In Vitro Binding of Purified Murine Ecotropic Retrovirus Envelope Surface Protein to Its Receptor, MCAT-1

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An amino-terminal portion of the Friend murine leukemia virus (MLV) envelope surface protein (SU, residues 1 to 236 [SU:(1-236)]) and its receptor, MCAT-1, were each purified from insect cells after expression by using recombinant baculoviruses. Friend SU:(1-236) bound specifically to Xenopus oocytes that expressed MCAT-1 with an affinity (Kₐ, 55 nM) similar to that of viral SU binding to permissive cells. Direct binding of Friend SU:(1-236) to purified MCAT-1 was observed in detergent and after reconstitution into liposomes. Analysis of binding demonstrated that MCAT-1 and Friend SU:(1-236) interact with a stoichiometry of near 1:1. These findings demonstrate that the amino-terminal domain from the SU of ecotropic murine retroviruses contains an MCAT-1 binding domain.

MATERIALS AND METHODS

Construction of expression plasmids encoding MCAT-1 and Friend SU. The vector pSP64T (16) was modified by (i) insertion of the BamHI-to-XhoI portion of the polylinker from pCDNA3 (Invitrogen) at the BglII/SalI site, (ii) reinsertion of the deleted BglII-to-SalI fragment of pSP64T at the BamHI/PvuII site and addition of a second polylinker containing XhoI, BglII, ApaI, and PstI sites at the PstI site, and (iii) insertion of a 140-bp fragment encoding (in tandem) a factor Xa protease site (amino acids IEGR), three copies of the hemagglutinin (HA) peptide (YPYDVPYA [10]) recognized by monoclonal antibody 12CA5 (Boehringer Mannheim), and a six-histidine motif at the XhoI/PstI site in the polylinker (Flu-his, tag). The new vector was designated pRD67/Flu-his.

A clone encoding envelope SU of Friend MLV (reference 12, GenBank accession no. J02192) in plasmid pUE13 (a gift from A. Finter) was modified by addition of a 5' EcoRI site at bp ~40 relative to the initiator ATG and a 3' XhoI site at bp 810 that permitted insertion into pRD67/Flu-his, with an in frame fusion at the 3' end to the sequences encoding the protease cleavage site and Flu-his, tag.

A cDNA encoding the ecotropic retrovirus receptor (MCAT-1) with the C-terminal protease cleavage site and Flu-his tag (MCAT-1t) was constructed by using PCR to place an EcoRI site at bp ~50 relative to the initiator ATG and to replace the stop codon of MCAT-1 (residue 622) with an in-frame XhoI recognition site and inserted into the polylinker of pRD67/Flu-his. To avoid the possibility of PCR-induced mutations, the BamHI-to-SphI fragment of this plasmid was excised and replaced by the equivalent fragment from the original MCAT-1 cDNA clone. The absence of Tag polymerase-induced changes in the remaining portion (<10% of the coding region) was confirmed by nucleotide sequencing.

Expression of recombinant proteins in oocytes and mammalian cells. Capped mRNAs encoding MCAT-1 or MCAT-1t were transcribed and injected into Xenopus laevis oocytes, and after 3 days, transport of L-[14C]arginine and exogenous binding of 125I-SU proteins were measured as described elsewhere (11). In some experiments, detergent extracts of oocytes were prepared and solubilized proteins separated on sodium dodecyl sulfate (SDS)-8% polyacrylamide gels, transferred to nitrocellulose filters, and immunoblotted with monoclonal antibody 12CA5 or a polyclonal antibody raised against a C-terminal peptide of the deleted EcoRI fragment of pCDNA3 (Invitrogen) at the EcoRI site at bp 40 relative to the initiator ATG and a XhoI site at bp 810 that permitted insertion into pRD67/Flu-his, with an in-frame fusion at the 3' end to the sequences encoding the protease cleavage site and Flu-his, tag.

For expression in mammalian cells, the cDNA encoding MCAT-1t was inserted into the expression vector pCDNA3 (Invitrogen), using EcoRI and ApcI sites. Receptor function was confirmed by using ecotropic β-galactosidase-encoding virus after transient expression in nonpermissive human 293 cells (2).

Protein expression in insect cells. Recombinant insect viruses encoding the recombinant envelope and receptor proteins were made by cloning the Friend SU or MCAT-1t insert from the pRD67/Flu-his into the baculovirus vector pVL1395 (Pharmingen) adjacent to the polh promoter element vector backbone, using the 5' EcoRI and 3' BglII sites. Each of the resulting plasmids was co-transfected with linear baculovirus virus DNA (Pharmingen) into Spodoptera frugiperda Sf9 insect cells, and recombinant viruses encoding the correct proteins were recovered and amplified in Hi5 cells (Invitrogen) propagated in Excel-405 medium (JRH Biosciences). For protein purification, Hi5 cells were grown in suspension cultures (400 ml/2-liter baffled flask) rotated at 100 rpm at 27°C. Cells in log growth phase were infected at a multiplicity of infection of 10.

Expression of truncated envelope proteins. Three days after baculovirus infection, culture supernatants were collected, filtered (0.2-μm-pore-size filter),
and incubated for 1 h at room temperature with Nε-ε-carboxymethyl lysine (20 μM of resin to 1 mg of resin) in 50 mM sodium phosphate buffer (pH 6.4). After digestion, the resin was washed four times with 10 column volumes of 50 mM sodium phosphate–500 mM NaCl–20 mM Tris-HCl (pH 7.4). The bound protein was eluted with 500 mM imidazole–500 mM NaCl–10 mM sodium phosphate (pH 6.4). Protein was dialyzed overnight against 500 mM NaCl–20 mM HEPES (pH 7.4) and then dialyzed for 4 h against 50 mM NaCl in the same buffer. A precipitate which contained the recombinant SU protein formed. This was pelleted, redissolved in 300 mM imidazole (pH 8.0) to 1 mg/ml, and digested with 1% (wt/wt) factor Xa protease (New England Biolabs) for 48 h at room temperature. After digestion, the sample was diluted with 10 volumes of 50 mM NaCl–20 mM morpholinooctanesulfonic acid (MES; pH 6.0) and added to a M2-anti-Mouse IgG Sepharose 4B column (Pharmacia). Elution of recombinant protein was performed by using an NaCl gradient (50 to 500 mM in 20 mM MES [pH 6.0]). Digested recombinant Friend SU, residues 1 to 236 (Fr SU:(1-236)), eluted as a broad peak. Envelope proteins were labeled by using iodo-beads as described by the manufacturer (Pierce). Typically, 1 μg of carrier-free Na251 (NEN-DuPont) was used per 0.1 mg of protein.

**Purification of MCAT-1**. The following protocol is based on a 1-liter culture; typically 4-liter batches were prepared. Two days after infection with baculovirus expressing the Friend 57 SU protein truncated just before the proline-rich region (residue 236) decreased MCAT-1-dependent L-arginine transport by less than 40% of the control value, consistent with binding-induced downregulation of MCAT-1 (11). Battini et al. reported that expression of the amino-terminal domain of Friend 57 SU product was purified to homogeneity by anion-exchange chromatography (lanes 5 and 6). Sequencing revealed that the amino-terminal residues of the purified SU protein were identified by using the Amersham ECL substrate. Protein concentration was determined from the absorbance at 550 nm.

**RESULTS**

Purification of the MCAT-1 Friend SU binding domain. It has been established that expression of MCAT-1 is required for ecotropic MLV binding (2) and for infection (20). Also, expression of ecotropic MLV SU blocked MCAT-1-dependent binding of exogenous SU and decreased L-arginine transport to less than 40% of the control value, consistent with binding-induced downregulation of MCAT-1 (11). Battini et al. reported that expression of the amino-terminal domain of Friend SU product was purified to homogeneity by anion-exchange chromatography (lanes 5 and 6). Sequencing revealed that the amino-terminal residues of the purified SU protein were identified by using the Amersham ECL substrate.

Reconstitution of purified proteins into phospholipid vesicles. A lipid mixture in chloroform [90% (wt/wt) phosphatidylcholine (bovine heart), 10% (wt/wt) cholesterol] was dried in a 18-gauge needle. After drying, the last 50% (wt/vol) solution of Biobeads SM-2 (Bio-Rad). A 50,000-molecular-weight cutoff dialysis membrane (Spectra/Por, Spectrum Medical Industries) was used. After dialysis, lipid content of samples was determined from the absorbance at 550 nm of the rhodamine-labeled lipid and also by lipid-derived phosphate determination (18).

**Detergent binding assay.** One hundred micromolars of purified Fr SU:(1-236) or amphotropic 4070 SU:(1-205) was reacted with 60 nmol of sulfo-NHS-Biotin (Pierce) for 30 min at room temperature in 100 mM sodium phosphate buffer (pH 8.0). Unincorporated biotin was removed by using a NAP-10 column (Pharmacia). Fifty microliters of Ultralink streptavidin beads (Pierce) was then primed by incubation (15 min) with the biotin-coupled protein. Before use, the beads were washed three times in 150 mM NaCl in 20 mM HEPES buffer (pH 7.4). Samples containing MCAT-1 dissolved in 10 mM CHAPS–10% glycerol–150 mM NaCl–1 mM CaCl2–0.1 mM MgCl2–20 mM Tris-HCl (pH 7.4) (assay buffer, unless specified otherwise) were incubated with the SU–primed or unprimed beads. After 1 h at room temperature, the beads were washed three times in assay buffer and solubilized by resuspension in 2% SDS–8 M urea–100 mM Tris-HCl (pH 6.8). Samples were then spotted onto nitrocellulose or separated by SDS-PAGE and analyzed by immunoblotting. The same assay was also used to analyze MCAT-1 reconstituted liposomes except that the assay buffer was modified by omitting detergent.

Liposome flotation assay. Biotin-coupled Fr SU:(1-236) (0.1 μg) was mixed with 5 μg of liposomes containing MCAT-1 or no protein in 300 μl of assay buffer and incubated at room temperature with gentle mixing. After 1 h, the reaction mixture was mixed with an equal volume of 20% (wt/vol) dextran (average molecular weight, 74,000, Sigma) in assay buffer and dispensed into the bottom of an SW55 ultracentrifuge tube (Beckman): 1.5 ml of 7.5% and 5% dextran solutions and 0.3 ml of assay buffer were sequentially layered above the reaction mixture. After centrifugation at 159,000 × g for 1 h at 15°C, 0.4-ml fractions were collected. Lipid content was determined by assaying for rhodamine-tagged phosphatidylcholine by absorbance at 550 nm.
The mobility of Fr SU:(1-236) on gel filtration chromatography (Superdex 75; Pharmacia) fell between those of the marker proteins lysozyme (12.5 kDa) and aprotinin (6.5 kDa), suggesting that Fr SU:(1-236) is a monomer (Fig. 2B).

125I-labeled Fr SU:(1-236) bound specifically to frog oocytes that expressed MCAT-1 (Fig. 2C, left panel). Binding to these membranes was saturable, and the affinity (Kd, 55 nM) was comparable to that of virus-purified Moloney MLV SU binding to permissive murine fibroblasts (7). In addition, Fr SU:(1-236) was able to compete with 125I-labeled Moloney MLV SU gp70 purified from virions for binding to membranes of frog oocytes that express MCAT-1 (Fig. 2C, right panel). We conclude that Fr SU:(1-236) contains a functional receptor binding domain.

Function and purification of recombinant MCAT-1. To facilitate purification, MCAT-1 was modified by addition of the carboxyl-terminal tag used for purification of Fr SU:(1-236). Addition of the tag had no detectable effect on MCAT-1-dependent L-arginine transport or 125I-SU binding in frog oocytes (data not shown). Also, expression of the tagged MCAT-1 (MCAT-1t) conferred susceptibility of human 293 cells to infection by the ecotropic MLV vector encoding β-galactosidase (BAG). The titer of the BAG virus infection on these cells is comparable to that on cells that express the unaltered receptor protein. Therefore, the presence of the carboxyl-terminal tag produced no detectable alteration in transport or receptor function.

MCAT-1t was expressed in insect cells by using a recombinant baculovirus. The efficiency of MCAT-1t extraction from the membrane fraction of these cells was monitored by immunoblotting using monoclonal antibody 12CA5, which recognizes the influenza virus HA epitope tag. Extraction of MCAT-1t in buffers containing either sodium deoxycholate (12 mM), CHAPS (10 mM), or octylglucoside (30 mM) was comparable to that with 2% SDS–8 M urea and exceeded that with lithium dodecyl sulfate (5 mM), Triton X-100 (0.5 mM), and dodecylmaltoside (0.5 mM) (Fig. 3A). The function of detergent-extracted MCAT-1t was monitored by measuring binding to biotin-coupled Fr SU:(1-236) bound to streptavidin beads. Preliminary experiments demonstrated that the streptavidin-biotin linkage was stable in detergent, but Fr SU:(1-236) was completely released from beads by addition of 8 M urea to 2% SDS (data not shown). After 1 h of incubation in detergent extracts containing MCAT-1t, the Fr SU:(1-236)-charged beads were washed and MCAT-1t and Fr SU:(1-236) was eluted with 2% SDS–8 M urea and detected by immunoblotting.
MCAT-1t to Fr SU:(1-236)-charged beads (Fig. 3B), but not uncharged beads (Fig. 3C), was observed in buffers containing sodium deoxycholate, CHAPS, or dodecylmaltoside, but binding was diminished in Triton X-100, octylglucoside, or lithium dodecyl sulfate. These experiments indicate that sodium deoxycholate and CHAPS are more effective than the other detergents in efficient extraction of functional MCAT-1t from insect cell membranes.

Purification of MCAT-1t was monitored by silver staining (Fig. 4A, left panel) and immunoblotting (right panel) after SDS-PAGE. A 450-mg membrane pellet from insect cells that express MCAT-1t was extracted in buffer containing sodium deoxycholate (lane 1). Ni²⁺-chelating resin was incubated with this extract and then washed with 10 column volumes of detergent buffer containing imidazole (50 mM) and citric acid (30 mM) to remove loosely bound proteins (lanes 2 and 3). The final wash contained CHAPS (8 mM) in place of sodium deoxycholate, which formed a gel under the conditions (pH 4) required to elute MCAT-1t (lane 4). The yield of MCAT-1t from this protocol was 100 µg (>90% homogeneity), as assessed by silver staining (lane 4; summarized in Table 1). In some preparations, MCAT-1t was purified further by using antibody (12CA5) affinity chromatography. After application of MCAT-1t in CHAPS buffer at pH 7.5, Sephadex beads coupled to 12CA5 were washed extensively at pH 6 (lane 5), and MCAT-1t was eluted from the antibody at pH 3 (lane 6). Brief (less than 1-h exposure of MCAT-1t to pH 3 to 10 did not noticeably alter its binding properties. The 30- and 34-kDa proteins that copurified with MCAT-1t were detected by antibody 12CA5 on the immunoblot (Fig. 4A, right panel) are probably proteolytic fragments of MCAT-1t. Proteolytic cleavage is likely to occur in insect cells, since these fragments were present in the initial membrane extracts, despite the presence of protease inhibitors. Since both retain the carboxyl-terminal tag, they copurified with MCAT-1t. These proteins migrated as a single broad peak between the protein standards bovine serum albumin (67 kDa) and cytochrome c (29 kDa) on a gel filtration column in the presence of CHAPS (Fig. 4B). No MCAT-1t was detected in the early column fractions containing proteins that would be multiples of the calculated molecular mass of MCAT-1t (67 kDa). Therefore, like Fr SU:(1-236), MCAT-1t was purified as a monomer.

**Binding of purified MCAT-1t to truncated envelope.** Purified MCAT-1t bound to Fr SU:(1-236)-charged streptavidin beads (Fig. 5A, lane 1, right panel) but not to beads charged with the equivalent amino-terminal region of amphotropic SU:(1-209) (lane 2, left panel) purified by using the same protocol (lane 2, left panel). The 30- and 34-kDa proteolytic fragments that copurified with MCAT-1t were not detected in the eluate obtained from Fr SU:(1-236)-charged beads, demonstrating that they lack binding activity. More than 90% of purified MCAT-1t could be adsorbed by excess Fr SU:(1-236)-charged beads (data not shown), demonstrating that less than 10% of purified MCAT-1t was irreversibly denatured during purification.

An indirect measure of the stoichiometry of SU binding to MCAT-1t was obtained using a batch of purified Fr SU:(1-236) that retained the influenza virus HA epitope tag. Beads charged with Fr SU:(1-236) were incubated with excess MCAT-1t in detergent; after washing, the relative amounts of eluted proteins were identified by densitometry of bands on an immunoblot (Fig. 5B). The ratios of eluted MCAT-1t to bisalbumin (Fr SU:(1-236)) were 1.0 in CHAPS, 0.9 in sodium deoxycholate, 0.3 in dodecylmaltoside, and near zero in either octylglucoside or Triton X-100. Since the same epitope tag is present at the carboxyl terminus of both proteins, they are likely to be detected equally well by antibody 12CA5. Given that both Fr SU:(1-236) and MCAT-1t were purified as monomers, the binding ratio of near 1:1 in CHAPS and sodium

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**TABLE 1. Purification of MCAT-1t from crude insect cell membranes**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (µg)</th>
<th>Titera</th>
<th>Fold enrichment (cumulative)</th>
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</thead>
<tbody>
<tr>
<td>Whole cells</td>
<td>45,000</td>
<td>4,096</td>
<td>1</td>
</tr>
<tr>
<td>Membranesb</td>
<td>14,840</td>
<td>4,096</td>
<td>3</td>
</tr>
<tr>
<td>Not adsorbed to Ni resinb</td>
<td>13,040</td>
<td>1,600</td>
<td>15</td>
</tr>
<tr>
<td>Wash eluted</td>
<td>1,200</td>
<td>1,600</td>
<td>18</td>
</tr>
<tr>
<td>Acid-eluted protein</td>
<td>90</td>
<td>1,024</td>
<td>41</td>
</tr>
<tr>
<td>Imidazole eluteddb</td>
<td>440</td>
<td>256</td>
<td>ND</td>
</tr>
<tr>
<td>Not adsorbed to antibody resinb</td>
<td>5</td>
<td>64</td>
<td>ND</td>
</tr>
<tr>
<td>Eluted from antibody resinb</td>
<td>35</td>
<td>512</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Determined by serially diluting samples 1:2-fold, applying the protein to nitrocellulose filters, and immunoblotting with 12CA5. The titer given is the reciprocal of the dilution factor and was normalized for the initial volume of the protein sample.

b Crude membranes were made from insect cells overexpressing MCAT-1t 48 h after infection with recombinant baculovirus.

c Membranes were dissolved in sodium deoxycholate buffer and applied to a nickel-charged chelating resin in batch.

d Material not eluted with imidazole following MCAT-1t elution.

e ND, not determined.

f Fifty micromolars of acid-eluted protein from the nickel resin was buffer exchanged and applied.

g No apparent increase in purity of protein acid eluted from the antibody column as determined by SDS-PAGE, but this step was included in some experiments to further ensure the homogeneity of the MCAT-1t protein.
MCAT-1t from this fraction was saturable (plateau reached at 10%, wt/vol) (Fig. 6A). Seventy percent of the lipid was recovered at the top of the gradient, as assessed in experiments that included during liposome formation) and by light scattering (0.1% rhodamine-tagged phosphatidylcholine was observed in these detergents.

Highly purified preparations of MCAT-1t have been obtained by sequential application of nickel chelation and antibody affinity chromatography in the presence of CHAPS and/or sodium deoxycholate. When purified in these detergents, more than 90% of MCAT-1t can bind to excess Fr SU:(1-236) coupled to beads. Given the high specific activity of the MCAT-1 preparation in these detergents, the existence of additional proteins that are required for SU binding and copurify with MCAT-1t is unlikely since these proteins were not identified by silver staining of purified MCAT-1t preparation after SDS-polyacrylamide gel electrophoresis. These studies reported here confirm direct binding between the amino-terminal domain of Friend 57 SU and the amino-terminal domain of Friend SU to membranes through interaction with residues 1 to 236.

**DISCUSSION**

The envelope protein of type C mammalian viruses can be divided into a variable amino-terminal portion, a proline-rich region similar to the immunoglobulin hinge region, and a conserved carboxyl-terminal domain (4). Chimeric SU proteins created by combining these regions from related mammalian type C retroviruses are functional, and receptor specificity is determined by the amino-terminal portion of SU (5, 17). Battini et al. (4) demonstrated that expression of the amino-terminal domain of Friend 57 SU is sufficient for superinfection interference, consistent with receptor binding. The studies reported here confirm direct binding between the amino-terminal domain of Friend SU to MCAT-1, using purified proteins.

The source of envelope protein in these studies was Friend 57 SU truncated (residues 1 to 236) just before the proline-rich region. After purification from insect cells, this protein bound to oocyte membranes that express MCAT-1 with an affinity comparable to that found for Moloney MLV SU gp70 binding to permissive cells (7). This finding suggests that Fr SU:(1-236) contains the entire MCAT-1 binding domain(s). Consistent with this conclusion, the equivalent portion of the amphotropic SU(1-209), purified by using the same protocol (Fig. 5), specifically binds to membranes that express its receptor, Pit2 (unpublished results).

Both Fr SU:(1-236) and MCAT-1 (in CHAPS) migrated as single peaks near their expected molecular weights on column chromatography and bound to each other with a stoichiometry of near 1:1. While it is possible that receptor-SU interaction could mediate oligomerization of either protein, the simplest
explanation of these findings is a direct interaction between a monomer of each protein. Indeed, amino acid substitutions in Fr SU:(1-236) that abrogate membrane binding and infection have been localized to a single region (variable region A) that is conserved in ecotropic MLV but not other subgroups of MLV (15). Analysis of the molecular structure of Fr SU:(1-236), recently obtained by X-ray crystallography (8), reveals that these residues are localized on one face of a loop at the top of the molecule, suggesting they define a discrete MCAT-1 binding domain. Determination of the molecular structure of Fr SU:(1-236) bound to the envelope binding domain from MCAT-1 will be required to confirm this conclusion.

At present, the Fr SU:(1-236) binding domain in MCAT-1 is not well defined. Residues within the extracellular loop connecting the third and fourth membrane-spanning domains are required for SU binding and infection, suggesting that they are part of the cognate SU binding site; however, to date, direct evidence in support of this conclusion has not been obtained. Specifically, competitive inhibition of SU binding by using peptides derived from this loop has not been observed (unpublished data). These peptides may fail to bind Fr SU:(1-236) because they cannot assume the conformation achieved in the context of the MCAT-1 loop. Indeed, conformation-dependent function of the SU binding domain of the receptor for the type C virus subgroup A avian leukemia virus has been reported (27). Alternatively, SU binding to MCAT-1 may require another, as yet unidentified domain whose conformation is critically influenced by residues in this loop. Indeed, detergent-dependent differences in MCAT-1 conformation may explain the reduced binding to Fr SU:(1-236) in octylglucoside or Triton X-100. Alternatively, those detergents that promote binding, such as sodium deoxycholate and CHAPS, may participate directly in SU binding by substituting for a specific lipid associated with MCAT-1 in the membrane. Indeed, enhancement of ecotropic MLV-induced syncytium formation in the presence of small amounts of amphoterocerin, a small hydrophobic molecule with detergent properties, has been reported (21).

To explore this hypothesis, conditions have been established for study of MCAT-1 binding to SU in the absence of detergent after reconstitution in liposomes. In the experiments reported here, binding of Fr SU:(1-236) to MCAT-1 was observed after reconstitution in liposomes containing total brain lipids (10%), which may contribute specific lipids required for MLV infection of mammalian cells. Additional experiments comparing the binding properties of MCAT-1-containing liposomes of specific lipid composition can now be performed to test this hypothesis.

Although Fr SU:(1-236) was purified as a monomer, in virions, SU forms a trimer and is associated with TM (9). The structure of MCAT-1 in the membrane is unknown, but assembly into trimers would allow binding of one subunit to each SU in a trimer on the virion surface. Alternatively, interaction with a single MCAT-1 protein may be sufficient to induce the conformational change in the trimeric SU-TM required for infection. Further studies of this issue are required to create a clearer picture of the interaction of MCAT-1 with the virion surface. These details are likely to be important since interactions between several adjacent SU and receptor molecules are likely required to initiate membrane pore formation necessary for infection.

How MCAT-1 binding to SU leads to the conformational change in the TM protein required for infection remains unclear. Recently, residues near the amino terminus domain of Friend SU that are required for membrane fusion (but not SU binding) have been identified (3). This observation could be explained by an interaction between the amino-terminal domain of SU and TM, similar to the interaction between the amino termini of HA1 and HA2 along the threefold axis of the
influenza virus HA trimer (6). Alternatively, the interaction between the amino-terminal domain of SU and MCAT-1 identified here could trigger additional interactions between envelope and MCAT-1 and/or unidentified coreceptors. If the latter, these protein(s) must be highly conserved, since cells from all mammalian and avian species can be made susceptible to ecotropic MLV infection by expression of MCAT-1. The reconstitution of MCAT-1 into liposomes provides a reagent that may be suitable to determine if MCAT-1 is sufficient to induce ecotropic MLV envelope-dependent fusion.

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