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Amphotropic Murine Leukemia Virus Entry Is Determined by Specific Combinations of Residues from Receptor Loops 2 and 4

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Pit2 is the human receptor for amphotropic murine leukemia virus (A-MuLV); the related human protein Pit1 does not support A-MuLV entry. Interestingly, chimeric proteins in which either the N-terminal or the C-terminal part of Pit2 was replaced by the Pit1 sequence all retained A-MuLV receptor function. A possible interpretation of these observations is that Pit1 harbors sequences which can specify A-MuLV receptor function when presented in a protein context other than Pit1, e.g., in Pit1-Pit2 hybrids. We reasoned that such Pit1 sequences might be identified if presented in the Neurospora crassa protein Pho-4. This protein is distantly related to Pit1 and Pit2, predicted to have a similar membrane topology with five extracellular loops, and does not support A-MuLV entry. We show here that introduction of the Pit1-specific loop 2 sequence conferred A-MuLV receptor function upon Pho-4. Therefore, we conclude that (i) a functional A-MuLV receptor can be constructed by combining sequences from two proteins each lacking A-MuLV receptor function and that (ii) a Pit1 sequence can specify A-MuLV receptor function when presented in another protein context than that provided by Pit1 itself. Previous results indicated a role of loop 4 residues in A-MuLV entry, and the presence of a Pit2-specific loop 4 sequence was found here to confer A-MuLV receptor function upon Pho-4. Moreover, the introduction of a Pit1-specific loop 4 sequence, but not of a Pit2-specific loop 4 sequence, abolished the A-MuLV receptor function of a Pho-4 chimera harboring the Pit1-specific loop 2 sequence. Together, these data suggest that residues in both loop 2 and loop 4 play a role in A-MuLV receptor function. A-MuLV is, however, not dependent on the specific Pit2 loop 2 and Pit2 loop 4 sequences for entry; rather, the role played by loops 2 and 4 in A-MuLV entry can be fulfilled by several different combinations of loop 2 and loop 4 sequences. We predict that the residues in loops 2 and 4, identified in this study as specifying A-MuLV receptor function, are to be found among those not conserved among Pho-4, Pit1, and Pit2.

Amphotropic murine leukemia virus (A-MuLV) and gibbon ape leukemia virus (GALV), both type C mammalian retroviruses, utilize related cell surface proteins for entry into cells. The human receptor for A-MuLV is Pit2 (formerly GLVR2) (31) and that for GALV is Pit1 (formerly GLVR1) (20). cDNAs encoding homologs of these proteins have been cloned from other species besides humans, e.g., Pit1 cDNAs have been cloned from mice (MusPit1, formerly Glvr-1) (8) and hamsters (HaPit1, formerly EGR) (35), and Pit2 cDNAs have been cloned from rats (RatPit2, formerly Ram-1) (18) and hamsters (HaPit2, formerly EAR) (35). Pit1 is also a receptor for feline leukemia virus subgroup B (FeLV-B) (29). In general, Pit2 homologs are not receptors for GALV or FeLV-B (6, 19, 22, 24, 27); however, one exception is HaPit2, which supports entry by GALV and the 90Z FeLV-B isolate (2, 35). For A-MuLV, only Pit2 homologs are efficient receptors, whereas the A-MuLV related isolate 10A1 can utilize all tested Pit1 and Pit2 homologs for entry (13, 19, 22, 35).

Pit1 and Pit2 have 62% overall amino acid identity (31) and are distantly related (both about 25% amino acid identity) to Pho-4, a sodium-dependent phosphate transporter from the filamentous fungus Neurospora crassa (8, 14, 32). The cellular function of Pit1 was suggested to be phosphate transport, based on its homology to Pho-4 (8), and both Pit1 and Pit2 were subsequently shown to be sodium-dependent phosphate transporters (10, 21, 33). On the basis of hydropathy plots, Pho-4, Pit1, and Pit2 are predicted to have 10 membrane-spanning domains, 5 extracellular loops, and 4 intracellular domains, of which the third is large and hydrophilic (8, 31). The cytoplasmic localization of the large hydrophilic domain is supported by recent biochemical data obtained on Pit2 (4).

Residues in the fourth extracellular loop of Pit1 have been shown to be critical for receptor function for GALV and FeLV-B. Chimeras between MusPit1, which does not support FeLV-B and GALV entry, and Pit1, as well as mutational analyses of these proteins, revealed that a stretch of nine amino acids, termed region A, in the C-terminal part of the fourth extracellular loop (Pit1 positions 550 through 558) was critical for receptor function for GALV and FeLV-B (9, 28). Studies on chimeras between Pit1 and Pit2 or RatPit2 confirmed the critical role of Pit1 region A in FeLV-B and GALV entry (19, 22, 27). Moreover, Eiden and colleagues showed that replacement of a lysine in the first position of Pit2 region A (position 522) by a glutamic acid (the corresponding Pit1 residue) allowed Pit2 to support GALV entry (6). Furthermore, we recently showed that the presence of 12 Pit1-specific amino acids, comprising region A, conferred GALV receptor function upon Pho-4 (25). These results demonstrate the importance of region A for infection by GALV and FeLV-B. In addition, we have recently obtained results indicating that region A also plays a role in 10A1 receptor function (13). However, as previously observed for FeLV-B (22), recent results indicate that entry by GALV and 10A1, besides region A, is also dependent on other receptor regions (3, 13).

Receptor regions involved in A-MuLV entry have also been
investigated by using chimeras between Pit1 and Pit2 homologs from different species (6, 19, 22, 27). Pit1-Pit2 chimeras harboring the region A sequence from Pit2 and RatPit2 were found to support A-MuLV entry (19, 22). Moreover, insertion of a threonine residue in MusPit1 region A (MusPit1 position 554) resulted in a functional A-MuLV receptor (13). Thus, region A in loop 4 also plays a role in A-MuLV receptor function (13). However, interestingly, chimeras in which the N-terminal two-thirds (comprising loops 1, 2, and 3 and the large intracellular domain) or the C-terminal third (comprising loops 4 and 5) of Pit2 or RatPit2 were exchanged for the corresponding Pit1 sequences all retained A-MuLV receptor function (6, 19, 22). Thus, identical region A sequences are present both in Pit1 with no A-MuLV receptor function and in a chimeric A-MuLV receptor. Moreover, comparable chimeras in which the N- and C-terminal parts were derived from HaPit2 only afforded low levels of A-MuLV infection compared to the Pit1-Pit2 and Pit1-RatPit2 hybrids, although HaPit2 is as efficient an A-MuLV receptor as is Pit2 (6).

To investigate whether Pit1 harbors sequences which can specify A-MuLV receptor function, we reasoned that a new protein context for Pit1 sequences should be provided by a protein which is structurally related to Pit1 but is not itself an A-MuLV receptor, requirements fulfilled by Pho-4 (8, 25). We therefore tested a series of chimeras with the backbone provided by Pho-4 and each harboring an individual Pit1 loop sequence (Fig. 1) for their ability to support A-MuLV entry. Indeed, the presence of a Pit1-specific sequence in loop 2, but not in loops 1, 3, 4, or 5, conferred A-MuLV receptor function upon Pho-4. As mentioned above, the presence of Pit2 region A in loop 4 of Pit1 was sufficient to make it a functional A-MuLV receptor. We therefore tested the influence of loop 4 sequences on A-MuLV receptor function in a Pho-4 backbone. A Pit2 loop 4 sequence was found to confer A-MuLV receptor function upon Pho-4. Moreover, the presence of a Pit1-specific loop 4 sequence, but not a Pit2-specific loop 4 sequence, together with the Pit1 loop 2 sequence in Pho-4, abolished A-MuLV receptor function. These results show that the ability of residues in Pit1 loop 2 to specify A-MuLV receptor function is dependent on the residues present in loop 4. We therefore suggest that both loop 2 and loop 4 are involved in A-MuLV entry and that specific combinations of loops 2 and 4 sequences specify A-MuLV receptor function.

MATERIALS AND METHODS

Expression plasmids. The plasmid pOJ72 (25) harbors the open reading frame of Pho-4, originally derived from the pB8005 plasmid (14) obtained from R. L. Metzenberg, in the eucaryotic expression vector pcDNA1A*KpnA (31). With pOJ72 as a template, Pho-4-Pit2 chimeras were constructed by replacing the
Pho-4 sequences indicated in Fig. 1 with the indicated Pit2 sequences via site-
directed mutagenesis according to the method of Kunkel et al. (11). Restriction enzyme fragments harboring the mutations were cloned back into pOJ72; the 
integrity of these fragments was verified by nucleotide sequence analyses. The 
designations used for the Pho-4-derived chimeras are to be read as follows, e.g., the 
chimera Pho/P1(2)/P2(4) harbors the Pit1 loop 2 and Pit2 loop 4 sequences indicated 
in Fig. 1 in the Pho-4 backbone.

To construct pA16, encoding Pho/P2(2), amino acids 117 through 149 in loop 2 
of Pho-4 were replaced by amino acids 117 through 143 in loop 2 of Pit2. A 
Bal3H HindIII fragment harboring the mutated sequence was then used to replace 
the corresponding pOJ72 fragment, yielding the pA16 plasmid. pA17, encoding 
Pho/P1(2)/P1(3), was constructed by replacing amino acids 486 through 496 in loop 4 
of Pho-4 by amino acids 517 through 531 in loop 4 of Pit2. An Afl III/HindIII fragment harboring the mutated sequence was then cloned back into an Afl III-
digested pOJ72 vector, resulting in the intermediate plasmid p9A9. Finally, a 
Kpn I/EcoRI fragment of p9A4 harboring the mutated sequence was cloned into 
a Kpn I/EcoRI-digested pOJ72 vector, resulting in pA17. To construct pA18, 
encoding Pho/P2(2)+4, an Afl III/HindIII fragment of pA17 was replaced by the 
corresponding pA16 fragment harboring the Pit2 loop 2 sequence. pA19, encoding 
Pho/P2(2)+P4(2), was constructed by cloning an Afl III/HindIII fragment of 
pA16 harboring the Pit2 loop 2 sequence into Afl III-digested pOJ79 (25) vector, 
encoding Pho/P1(4) and thus harboring the Pit1 loop 4 sequence. To construct 
pA20, encoding Pho/P1(2)/P2(4), Pho/P1(3), and Pho/P1(5), respectively, (23); pOJ79 and pOJ86, which encode 
Pit2 loop 2 sequence in Pho-4, were tested 
(Pit1-Pit2 chimeras and a MusPit1 mutant, showing that resi-
sides in loop 4 can specify A-MuLV receptor function (13, 19,
558 (region A) for the corresponding Pit2 residues resulted in 
the lack of A-MuLV receptor function observed with these con-
structs was not due to lack of chimeric proteins on the cell 
surface, in that the Pho-4-Pit1 chimeras shown in Fig. 2 all 
support infection by 10A1 (23); furthermore, chimera Pho/ 
P1(4) supports GALV entry (25). Interestingly, the Pho-4 chi-
mera harboring the Pit1 loop 2 sequence, Pho/P1(2), sup-
ported A-MuLV infection at about 4.5% of the Pit2 level, 
which is more than 200 times above the background level (the 
level obtained on cells transfected with pOJ72 which encodes 
Pho-4) (Fig. 2). Therefore, a Pit1 loop 2 sequence can confer 
A-MuLV receptor function upon Pho-4, although neither 
Pho-4 nor Pit1 themselves are efficient A-MuLV receptors. 
Furthermore, this result indicates that residues in loop 2 play 
a role in A-MuLV receptor function.

To further investigate the role of loop 2 in A-MuLV entry, we 
tested whether the corresponding Pit2-specific sequence 
conferring A-MuLV receptor function upon Pho-4. Interest-
ingly, the chimera Pho/P2(2) featuring the Pit2 loop 2 
sequence did not efficiently support A-MuLV entry (Fig. 1 and 
3). When tested in parallel for 10A1 and A-MuLV receptor 
function in CHO K1 cells in an independent experiment, Pho/ 
P2(2) supported entry by 10A1 at a level comparable to that of 
Pho-4, whereas none of Pit1 (data not shown); thus, the inability of Pho/P2(2) to allow 
A-MuLV infection is not due to the lack of chimeric protein on the 
cell surface.

**RESULTS**

In an attempt to assess whether Pit1 harbors sequences 
which can specify A-MuLV receptor function when presented 
in a protein context different from Pit1 itself, Pho-4 chimeras 
each harboring a different Pit1 loop sequence, i.e., Pho/P1(1), 
Pho/P1(2), Pho/P1(3), Pho/P1(4), and Pho/P1(5), were tested 
for their ability to support A-MuLV infection. As shown in Fig. 
1, the loop sequences exchanged in the chimeras were those 
least conserved between Pho-4 and Pit1. A-MuLV receptor 
function of the chimeras was investigated by transfecting ex-
pression plasmids encoding chimeric or wild-type proteins into 
nonpermissive cells and assaying the susceptibility of these 
cells to infection with A-MuLV pseudotypes of a β-galactosi-
dase-encoding vector. The results obtained are shown in Fig. 2. 

A chimeric A-MuLV receptor derived from two proteins 
each lacking A-MuLV receptor function. As previously 
observed, Pho-4 did not support A-MuLV infection (Fig. 2) (25). 
Pho-4 chimeras harboring Pit1 sequences in loops 1, 3, 4, or 5 
(chimeras Pho/P1(1), Pho/P1(3), Pho/P1(4), and Pho/P1(5), 
respectively) also were not efficient A-MuLV receptors. The 
lack of A-MuLV receptor function observed with these con-
structs was not due to lack of chimeric proteins on the cell 
surface, in that the Pho-4-Pit1 chimeras shown in Fig. 2 all 
support infection by 10A1 (23); furthermore, chimera Pho/ 
P1(4) supports GALV entry (25). Interestingly, the Pho-4 chi-
mera harboring the Pit1 loop 2 sequence, Pho/P1(2), sup-
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level obtained on cells transfected with pOJ72 which encodes 
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function in CHO K1 cells in an independent experiment, Pho/ 
P2(2) supported entry by 10A1 at a level comparable to that of 
Pho-1 (data not shown); thus, the inability of Pho/P2(2) to allow 
A-MuLV infection is not due to the lack of chimeric protein on the 
cell surface.

**Pit1 loop 4, but not Pit2 loop 4, interferes with A-MuLV 
receptor function in a Pho-4 backbone.** The presence of a 
Pit1-specific receptor sequence conferred A-MuLV receptor 
function upon Pho-4, although Pit1 itself does not efficiently 
support A-MuLV entry (Fig. 2). This result suggests the existence 
of a Pit1 sequence(s) which is incompatible with A-
MuLV receptor function. We and others previously found that 
a C-terminal A-MuLV receptor determinant is positioned in 
loop 4, in that replacement of Pit1 loop 4 residues 550 through 
558 (region A) for the corresponding Pit2 residues resulted in 
an efficient A-MuLV receptor with a Pit1 backbone (19, 22). 
We therefore wanted to investigate the possible influence of 
loop 4 sequences on the receptor function of chimeras with a 
Pho-4 backbone.

A chimera harboring a Pit2-specific loop 4 sequence, Pho/ 
P2(4), was found to support A-MuLV entry at a level of 3.5% 
of the Pit2 level (Fig. 1 and 3). Thus, the presence of the 
Pit2 loop 4 sequence conferred A-MuLV receptor function upon 
Pho-4, in agreement with the previous results obtained with 
Pit1-Pit2 chimeras and a MusPit1 mutant, showing that resi-
dues in loop 4 can specify A-MuLV receptor function (13, 19, 
22). Moreover, a chimera harboring Pit2 loop 2 sequence 
together with the Pit2 loop 4 sequence, Pho/P2(2)+4, supported 
A-MuLV entry at a level of 7.4% of that obtained by Pit2 (Fig. 3). In addition, the presence of the Pit2 loop 4 sequence 
together with Pit1 loop 2 sequence in Pho-4, chimera Pho/P1(2)/ 
P2(4), was compatible with A-MuLV receptor function, in that 
the chimera supported A-MuLV entry at a level of 3.9% of the
Pit2 level (Fig. 3). Thus, all of the tested chimeras harboring the Pit2-specific loop 4 sequence were A-MuLV receptors. We also tested the receptor function of chimeras harboring a Pit1-specific loop 4 sequence. As mentioned above, chimeras harboring either a Pit1 loop 4 sequence, Pho/P1(4), or a Pit2 loop 2 sequence, Pho/P2(2), were not efficient A-MuLV receptors, and a chimera harboring Pit1 loop 4 and Pit2 loop 2 sequences together, Pho/P2(2)/P1(4), did also not support A-MuLV entry (Fig. 3). Pho/P2(2)/P1(4) supported entry by GALV (Fig. 3), thus demonstrating the presence of chimeric protein on the cell surface. Whether the presence of a Pit1-specific loop 4 sequence would influence the A-MuLV receptor function introduced by the presence of the Pit1-specific loop 2 sequence in Pho-4 was investigated by analyzing the receptor function of the chimera Pho/P1(214), which harbors the Pit1 loop 4 sequence together with Pit1 loop 2. Cells transfected with a construct encoding this chimera did not support A-MuLV entry (Fig. 3). The chimera supports 10A1 entry (23); moreover, it was shown to be an efficient GALV receptor in a parallel experiment (Fig. 3), and the lack of A-MuLV receptor function of chimera Pho/P1(2+4), which harbors the Pit1 loop 4 sequence together with Pit1 loop 2, Cells transfected with a construct encoding this chimera did not support A-MuLV entry (Fig. 3). The chimera supports 10A1 entry (23); moreover, it was shown to be an efficient GALV receptor in a parallel experiment (Fig. 3), and the lack of A-MuLV receptor function of chimera Pho/P1(2+4) is therefore not due to the lack of cell surface expression of the protein. Thus, the introduced Pit1 loop 4 sequence manifests a negative effect on the A-MuLV receptor function introduced by the Pit1 loop 2 sequence, and it may thus indeed represent a Pit1 sequence that is incompatible with A-MuLV receptor function when present in Pit1.

Specific combinations of loop 2 and loop 4 sequences determine A-MuLV receptor function. The results obtained from the receptor function analyses of Pho-4 chimeras harboring loop sequences from Pit1 and/or Pit2 are summarized in Table 1. All Pho-4 chimeras harboring Pit2 sequence in loop 4 were functional A-MuLV receptors, while the presence of Pit1 sequence in loop 4 was incompatible with A-MuLV receptor function. The Pho-4 loop 4 sequence was compatible with A-MuLV receptor function only when the divergent part of loop 2 was derived from Pit1.

DISCUSSION

Pit2 is the human receptor for A-MuLV, while the related protein Pit1 supports entry by GALV and FeLV-B but not by A-MuLV. We (22) and others (6, 19) have observed that when the N-terminal two-thirds or the C-terminal third of Pit2 or RatPit2 were exchanged for the corresponding Pit1 sequences, all chimeric proteins retained A-MuLV receptor function. A possible interpretation of these results is that Pit1 harbors sequences which can specify A-MuLV receptor function when presented in a protein context other than that provided by Pit1 itself, e.g., in Pit1-Pit2 hybrids. We have shown here that the introduction of the Pit1-specific loop 2 sequence conferred A-MuLV receptor function upon Pho-4 (Fig. 2), although neither Pit1 nor Pho-4 are efficient A-MuLV receptors. Therefore, first, a Pit1 sequence can specify A-MuLV receptor function when presented in a protein context other than that provided by Pit1 itself. Second, a functional retroviral receptor can be constructed by combining sequences from two proteins, each lacking receptor function for the retrovirus in question. Finally, as previously shown for GALV (25), the fungal phosphate transporter Pho-4 can serve as a receptor backbone for the mammalian retrovirus A-MuLV.

The observation that the introduction of a Pit1-specific loop 2 sequence confers A-MuLV receptor function upon Pho-4 indicates a role of loop 2 in A-MuLV entry. Recent studies by Leverett and coworkers showed that A-MuLV receptor function is conferred upon Pit1 by introduction of C-terminal Pit2-specific loop 2 residues (Pit2 positions 121 through 141) (chi-
Moreover, we find that exchange of the C-terminal part of Pit1 loop 2 for the corresponding Pit2 sequence (Pit2 positions 130 through 141) results in a functional A-MuLV receptor (5). These results demonstrate that residues in the second extracellular loop are important for A-MuLV receptor function and suggest that loop 2 harbors an N-terminal determinant of A-MuLV receptor function.

Previous results indicate that sequences in loop 4 constitute a C-terminal determinant of A-MuLV receptor function, e.g., Pit2 and RatPit2 region A in the C-terminal part of loop 4 was found to confer A-MuLV receptor function upon Pit1 (19, 22), and a single amino acid insertion in MusPit1 region A resulted in an A-MuLV receptor (13). We have here tested whether loop 4 sequences also play a role in A-MuLV receptor function in chimeras with a Pho-4 backbone. We found that the presence of a Pit2 loop 4 sequence conferred A-MuLV receptor function upon Pho-4, chimera Pho/P2(4) (Fig. 3), thus confirming the involvement of loop 4 sequences in receptor function for A-MuLV. The importance of loop 4 sequences in A-MuLV entry was further demonstrated by the result obtained with the chimera Pho/P1(2+4). This chimera supports infection by GALV and 10A1 and is thus present on the cell surface; however, it does not support A-MuLV infection in spite of the Pit1 loop 2 sequence present. Thus, loop 4 sequences can not only specify but also interfere with A-MuLV receptor function. Interestingly, the presence of the Pit2 loop 4 sequence or the Pho-4 loop 4 sequence did not interfere with the A-MuLV receptor function introduced by the Pit1 loop 2 sequence, chimeras Pho/P1(2)P2(4) and Pho/P1(2), respectively, as both of these chimeras support A-MuLV entry. The ability of the Pit1 loop 2 sequence to confer receptor function on Pho-4 is thus dependent on the sequence present in loop 4. Moreover, comparison of the A-MuLV receptor function of all the tested chimeras with a Pho-4 backbone revealed that whether a given loop 4 sequence will support A-MuLV entry may also be dependent on the sequence present in loop 2 (Table 1). Thus, although the presence of the Pit2 loop 4 sequence was compatible with A-MuLV infection and the presence of the Pit1 loop 4 sequence was incompatible with A-MuLV entry in all chimeras, the Pho-4 loop 4 sequence was

![TABLE 1. Ability of combinations of loop sequences in a Pho-4 backbone to support A-MuLV entry](https://example.com/table1)

<table>
<thead>
<tr>
<th>Loop 4 sequence origin</th>
<th>Pho-4</th>
<th>Pit1</th>
<th>Pit2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pho-4</td>
<td>No†</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Pit1</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Pit2</td>
<td>No†</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

† The loop sequences present in the chimeras are indicated in Fig. 1. The loop 2 and loop 4 sequences were derived from receptors Pit1 and Pit2 and N. crassa protein Pho-4 as indicated in the table.

† No, this combination of loop sequences does not confer A-MuLV receptor function upon Pho-4.
compatible with A-MuLV receptor function only when the loop 2 sequence was derived from Pit1.

To summarize, we suggest that, first, A-MuLV receptor function is defined by sequences in both loop 2 and loop 4. Second, specific combinations of loop 2 and 4 sequences specify A-MuLV receptor function, i.e., whether a given loop 2 sequence will specify A-MuLV receptor function is dependent on the sequence present in loop 4 and vice versa; thus, sequences in loops 2 and 4 interdependently determine A-MuLV receptor function. Third, the observed interdependency of sequences in loops 2 and 4 in determining A-MuLV receptor function suggests that residues in loops 2 and 4 are spatially proximate.

Presently, the specific roles of loops 2 and 4 in A-MuLV entry are not known. It is possible that residues from loops 2 and 4 together create a nonlinear viral recognition site. However, it is also possible that the loop 2 and 4 sequences compatible with A-MuLV entry allow the SU protein to interact with another part(s) of the receptor, which then is directly critical for binding of the SU protein. Such a sequence(s) may be conserved between all wild-type and chimeric A-MuLV receptors, including Pho-4-derived chimeras. Interestingly, there is complete amino acid identity between all Pit1 and Pit2 homologs and Pho-4 in the six most N-terminal residues of loop 1 (Fig. 1). Moreover, the 10, 9, and 7 most N-terminal residues of loops 2, 4, and 5 in these proteins show 5, 7, and 5 conserved residues, respectively (Fig. 1) (8, 18, 20, 26, 31, 34, 35). However, other loop residues are also conserved between all known wild-type and chimeric A-MuLV receptors, and further investigations need to be performed in order to assess the possible role of conserved loop residues in A-MuLV entry. Besides, since the conserved N-terminal loop sequences are present in all Pit1 and Pit2 homologs and Pho-4, it is also possible that these might play a common role in receptor function for all viruses which can utilize wild-type and/or chimeric Pit proteins as receptors, that is, A-MuLV, 10A1, GALV, and FeLV-B (2, 3, 6, 8, 9, 12, 13, 19, 20, 22, 23, 25–29, 31, 34, 35).

An interesting observation comes from a comparison of data presented here with recent data published by Leverett and coworkers (12). As mentioned above, the presence of Pit2 loop 2 residues 121 through 141 can confer A-MuLV receptor function upon Pit1 (12); however, the presence of the same Pit2 loop 2 sequence cannot confer this function upon the chimera Pho/Pit1(P4)P2(2) (Fig. 1 and 3). Thus, in the Pit1 backbone, the Pit2 loop 2 and the Pit1 loop 4 sequences are compatible with A-MuLV receptor function, but in the Pho-4 backbone, they are incompatible. This observation is not trivial, in that other combinations of loop 2 and 4 sequences in Pho-4 backbones support A-MuLV infection. This observation therefore suggests that the ability of specific combinations of loop 2 and 4 sequences to support A-MuLV entry also is dependent on the receptor backbone, which here means the sequences outside the divergent parts of loops 2 and 4. The receptor backbone is the major determinant of the structural organization of the receptor proteins and may thus influence the relative positioning of residues in loop 2 and loop 4 with respect to each other. Therefore, the backbone of the receptor might be expected to influence the A-MuLV receptor function no matter whether loop 2 and 4 residues are directly involved in binding of the viral SU protein or create a binding site in another part of the receptor as discussed above.

A role of protein backbone in modulating receptor function is also supported by data previously published by Miller and Miller (19). They reported on a chimera (RGG) harboring the N-terminal part of RatPit2 (encompassing loops 1 and 2) and supporting low levels of infection by A-MuLV. However, when the middle part of RatPit2 (encompassing loop 3 and the large intracellular domain) was introduced into Pit1 in addition to the N terminus of RatPit2, the resulting chimera (GRG) supported A-MuLV infection at the wild-type level, although the middle part of RatPit2 alone did not confer A-MuLV receptor function upon Pit1 (chimera GRG). Recently, Leverett and coworkers (12) obtained similar results with chimeras between Pit1 and Pit2. Thus, since the Pit2 sequence encompassing loop 3 and the large intracellular domain alone cannot confer A-MuLV receptor function upon Pit1, we suggest that these sequences influence receptor function by modulating the structural organization of the proteins. In line with this hypothesis, Tailor and Kabat (27) have pointed out the possibility that the large intracellular domain plays a role in receptor function due to its conceivable influence on overall receptor folding. It has previously been suggested by us (13, 22) and others (12), based on results obtained on Pit1-Pit2, Pit1-RatPit2, and Pit1-HaPit2 chimeras and on a MusPit1 mutant, that A-MuLV receptor function is defined by a combination of sequences in the N- and C-terminal parts of the receptors. We have here elaborated on this model and suggest that specific combinations of loop 2 and loop 4 sequences determine A-MuLV receptor function. Moreover, as discussed above, comparison of the data presented here on Pho-4-derived chimeras with the result obtained with a Pit1-Pit2 chimera harboring a Pit2 loop 2 sequence (12) suggests that the receptor backbone, which here means receptor sequences outside the divergent regions of loops 2 and 4, modulates A-MuLV receptor function determined by sequences in these two loops.

Recently, Tailor and Kabat (27) studied the ability of viruses carrying chimeric A-MuLV-FeLV-B SU proteins to utilize Pit1-RatPit2 chimeras as receptors. For some of these chimeras, they found that the presence of FeLV-B and A-MuLV VRAs and the C-terminal parts (comprising loops 4 and 5) from Pit1 and Pit2, respectively, correlated with high infection levels. A similar correlation was found between the presence of FeLV-B and A-MuLV VRBs and the N-terminal parts (comprising loops 1 and 2) from Pit1 and Pit2, respectively. Based on these observations the authors suggested that A-MuLV VRAs and the C-terminal parts (comprising loops 4 and 5) and A-MuLV VRBs and the N-terminal parts (comprising loops 1 and 2) from Pit1 and Pit2, respectively. Based on these observations the authors suggested that A-MuLV VRAs and the C-terminal parts (comprising loops 4 and 5) and A-MuLV VRBs interact with Pit2 loop 2 and that both these interactions are required for A-MuLV entry. The majority of the chimeric receptors were not tested for A-MuLV receptor function in this study. However, several observations on A-MuLV receptor function of the same Pit1-RatPit2 chimeras by Miller and Miller (19) and of similar chimeras between Pit1 and Pit2 by us (22) and others (6) appear to be in disagreement with the model proposed by Tailor and Kabat (27). For example, a chimera harboring RatPit2 loops 1, 2, and 3 and Pit1 loops 4 and 5 support wild-type level of infection by A-MuLV (chimera RGG in Miller and Miller [19]). Moreover, a chimera harboring Pit1 loops 1, 2, and 3 and RatPit2 loops 4 and 5 is also an efficient A-MuLV receptor (chimera GGR in Miller and Miller [19]). In addition, introduction of a Pit2 loop 2 sequence only (5, 12) or of a Pit2 loop 4 sequence only (19, 22) in Pit2 both resulted in functional A-MuLV receptors. Thus, A-MuLV can use these chimeras for entry, although neither of them harbors both loop 2 and loops 4 and 5 from Pit2. These observations can, however, be explained by the elaborated model on A-MuLV receptor function presented here. Thus, although we also suggest that loop 2 and 4 sequences both are involved in A-MuLV receptor function, we furthermore suggest, based on the data presented here, that A-MuLV is not dependent on the specific Pit2 loop 2 and Pit2 loop 4 sequences for entry. Rather, loops 2 and 4 interdependently determine A-MuLV
receptor function, and the role played by loops 2 and 4 in A-MuLV entry can be fulfilled by several different combinations of loop 2 and loop 4 sequences. We also predict that the residues in loop 2 and loop 4, identified in this study as specifying A-MuLV receptor function, are to be found among those not conserved among Pho-4, Pit1, and Pit2.

As mentioned above, chimeric proteins in which either the N-terminal or the C-terminal parts of Pit2 or RatPit2 were replaced by the corresponding Pit1 sequences retained A-MuLV receptor function (6, 19, 22). However, comparable chimeras between Pit1 and HaPit2 supported A-MuLV infection less efficiently, although HaPit2 is as efficient an A-MuLV receptor as is Pit2 (6). Interestingly, in the extracellular loops, HaPit2 only differs from both Pit2 and RatPit2 in loops 2, 4, and 5 (18, 35). It remains to be investigated whether the differences in loops 2 and 4 can account for the lower efficiency by which Pit1-HaPit2 chimeras support A-MuLV entry compared to Pit1-Pit2 and Pit1-RatPit2 chimeras. However, it is possible that the study of the loop 2 and 4 sequence differences among naturally occurring efficient A-MuLV receptors might provide further insight into which combinations of loop 2 and 4 residues will allow for A-MuLV receptor function and thus also elucidate the role of loops 2 and 4 sequences in A-MuLV entry.

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