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Identification of Envelope Protein Residues Required for the Expanded Host Range of 10A1 Murine Leukemia Virus

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The 10A1 murine leukemia virus (MuLV) is a recombinant type C retrovirus isolated from a mouse infected with amphotropic MuLV (A-MuLV). 10A1 and A-MuLV have 91% amino acid identity in their envelope proteins yet display different host ranges. For example, CHO-K1 cells are resistant to A-MuLV but susceptible to infection by 10A1. We have now determined that retroviral vectors bearing altered A-MuLV envelope proteins containing 10A1-derived residues at positions 71 (A71G), 74 (Q74K), and 139 (V139M) transduce CHO-K1 cells at efficiencies similar to those achieved with 10A1 enveloped vectors. A-MuLV enveloped retroviral vectors with these three 10A1 residues were also able to transduce A-MuLV-infected NIH 3T3 cells. This observation is consistent with the ability of vectors bearing this altered A-MuLV envelope protein to recognize the 10A1-specific receptor present on NIH 3T3 cells and supports the possibility that residues at positions 71, 74, and 139 of the 10A1 envelope SU protein account for the expanded host range of 10A1.

Murine leukemia viruses (MuLVs) infect susceptible cells through an interaction between the viral envelope glycoprotein and a specific surface protein on the target cell. This interaction is followed by viral membrane-cell membrane fusion, thereby allowing entry of the viral core into the cell (7, 33). Once a productive infection is established, the endogenous expression of viral envelope protein causes the host cell receptors to be unavailable to exogenous viruses, and the cell becomes resistant to superinfection by viruses utilizing the same receptor. MuLVs have been categorized into five classes on the basis of their interference properties: ecotropic, polytropic (mink cell focus forming), xenotropic, amphotropic, and 10A1 (26, 27, 29).

10A1 is a class of MuLV isolated from a mouse infected with amphotropic MuLV (A-MuLV) (25). Its envelope protein appears to be derived from a recombination between A-MuLV and an endogenous retroviral sequence (21). It retains close sequence identity with the A-MuLV envelope in the N terminus of the surface protein (SU), differing by only the sequences encoded by six codons in the first 200 amino acids from the sequences of A-MuLV isolate 4070A (22). However, the hypervariable region immediately downstream of the N terminus resembles that in polytropic MuLV (21). 10A1 virus retains an ability to interact with the A-MuLV receptor, Pit2, but can also enter cells through another receptor, Pit1, that A-MuLV is not able to use (13, 18, 35). Pit1 serves as a receptor for a number of retroviruses, including gibbon ape leukemia viruses, simian sarcoma-associated virus, and feline leukemia viruses of subgroup B (31). Pit1 and Pit2 are both phosphate symporters and have approximately 60% amino acid identity (8, 9, 15, 20, 32, 34, 35). 10A1 is the only class of murine type C retrovirus that has been shown to utilize two different receptors.

10A1 and 4070A exhibit nonreciprocal interference patterns on mouse cells, consistent with their receptor utilization (22, 28). Existing 10A1 infection blocks both 4070A and 10A1 superinfection, while 4070A infection prevents only 4070A superinfection. Previous studies have demonstrated that the distinct interference properties of 10A1 MuLV are conferred by the N terminus of SU, as viruses containing 4070A envelope proteins in which the first 169 amino acids of the processed SU are replaced by 10A1 sequences display the 10A1 interference properties (22).

Studies with chimeric envelope proteins and recombinant envelope protein fragments have suggested that the N-terminal domain of MuLV SU is responsible for receptor recognition, possibly through the two variable regions A and B (VRA and VRB) (1–3, 5, 19, 22, 24). Previous work in our laboratory has demonstrated that changes in a single residue in VRA of the ecotropic Moloney MuLV (Mo-MuLV) SU can severely decrease the binding affinity of the envelope glycoprotein, implying the presence of a direct receptor contact point (11). Therefore, we sought to determine whether one or more of the six amino acids in the N terminus of SU that differ between the 10A1 and 4070A envelope proteins were sufficient to confer the 10A1 receptor recognition properties to 4070A, possibly by acting as contact points for Pit1.

MATERIALS AND METHODS

Cell culture. The cell line 293T/17 was obtained from the American Type Culture Collection (CRL 11268). The cell lines 293T/17 and NIH 3T3 were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (HyClone, Logan, Utah) and 2 mM glutamine (Gibco BRL, Grand Island, N.Y.). The cell line CHO-K1 was obtained from M. Kaden (Genetic Therapy, Inc., Gaithersburg, Md.) and was maintained in α-modified minimal essential medium (Irvine Scientific, Santa Ana, Calif.) supplemented with 10% fetal calf serum and 2 mM glutamine. The producer cell line G1nBgSvNa/PE501 is the ecotropic packaging cell line PE501 (14) containing the retroviral vector G1nBgSvNa (provided by Genetic Therapy, Inc.), which carries the *neo* and *lacZ* genes.

Plasmids and mutagenesis. Plasmids pMo(4070A) and pMo(10A1) are infectious clones of Mo-MuLV containing the 4070A and 10A1 *env* genes, respectively (23). These plasmids were kindly provided by A. Rein (NCI-Frederick Cancer Research Facility). Plasmids pRV109 and pHIT60 (30) were obtained from A. Kingsman (University of Oxford). pHIT60 is a cytomegalovirus (CMV)-

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driven plasmid expressing Mo-MuLV Gag and Pol. pCnBg is a retroviral vector with a hybrid 5′ CMV long terminal repeat promoter produced by inserting the *Kpn*I fragment of the vector G1nBgSvNa into pRV109.

Plasmids pSCA and pSC10 are 4070A and 10A1 *env* expression plasmids, respectively, each containing a CMV promoter and a simian virus 40 origin of replication. The 4070A *env* gene was derived from pCAE (19), while the 10A1 *env* gene was derived from pMo(10A1). The chimeric envelope plasmids pSCA/10 and pSC10/A were constructed by using a conserved *Xho*I restriction site located at the codon for amino acid 158 of the processed envelope glycoprotein. For site-directed mutagenesis, PCR splice overlap mutagenesis (6) was used to introduce mutations. Two complementary mutagenic primers containing single or double point mutations were used to introduce mutations, and the two amplified segments of the envelope protein were then ligated in a subsequent PCR. Convenient restriction enzyme sites were used to insert the PCR product into intermediate plasmids, which were completely sequenced. The sequenced fragments were then introduced into the parental *env* expression vector pSCA. Mutants are designated by the amino acid in the 4070A envelope protein followed by the residue number and the amino acid found in the mutant protein. The amino acid residues are numbered from the N terminus of SU after the signal peptide is cleaved.

Virus production and titer determination. Retroviral vectors were produced by transient transfection of plasmid pHIT60, plasmid pCnBg, and an *env* expression plasmid (5 μg each) into 293T/17 cells (5×10^5 cells in a 60-mm-diameter dish) by calcium phosphate precipitation, essentially as described previously (30). Sixteen hours posttransfection, the precipitate was removed and replaced with 5 ml of medium containing 10 mM sodium butyrate (Sigma, St. Louis, Mo.) for 12 h. The cells were then incubated in 3 ml of fresh medium to allow production of retroviral vectors, which were harvested after a further 12 h of incubation at 37°C and used immediately. Retroviral vectors containing the ecotropic Mo-MuLV envelope glycoproteins were collected from G1nBgSvNa/PE501 cells as previously described (36).

For viral transduction, 3×10^4 cells were seeded in the 30-mm-diameter wells of a six-well plate in 1 ml of medium. Twenty-four hours later, the medium was replaced with 1 ml of appropriately diluted viral supernatant containing 8 μg of Polybrene (Sigma) per ml and incubated at 37°C. Eighteen to twenty-four hours posttransduction, the culture medium was replaced with fresh medium, and the cells were incubated for another 48 h. Cells were stained for β-galactosidase expression as previously described (11).

Interference assays. Stocks of infectious viruses were produced by transfecting 15 μg of pMo(4070A) or pMo(10A1) into 293T/17 cells (5×10^5 cells in a 60-mm-diameter dish) by calcium phosphate precipitation. Sixteen hours posttransfection, the precipitate was removed and replaced with 5 ml of medium. The viral supernatant was collected after a further 24 h of incubation at 37°C. NIH 3T3 cells were infected with the resulting filtered supernatants, and the infections were monitored by a reverse transcriptase (RT) assay (4) of the culture supernatants until chronically infected populations were obtained. The RT activities of the supernatants were calculated from a standard curve generated with a serial dilution of recombinant MuLV RT (Promega, Madison, Wis.). Typical chronically infected populations had supernatant RT activities within the range of 0.01 to 0.1 U/ml.

Interference assays were performed on the infected NIH 3T3 cells with retroviral vector supernatants, produced as described above. A total of 3×10^4 NIH 3T3 cells chronically infected with either Mo(4070A) or Mo(10A1) MuLV were seeded in the 30-mm-diameter wells of a six-well plate in 1 ml of medium. Transduction and staining for β-galactosidase expression were done as described above for uninfected cells.

RESULTS

10A1 host range can be conferred by the N-terminal 158 amino acids of SU. In order to determine more precisely the region within the SU of 10A1 MuLV that accounts for its host range, we constructed chimeras between the 4070A and 10A1 envelope proteins (Fig. 1) similar to those made by Ott and Rein (22). A conserved *Xho*I site located at the codon for amino acid 158 of processed SU was used to generate these constructs. The resulting chimeric envelope proteins were incorporated into retroviral vector particles, and the particles were assayed for the ability to transduce NIH 3T3 and CHO-K1 cells.

As expected, vectors containing 10A1 envelope proteins were able to transduce CHO-K1 and NIH 3T3 cells, whereas the 4070A vectors were able to transduce only NIH 3T3 cells (Table 1). Vectors containing the 10/A chimeric protein, which contains the 10A1 envelope protein N terminus, gave titers on CHO-K1 cells that were approximately 60% of the titers observed for the 10A1 vectors. The chimera with the 4070A envelope protein N terminus, A/10, gave titers on CHO-K1 cells that were just under 10% of the titers obtained with 10A1.

The host ranges of the vectors containing the wild-type and chimeric envelope proteins were further tested by measuring the titer of the retroviral vectors on NIH 3T3 cells chronically infected with either Mo(4070A) or Mo(10A1) (Table 1). As expected, infection with Mo(10A1) blocked subsequent transduction by all vectors except for those carrying ecotropic Mo-MuLV envelope proteins. Mo(4070A) infection blocked transduction by vectors with 4070A envelope proteins but still allowed transduction by vectors containing 10A1 envelope protein and the 10/A chimeric proteins. In contrast, vectors containing the A/10 chimeric proteins were unable to transduce cells chronically infected by Mo(4070A). These results are in agreement with those reported by Ott and Rein (22).

Single substitutions in the 4070A envelope protein do not allow efficient transduction of CHO-K1 or Mo(4070A)-infected cells. Because the host ranges of vectors with the 10/A chimeric envelope protein closely resembled those of 10A1, we investigated, in more detail, the six residues within the first 158 amino acids that differ between 4070A and 10A1 SU (Fig. 1). Mutant 4070A envelope proteins were produced by site-directed mutagenesis in which each residue at positions 41, 71, 74, 97, 103, and 139 was replaced with the corresponding residue of the 10A1 envelope protein. The mutant proteins were then incorporated into retroviral vectors, and the vectors were tested for the ability to transduce NIH 3T3 and CHO-K1 cells.

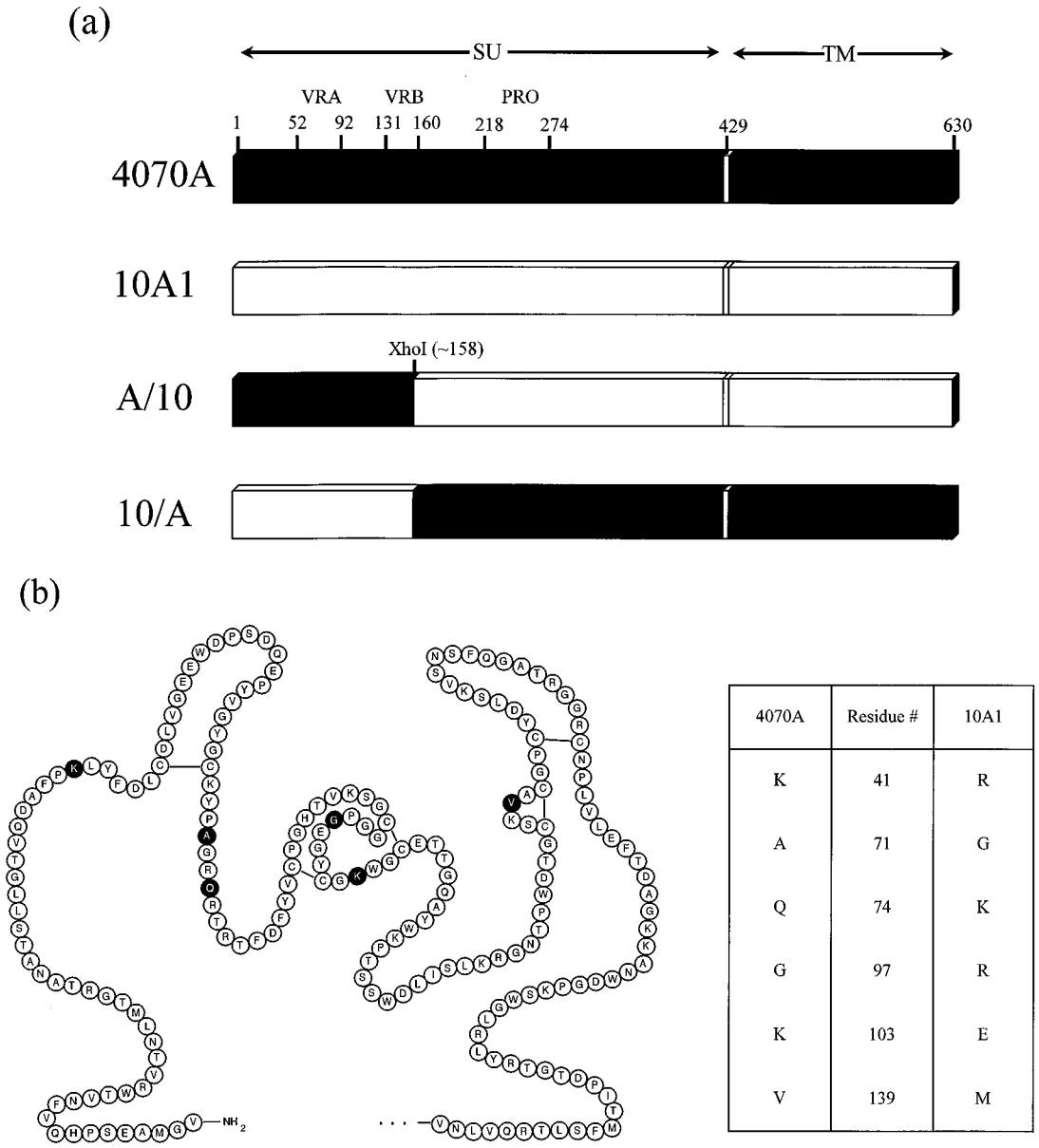
All of the retroviral vectors with envelope proteins containing single 10A1 substitutions transduced NIH 3T3 cells at levels comparable to those of vectors with wild-type 4070A envelope protein, indicating that the mutant proteins were biologically active (Table 2). However, no vectors containing these single-substitution proteins were able to transduce CHO-K1 cells.

We also examined the ability of the vectors to transduce NIH 3T3 cells chronically infected with Mo(4070A). Vectors bearing envelope proteins with the substitution Q74K were able to transduce those cells, although at a level that was approximately 5 orders of magnitude lower than that obtained with wild-type 10A1 (Table 2). None of the other single substitutions allowed transduction of Mo(4070A)-infected NIH 3T3 cells.

Multiple amino acid substitutions are required for efficient transduction of CHO-K1 cells. Since none of the single substitutions were sufficient to allow transduction of CHO-K1 cells, we tested whether multiple amino acid substitutions would allow transduction. In particular, we were interested in combinations of residue substitutions at positions 71, 74, and 139, as these three residues lie in the two variable regions VRA and VRB (Fig. 1). In addition, we made combinations that included substitutions of residues 97 and 103, as these represented the least conservative changes between the 10A1 and 4070A residues (Fig. 1).

All of the mutant envelope proteins were incorporated into retroviral vectors, and the vectors were tested for the ability to transduce CHO-K1 cells (Table 3). The double substitution of residues 74 and 139 resulted in titers of 140 CFU/ml on CHO-K1 cells, which is within 1 order of magnitude of the titer of wild-type 10A1. The combined substitution of all three of the VRA and VRB residues (A71G-Q74K-V139M) resulted in the highest titers, yielding 73% of the titer of wild-type 10A1 on CHO-K1 cells. None of the other combinations resulted in transduction of CHO-K1 cells. However, it cannot be ruled out that different combinations than those tested may allow a 10A1-like host range.

Envelope proteins with multiple substitutions allow transduction of Mo(4070A)-infected NIH 3T3 cells. The ability of a



4070A	Residue #	10A1
K	41	R
A	71	G
Q	74	K
G	97	R
K	103	E
V	139	M

FIG. 1. (a) Structures of the 4070A, 10A1, and chimeric envelope proteins. Numbers correspond to the positions of the amino acid residues delineating domains in the mature 4070A envelope protein after removal of the signal peptide. VRA and VRB reside within the receptor binding domain (2). A conserved *Xho*I site corresponding to amino acid 158 was used to generate the chimeras. PRO, proline-rich hypervariable domain. (b) Schematic representation of the N-terminal 209 residues of the A-MuLV 4070A SU protein. Solid lines indicate disulfide bonds, based on the proposed structure of mink cell focus-forming MuLV SU (10). The six residues which differ between 4070A and 10A1 are indicated as dark circles, and the identities and positions of these residues in the 4070A and 10A1 proteins are presented in the box on the right.

vector with an envelope protein to transduce CHO-K1 cells did not necessarily correlate with its ability to utilize the murine Pit1 receptor. Vectors bearing the A/10 chimeras could transduce CHO-K1 cells but were unable to transduce Mo(4070A)-infected NIH 3T3 cells, whereas the opposite case was seen for vectors with the Q74K substitution. Therefore, we were interested in determining whether the vectors bearing the envelope proteins with multiple substitutions that were able to transduce CHO-K1 cells were also capable of transducing Mo(4070A)-infected NIH 3T3 cells.

The same panel of envelope proteins with substitutions was used in an interference assay (Table 3). Mo(4070A) infection of NIH 3T3 cells did not block transduction by vectors containing the double substitution Q74K-V139M or the triple substitution A71G-Q74K-V139M. Somewhat unexpectedly, vectors bearing envelope proteins with the double substitution A71G-Q74K were also able to transduce Mo(4070A)-infected NIH 3T3 cells, despite the absence of titers of such vectors on CHO-K1 cells. Although residues 97 and 103 represent the least conservative changes between the 4070A and 10A1 proteins (Fig. 1), the substitution of these residues either singly or in combination with other changes failed to enhance the titer on either CHO-K1 or Mo(4070A)-infected cells. As expected, Mo(10A1) infection blocked subsequent transduction by all of the vectors containing 4070A- and 10A1-derived envelope proteins.

TABLE 1. Host ranges and interference properties of vectors with chimeric envelope proteins

Envelope protein	Titer (CFU/ml)[a] of vector on cell line			
	NIH 3T3	CHO-K1	Mo(4070A)-3T3[c]	Mo(10A1)-3T3[d]
None	<50	<50	<50	<50
4070A	$(7.5 \pm 0.8) \times 10^6$	<50	<50	<50
10A1	$(5.8 \pm 1.5) \times 10^6$	$(6.3 \pm 4.4) \times 10^3$	$(6.3 \pm 0.9) \times 10^5$	<50
A/10	$(6.0 \pm 0.8) \times 10^6$	$(1.4 \pm 1.0) \times 10^2$	<50	<50
10/A	$(4.1 \pm 0.8) \times 10^6$	$(3.6 \pm 2.6) \times 10^3$	$(4.8 \pm 2.0) \times 10^5$	<50
Mo-MuLV[b]	$(2.7 \pm 1.7) \times 10^6$	<50	$(7.2 \pm 1.3) \times 10^5$	$(4.9 \pm 0.7) \times 10^5$

[a] The titers were averaged from at least three independent experiments and are expressed as mean number of β-galactosidase-expressing colonies ± the standard error of the mean.
[b] Ecotropic Mo-MuLV vectors were collected from G1nBgSvNa/PE501 cells.
[c] NIH 3T3 cells chronically infected with Mo(4070A).
[d] NIH 3T3 cells chronically infected with Mo(10A1).

DISCUSSION

It has been previously demonstrated that 10A1 MuLV can utilize both Pit1 and Pit2 receptors to infect NIH 3T3 cells, whereas infection by 4070A MuLV is restricted to Pit2 (13, 18, 35). Our analysis has revealed that two or more substitutions within VRA and VRB of 4070A SU (A71G, Q74K, and V139M) are sufficient to allow transduction of Mo(4070A)-infected NIH 3T3 cells by retroviral vectors at 7 to 21% of the level attained by vectors containing the 10A1 envelope protein. Therefore, it appears that these changes allow vectors with 4070A envelope proteins to utilize murine Pit1.

Unlike vectors with 10A1, vectors with the 4070A envelope protein cannot transduce CHO-K1 cells. We therefore also examined the minimal changes to 4070A SU needed to allow transduction of CHO-K1 cells. In general, there was a good correlation between transduction of Mo(4070A)-infected NIH 3T3 cells and CHO-K1 cells. Certain combinations of two changes from the VRA-VRB trio allowed transduction of both CHO-K1 and Mo(4070A)-infected cells, with the triple substitution leading to the highest titers in both cases. However, we have also discovered that it is possible to construct envelope proteins that exhibit 10A1 interference properties in NIH 3T3 cells but fail to allow transduction of CHO-K1 cells (A71G-Q74K). Conversely, vectors bearing the A/10 chimeric proteins transduced CHO-K1 cells at a low efficiency but failed to transduce 4070A-infected NIH 3T3 cells. These findings suggest that different regions within the 10A1 SU may contribute to the ability of this virus to utilize murine Pit1 and to transduce CHO-K1 cells.

It is not known, at present, which receptor(s) is utilized by 10A1 to gain entry into CHO-K1 cells. Since 10A1 can efficiently utilize murine Pit1, it is possible that 10A1 uses the hamster homolog of Pit1 on CHO-K1 cells. Alternatively, 10A1 may be able to use hamster Pit2 as a receptor. Although CHO-K1 cells express Pit2, they are unable to support infection by A-MuLV, and it has been suggested that this could be due to an additional N-linked glycosylation site in the second extracellular region of the hamster protein (35). 10A1 may be able to circumvent this proposed glycosylation block, and the mutated 4070A envelope proteins with as few as two 10A1-derived residues may similarly have acquired an ability to interact with CHO-K1 cell Pit2. A precedent for such a mechanism exists in the case of the ecotropic MuLV PVC211, which can infect CHO-K1 cells despite an N-linked glycosylation of the hamster cell receptor that prevents infection by other ecotropic viruses (12). Furthermore, the placement of two amino acid residues from PVC211 into the envelope protein of Friend MuLV enabled this virus to efficiently infect CHO-K1 cells (12).

Retroviral vectors containing 10A1 envelope proteins transduced CHO-K1 cells less efficiently than they did NIH 3T3 cells (typically 3 orders of magnitude less), although the absolute titer obtained for 10A1 retroviral vectors varied between two different lab strains of CHO-K1 cells (data not shown). It has been reported that CHO-K1 cells secrete a protein factor(s) that can inhibit infections by both A-MuLV and 10A1, but not ecotropic-MuLV (16–18). The reduced titer of 10A1 retroviral vectors on CHO-K1 cells compared to that on NIH 3T3 cells could therefore be due to this inhibitory factor.

TABLE 2. Host ranges of 4070A enveloped vectors with single residue substitutions

Envelope protein[a]	Titer (CFU/ml)[b] of vector on cell line			
	NIH 3T3	CHO-K1	Mo(4070A)-3T3[c]	Mo(10A1)-3T3[d]
None	<50	<50	<50	<50
4070A	$(3.1 \pm 2.2) \times 10^6$	<50	<50	<50
10A1	$(2.2 \pm 1.1) \times 10^6$	$(7.5 \pm 2.2) \times 10^2$	$(4.5 \pm 2.0) \times 10^7$	<50
K41R	$(4.0 \pm 2.8) \times 10^6$	<50	<50	<50
A71G	$(1.6 \pm 0.7) \times 10^6$	<50	<50	<50
Q74K	$(3.7 \pm 2.4) \times 10^6$	<50	$(4.9 \pm 1.3) \times 10^2$	<50
G97R	$(3.4 \pm 2.0) \times 10^6$	<50	<50	<50
K103E	$(2.6 \pm 1.5) \times 10^6$	<50	<50	<50
V139M	$(2.6 \pm 1.2) \times 10^6$	<50	<50	<50

[a] Mutants are identified by the residue in the 4070A envelope protein, followed by its position and the substituted 10A1 residue.
[b] The titers are averaged from at least three independent experiments and are expressed as mean number of β-galactosidase-expressing colonies ± the standard error of the mean.
[c] NIH 3T3 cells chronically infected with Mo(4070A).
[d] NIH 3T3 cells chronically infected with Mo(10A1).

TABLE 3. Host ranges and interference properties of 4070A enveloped vectors with multiple residue substitutions

Envelope protein[a]	Titer (CFU/ml)[b] of vector on cell line			
	NIH 3T3	CHO-K1	Mo(4070A)-3T3[c]	Mo(10A1)-3T3[d]
None	<50	<50	<50	<50
4070A	$(7.6 \pm 2.0) \times 10^6$	<50	<50	<50
10A1	$(1.7 \pm 1.1) \times 10^7$	$(1.5 \pm 0.0) \times 10^3$	$(1.7 \pm 0.6) \times 10^6$	<50
A71G-Q74K	$(5.1 \pm 1.3) \times 10^6$	<50	$(1.2 \pm 0.4) \times 10^5$	<50
A71G-Q74K-V139M	$(5.6 \pm 0.8) \times 10^6$	$(1.1 \pm 0.2) \times 10^3$	$(3.5 \pm 1.0) \times 10^5$	<50
Q74K-V139M	$(7.3 \pm 1.2) \times 10^6$	$(1.4 \pm 0.4) \times 10^2$	$(3.5 \pm 0.3) \times 10^5$	<50
G97R-K103E	$(5.4 \pm 2.3) \times 10^6$	<50	<50	<50
G97R-K103E-V139M	$(6.1 \pm 3.2) \times 10^6$	<50	<50	<50
G97R-V139M	$(5.1 \pm 1.1) \times 10^6$	<50	<50	<50
K103E-V139M	$(5.6 \pm 1.0) \times 10^6$	<50	<50	<50

[a] Mutants are identified by the residue in the 4070A envelope protein, followed by its position and the substituted 10A1 residue.
[b] The titers were averaged from at least three independent experiments and are expressed as mean number of β-galactosidase-expressing colonies ± the standard error of the mean.
[c] NIH 3T3 cells chronically infected with Mo(4070A).
[d] NIH 3T3 cells chronically infected with Mo(10A1).

The finding that both the A/10 and 10/A chimeras allowed transduction of CHO-K1 cells was unexpected. However, there is some evidence suggesting that sequences downstream of the sequences encoding the N-terminal domain in the nonecotropic MuLV envelope proteins can influence the receptor recognition properties of the N-terminal domain (1, 22). For example, Ott and Rein have demonstrated that chimeric 4070A or 10A1 envelope proteins whose genes contain polytropic sequences downstream of the *Eco*RI site corresponding to amino acid 169 in 4070A SU were able to block superinfection by polytropic MuLV, presumably by interfering with the polytropic receptor (22). Thus, the replacement of the C-terminal domain of 4070A by 10A1 sequences in the A/10 chimera may have influenced the ability of the N-terminal domain to interact with a receptor on CHO-K1 cells that 4070A cannot normally utilize.

Since the host range of vectors bearing 4070A envelope protein could be altered either by introducing different multiple amino acid substitutions into the N terminus of 4070A SU or by changing the carboxy-terminal sequences of the SU protein, it is likely that no single one of the six mutated residues serves individually as a molecular contact for the 10A1 receptor. This result contrasts with the situation in Mo-MuLV, where a single residue, D84, has been identified as critical for binding to the ecotropic receptor (11). It seems likely that the same region in the 10A1 envelope protein interacts with both the murine Pit1 and Pit2 proteins, especially given the high degree of homology between these two receptors and also with the 10A1 receptor on CHO-K1 cells. This common binding moiety is probably also utilized in the A-MuLV–Pit2 interaction. Our mutations of the 4070A protein that allowed murine Pit1 utilization and CHO-K1 transduction could have altered the overall structure of this receptor binding domain or increased the flexibility of the region, allowing for the dynamic recognition of the 10A1 receptors through an induced-fit process. Alternatively, the original 4070A protein residues may have sterically hindered the interaction with the 10A1 receptors. The different presentation of this putative Pit1/Pit2 binding domain, caused by either multiple substitutions in the 4070A protein or the replacement of the C-terminal region of the 4070A protein with that of the 10A1 protein, could thereby allow a more promiscuous interaction with these host cell receptors.

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