled for 3 h with [^{35}S]cysteine, and a polyclonal goat anti-Rauscher gp70 antibody was used to detect envelope molecules in cell extracts and culture supernatants by immunoprecipitation (Fig. 2).

Precursor molecules of 85 kDa (gpr85) and cleaved *env* products of 70 kDa (gp70) were detected in cells expressing the GP constructs. Significant amounts of shed gp70 were present in supernatants of cells expressing A-GP and X-GP. Shedding was less pronounced for E and P gp70.

Two forms of A-SU appeared in cell extracts. A gp70 form, which was secreted into the medium, showed a molecular mass slightly higher than that of the cleaved product of A-GP, suggesting that cleavage of 16 additional amino acids at the carboxy-terminal extremity of A-SU had not occurred. This was confirmed by removing the oligosaccharide chains by peptide N-glycosidase F digestion (data not shown). The lowest-molecular-mass form that corresponded to an underglycosylated species was not secreted. E-SU was detected in cell extracts as a 70-kDa species efficiently secreted in the medium, where it appeared with a slightly increased molecular mass.

In cell extracts, PRO fragments appeared as two molecular species. The highest-molecular-mass form (49, 43, and 40 kDa for A-PRO, P-PRO, and X-PRO, respectively) were efficiently secreted in cell supernatants. The lowest-molecular-mass forms probably corresponded to underglycosylated molecules and were mostly (A-PRO) or totaly (P-PRO and X-PRO) retained in cells.

All ST fragments were detected in cell extracts and secreted in medium. P-ST and X-ST appeared as single 32-kDa species in cell extracts. Secreted molecules showed the same size. A-ST and E-ST were detected as two bands. Both forms of A-ST (30 and 32 kDa) and E-ST (32 and 35 kDa) were secreted. To document the intra- and extracellular stability of ST fragments, 40-min pulse-labeling periods were followed by chase periods of various times (Fig. 3). Synthesis of ST proteins appeared to be roughly equivalent for the four subgroups. However, the intracellular half-lives of the A-ST and X-ST fragments were significantly shorter than those of the E-ST and

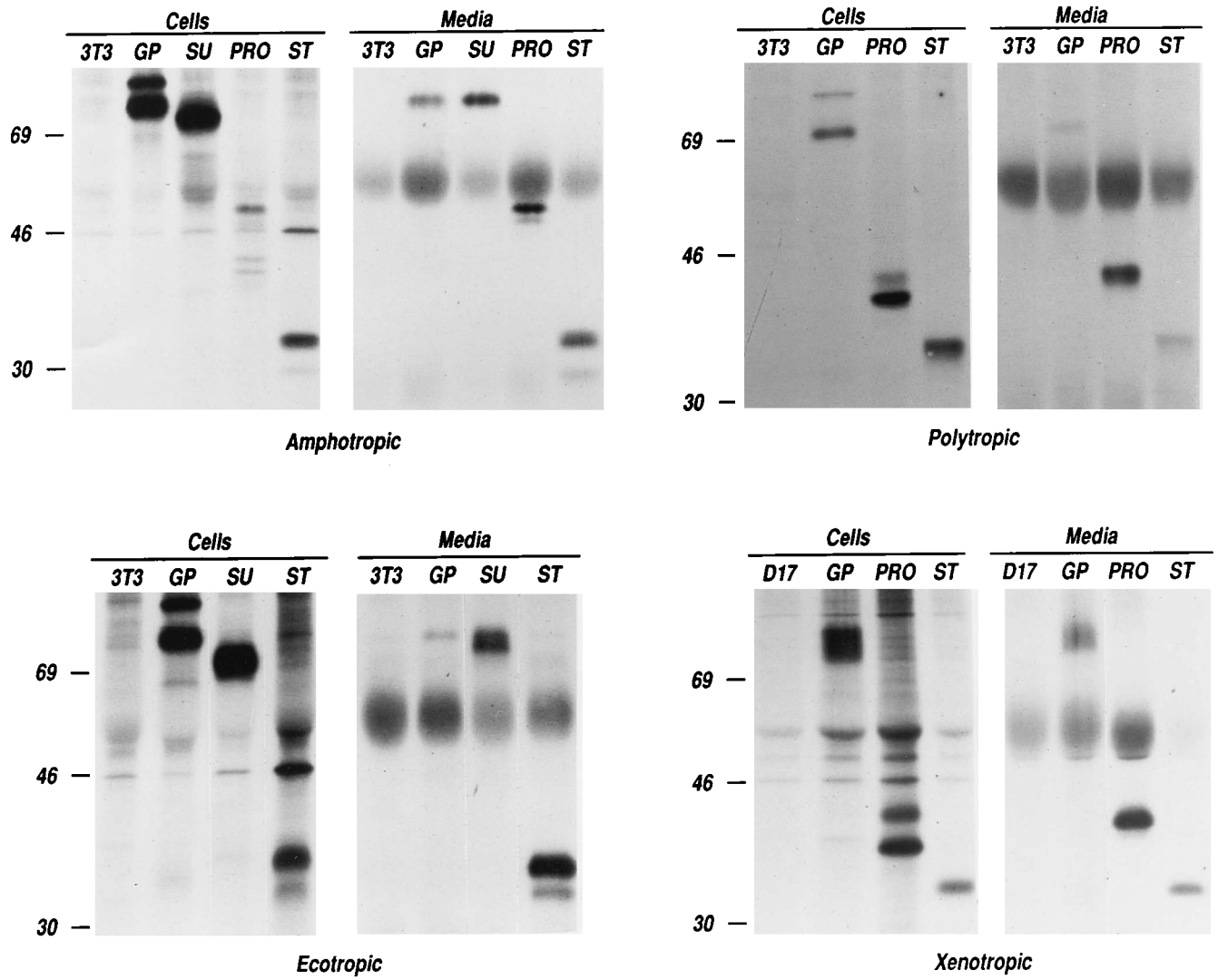

FIG. 2. Detection of envelope glycoproteins in transfected cells. Cells stably expressing the GP, SU, PRO, and ST constructs were labeled with [^{35}S]cysteine for 3 h. Goat anti-MuLV gp70 serum was used to form immune complexes which were analyzed by SDS–12% polyacrylamide gel electrophoresis. 3T3 and D17 were negative controls. Molecular size markers are indicated in kilodaltons.

P-ST fragments. For each construct, secreted molecules became detectable after 1.5 h and remained at the same levels in media for at least 6 h, indicating that ST fragments were secreted with similar efficiencies and were similarly stable in culture supernatants.

Interference with virus entry in envelope-expressing cells. The receptor-binding capacity of full-length and truncated SUs was assayed by determining their capacity to compete with retrovirus particles for receptor occupancy and to interfere with infection. For that purpose, replication-deficient particles pseudotyped with envelope proteins of the different MuLV subgroups were prepared, including two different E pseudotypes coated with the Moloney or Friend MuLV envelope (referred to as ME and FE, respectively). All of these particles contained the same genome coding for the *nls-lacZ* marker.

In a first experiment, cells expressing ST, SU, PRO, and GP were used as targets and susceptibility to infection was expressed as a percentage of β-gal-positive FFU scored in nontransfected cells. Control infections were performed with pseudotypes bearing nonrelevant envelope glycoproteins, for which no interference was expected. Results are shown in Fig. 4.

NIH 3T3 cells expressing A-GP, A-SU, A-PRO, and A-ST were resistant to infection with the A pseudotype (3.7% ± 1.1%, 9.2% ± 0.6%, 9.9% ± 1.3%, and 10% ± 2.7% of the nontransfected cells, respectively), whereas infection with the E pseudotype was not affected. These data suggested that A-ST binds the A receptor as efficiently as does A-SU. Similarly, NIH 3T3 cells expressing E-GP, E-SU, and E-ST were resistant to the ME pseudotype (3.6% ± 0.5%, 9.4% ± 1.7%, and 10.2% ± 0.2% of the nontransfected cells, respectively) but permissive to the A pseudotype. These data indicated that all of the receptor-binding determinants of the A and E envelopes are located upstream of PRO and that fragments of 221 and 245 amino acids, respectively, exhibit full biological activity.

The situation was different in NIH 3T3 cells expressing the P constructs and in D17 cells expressing the X constructs. Cells expressing P-GP and X-GP showed decreased susceptibility to the cognate pseudotype. Interference values (25.9% ± 10.1%

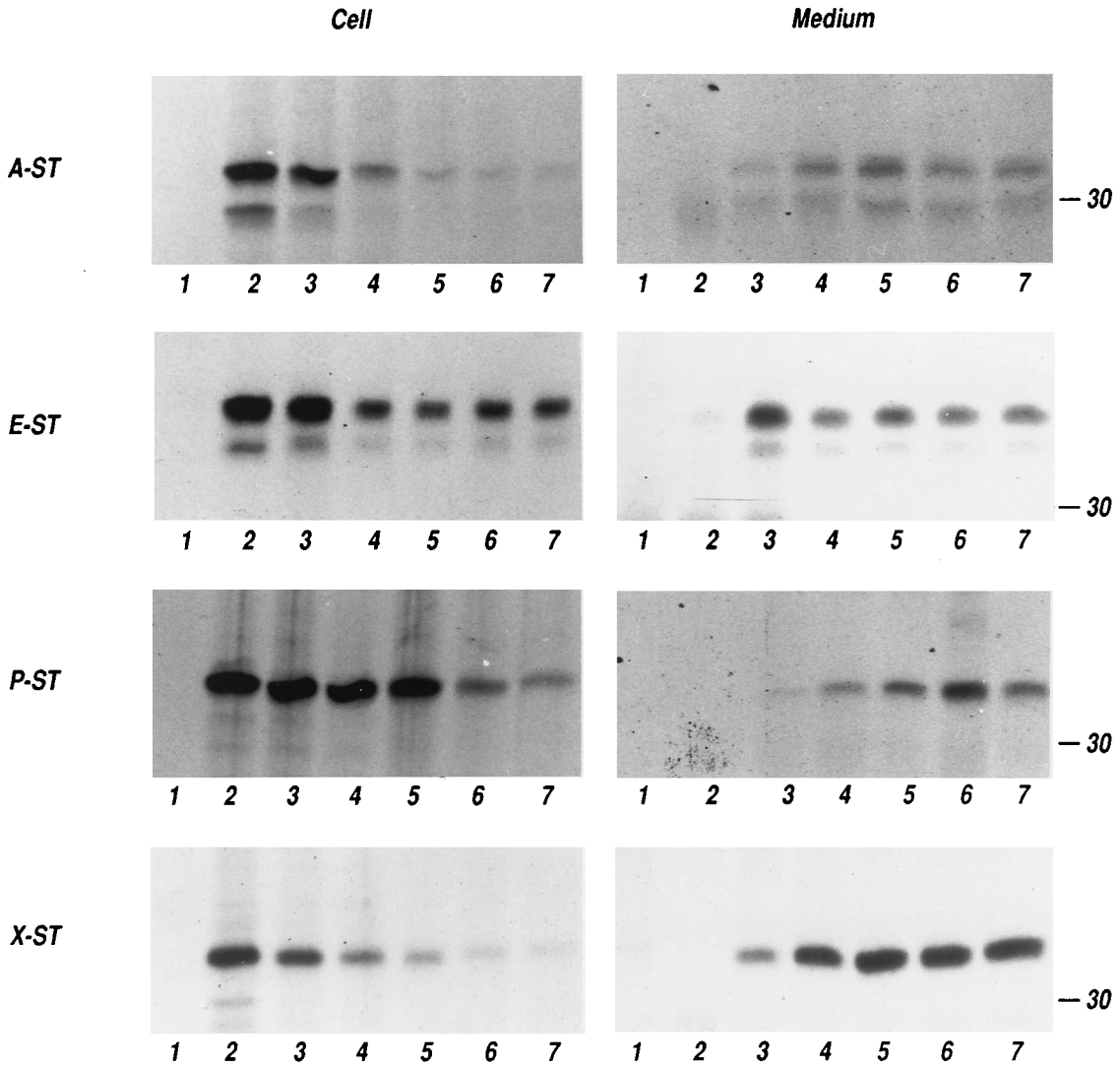

FIG. 3. Pulse-chase experiments with cells expressing ST envelope fragments. Cells were labeled with [^{35}S]cysteine for 40 min (lanes 1 and 2) and incubated with unlabeled cysteine medium for 30 min (lane 3), 1 h (lane 4), 2 h (lane 5), 4 h (lane 6) or 6 h (lane 7). Cell extracts and culture supernatant from NIH 3T3 cells (D17 cells for X fragments) (lane 1) or ST fragments (lanes 2 to 7) were immunoprecipitated with goat anti-gp70 serum before analysis by SDS-polyacrylamide gel electrophoresis. Molecular size markers are indicated in kilodaltons.

and 23.3% ± 8.7% of nontransfected cells for P-GP and X-GP, respectively) were lower than for A-GP and E-GP. Similar observations were made with cells expressing P-PRO (19.3% ± 0.8% of nontransfected cells) and X-PRO (23.6% ± 6.7% of nontransfected cells). In contrast, expression of P-ST or X-ST had no or little effect on susceptibility to infection with the relevant pseudotype (95.8% ± 17.1% and 72.9% ± 23.8% of nontransfected cells, respectively). We concluded that addition

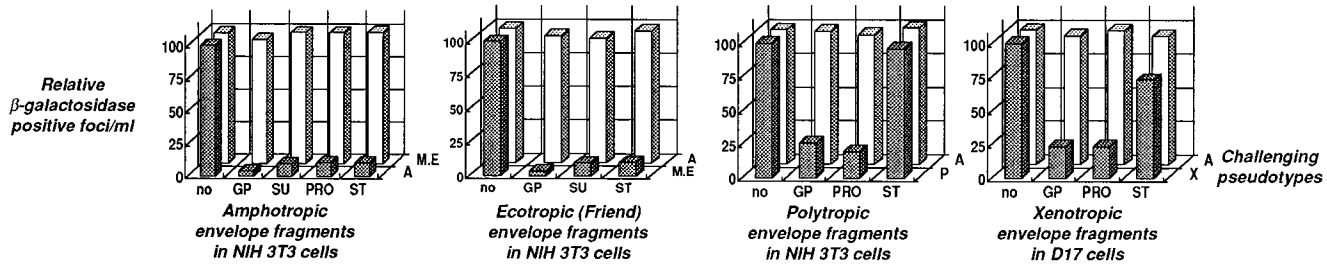

FIG. 4. Interference capacity of wild-type and truncated envelopes. Cells (NIH 3T3 or D17) expressing wild-type (GP) or envelope (SU, PRO, and ST) fragments of A, E, P, or X origin were infected with the A, ME, P, or X MuLV pseudotype of a helper-free retroviral vector coding for *Escherichia coli* β-gal. The numbers of β-gal-positive foci scored for envelope-expressing cells were compared to the numbers scored for the negative control, and the resulting percentages are shown here. Grey columns are the data from infection with the relevant pseudotype (same origin as the expressed envelope constructions), and white columns are data from control infections with nonrelevant pseudotypes.

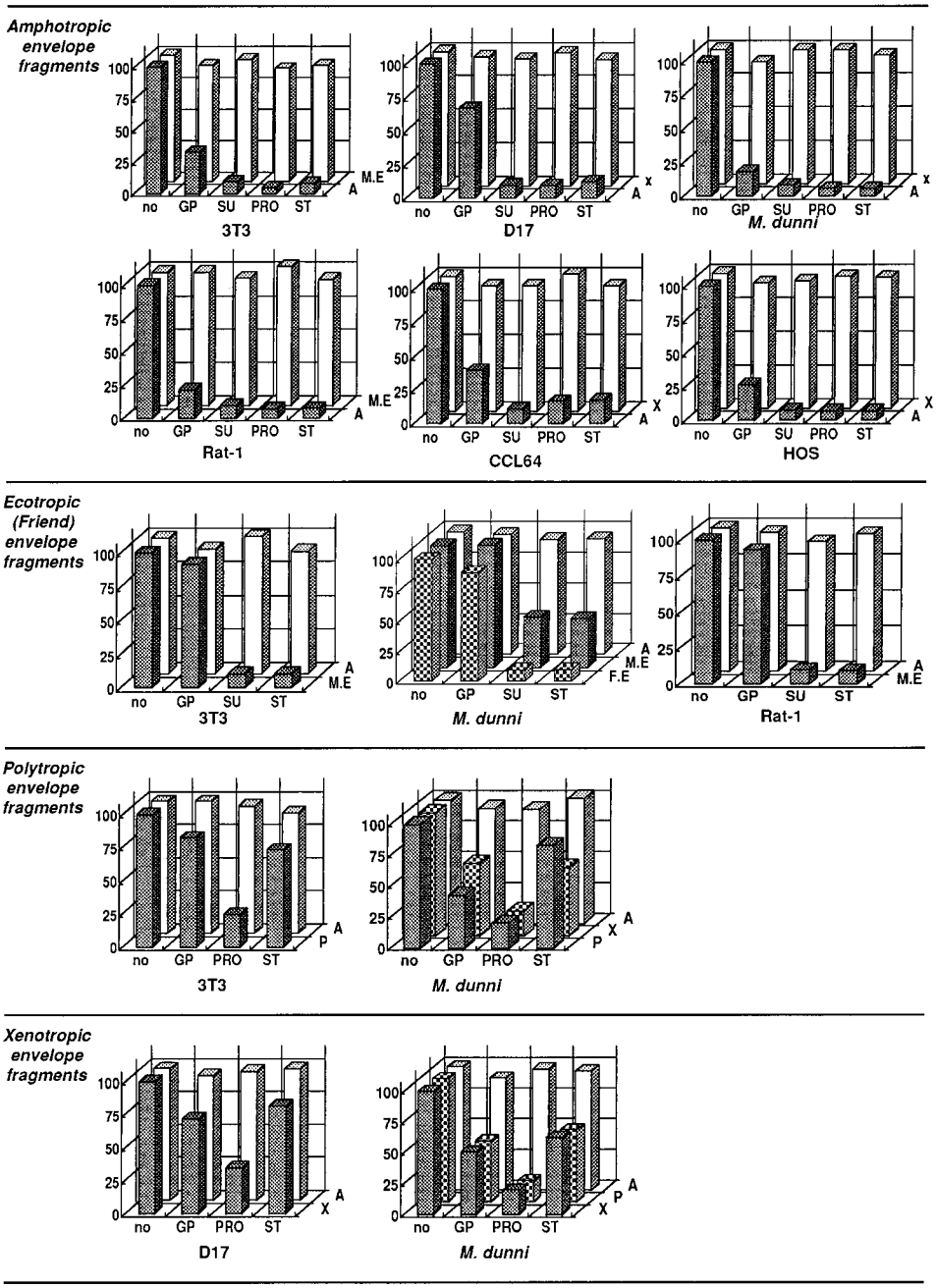

FIG. 5. Interference induced in naive cells by CM. Mouse NIH 3T3, dog D17, wild mouse (*M. dunni*), rat Rat-1, mink CCL64, and human HOS cells were incubated with CM from control cells (left column) or from cells expressing full-length (GP) or truncated (SU, PRO, and ST) envelope fragments and then infected with the A, ME, Friend ecotropic (FE), P, or X MuLV pseudotype of a helper-free retroviral vector coding for *E. coli* β-gal. Data are expressed as in Fig. 4. White columns shows the data from control infections with nonrelevant pseudotypes.

of PRO is essential for the activity of X- and P-based SUs. This confirmed our previous observations made with virions coated with chimeric envelopes (1). It is noticeable that spontaneous recombinants between exogenous E and endogenous P envelope sequences (MCF viruses) always exchange PRO together with the envelope amino-terminal domain.

Interference in naive cells preincubated with soluble envelope fragments. In a second experiment, we examined whether soluble envelope fragments secreted in culture media could compete with virus particles for occupancy of viral receptors expressed at the surface of naive cells. Cells from different species were used as targets to reveal cell type-specific variations in the envelope-receptor interaction. For that purpose, mouse NIH 3T3, dog D17, wild mouse (*M. dunni*), rat Rat-1, mink CCL64, and human HOS cells were incubated for 1 h with culture supernatants from NIH 3T3 or D17 cells expressing the various envelope constructs and then infected with the relevant virus pseudotype or with a nonrelevant control. Susceptibility to infection was expressed as a percentage of β-gal-positive FFU scored in cells pretreated with culture superna-

tants from nontransfected NIH 3T3 or D17 cells. Results are shown in Fig. 5.

Pretreatment of the different target cell lines with A-ST, A-PRO, and A-SU conditioned media (CM) resulted in reduced susceptibility to infection with the A pseudotype (4.4 to 17.9% of cells pretreated with NIH 3T3 CM), whereas infection with a nonrelevant pseudotype (ME or X) was not affected. Consistent with the observed shedding of A-GP, interference was also observed after incubation with A-GP CM (18.5 to 40.3% of cells pretreated with NIH 3T3 CM). One exception was D17 cells (67.1% of cells pretreated with D17 CM), suggesting that shed A gp70 could not saturate or bind the dog A receptor as efficiently as the A receptor of other species.

Pretreatment of NIH 3T3 and Rat-1 cells with E-SU or E-ST resulted in reduced susceptibility to infection with the ME pseudotype (9.5 to 10.9% of cells pretreated with NIH 3T3 CM) without alteration of susceptibility to the A pseudotype. Interestingly, preincubation of *M. dunni* cells with fragments of the FE envelope induced a much higher resistance to the FE pseudotype (9.4 to 10% of cells pretreated with NIH 3T3 CM) than to the ME pseudotype (40.2 to 45.7% of cells pretreated with NIH 3T3 CM). Consistent with the absence of shed E-GP, incubation with CM from E-GP-expressing cells did not induce interference.

Pretreatment of NIH 3T3, D17, *M. dunni*, or CCL64 (data not shown) cells with media conditioned with P-ST or X-ST fragments did not result in significant interference. Media containing PRO fragments induced a low level of resistance to infection with the relevant P or X virus in NIH 3T3 and D17 cells (24.5 to 34.2% of cells pretreated with NIH 3T3 or D17 CM) and with both P and X viruses in *M. dunni* cells (17.5 to 20.6% of cells pretreated with NIH 3T3 or D17 CM). This is consistent with a previous report of cross-interference between the P and X MuLVs in *M. dunni* cells (4). It confirmed that binding to the P and X receptors requires determinants located in PRO. The small amounts of gp70 shed from P-GP- and X-GP-expressing cells also induced very weak interference with both P and X infections of *M. dunni* cells, whereas the susceptibility of NIH 3T3 cells was not significantly affected.

Role of PRO in envelope-receptor interactions. The requirement for PRO determinants in the binding of P or X envelope glycoproteins to their receptors has been previously suggested (1, 17). PRO can be implicated in envelope-receptor interactions by different nonexclusive mechanisms. It may directly bind the receptor, or alternatively, it may have an indirect effect by inducing appropriate folding of the adjacent receptor-binding domain. We tested these hypothesis with the P envelope SU by using chimeric constructions in which the amino-terminal envelope region upstream of PRO and PRO itself were from different MuLV subgroups. AP-PRO associates an A amino-terminal domain with a P PRO. PA-PRO associates a P amino-terminal domain with an A PRO. The chimeras were stably introduced into NIH 3T3 cells. Both gave rise to protein products that were detected in cell extracts and efficiently secreted in culture supernatants (data not shown). Cells expressing PA-PRO (Fig. 6A) or incubated with the supernatant of PA-PRO (Fig. 6B) were equally susceptible to infection with the A or P pseudotype, suggesting that this envelope fragment was unable to bind those receptors. In contrast, cells expressing AP-PRO (Fig. 6A) or pretreated with AP-PRO CM (Fig. 6B) were resistant to infection with the A pseudotype but still susceptible to infection with the P pseudotype, as well as with the ME pseudotype. These data suggested that P PRO does not bind the P receptor but rather induces appropriate allosteric shaping of the amino-terminal receptor-binding domain. In agreement with our previous finding (1), they indicated that a soluble envelope fragment of P origin (like P-ST) requires a homologous PRO for receptor interaction. However, we cannot exclude the possibility that the presence of an A amino-terminal domain destabilizes a direct interaction between P PRO and the receptor.

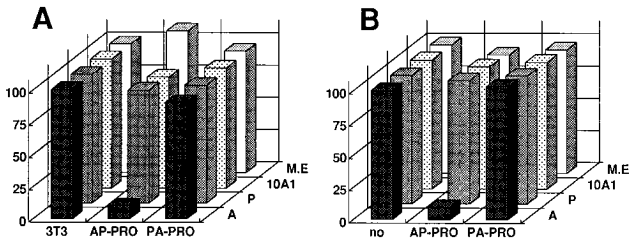

FIG. 6. Interference capacity of chimeric truncated SUs. Control mouse NIH 3T3 cells (3T3), and cells expressing the AP-PRO or PA-PRO construct (A), and cells incubated with CM from cell expressing (B) the AP-PRO or PA-PRO construct were infected with the A, ME, P, or 10A1 MuLV pseudotype of a helper-free retroviral vector coding for *E. coli* β-gal. Data are expressed as in Fig. 4.

Since the juxtaposition of an A amino-terminal domain to a P PRO is reminiscent of the structure of the 10A1 MuLV envelope, we wondered whether the AP-PRO fragment will interfere with the entry of a 10A1 pseudotype. The data in Fig. 6 indicate that the susceptibility of NIH 3T3 cells to 10A1 pseudotype infection was unaffected by AP-PRO expression or by treatment with AP-PRO CM. Therefore, AP-PRO did not compete with the binding of 10A1 to its receptor. The conclusions that can be drawn from this experiment are, however, limited, since the chimeric envelope of 10A1 and the AP-PRO molecule associate fragments from different A (1504 versus 4070A) and P (Moloney MCF versus MCF 247) isolates (16).

ACKNOWLEDGMENTS

We are grateful to Alan Rein for the gift of the 10A1 molecular clone. We thank Nicolas Ferry, Valérie Maréchal, Christophe Perez, and Olivier Schwartz for help and discussions.

This work was supported by grants from the Agence Nationale de Recherches sur le Sida (ANRS) and the Institut Pasteur. J.-L.B. is a fellow of the Ministère de l'Enseignement Supérieur et de la Recherche.

REFERENCES

1. **Battini, J. L., J. M. Heard, and O. Danos.** 1992. Receptor choice determinants in the envelope glycoproteins of amphotropic, xenotropic, and polytropic murine leukemia viruses. J. Virol. **66:**1468–1475.
2. **Bosselman, R. A., F. V. Straafen, C. V. Beveren, I. M. Verma, and M. Vogt.** 1982. Analysis of the *env* gene of a molecularly cloned and biologically active Moloney mink cell focus-forming proviral DNA. J. Virol. **44:**19–31.
3. **Brojatsch, J., B. S. Kristal, G. A. Viglianti, R. Khiroya, E. A. Hoover, and J. I. Mullins.** 1992. Feline leukemia virus subgroup C phenotype evolves through distinct alterations near the N terminus of the envelope surface glycoprotein. Proc. Natl. Acad. Sci. USA **89:**8457–8461.
4. **Chesebro, B., and K. Wehrly.** 1985. Different murine cell lines manifest unique patterns of interference to superinfection by murine leukemia viruses. Virology **141:**119–129.
5. **Gray, K. D., and M. Roth.** 1993. Mutational analysis of the envelope gene of Moloney murine leukemia virus. J. Virol. **67:**3489–3496.
6. **Heard, J. M., and O. Danos.** 1991. An amino-terminal fragment of the Friend murine leukemia virus envelope glycoprotein binds the ecotropic receptor. J. Virol. **65:**4026–4032.
7. **Holland, C. A., J. Wozney, and N. Hopkins.** 1983. Nucleotide sequence of the gp70 gene of murine retrovirus MCF 247. J. Virol. **47:**413–420.
8. **Hunter, E., and R. Swanstrom.** 1990. Retrovirus envelope glycoproteins. Curr. Top. Microbiol. Immunol. **157:**187–253.
9. **Koch, W., G. Hunsmann, and R. Friedrich.** 1983. Nucleotide sequence of the envelope gene of Friend murine leukemia virus. J. Virol. **45:**1–9.
10. **Koch, W., W. Zimmermann, A. Oliff, and R. Friedrich.** 1984. Molecular analysis of the envelope gene and long terminal repeat of Friend mink cell

focus-inducing virus: implications for the functions of these sequences. J. Virol. **49:**828–840.
11. **Linder, M., D. Linder, J. Hahnen, H. H. Schott, and S. Stirm.** 1992. Localization of the intrachain disulfide bonds of the envelope glycoprotein 71 from Friend murine leukemia virus. Eur. J. Biochem. **203:**65–73.
12. **Linder, M., V. Wenzel, D. Linder, and S. Stirm.** 1994. Structural elements in glycoprotein 70 from polytropic Friend mink cell focus-inducing virus and glycoprotein 71 from ecotropic Friend murine leukemia virus, as defined by disulfide-bonding pattern and limited proteolysis. J. Virol. **68:**5133–5141.
13. **Miller, A. D., and G. J. Rosman.** 1989. Improved retroviral vectors for gene transfer and expression. BioTechniques **7:**980–990.
14. **Morgan, R. A., O. Nussbaum, D. D. Muenchau, L. Shu, L. Couture, and W. F. Anderson.** 1993. Analysis of the functional and host range-determining regions of the murine ecotropic and amphotropic retrovirus envelope proteins. J. Virol. **67:**4712–4721.
15. **O'Neill, R. R., C. E. Buckler, T. S. Theodore, M. A. Martin, and R. Repaske.** 1985. Envelope and long terminal repeat sequences of a cloned infectious NZB xenotropic murine leukemia virus. J. Virol. **53:**100–106.
16. **Ott, D., R. Friedrich, and A. Rein.** 1990. Sequence analysis of amphotrophic and 10A1 murine leukemia viruses: close relationship to mink cell focus-inducing viruses. J. Virol. **64:**757–766.
17. **Ott, D., and A. Rein.** 1992. Basis for receptor specificity of nonecotropic murine leukemia virus surface glycoprotein $gp70^{SU}$. J. Virol. **66:**4632–4638.
18. **Rigby, M. A., J. L. Rojko, M. A. Stewart, G. J. Kociba, C. M. Cheney, L. J. Rezanka, L. E. Mathes, J. R. Hartke, O. Jarrett, and J. C. Neil.** 1992. Partial dissociation of subgroup C phenotype and *in vivo* behaviour in feline leukaemia viruses with chimeric envelope genes. J. Gen. Virol. **73:**2839–2847.
19. **Stoye, J. P., and J. M. Coffin.** 1987. The four classes of endogenous murine leukemia virus: structural relationships and potential for recombination. J. Virol. **61:**2659–2669.
20. **Stoye, J. P., C. Morini, and J. M. Coffin.** 1991. Virological events leading to spontaneous AKR thymomas. J. Virol. **65:**1273–1285.
21. **Weiss, R. A.** 1993. Cellular receptors and viral glycoproteins involved in retrovirus entry, p. 1–108. *In* J. A. Levy (ed.), The Retroviridae. Plenum Press, New York.