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An Amino-Terminal Fragment of the Friend Murine Leukemia Virus Envelope Glycoprotein Binds the Ecotropic Receptor

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Retrovirus entry into cells is mediated by specific binding of the envelope glycoprotein to a cell membrane receptor. Constitutive envelope gene expression prevents infection by interfering with the binding of viruses which recognize the same receptor. We have used this property to investigate the receptor binding capacities of deleted or truncated murine leukemia virus ecotropic envelope glycoproteins. Friend murine leukemia virus envelope glycoproteins bearing internal amino-terminal deletions, or a soluble 245-amino-acid gp70 amino-terminal fragment, were expressed in NIH 3T3 cells. The susceptibility of these cells to ecotropic and amphotropic virus infection was determined. We observed that both membrane-bound and soluble forms of the gp70 245-amino-acid amino-terminal domain induced resistance to ecotropic virus, indicating that this fragment binds the ecotropic receptor. Binding occurs both at the cell surface and in the endoplasmic reticulum, as shown by the use of soluble envelope fragments either secreted in the culture supernatants or retained in the endoplasmic reticulum lumen by a KDEL sequence. These results suggest that the gp70 amino-terminal domain folds into a structure which recognizes the ecotropic receptor regardless of the carboxy-terminal part of the molecule.

Entry of retroviruses into cells involves the binding of the viral envelope glycoprotein to a receptor on the cell surface. Different retroviruses usually bind to different cell surface components. Viruses using the same receptor can be identified because they exhibit similar host ranges and interfere with each other for entry into cells (43, 45). Those properties specify retrovirus subgroups. Five different murine retrovirus (murine leukemia virus [MLV]) subgroups have been described and are referred to as the ecotropic, amphotropic, xenotropic, polytropic (mink cell focus-forming virus), and 10A1 subgroups (35, 36).

The nature of cell surface components used as receptors still remains undetermined for most retroviruses, with the exceptions of the human and simian immunodeficiency viruses (20, 39), the ecotropic subgroup of MLVs (1), and the gibbon ape leukemia virus (27).

The envelope-receptor interactions occur in two different contexts: as the first step of virus entry into cells and in cells constitutively expressing envelope glycoproteins. Multiple functions of the envelope glycoprotein are required for virus entry, including the correct insertion of spike glycoproteins into the virion particle, recognition and binding of the receptor, and membrane fusion activity. Therefore, it may be difficult to interpret the functional consequences of structural modifications introduced in envelope glycoproteins by using virus entry assays. On the other hand, in cells constitutively expressing envelope glycoproteins, the interference phenomenon results from envelope-receptor interactions alone. Therefore, the investigation of cell resistance to further entry of virus particles that bind the same receptor provides a functional receptor binding assay. In this study, we have used interference assays to examine the interactions between the ecotropic MLV envelope glycoprotein and its cellular receptor.

The 80-kDa murine ecotropic envelope glycoprotein precursor (gPr80) is synthesized and cotranslationally glycosylated in the endoplasmic reticulum (ER). As the molecule is transported through the Golgi vesicles, processing of oligosaccharides results in a product with an apparent molecular mass of 90 kDa (gPr90). During its transport to the cell surface, the molecule is cleaved into two subunits which are incorporated into virions as heteropolymers. The nonglycosylated Pr15E (transmembrane [TM]) anchors the envelope in the plasma membrane and is subsequently shortened by 16 amino acids during virus assembly to give p15E (15). The highly glycosylated extracellular gp70 (surface [SU]) carries most of the antigenic determinants, binds the receptor, and is responsible for interference (7).

A comparison of envelope glycoprotein sequences indicates that MLV gp70 amino-terminal domains differ according to subgroups, whereas the carboxy-terminal domains and p15Es are highly conserved (3, 22). A 50-amino-acid hypervariable, proline-rich region is located between these domains. In some polytropic recombinant viruses spontaneously arising after infection with ecotropic viruses, the gp70 amino-terminal domain and the central proline-rich region have been exchanged with endogenous retroviral sequences (18, 22). Since those viruses bind the polytropic receptor instead of the ecotropic receptor, it was presumed that the receptor binding domain might be contained within the exchanged fragment. In addition to the proline-rich region, two variable segments in this fragment have been described previously (29). The respective roles of these different regions in receptor binding are still unclear (29).

To establish more precisely the structural requirements for binding the ecotropic receptor, we modified the Friend MLV (FMLV) envelope glycoprotein structure. Constructions included in-frame deletions within the amino-terminal domain or the removal of the proline-rich region and the carboxy-terminal domain to produce either a soluble amino-terminal fragment or a hybrid protein in which the gp70 amino-terminal domain was fused to a nonrelated transmembrane protein. By using immunoprecipitations and interference assays, we show in this study that the gp70 amino-terminal domain folds into a structure which recognizes the

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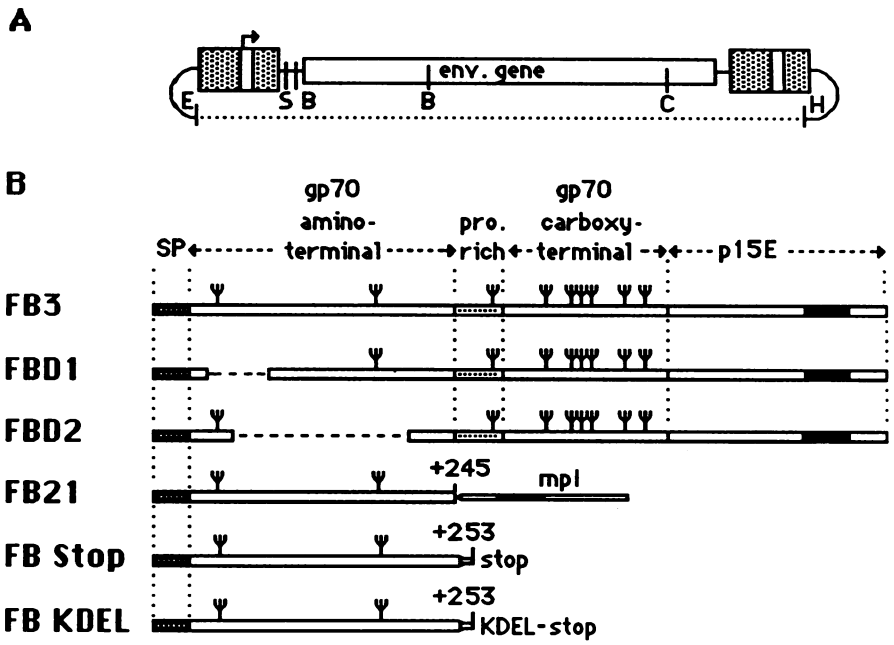

FIG. 1. Structures of wild-type, modified, and truncated FMLV envelope expression vectors. (A) Genetic map of the FB plasmid used for eucaryotic cell expression. Restriction enzyme site abbreviations: E, *Eco*RI; S, *Spe*I; B, *Bam*HI; C, *Cla*I; H, *Hin*dIII. Stippled boxes represent the upstream and downstream LTRs, and the dotted line shows pUC19 sequences. (B) Schematic representations of wild-type and modified envelope proteins. Abbreviations: SP, signal peptide; pro. rich, proline-rich region; mpl, fragment of the *mpl* gene product (42); ψ, potential N-linked glycosylation sites. Black boxes represent transmembrane domains.

ecotropic receptor regardless of the carboxy-terminal part of the molecule.

MATERIALS AND METHODS

Construction of FMLV envelope glycoprotein expression vectors. The FB3 envelope expression vector was constructed by ligating a 2,995-bp *Bam*HI-*Spe*I fragment from the permuted FMLV clone 57 (28), encompassing the *env* gene and the long terminal repeat (LTR), to a 1,012-bp *Cla*I-*Bam*HI fragment of the permuted FMLV clone FB29 (40), encompassing the LTR but not the packaging sequences (24). The FB3 *env* gene was transcribed from the FMLV clone FB29 LTR as an unspliced mRNA, and a polyadenylation signal was provided by the FMLV clone 57 LTR (Fig. 1A). All the envelope glycoproteins used in this study were expressed with this vector. Numbering of the FMLV envelope amino acids starts at the amino-terminal alanine residue of the mature protein after cleavage of the 34-amino-acid signal peptide. The deletion of amino acids 16 to 69 carried by the FBD1 glycoprotein was engineered by removing a 175-bp *Acc*I-*Apa*I fragment from the FB3 *env* gene and inserting a synthetic double-stranded oligonucleotide (5′-CTCGAGCAGGCC-3′). Deletion of amino acids 30 to 209 was originally observed in a defective and highly pathogenic isolate of FMLV referred to as myeloproliferative leukemia virus (MPLV) (42). In addition to the amino-terminal deletion, MPLV has exchanged the envelope proline-rich region (from amino acid 245) and the carboxy-terminal domain with cellular sequences homologous to growth factor receptors (referred to as the *mpl* gene product) in which a putative transmembrane domain was identified. To assemble the FBD2 deleted envelope glycoprotein (Fig. 1B), a 1,153-bp *Bam*HI-*Cla*I fragment containing the *mpl* sequences was removed and replaced by the original *env* sequences from the FB3 vector. The MPLV genome was also used to generate the FB21 hybrid protein in which the gp70 amino-terminal domain was fused to the *mpl* gene product. This 428-amino-acid protein possesses two potential N-linked glycosylation sites in the extracellular envelope domain and a characteristic transmembrane segment in the Mpl domain (Fig. 1B). The FB21 construction was done by inserting a 830-bp *Bam*HI fragment from the wild-type FMLV *env* gene in place of the amino-terminal deleted fragment of the MLPV genome. The FB-KDEL and the FB-stop envelope fragments were obtained by inserting double-stranded synthetic oligonucleotides (5′-TCGATCGT TCCTGCTCGAAATTAGC-3′ [FB-KDEL] and 5′-TCGAT CGATTAGC-3′ [FB-stop]) in a *Sac*I site located 20 bp downstream of the *env-mpl* junction in FB21 (42).

Cells and virus infection. Envelope expression vectors were cotransfected into NIH 3T3 cells along with pSV2neo, and neomycin-resistant clones were selected by using 1 mg of G418 per ml. Amphotropic and ecotropic helper-free stocks of the MFG-NB retroviral vector used in the interference assays were prepared from ψ-CRIP and ψ-CRE packaging cell lines (6). The MFG-NB retroviral vector contains a modified *lacZ* gene (*nls-lacZ*) which codes for the *Escherichia coli* β-galactosidase sequence fused to a 21-amino-acid nuclear localization sequence from the simian virus 40 large T antigen (14). Target cells were plated in six-well plates, infected with 1 ml of virus suspension (10^2 to 10^3 β-galactosidase foci per ml) in 8 μg of Polybrene per ml, grown to confluence, and then fixed with 0.5% glutaraldehyde in phosphate-buffered saline before X-Gal staining (38). Conditioned media were prepared as follows: cells were grown to confluence, and 48-h culture supernatants were harvested, filtered, and incubated with target cells for 1 h at 37°C before virus infection.

Protein analysis. After starvation in methionine- and cysteine-free culture media, cells were metabolically labeled with 40 μCi of [^{35}S]methionine and 40 μCi of [^{35}S]cysteine per ml, followed by a chase with a 500-fold excess of unlabeled amino acids for various lengths of time. Cells were disrupted in 0.5% Triton X-100–150 mM NaCl–20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.4), and cell extracts were clarified by centrifugation. Culture supernatants and cell extracts were incubated overnight with goat anti-Rauscher gp70 antibody or nonimmune serum, and immune complexes were precipitated by using protein A-Sepharose (Pharmacia). After being washed in 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-polyacrylamide gels followed by fluorography. To determine the carbohydrate composition of immunoprecipitated glycoproteins, immune complexes were digested as described previously (34) with endoglycosidase H (endo-H; Boehringer) prior to the analysis of labeled products on gels.

Labeling of cell surface proteins with ^{125}I by using lactoperoxidase was carried out as described previously (37).

RESULTS

Receptor binding domain of the FMLV envelope glycoprotein. Previous studies have defined different regions in the FMLV ecotropic envelope glycoprotein (21) as follows: the gp70 amino-terminal domain extends from amino acid 1 to 243, the central proline-rich region extends from amino acid 244 to 283, the gp70 carboxy-terminal domain extends from amino acid 284 to 445, and p15E extends from amino acid 446 to 641. Figure 1B shows the FMLV *env*-derived constructs used in this study. FB3 is a wild-type envelope glycoprotein, whereas FBD1 and FBD2 carry deletions in their amino-terminal domains (from amino acids 16 to 69 and 30 to 209, respectively). In the FB21 hybrid protein, the gp70 amino-terminal domain (from amino acids 1 to 245) was fused to the *mpl* gene product, which possesses a characteristic transmembrane segment (42) (see Materials and Methods).

(i) Synthesis, maturation, and transport of modified envelope glycoproteins. Envelope expression vectors were transfected into NIH 3T3 cells together with a *neo* gene, and clones were isolated by G418 selection. A polyclonal goat anti-Rauscher gp70 antibody was used to detect the *env* gene products in these cells after [^{35}S]methionine and [^{35}S]cysteine metabolic labeling. Figure 2A shows the envelope precursors analyzed by SDS electrophoresis after a 20-min pulse labeling. In lane 2, the 90-kDa band corresponds to the glycosylated envelope precursor (gPr90) expressed from the wild-type FB3 envelope gene. In lanes 4, 6, and 8, the bands migrating with the apparent molecular masses of 52, 68, and 81 kDa, respectively, are consistent with the expected sizes of the glycosylated FB21, FBD2, and FBD1 uncleaved proteins. Pulse-chase labeling was used to examine the cleavage and stability of these proteins (Fig. 2B). The wild-type FB3 gPr90 precursor was rapidly cleaved, and a 70-kDa band (gp70) was detectable during the 4-h chase. In contrast, no cleavage occurred in deleted envelope molecules which exhibited the same 81- and 68-kDa bands (FBD1 and FBD2, respectively) throughout the chase period. The FB21 52-kDa product was not expected to be cleaved. It was not detectable after a 2-h chase, indicating that the hybrid protein has a reduced half-life.

The FMLV envelope glycoprotein is efficiently transported to the cell surface, where it can be detected as mature gp70 and Pr15E. We examined whether the modified envelope glycoprotein could be detected at the cell surface. Cell surface proteins were radio-iodinated with ^{125}I by using lactoperoxidase, and cell lysates were immunoprecipitated with anti-gp70 antibody before analysis on SDS gels. Figure 3 shows large amounts of wild-type FB3 gp70 at the cell surface. The 52-kDa FB21 hybrid molecule was detected in smaller amounts, which is consistent with the short half-life of this molecule. This suggests that the putative transmembrane segment found in the *mpl* gene product is actually functional. The FBD1 81- and FBD2 68-kDa deleted envelope proteins were detectable at the cell surface only after prolonged film exposure (Fig. 3B). Inefficient transport of uncleaved envelope molecules has been reported previously (13, 31, 41).

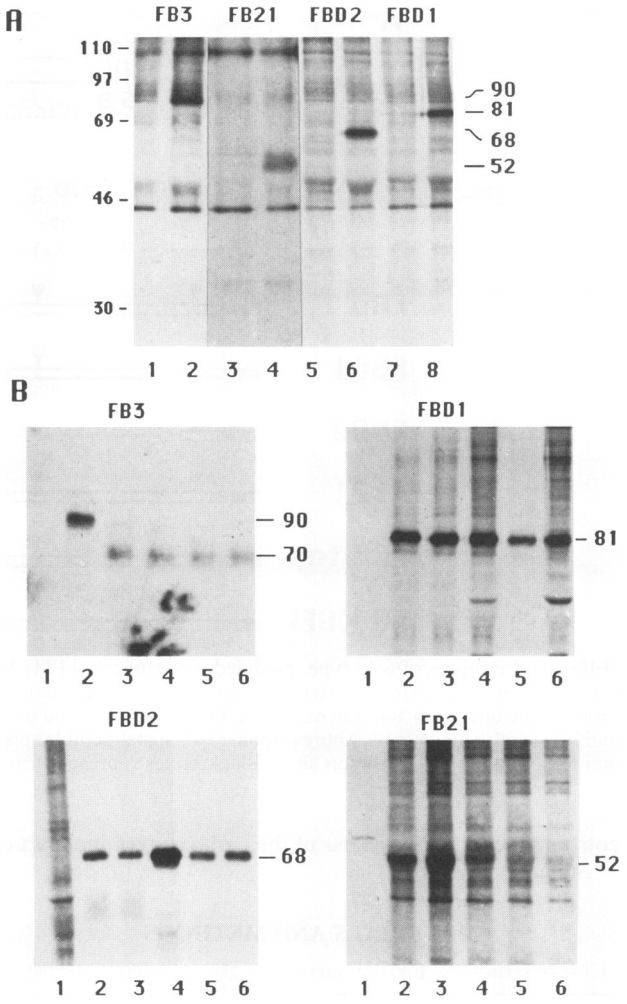

FIG. 2. Metabolic labeling of cells expressing wild-type and modified envelope proteins. (A) After 20-min labeling with [^{35}S]methionine and [^{35}S]cysteine, cells were disrupted and cell extracts were immunoprecipitated with goat nonimmune serum (lanes 1, 3, 5, and 7) or goat anti-gp70 antibodies (lanes 2, 4, 6, and 8). (B) Pulse-chase experiments. Cells were labeled for 30 min (lanes 1 and 2) and then chased with excess unlabeled amino acids for 30 min (lanes 3), 1 h (lanes 4), 2 h (lanes 5), and 4 h (lanes 6). Cell lysates were precipitated with nonimmune goat serum (lanes 1) or goat anti-gp70 antibodies (lanes 2 to 6). The apparent molecular sizes of the precipitated proteins and markers are indicated in kilodaltons.

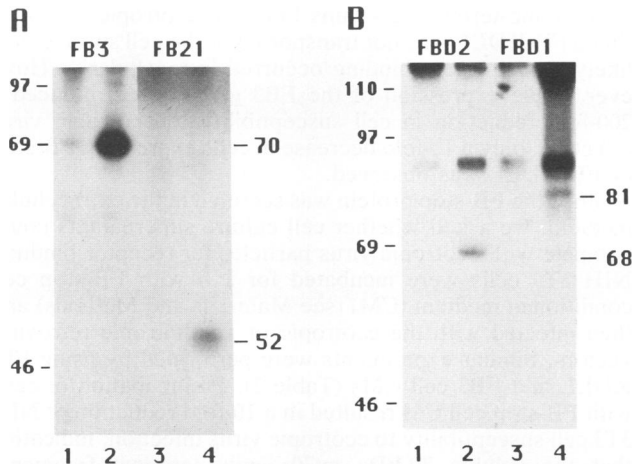

FIG. 3. Radioiodination of cell surface proteins by using lactoperoxidase. After labeling with ^{125}I, cells were disrupted and extracts were precipitated with nonimmune goat serum (lanes 1 and 3) or goat anti-gp70 antibodies (lanes 2 and 4). Immune complexes were analyzed on a 12% SDS–polyacrylamide gel (24-hour exposure; A) or an 8% SDS-polyacrylamide gel (8-day exposure; B). The apparent molecular sizes of the precipitated proteins and markers are indicated in kilodaltons.

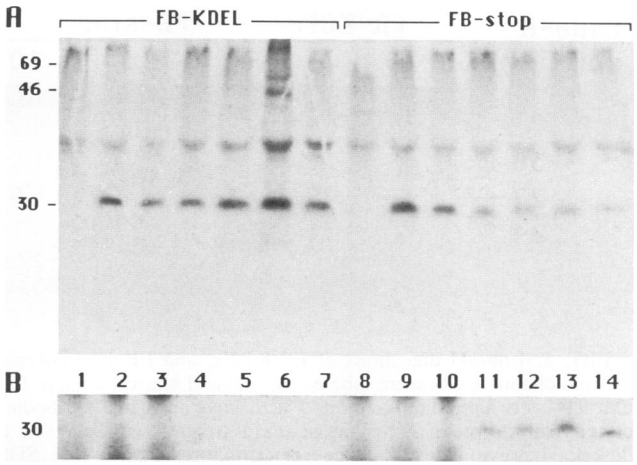

FIG. 4. Pulse-chase experiments on cells expressing the FB-KDEL and FB-stop gp70 amino-terminal fragments. (A) Cell extracts; (B) culture supernatants. Cells were labeled for 20 min (lanes 1, 2, 8, and 9) followed by 30-min (lanes 3 and 10), 1-h (lanes 4 and 11), 2-h (lanes 5 and 12), 4-h (lanes 6 and 13), and 6-h (lanes 7 and 14) chases. Amino-terminal gp70 fragments were immunoprecipitated with nonimmune goat serum (lane 1 and 8) or goat anti-gp70 antibodies (lane 2 through 7 and 9 through 14) and analyzed on 15% SDS-polyacrylamide gels. The apparent molecular sizes of the precipitated proteins and markers are indicated in kilodaltons.

(ii) Interference of modified envelope glycoproteins with ecotropic virus infection. We used an interference assay to examine whether the modified envelope glycoproteins were still capable of binding the ecotropic receptor. Defective retroviral vectors transducing a modified *E. coli lacZ* gene were used to infect cells expressing wild-type or modified gp70s. Susceptibilities to infection with either ecotropic or amphotropic pseudotypes were determined by counting X-Gal-positive, infected cell foci (Table 1). The number of cells infected with amphotropic pseudotypes was not modified by the expression of the FB3, FBD1, FBD2, and FB21 envelope glycoproteins. As expected, susceptibility to ecotropic pseudotypes was 200-fold lower in cells expressing the wild-type FMLV envelope glycoprotein (FB3) than in controls. In contrast, envelope glycoproteins with amino-terminal deletions (FBD1 and FBD2) did not interfere with ecotropic viruses, indicating that the integrity of the gp70 amino-terminal domain was necessary for interference. Cells expressing the hybrid FB21 protein were as resistant to ecotropic virus infection as those expressing the wild-type gp70. The presence of an intact amino-terminal domain was therefore sufficient to interfere with ecotropic virus, indicating that the ecotropic receptor binding domain is located within the first 245 amino acids of the FMLV gp70.

TABLE 1. Interference of wild-type or modified FMLV envelope glycoproteins expressed in NIH 3T3 cells with ecotropic viruses

Target cell envelope glycoprotein	No. of X-Gal-positive cell foci[a]	
	Ecotropic pseudotype	Amphotropic pseudotype
None	390	360
FB3	2	280
FBD1	400	160
FBD2	310	260
FB21	3	120

[a] Values shown are from a representative experiment.

Receptor binding of soluble gp70 amino-terminal fragments. Proteolytic analysis of the FMLV gp70 has previously suggested that the amino- and carboxy-terminal domains fold as two independent globular subunits (33). The receptor binding capacity of the hybrid protein FB21 also suggested that the gp70 amino-terminal domain is structured independently of the carboxy-terminal domain. Consequently, we asked whether a truncated gp70 amino-terminal domain could be produced as a soluble protein and still be capable of binding the ecotropic receptor. An in-frame stop codon was inserted in the FB21 coding sequence seven codons downstream of the *mpl* gene junction (see Materials and Methods). The mature 253-amino-acid truncated *env* gene product (Fig. 1B, FB-stop) possessed no transmembrane domain and was therefore expected to be secreted in the culture supernatant of transfected cells. We also inserted a KDEL sequence after residue 253 of the truncated protein, with the aim of retaining this product (FB-KDEL) in the ER (25).

(i) Synthesis and transport of truncated envelope glycoproteins. Genes coding for truncated envelope proteins were introduced into NIH 3T3 cells, and after a short metabolic labeling and chase, cell extracts (Fig. 4A) and culture supernatants (Fig. 4B) were immunoprecipitated. The FB-stop product was rapidly detected as a 30-kDa cellular protein (Fig. 4A, lanes 9 to 14), which was progressively and quantitatively secreted in the culture supernatant during the 6-h chase period (Fig. 4B, lanes 9 to 14). In contrast, the 30-kDa FB-KDEL product was stably detected in the cytoplasm (Fig. 4A, lanes 2 to 7), and remained undetectable in the culture supernatant (Fig. 4B, lanes 2 to 7).

Conversion of high-mannose oligosaccharide chains to complex sugars occurs during the transport of envelope glycoproteins through the Golgi vesicles (26). Molecules retained in the ER are not subjected to these modifications and remain sensitive to endo-H digestion. Since two sites for

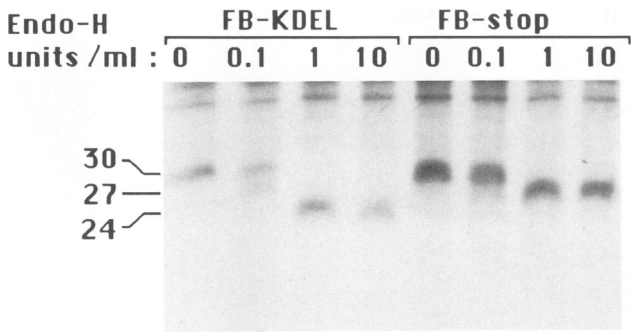

FIG. 5. Endo-H digestions of FB-KDEL and FB-stop polysaccharide chains. Cells were labeled for 1 h and chased for 1 h. Cell extracts were immunoprecipitated with goat anti-gp70 antibodies, eluted from protein A-Sepharose, and treated with endo-H as described previously (34) before electrophoresis on a 15% SDS-polyacrylamide gel. The apparent molecular sizes of the precipitated proteins are indicated in kilodaltons.

N-linked glycosylation exist in the FB-stop and FB-KDEL products (Fig. 1B), we used endo-H digestions to examine the subcellular transport of these molecules. Cells were submitted to a 1-h labeling followed by a 1-h chase, and cell extracts were immunoprecipitated with anti-gp70 antibody and digested with various amounts of endo-H. The 30-kDa FB-KDEL protein was converted successively to 27 and 24 kDa by increasing amounts of endo-H (Fig. 5). This result is consistent with the presence of two carbohydrate chains on this molecule, both of them being sensitive to endo-H digestion. It indicates that the FB-KDEL molecule is not transported through the Golgi apparatus. In contrast, one carbohydrate chain on the FB-stop molecule acquired resistance to endo-H (lane 7 and 8), as expected for a protein transported to the extracellular medium. The persistence of one endo-H-sensitive oligosaccharide chain on the amino-terminal domain of mature gp70 proteins has been observed previously (32, 33).

(ii) Interference of truncated envelope glycoproteins with ecotropic virus infection. Cells expressing FB-stop and FB-KDEL were infected with ecotropic and amphotropic retroviral vectors along with control fibroblasts and cells expressing the FB3 wild-type envelope glycoprotein (Table 2). Susceptibilities to amphotropic pseudotype infection were equivalent. Interference with ecotropic pseudotypes could be demonstrated in all the cell lines expressing the gp70 amino-terminal domain. These results showed that soluble gp70 amino-terminal domains bind the ecotropic receptor. Since FB-KDEL was not transported to the cell surface, it is likely that receptor binding occurred intracellularly. However, while expression of the FB3 glycoprotein induced a 200-fold reduction in cell susceptibility to ecotropic virus infection, only a 10-fold decrease in cells expressing FB-stop or FB-KDEL was observed.

TABLE 2. Interference of wild-type or truncated FMLV envelope glycoproteins expressed in NIH 3T3 cells and secreted in culture supernatants with ecotropic viruses

Target cell envelope glycoprotein and treatment	No. of X-Gal-positive cell foci[a]	
	Ecotropic pseudotype	Amphotropic pseudotype
None	346	312
FB3	1	272
FB-KDEL	44	192
FB-stop	48	452
None + FB3 cell CM	316	308
None + FB-KDEL cell CM	120	284
None + FB-stop cell CM	27	392

[a] Values shown are from a representative experiment.

Since the FB-stop protein was secreted in the extracellular medium, we asked whether cell culture supernatants could compete with ecotropic virus particles for receptor binding. NIH 3T3 cells were incubated for 1 h with FB-stop cell conditioned medium (CM) (see Materials and Methods) and then infected with the ecotropic or amphotropic retroviral vectors. Similar experiments were performed by using FB-KDEL and FB3 cell CMs (Table 2). Preincubation of cells with FB-stop cell CM resulted in a 10-fold reduction of NIH 3T3 cell susceptibility to ecotropic virus infection, indicating that the soluble 30-kDa gp70 amino-terminal fragments which are secreted in the cell culture supernatants bind the ecotropic receptors present at the cell surface. Since no material could be precipitated with anti-gp70 antibodies in culture supernatants of FB3 cells (data not shown) and FB-KDEL cells (Fig. 4B, lanes 2 to 7), target NIH 3T3 cells were preincubated with these CMs as negative controls. As expected, FB3 CM had no effect. In contrast, FB-KDEL cell CM slightly reduced the susceptibility of target cells to ecotropic infection. This could result from trace amounts of gp70 amino-terminal fragments not detected by immunoprecipitation which escape the KDEL retention mechanism, as previously observed in other systems (11, 30).

DISCUSSION

The data reported here indicate that the FMLV envelope glycoprotein binds the ecotropic virus cell surface receptor through its amino-terminal domain. Demonstration was provided by isolating a 245-amino-acid fragment from the rest of the envelope molecule and showing that this fragment interferes with ecotropic virus infection.

In our experiments, cell susceptibilities to infection were measured by using replication-defective retroviral vectors packaged into either ecotropic or amphotropic particles and containing a β-galactosidase gene. In the absence of the spread of virus, interference levels were quantified from single-hit events, and consequently, the observed inhibition never exceeded 200-fold.

The interference phenomenon results from envelope-receptor interactions which hamper further binding and penetration of infecting virus particles (16, 19, 43). Several observations suggest that the envelope glycoprotein region involved in receptor binding is the same for both interference and virus entry. (i) Classifications of MLV subgroups according to either cross-interferences or host ranges are largely overlapping (35). (ii) In polytropic virus genomes, recombinations within the *env* gene have modified both cell tropisms and interference specificities (18, 22). (iii) By exchanging limited regions of the gp70 amino-terminal domain between different MLV subgroups, we have shown that the acquisition of a different virus host range is always associated with the acquisition of the corresponding interference specificity (2). However, although our data show that all the structural elements involved in interference with ecotropic virus infection, and therefore in binding the ecotropic receptor, are present in the FMLV gp70 amino-terminal domain, it is obvious that additional envelope structures are required for virus penetration into cells.

A comparison of envelope sequences shows that MLV gp70s differ in two limited regions in their amino-terminal domains (amino acids 50 to 116 and 170 to 183 of the FMLV gp70) and in the proline-rich segment (amino acids 244 to 283) (29). These three hypervariable regions are candidate receptor binding sites, by analogy with the avian sarcoma and leukemia virus envelope glycoproteins, in which determinants for receptor interaction have been ascribed to short hypervariable sequences (10). Since the FB21 protein does not contain the proline-rich region and since it induced interference as strongly as the wild-type envelope, this fragment appears dispensable for receptor binding. In our FBD2 deletant, both amino-terminal variable regions were removed, while in FBD1 amino acids 170 to 183 were conserved. In agreement with the hypothesis that these regions may play a role in receptor binding, we observed that interference with ecotropic viruses was lost when they were deleted. However, in the FBD1 and FBD2 proteins, deletions hampered proper proteolytic cleavage of the modified envelope precursor. It was previously reported that even short deletions or point mutations can deeply alter envelope protein folding (44). Altered folding probably accounts for the absence of cleavage of the deleted envelope glycoproteins and is likely to disturb the receptor binding properties as well. This suggests that the functional consequences of structural modifications introduced in the envelope glycoprotein cannot be unambiguously interpreted in the context of the full-length, multifunctional molecule.

In contrast, the receptor-binding property of the gp70 amino-terminal domain was conserved in the FB-stop construct, where the proline-rich and carboxy-terminal regions had been removed. This truncation resulted in the synthesis of soluble gp70 fragments which were efficiently secreted in the extracellular medium, which bound the cell surface ecotropic receptor, and which competed with the ecotropic virus entry into target cells. The same truncated gp70 fragments could also be retained in the ER by the addition of a KDEL motif at its carboxy-terminal extremity and still interfered with ecotropic virus infection. This observation suggests that the ecotropic virus envelope-receptor binding reaction can take place in the ER, as previously shown for the avian reticuloendotheliosis virus (9) and for the binding of human immunodeficiency virus type 1 gp160 to the CD4 molecule (4, 5).

A 20-fold-lower interference was observed in cells expressing the truncated soluble gp70 amino-terminal molecules (FB-stop or FB-KDEL) compared with cells expressing the membrane-bound form of the same fragment (FB21). This difference might result from an increased affinity of the gp70 amino-terminal domain for binding the ecotropic receptor in the context of the FB21 hybrid protein. However, direct binding of the Mpl domain of FB21 to the receptor is unlikely, since the wild-type chimeric Env-Mpl protein, which contains all the sequences present in FB21 except gp70 amino acids 30 to 209 (42), does not affect cell susceptibility to ecotropic virus infection (our unpublished results). On the other hand, the possibility that, in FB21, the Mpl sequences indirectly facilitate the binding of gp70 amino-terminal determinants to the ecotropic receptor cannot be excluded. A more likely explanation for the decreased interference efficiency of the truncated gp70 molecules is that efficient receptor binding depends on high levels of membrane-bound forms of the gp70 amino-terminal fragment. It is expected that cells producing membrane-bound molecules accumulate more gp70 amino-terminal fragments in the vicinity of the receptor than those secreting the ligand in the culture medium or in the ER lumen. A similar mechanism was proposed to account for the decreased capacity of truncated soluble colony-stimulating factor 1 (CSF-1) molecules to induce the autocrine transformation of fibroblasts expressing the human CSF-1 receptor (17).

Autonomous folding of the FMLV gp70 amino-terminal domain has been suggested by biochemical analysis indicating that this protein, as well as the polytropic spleen focus-forming virus gp55, includes two independent globular domains (23, 33). The central, probably nonstructured, proline-rich region may function as a linker between the amino- and carboxy-terminal domains. Comparable sequence organization of envelope glycoproteins exists in other mammalian type C retroviruses like the feline leukemia virus and gibbon ape leukemia virus (8, 12). We suggest that all these viruses, like FMLV, bind specific cell surface receptors through the amino-terminal domains of their surface glycoproteins. The availability of large amounts of functional, soluble amino-terminal fragments of the surface envelope glycoprotein might help in studying the spatial configuration required for envelope-receptor interactions. We are currently investigating the respective roles in receptor recognition of the variable regions located in the gp70 amino-terminal domains of different MLVs by using secreted envelope glycoprotein fragments and interference assays.

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