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The Japanese Feral Mouse Pit1 and Pit2 Homologs Lack an Acidic Residue at Position 550 but Still Function as Gibbon Ape Leukemia Virus Receptors: Implications for Virus Binding Motif

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Murine cells are typically resistant to gibbon ape leukemia virus (GALV). MMMol, a Japanese feral mouse cell line, is an exception in that these cells are susceptible to infection by GALV. We show here that MMMol cells are further distinguished by their unusual receptor properties. MMMol cells infected by GALV are resistant to subsequent infection not only by GALV but also by amphotropic murine leukemia virus. This suggests that GALV can enter MMMol via not only the GALV receptor (MolPit1) but also the amphotropic murine leukemia virus receptor (MolPit2). Therefore, MolPit2 was cloned, sequenced, and compared with the previously reported sequence of MolPit1. Earlier studies have shown that a stretch of nine residues (position 550 to 558) in the fourth extracellular domain of Pit1 is crucial for GALV entry and that an acidic residue at position 550 is indispensable. However, MolPit1 has isoleucine at this position and MolPit2 has glutamine at the corresponding position (position 522), thus breaking this consensus. To determine what effect these specific changes in the fourth extracellular domain of MolPit1 and MolPit2 have on GALV receptor function, chimeric receptors were made by substituting the fourth extracellular domain of either MolPit1 or MolPit2 for the same region of Pit2, a nonfunctional receptor for GALV. These chimeras were then tested in MDTF, a cell line that lacks functional GALV receptors and is resistant to GALV. Results show that MDTF expressing these chimeras became susceptible to GALV, whereas cells expressing wild-type Pit2 remained resistant. Further, the MolPit1 chimera was identical to Pit1 in efficiency, but the MolPit2 chimera proved substantially less efficient.

The cDNAs that encode the human, hamster, mouse, and rat receptors for gibbon ape leukemia virus (GALV) (3, 11, 14, 19, 20) and the human, hamster, and rat amphotropic murine leukemia virus (A-MuLV) receptors have been cloned (9, 15, 20). These receptors are highly related phosphate symporters (5, 12, 17), and their human homologs are now designated Pit1 (GALV receptor) and Pit2 (A-MuLV receptor). Although the GALV and A-MuLV receptors are coexpressed on most cells, typically each virus uses only its own receptor to enter cells.

Not all naturally occurring GALV receptors are functional, and the murine forms are typically inactive for the virus. Sequence comparison of functional and nonfunctional GALV receptors has permitted the identification of regions in Pit1 critical for viral infection. These comparisons and studies using chimeric receptors formed between Pit1 and various nonfunctional receptors have shown that a stretch of nine residues (position 550 to 558) in the fourth extracellular domain of Pit1 is crucial for GALV entry into cells (2, 4, 10, 13, 20). Certain mutations within this region of rat Pit1, normally functional for GALV, abrogate GALV receptor function (14). Likewise, certain mutations in mouse Pit1, normally inactive for GALV, restore receptor function (4). Previously we reported that the hamster A-MuLV receptor (HaPit2) allows entry by both A-MuLV and GALV (20). The cDNA encoding HaPit2 was cloned and sequenced. HaPit2 has 93% amino acid identity with Pit2, which does not permit GALV infection. The molecular basis for the expanded viral recognition properties of HaPit2 was determined by using chimeric receptors in which regions of HaPit2 were substituted for the corresponding regions of Pit2. Thus, residues that differ between HaPit2 and Pit2 and accounted for the functional difference between the two receptors were identified. A notable difference is that Pit2 has a lysine at position 522 in the fourth extracellular domain whereas HaPit2 has a glutamic acid at the same position. Site-directed mutagenesis revealed that replacing this lysine with glutamic acid within Pit2 is sufficient to impart GALV receptor function (2). It remains unclear, however, whether an acidic residue at position 522 is required for GALV receptor function or whether this function is inhibited by the presence of a positively charged residue.

Most murine cells express Pit1 homologs that do not function as GALV receptors. We recently identified a Japanese feral mouse (*Mus musculus molossinus*) kidney cell line, MMMol, that is susceptible to GALV (19). The cDNA encoding the MMMol homolog of Pit1 (MolPit1) has been cloned and sequenced (19). In contrast to all of the previously characterized functional GALV receptors, MolPit1 contains isoleucine instead of a negatively charged residue at position 550 in the fourth extracellular domain (19).

The present work reports that MMMol cells infected by GALV are resistant to subsequent infection by A-MuLV, sug-

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gesting that GALV can use the MMMol homolog of Pit2 (MolPit2) as a receptor. Indeed, chimeric Pit2 containing the fourth extracellular domain of MolPit2 functioned, albeit inefficiently, as a GALV receptor. The chimeric Pit2 carrying the fourth extracellular domain of MolPit1 proved identical to Pit1 in efficiency for GALV infection. Thus, an acidic residue at position 550 in at least MolPit1 is dispensable for efficient GALV receptor function.

MATERIALS AND METHODS

Cells and viruses. The following cells were used in these studies: NIH 3T3 (ATCC CRL 1658), *M. musculus molossinus* kidney fibroblasts MMMol (ATCC CRL 6439), mink lung fibroblasts Mv1Lu (ATCC CCL 64), PA317 (ATCC CRL 9078) (7), PG13 (ATCC 10686) (8), PE501 (obtained from A. D. Miller, Seattle, Wash.), CHO K1 (ATCC 61), and *Mus dunni* tail fibroblasts (MDTF) (obtained from Olivier Danos, Institut Pasteur, Paris, France). All cell lines were grown in Dulbecco's modified Eagle's medium (BioWhittaker, Walkersville, Md.) containing 5% fetal bovine serum, except for CHO K1 cells, which were grown in alpha-modified Eagle's medium containing 10% heat-inactivated fetal bovine serum. Cells were grown at 35°C in the presence of 5% CO_2 in humidified chambers.

Supernatant containing specific virus was collected from cells infected with that virus. For GALV-SEATO, the cells were Mv1Lu transfected with p386, a molecular clone of GALV-SEATO. For A-MuLV, 4070A-infected Mv1Lu cells were used. For ecotropic MuLV (E-MuLV), NIH 3T3 cells productively infected with an infectious molecular clone of Friend MuLV, p57A (obtained from Marc Sitbon, Institut Pasteur), were used. 10A1 was collected from DC8/3T3 cells (provided by A. Rein, National Cancer Institute, Bethesda, Md.).

Retroviral vectors containing the G1 genome were produced by PA317 and PG13 packaging cell lines exposed to supernatant from PE501 cells transfected with the G1 plasmid. The G1 genome contains genes for β-galactosidase and neomycin resistance (6). 10A1/G1 pseudotypes were produced by transducing 10A1-infected MDTF with PA317/G1 vectors. Neomycin-resistant MDTF and CHO K1 cells were selected with G418 (500 μg/ml; Life Technology, Gaithersburg, Md.). MDTF expressing Pit1, Pit2, HaPit2, Pit2(K522E), HaPit2/Pit2, or Pit1/Pit2 (formerly designated glvr1, glvr2, EAR, AAAA*A, AAAEE, and GAAGG, respectively) have been previously described (2).

Cloning and sequencing of MolPit2. The cDNA encoding MolPit2 was cloned by a reverse transcription-PCR strategy (2). Polyadenylated mRNA was isolated from MMMol cells with the Fast Track 2.0 kit (Invitrogen Corp., San Diego, Calif.), and the cDNA was synthesized with random hexanucleotides and avian myeloblastosis virus reverse transcriptase (Promega Corp., Madison, Wis.). The primers were derived from either Pit2 or sequenced regions of MolPit2 cDNA. The PCR products were ligated directly into the pCRII TA cloning vector (Invitrogen). Several PCR products were sequenced to determine a consensus sequence for MolPit2 cDNA and to ensure that no PCR-induced changes were present. The accession numbers for the MolPit2 cDNA sequences are U62559 (the sequence of the region spanning extracellular domains one to three) and U62560 (the sequence of the region spanning extracellular domains four to five).

Construction of MolPit1/Pit2 and MolPit2/Pit2 chimeras. An *Xho*I site was introduced by PCR mutagenesis in MolPit1 cDNA to facilitate subcloning into Pit2 cDNA, which has an existing unique *Xho*I site at the corresponding position. Chimeric Pit2 receptors containing the fourth extracellular domain of either MolPit1 or MolPit2 were then made by exchanging the region between *Xho*I and *Pfl*MI sites of Pit2 with the corresponding region of either MolPit1 or MolPit2 (see Fig. 2).

Infections and interference analysis. MMMol cells were exposed to GALV, A-MuLV, 10A1, or Friend MuLV in the presence of Polybrene (5 μg/ml), and cultured for 2 weeks. Productive infections were confirmed by assaying the reverse transcriptase activity in supernatants (18). For interference assays, 3×10^4 cells per well were seeded in 12-well assay plates, and uninfected MMMol cells were always included as controls. Later (after 24 h), the cells were exposed to 10-fold serial dilutions of supernatant containing PA317/G1, PG13/G1, 10A1/G1 pseudotypes, or PE501/G1. All infections were in the presence of Polybrene (5 μg/ml). Cells were processed for β-galactosidase activity 72 h after infection, as described previously (16). Titers were determined by counting the blue foci (BFU) in triplicate wells, and two dilutions were used for each determination. The titers reported are means plus or minus the standard errors of the means. Similar assays were employed to assess GALV infection of various native and chimeric receptors expressed in MDTF, and uninfected cells were always included as the negative control.

RESULTS

Interference assays are based on the observation that retroviral infection of a cell results in the specific loss of viral receptor function and the acquisition of resistance to subsequent infection by viruses dependent on that receptor for entry. However, the infected cells remain susceptible to viruses that utilize other receptors. To determine whether A-MuLV and GALV use the same receptor to infect MMMol cells, we performed interference assays. The results show that MMMol cells infected with A-MuLV were resistant to subsequent infection by A-MuLV, as expected, but remained susceptible to GALV (Table 1). In contrast, GALV-infected MMMol cells were resistant to infection by both GALV and A-MuLV (Table 1), suggesting that GALV may enter these cells via either the A-MuLV or the GALV receptors. To further assess this possibility, we did interference assays with 10A1, a murine retrovirus that is known to use both Pit1 and Pit2 to enter cells and that exhibits reciprocal interference with GALV (10, 20). We reasoned that if 10A1 used the Pit1 and Pit2 homologs in MMMol cells, GALV and 10A1 would show reciprocal interference with these cells. Predictably, 10A1-infected MMMol cells were resistant to both GALV and A-MuLV, and GALV-infected cells were resistant to both 10A1 and A-MuLV (Table 1).

TABLE 1. Interference properties of A-MuLV, GALV, 10A1, and E-MuLV with MMMol cells

Challenge pseudovirus or retroviral vector[a]	Virus titer ratio on MMMol cells infected with[b]:			
	A-MuLV	GALV	10A1	E-MuLV
A-MuLV	<0.0001	<0.0001	<0.0001	0.45
GALV	0.35	<0.0001	<0.0001	0.41
10A1	0.41	0.0003	<0.0001	0.33
E-MuLV	0.32	0.19	0.17	<0.0001

[a] PG13/G1, PA317/G1, or PE501/G1 retroviral vectors were used as A-MuLV, GALV, or E-MuLV challenge vectors, as described in Materials and Methods. 10A1/G1 pseudovirions were used to superinfect infected MMMol cells.

[b] The 4070A strain of A-MuLV, the SEATO strain of GALV, and the Friend strain of E-MuLV were used to infect MMMol cells. Virus titer ratio = titer of challenge pseudovirus or retroviral vector on infected cells/titer of challenge pseudovirus or retroviral vector on uninfected cells.

A-MuLV, GALV, and 10A1 do not use the E-MuLV receptor to enter cells. Therefore, interference assays with E-MuLV-infected MMMol cells were included as controls to assess nonspecific effects of productive infection on subsequent challenge infection. As expected, E-MuLV-infected MMMol cells resisted superinfection by E-MuLV but maintained susceptibility to GALV, A-MuLV, and 10A1 (Table 1).

The deduced amino acid sequences of the five extracellular domains of MolPit2 are presented in Fig. 1. MolPit2 is very similar to the previously identified Pit2 homolog expressed in rats (10). Differences between the extracellular domains of MolPit2 and Pit2 include proline at position 132 in the second domain, serine at position 209 and isoleucine at position 212 in the third domain, and glutamine at position 522 in the fourth domain (Fig. 1). Unlike MolPit2, Pit2 has lysine at position 522. Previous evidence has demonstrated that it is this lysine that renders Pit2 nonfunctional for GALV (2).

To determine whether the fourth extracellular domains of MolPit1 and MolPit2 are compatible with GALV receptor function, Pit2 chimeras carrying the fourth domain of either MolPit1 or MolPit2 were constructed (Fig. 2). These chimeras were then tested for GALV infection in MDTF, a cell line that lacks functional GALV receptors. Both chimeras allowed GALV infection, demonstrating that the fourth extracellular domains of MolPit1 and MolPit2 indeed confer susceptibility to GALV. CHO K1 cells, normally resistant to A-MuLV, were rendered susceptible to PA317/G1 when either MolPit1/Pit2 or MolPit2/Pit2 chimeric receptors were expressed in them (data not shown).

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DOMAIN I      27-44

PiT2          NDVANSFGTAVGSGVVTL
RatPiT2       NDVANSFGTAVGSGVVTL
MolPiT2       NDVANSFGTAVGSGVVTL

DOMAIN II     108-141

PiT2          LRLFISGTHCIVGSTIGFSLVAIGTKGVQWMELV
RatPiT2       LRLFISGTHCIVGSTIGFSLVAIGPKGVQWMELV
MolPiT2       LRLFISGTHCIVGSTIGFSLVAIGPKGVQWMELV

DOMAIN III    205-214

PiT2          VLGL VLPMWA
RatPiT2       VLGL-SLPIWA
MolPiT2       VLGL SLPIWA

DOMAIN IV     505-530

PiT2          NDVSNAIGPLVALWLIYKQGGVTQEA
RatPiT2       NDVSNAIGPLVALWLIYKQGGVTQEA
MolPiT2       NDVSNAIGPLVALWLIYQQGGVTQEA

DOMAIN V      590-622

PiT2          LPVSTTHCKVGSVVAVGWIRSRKAVDWRLFRNI
RatPiT2       LPVSTTHCKVGSVVAVGWIRSRKAVDWRLFRNI
MolPiT2       LPVSTTHCKVGSVVAVGWIRSRKAVDWRLFRNI
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FIG. 1. Comparison of the deduced amino acid sequences of extracellular domains of the Pit2 homolog from *M. musculus molossinus* (MolPit2), Pit2 (15), and RatPit2 (11). The residues present in MolPit2 and RatPit2 but not in Pit2 are underlined. Glutamine 522 of MolPit2 is in boldface type.

The efficiency of the GALV receptor function of the MolPit1/Pit2 and MolPit2/Pit2 chimeras was compared with that of Pit1, Pit2, HaPit2/Pit2, Pit1/Pit2, and Pit2(K522E) (Fig. 2). The native receptors and the chimeras were expressed in MDTF, and these cells were then exposed to PG13/G1 to assess their susceptibility to GALV (Fig. 3; Table 2). The expression of Pit1 in MDTF resulted in PG13/G1 titers of over 2×10^5 BFU/ml, whereas the expression of Pit2 yielded no BFU. All the chimeric receptors allowed GALV entry, with MolPit1/Pit2 (1.8×10^5) and Pit2(K522E) (2×10^5) functioning with efficiencies nearly identical to that of wild-type Pit1 (Fig. 3; Table 2). Two of the chimeric receptors, HaPit2/Pit2 (4.9×10^3) and Pit1/Pit2 (1.8×10^4), were less efficient than Pit1. The chimeric receptor MolPit2/Pit2 afforded the lowest titer, approximately 20 BFU/ml (Fig. 3; Table 2).

Because 10A1 exhibited reciprocal interference with GALV in MMMol cells, we also determined whether 10A1 blocked infection of MDTF by GALV. MDTF expressing Pit1, HaPit2/Pit2, Pit1/Pit2, MolPit1/Pit2, MolPit2/Pit2, and Pit2(K522E) were productively infected with 10A1 and then exposed to PG13/BSN vectors. In each case, the cells were resistant to PG13 challenge infection, suggesting that 10A1 can use not only Pit1 but also each of the chimeric receptors (data not shown).

DISCUSSION

The nonreciprocal receptor interference between A-MuLV and GALV with MMMol cells is identical to the phenomenon exhibited by these viruses with hamster E36 cells (1). In both cases, GALV-infected cells are resistant to A-MuLV but A-MuLV-infected cells remain susceptible to GALV. In the case of E36 cells, nonreciprocal receptor interference is a consequence of the presence of two distinct receptors for GALV; one interacts specifically with GALV, and the other interacts with both GALV and A-MuLV (20). We report here that MMMol cells, like E36 cells but unlike other murine cells,

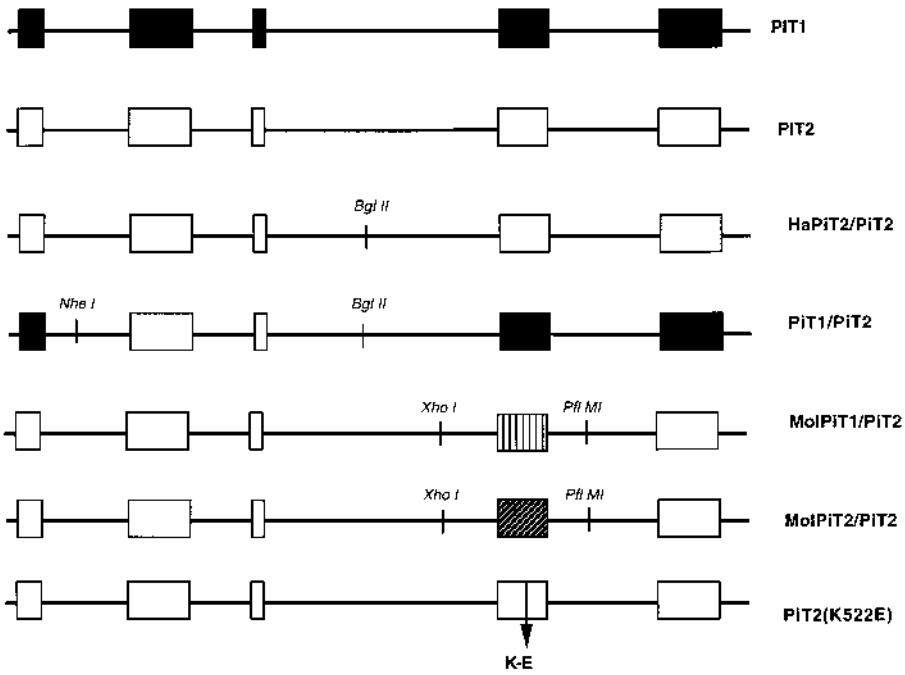

FIG. 2. Linear representation of receptors. Relative positions of the five extracellular domains of Pit1, the human GALV receptor, are shown by black boxes, and those of Pit2, the human A-MuLV receptor, are shown by white boxes. The chimeric receptor HaPit2/Pit2 contains the first three domains of Pit2 and the last two of the E36 hamster homolog of Pit2 (HaPit2 [shaded boxes]). The chimeric receptor Pit1/Pit2 contains domains one, four, and five of Pit1 and domains two and three of Pit2. The MolPit1/Pit2 chimera contains domains one, two, three, and five of Pit2 and domain four of MolPit1 (box with vertical bars). The MolPit2/Pit2 chimera is similar to MolPit1/Pit2 but contains domain four of MolPit2 (hatched box). Pit2(K522E) is a variant of Pit2 that has lysine 522 replaced by glutamic acid. The relative positions of the unique restriction sites for *Bgl*II, *Nhe*I, *Pfl*MI, and *Xho*I used to assemble the chimeras are shown.

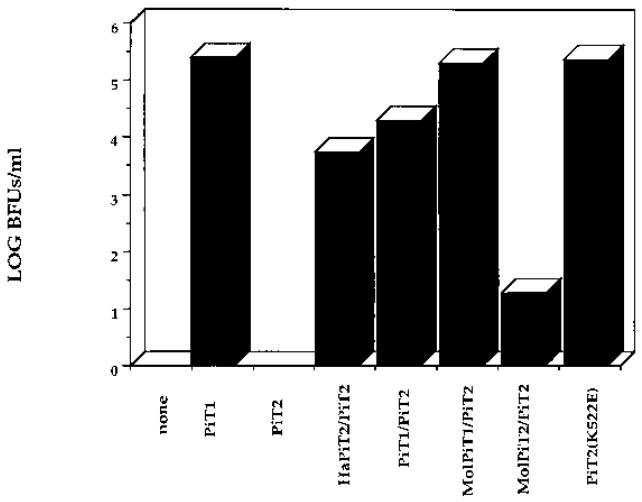

FIG. 3. Susceptibility of MDTF expressing various receptors to the vector PG13/BSN. Cells expressing the receptors were exposed to PG13/BSN and processed for β-galactosidase activity 72 h later, as described in Materials and Methods. The bar graph shows the PG13 titers determined on MDTF bearing the indicated receptors. MDTF expressing Pit1 were included as the positive control, and MDTF expressing no exogenous receptor were the negative control (none). The titers are plotted as the log of BFU per milliliter.

express an A-MuLV receptor that also functions as a GALV receptor.

The fourth extracellular domain of the GALV receptor is critical for GALV entry (2, 4, 10, 13, 14, 20). The Pit2 chimeric receptor containing the fourth extracellular domain of MolPit1 is nearly 10,000-fold more efficient than the Pit2 chimera containing the fourth domain of MolPit2. We investigated the basis for this functional difference between MolPit1 and MolPit2 by comparing sequences of the fourth extracellular domains of various functional and nonfunctional GALV receptors (Fig. 4). Such analyses of naturally occurring receptors, supported by studies with genetically modified receptor variants, had previously suggested that GALV receptor function requires a negatively charged residue at position 550 in the fourth extracellular domain. This notion is supported by several observations. (i) Pit1 (11) and RatPit1 (14), both functional for GALV, each contains an acidic residue at position 550. (ii) All Pit1 homologs that have lysine at position 550 are nonfunctional as GALV receptors (3, 14). (iii) Pit2 and its homologs that lack GALV receptor function contain lysine at position 522, which corresponds to position 550 in Pit1 (10, 15). But the hamster Pit2 (HaPit2), which has glutamic acid at position 522, functions as a GALV receptor (20). (iv) Substitution of glutamic acid for lysine 522 in Pit2 is sufficient to render the receptor functional for GALV (2).

TABLE 2. Summary of PG13 titers plotted in Fig. 3

Receptor	Titer (BFU/ml)[a]
None	<1.0
Pit1	218,333 ± 15,300
Pit2	<1.0
HaPit2/Pit2	4,900 ± 200
Pit1/Pit2	17,633 ± 200
MolPit1/Pit2	184,666 ± 3,400
MolPit2/Pit2	18.0 ± 2.0
Pit2(K522E)	203,333 ± 2,700

[a] Each titer is the mean of triplicate values plus or minus the standard error of the mean.

Receptor	GaLV Recognition	Amino Acid Sequence and Charges								
PiT1 numbering		**550**	551	552	**553**	554	555	556	557	558
PiT2 numbering		**522**	523	524	**525**	526	527	528	529	530
PiT1	YES	− **D**	T	G	− **D**	V	S	S	+ K	V
MolPiT1	YES	**I**	T	G	**D**	V	S	S	K	M
HaPiT2	YES	− **E**	Q	G	**G**	V	M	Q	− E	A
PiT2(K522E)	YES	− **E**	Q	G	**G**	V	T	Q	− E	A
MolPiT2	YES	**Q**	Q	G	**G**	V	T	Q	− E	A
MusPiT1	NO	+ **K**	Q	-	− **E**	A	S	T	+ K	A
DunniPiT1	NO	+ **K**	Q	-	− **D**	A	S	T	+ K	A
PiT2	NO	+ **K**	Q	G	**G**	V	T	Q	− E	A
RatPiT2	NO	+ **K**	Q	G	**G**	V	T	Q	− E	A

FIG. 4. The carboxyl-terminal nine residues of domain four of various functional and nonfunctional GALV receptors. Pit1, the human GALV receptor (11); MusPit1, the *M. musculus musculus* GALV receptor (4); DunniPit1, the *M. dunni* GALV receptor (19); MolPit1, the *M. musculus molossinus* GALV receptor (19); Pit2, the human A-MuLV receptor (15); RatPit2, the rat A-MuLV receptor (11); HaPit2, the E36 Chinese hamster A-MuLV receptor (11); MolPit2, the *M. musculus molossinus* A-MuLV receptor; and Pit2(K522E), Pit2 with the lysine-to-glutamic acid change at position 522 (2). The first 17 residues of the fourth extracellular domain, not shown, are highly conserved among the different receptors (Fig. 2). + and −, positively and negatively charged residues, respectively.

Our current findings suggest that an acidic residue at position 550 (Pit1) or 522 (Pit2) is dispensable for GALV receptor function. MolPit1 has isoleucine at position 550, and yet its ability to function as a GALV receptor is identical to that of Pit1. MolPit2 has glutamine at position 522, but still allows GALV entry. It is important, however, that at least in the chimeric form this receptor proved inefficient. Nevertheless, the two MMMol receptors break the presumptive consensus requirement for an acidic residue at position 550 for GALV receptor function.

What constitutes the GALV binding motif on these receptors remains unclear. It is likely that for optimal GALV receptor activity an acidic residue is required in the nine-residue stretch in domain four, but not necessarily at position 550. The receptors that function efficiently for GALV have an acidic residue at either position 550 (522 in Pit2) or position 553 (525 in Pit2) or both. However, lysine at position 550 (522 in A-MuLV receptor) abrogates GALV receptor function, even when an acidic residue is present at position 553 (Fig. 4). Thus, MolPit2 may be inefficient because it lacks an acidic residue at either position. It is possible that glutamine 522 in MolPit2 affords partial negative charge, enabling the receptor to weakly function for GALV. Mutational analysis of the various receptors to assess these possibilities is now under way in our laboratory.

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REFERENCES

1. **Eglitis, M. A., M. V. Eiden, and C. A. Wilson.** 1993. Gibbon ape leukemia virus and the amphotropic murine leukemia virus 4070A exhibit an unusual interference pattern on E36 Chinese hamster cells. J. Virol. **67:**5472–5477.
2. **Eiden, M. V., K. B. Farrell, and C. A. Wilson.** 1996. Substitution of a single amino acid residue is sufficient to allow the human amphotropic murine leukemia virus receptor to function as a gibbon ape leukemia virus receptor. J. Virol. **70:**1080–1085.
3. **Johann, S. V., J. J. Gibbons, and B. O'Hara.** 1992. GLVR1, a receptor for gibbon ape leukemia virus, is homologous to a phosphate permease of *Neurospora crassa* and is expressed at high levels in the brain and thymus. J. Virol. **66:**1635–1640.
4. **Johann, S. V., M. van Zeijl, J. Cekleniak, and B. O'Hara.** 1993. Definition of a domain of GLVR1 which is necessary for infection by gibbon ape leukemia virus and which is highly polymorphic between species. J. Virol. **67:**6733–6736.
5. **Kavanaugh, M. P., D. G. Miller, W. Zhang, W. Law, S. L. Kozak, D. Kabat, and A. D. Miller.** 1994. Cell-surface receptors for gibbon ape leukemia virus and amphotropic murine retrovirus are inducible sodium-dependent phosphate symporters. Proc. Natl. Acad. Sci. USA **91:**7071–7075.
6. **McLachlin, J. R., N. Mittereder, M. B. Daucher, M. Kadan, and M. Eglitis.** 1993. Factors affecting vector function and structural integrity. Virology **195:**1–5.
7. **Miller, A. D., and C. Buttimore.** 1986. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. Mol. Cell. Biol. **6:**2895–2902.
8. **Miller, A. D., J. V. Garcia, N. von Suhr, C. M. Lynch, C. Wilson, and M. V. Eiden.** 1991. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. J. Virol. **65:**2220–2224.
9. **Miller, D. G., R. H. Edwards, and A. D. Miller.** 1994. Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for gibbon ape leukemia virus. Proc. Natl. Acad. Sci. USA **91:**78–82.
10. **Miller, D. G., and A. D. Miller.** 1994. A family of retroviruses that utilize related phosphate transporters for cell entry. J. Virol. **68:**8270–8276.
11. **O'Hara, B., S. V. Johann, H. P. Klinger, D. G. Blair, H. Rubinson, K. J. Dunne, P. Sass, S. M. Vitek, and T. Robins.** 1990. Characterization of a human gene conferring sensitivity to infection by gibbon ape leukemia virus. Cell Growth Differ. **1:**119–127.
12. **Olah, Z., C. Lehel, W. B. Anderson, M. V. Eiden, and C. A. Wilson.** 1994. The cellular receptor for gibbon ape leukemia virus is a novel high affinity phosphate transporter. J. Biol. Chem. **269:**25426–25431.
13. **Pedersen, L., S. V. Johann, M. van Zeijl, F. S. Pedersen, and B. O'Hara.** 1995. Chimeras of receptors for gibbon ape leukemia virus/feline leukemia virus B and amphotropic murine leukemia virus reveal different modes of receptor recognition by retrovirus. J. Virol. **69:**2401–2405.
14. **Tailor, C. S., Y. Takeuchi, B. O'Hara, S. V. Johann, R. A. Weiss, and M. K. L. Collins.** 1993. Mutation of amino acids within the gibbon ape leukemia virus (GALV) receptor differentially affects feline leukemia virus subgroup B, simian sarcoma-associated virus, and GALV infection. J. Virol. **67:**6737–6741.
15. **von Zeijl, M., S. V. Johann, E. Cross, J. Cunningham, R. Eddy, T. B. Shows, and B. O'Hara.** 1994. An amphotropic virus receptor is a second member of the gibbon ape leukemia virus receptor family. Proc. Natl. Acad. Sci. USA **91:**1168–1172.
16. **Wilson, C., and M. Eiden.** 1991. Viral and cellular factors governing hamster cell infection by murine and gibbon ape leukemia viruses. J. Virol. **65:**5975–5982.
17. **Wilson, C. A., M. V. Eiden, W. B. Anderson, C. Lehel, and Z. Olah.** 1995. The dual-function hamster receptor for amphotropic murine leukemia virus (A-MuLV), 10A1 MuLV, and gibbon ape leukemia virus is a phosphate symporter. J. Virol. **69:**534–537.
18. **Wilson, C. A., M. V. Eiden, and J. W. Marsh.** 1994. Quantitative micro p30 and reverse transcriptase assays for Moloney murine leukemia virus. J. Virol. Methods **48:**109–118.
19. **Wilson, C. A., K. B. Farrell, and M. V. Eiden.** 1994. Comparison of cDNAs encoding the gibbon ape leukaemia virus receptor from susceptible and non-susceptible murine cells. J. Gen. Virol. **75:**1901–1908.
20. **Wilson, C. A., K. B. Farrell, and M. V. Eiden.** 1994. Properties of a unique form of the murine amphotropic leukemia virus receptor expressed on hamster cells. J. Virol. **68:**7697–7703.