JOURNAL OF VIROLOGY, Aug. 1992, p. 4632–4638
0022-538X/92/084632-07$02.00/0

Vol. 66, No. 8

Basis for Receptor Specificity of Nonecotropic Murine Leukemia Virus Surface Glycoprotein gp70^{SU}

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Received 14 February 1992/Accepted 21 April 1992

Murine leukemia viruses (MuLVs) initiate infection of NIH 3T3 cells by binding of the viral envelope (Env) protein to a cell surface receptor. Interference assays have shown that MuLVs can be divided into four groups, each using a distinct receptor: ecotropic, polytropic, amphotropic, and 10A1. In this study, we have attempted to map the determinants within viral Env proteins by constructing chimeric *env* genes. Chimeras were made in all six pairwise combinations between Moloney MCF (a polytropic MuLV), amphotropic MuLV, and 10A1, using a conserved *Eco*RI site in the middle of the Env coding region. The receptor specificity of each chimera was determined by using an interference assay. We found that amphotropic receptor specificity seems to map to the N-terminal portion of surface glycoprotein gp70^{SU}. The difference between amphotropic and 10A1 receptor specificity can be attributed to one or more of only six amino acid differences in this region. Nearly all other cases showed evidence of interaction between Env domains in the generation of receptor specificity. Thus, a chimera composed exclusively of MCF and amphotropic sequences was found to exhibit 10A1 receptor specificity. None of the chimeras were able to infect cells by using the MCF receptor; however, two chimeras containing the C-terminal portion of MCF gp70^{SU} could bind to this receptor, while they were able to infect cells via the amphotropic receptor. This result raises the possibility that receptor binding maps to the C-terminal portion of MCF gp70^{SU} but requires MCF N-terminal sequences for a functional interaction with the MCF receptor.

Like all retroviruses, murine leukemia viruses (MuLVs) initiate infection by a specific interaction between the MuLV surface glycoprotein (gp70^{SU}) and a host cell surface protein that acts as a receptor. The attached virions are subsequently internalized into the cell, where infection proceeds. In productively infected cells, envelope protein (Env) molecules present on cellular membranes bind the host cell receptor, effectively blocking further viral infection (20). Assembling virions receive their Env coat when they bud through the host plasma membrane (5).

While many different MuLVs, with a wide variety of properties, have been isolated, categorizing the known MuLVs by receptor specificity yields a limited number of groups. The use of competitive interference to superinfection as an assay for receptor specificity has established only five classes of MuLVs into which all known MuLVs can be placed (17, 18). Four of these five classes, ecotropic, polytropic (MCF), amphotropic, and 10A1, all utilize different receptors on mouse cells. The remaining class, the xenotropic MuLVs, do not have a functional receptor on laboratory mouse strains.

One way of approaching a better understanding of gp70^{SU} function would be to determine which regions of gp70^{SU} participate in the specific interaction with cell surface receptors. A variety of studies have suggested that the determinants for receptor specificity lie in the N-terminal two-thirds of gp70^{SU} (13, 16); in fact, Heard and Danos (11) have recently shown that an Env fragment containing most of this region of Friend MuLV gp70^{SU} can bind to the ecotropic receptor in NIH 3T3 cells.

We have previously compared Env amino acid sequences from each of the five classes of MuLV. We found (15) that four of the five classes (polytropic, xenotropic, amphotropic, and 10A1) were remarkably similar in the N-terminal two-thirds of gp70^{SU}; however, two blocks of sequence difference in this group (the polytropic-related MuLV [PRM] group) were noted. The remainder of Env is highly conserved for all MuLVs.

One of the polymorphic regions is located in the first 200 amino acids of the envelope polyprotein gene (*env*); this region of amphotropic and 10A1 gp70^{SU} contains two insertions of amino acid sequences relative to the MCF gp70^{SU} sequence. Xenotropic MuLV lacks one of these insertions and has a unique sequence at the other insertion. The second region consists of a proline-rich sequence named the hypervariable region (12, 15). Besides these two regions, there are scattered point differences between the PRM gp70^{SU} sequences.

In this study, we have attempted to map receptor specificity in gp70^{SU} by constructing a series of chimeric *env* genes, using Moloney MCF (Mo-MCF), 10A1, and amphotropic gp70^{SU} sequences. The analysis of MuLVs containing these chimeric gp70^{SU} gave both simple and complex results. In some cases, receptor specificity could be mapped to a single region of gp70^{SU}. In other cases, receptor specificity is determined by the interaction of two regions of gp70^{SU}. Finally, some combinations seem to be capable of a fully functional interaction with one receptor but also a partial or abortive interaction with one or two other receptors. The implications of these observations are considered in Discussion.

MATERIALS AND METHODS

DNA constructions. Chimeric MuLVs were based on Moloney MuLV (Mo-MuLV), with the placement of the chimeric *env* gene sequences into the virus by use of

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conserved *Sal*I (at Mo-MuLV nucleotide 3705 [19]) and *Cla*I (Mo-MuLV nucleotide 7674 [19]) restriction sites found in Mo-MuLV, Mo-MCF, amphotropic 4070A, and 10A1 MuLV sequences. The chimeric *env* gene sequences were constructed by the use of an *Eco*RI restriction site (amphotropic *env* nucleotide 625 [15]) that is conserved in amphotropic 4070A, Moloney-MCF, and 10A1 sequences. The *Eco*RI site is located between the two major variable regions of the $gp70^{SU}$. A Mo-MuLV infectious molecular clone in a pSV2Neo-derived plasmid that encodes G418 resistance (8) had its 3' *pol* and *env* gene sequences removed by *Sal*I and *Cla*I restriction endonuclease digestion. PRM sequences were then inserted in this region by ligation of *Sal*I-*Eco*RI and *Eco*RI-*Cla*I PRM fragments in order to construct a chimeric *env* gene around the conserved *Eco*RI site. Sources of PRM sequences were a full-length clone of Mo-MCF (1) (a kind gift from M. Vogt, Salk Institute, San Diego, Calif.), a circularly permuted clone of 10A1 (15), and an unpublished molecular clone of 4070A (14). This clone has the same receptor specificity as does the 4070A clone of Chattopadhyay et al. (3). We have determined its *env* gene sequence over the coding region for amino acid residues 1 to 190, 367 to 486, and 631 to 658 (14). Over these regions, the sequence was identical at the nucleotide level to that of the latter clone (15) except for two nucleotide changes in the coding region for the Env leader peptide. Some chimeras were further modified by exchanging 10A1 and amphotropic hypervariable regions. Two restriction endonuclease sites that flank the hypervariable regions, *Eco*RI (amphotropic *env* nucleotide 625 [15]) and *Bgl*I (amphotropic *env* nucleotide 964 [15]) were used to produce these recombinants.

Cells and viruses. CHO cells were cultured, infected, and harvested as described by Ott et al. (15). Genomes of chimeric MuLVs were introduced into CHO cells (which lack endogenous MuLV sequences) or NIH 3T3 cells by the calcium phosphate technique as described by Graham and van der Eb (9) and selected for stable integration of chimeric MuLV constructs by cultivation in G418 (GIBCO, Grand Island, N.Y.). Since chimeric MuLV plasmids also contain the *neo* gene from pSV2Neo (8), stable Neo^r expression ensures stable chimeric MuLV integration. Viruses produced by CHO cells transfected with the chimeras were either used to infect NIH 3T3 cells or used to produce Harvey sarcoma virus (HaSV) pseudotypes by superinfection of HaSV-transformed NIH 3T3 cells (17).

Interference tests. Superinfection interference assays were performed as described previously (17). NIH 3T3 cells (10^5) productively infected with an MuLV were exposed to approximately 10^4 focus-forming units of HaSV. The cells were incubated for 5 to 7 days to allow the superinfected NIH 3T3 cells to become confluent. Cells were examined for the presence of HaSV-transformed foci. Presence of $>10^3$ foci is interpreted to indicate lack of interference between the resident MuLV in the NIH 3T3 cells and the superinfecting HaSV pseudotype; presence of less than three foci induced by the 10^4 focus-forming particles on the challenged cells is interpreted to indicate superinfection interference between the two viruses.

Radioimmunoprecipitation. Chimeras that did not produce infectious virus were examined for the production of retroviral particles by assaying culture supernatants for pelletable capsid protein $p30^{CA}$ and $gp70^{SU}$, using radioimmunoprecipitation. CHO cells stably transfected with chimeric constructions were labeled for 16 h with [^{35}S]methionine. Culture supernatants were collected, clarified through a 0.45-μm-pore-size filter, and centrifuged in an SW27 rotor (Beckman Instruments, Palo Alto, Calif.) at 25,000 rpm for 90 min at 4°C in order to pellet viral particles. Viral pellets were resuspended in TNT buffer (20 mM Tris-Cl [pH 7.5], 0.2 M NaCl, 0.1% [vol/vol] Triton-X100). Immunoprecipitation was performed by addition of protein A-linked Sepharose (Pharmacia-LKB, Piscataway, N.J.) and either anti-$p30^{CA}$ or anti-$gp70^{SU}$ antiserum (both described in reference 21) to the resuspended pellets. After incubation at 4°C for 2 h, the mixture was microcentrifuged for 30 s. The Sepharose beads were washed three times with ice-cold TNT before the beads were combined with gel loading buffer (1% sodium dodecyl sulfate [SDS], 10% glycerol, 1% β-mercaptoethanol, 0.001% bromophenol blue, 100 mM Tris-Cl [pH 8.6]), boiled for 5 min, and subjected to SDS-polyacrylamide gel electrophoresis. Gels were treated with the fluor 2,5-diphenyloxazole in dimethyl sulfoxide and dried before exposure to X-ray film at −70°C.

Nucleotide sequence accession numbers. The Env sequences of the 4070A clone of Chattopadhyay et al. and of 10A1 are listed in GenBank under accession numbers M33469 and M33470, respectively. The long terminal repeat sequences of the 4070A clone of Chattopadhyay et al. (3) and of 10A1 are listed under accession numbers M55597 and M55596, respectively.

RESULTS

To map regions of the $gp70^{SU}$ molecule that contribute to the receptor specificity of the Env protein complex, we constructed chimeric *env* genes from amphotropic, Mo-MCF, and 10A1 *env* sequences (Fig. 1). The constructions recombined these sequences at a conserved *Eco*RI restriction enzyme site which is located just after amino acid 200 of the Env polypeptide. Thus, chimeric $gp70^{SU}$s had the amino half of MuLV $gp70^{SU}$ from one MuLV fused to the carboxy half of $gp70^{SU}$ and the majority of transmembrane protein $p15E^{TM}$ of the other MuLV. Designations for these chimeras are abbreviated as follows: A for amphotropic 4070A, M for Mo-MCF, and 10 for 10A1, with chimeric *env*s denoted by simple combinations of the abbreviations. For example, A.10 has the N-terminal portion of amphotropic MuLV and the C-terminal portion of 10A1 MuLV.

The chimeric MuLVs were tested for receptor specificity by testing HaSV pseudotypes for superinfection interference on NIH 3T3 cells previously infected with either ecotropic, MCF, amphotropic, or 10A1 MuLV. The assay was also performed reciprocally: NIH 3T3 cells infected with chimeric MuLVs were challenged with HaSV pseudotyped by an MuLV from one of the four classes that infect mouse cells.

Most chimeric MuLVs were also tested for their interference properties with each other. In all cases tested, the interference patterns observed between pairs of chimeras were consistent with the receptor specificities assigned to each chimera by interference patterns determined against ecotropic, amphotropic, Mo-MCF, and 10A1 viruses.

Amphotropic MuLV and 10A1 exchanges. While most classes of MuLV have straightforward interference properties, 10A1 receptor specificity is more complicated. Previously, 10A1 MuLV has been shown to interfere with both amphotropic and 10A1 MuLVs (18). However, only 10A1, not amphotropic MuLV, interferes with 10A1 superinfection. These data indicate that 10A1 MuLV has dual receptor specificity: it can apparently bind to both amphotropic and 10A1 receptors.

Chimeric Envs that exchanged sequences between am-

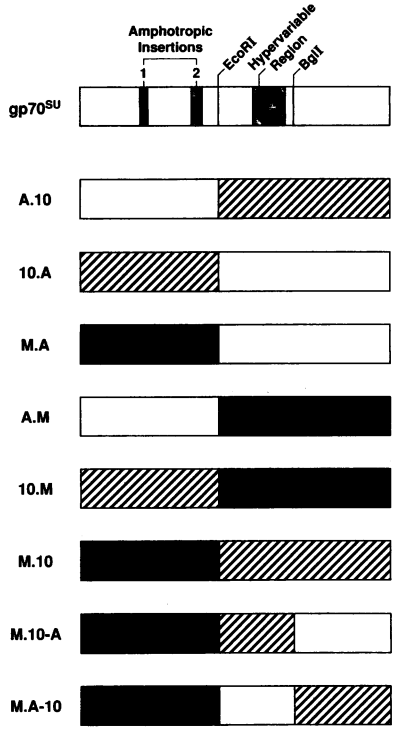

FIG. 1. Maps of chimeric gp70^{SU}s. Stippled regions denote highly variable regions of gp70^{SU} as described by Ott et al. (15). Restriction enzyme sites used for the constructions within gp70^{SU} are also shown. The different sequences are denoted as follows: open box for amphotropic; black box for Mo-MCF; and hatched box for 10A1.

photropic and 10A1 gp70^{SU}s were assayed for their receptor specificities (Table 1). In interference assays, A.10 MuLV interfered with superinfection by the amphotropic pseudotype of HaSV [HaSV(ampho)], while both amphotropic MuLV and 10A1 interfered with HaSV(A.10) superinfection. Thus, the interference properties of A.10 were identical to those of amphotropic MuLV. Our results with chimeras are summarized in Fig. 2.

In contrast, infection by the 10.A chimera rendered NIH 3T3 cells resistant to superinfection by both HaSV(10A1) and HaSV(ampho) pseudotypes, while only 10A1-infected

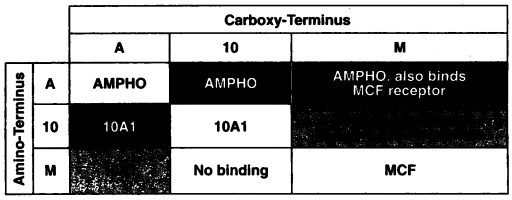

FIG. 2. Three-by-three matrix showing the receptor specificities of the six chimeric gp70^{SU}s. N termini of amphotropic (AMPHO), 10A1, and MCF gp70^{SU}s are listed at the left, and the C termini are listed above the matrix. The receptor specificities of the combinations are noted inside the boxes. Wild-type combinations are in white boxes; combinations for which receptor specificity is determined combinatorially are stippled; combinations for which receptor specificity maps to the amino half of gp70^{SU} are shown in black.

cells were resistant to HaSV(10.A) superinfection (Table 1). Thus, the 10.A chimera had the interference properties of 10A1 MuLV (Fig. 2). Therefore, in these two cases, the N termini determine the receptor specificity of these chimeric gp70^{SU}s.

Amphotropic MuLV and Mo-MCF exchanges. The amphotropic and Mo-MCF recombinations produced chimeras with intriguing properties (Table 1; Fig. 2). Prior infection of NIH 3T3 cells with the A.M chimera rendered them resistant to superinfection by HaSV(ampho). Conversely, amphotropic MuLV interfered with superinfection by HaSV(A.M). Thus, A.M clearly has amphotropic receptor specificity. However, the A.M virus also displayed nonreciprocal interference with Mo-MCF; that is, it interfered with Mo-MCF superinfection, although it was able to efficiently superinfect cells infected with Mo-MCF. These results suggest that the chimeric A.M gp70^{SU} is able to bind the MCF receptor as well as the amphotropic receptor but can use only the amphotropic receptor for entry into the cell (Fig. 2).

The M.A chimera also had an unexpected property: this chimera had the same interference properties as did 10A1 (Table 1; Fig. 2). Thus, HaSV(M.A) was able to infect all cell lines except those infected with 10A1 MuLV, while cells infected with M.A MuLV were resistant to either HaSV(10A1) or HaSV(ampho) but not HaSV(MCF).

Mo-MCF and 10A1 exchanges. The Mo-MCF and 10A1 recombinations also gave unexpected results. CHO cells transfected with the M.10 construct did not produce virus particles capable of infecting mouse, CHO, mink, or rat cell

TABLE 1. Interference testing of MuLVs containing chimeric envelopes

NIH 3T3 cells infected with:	Foci present after infection with HaSV pseudotyped by[a]:								
	Moloney	Mo-MCF	Amphotropic (4070A)	10A1	10.A	A.10	A.M	M.A	10.M
No virus	+	+	+	+	+	+	+	+	+
Moloney	−	+	+	+	+	+	+	+	+
Mo-MCF	+	−	+	+	+	+	+	+	+
Amphotropic (4070A)	+	+	−	+	+	−	−	+	−
10A1	+	+	−	−	−	−	−	−	−
10.A	+	+	−	−	−	−	−	−	ND
A.10	+	+	−	+	+	−	−	+	ND
A.M	+	−	−	+	+	−	−	+	ND
M.A	+	+	−	−	−	−	−	−	ND
10.M	+	−	−	−	ND	ND	ND	ND	−
M.10[b]	+	+	+	+	+	+	+	+	ND

[a] +, foci present; −, foci absent; ND, not done.
[b] NIH 3T3 cells transfected with plasmid M.10 and selected with G418.

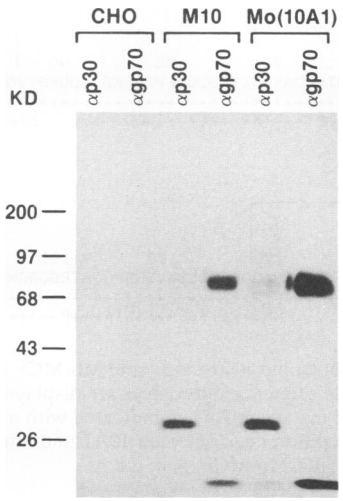

FIG. 3. Immunoprecipitation of $p30^{CA}$ and $gp70^{SU}$. Cell lines used are CHO, CHO transfected with M.10, and CHO infected with Mo(10A1), a chimeric Mo-MuLV that has a 10A1 *env* gene (14). Antisera used for immunoprecipitation are indicated above the lanes.

lines (data not shown). Further, NIH 3T3 cells stably transfected with the M.10 MuLV construct were permissive for all of the natural or chimeric viruses tested (Table 1). By virtue of the transfection and the G418 selection procedure, all of the challenged cells should have contained the M.10 construct (as noted in Materials and Methods). The absence of any superinfection interference by these cells and the absence of infectious virus suggest that the M.10 Env was functionally inactive.

The presence of both *gag* and *env* gene products in supernatants of CHO cells transfected with M.10 was examined to determine whether Env is expressed and is associated with virus particles. [^{35}S]methionine-labeled virions were analyzed by radioimmunoprecipitation. As shown in Fig. 3, CHO cells transfected with the M.10 construct produced virions that contained $gp70^{SU}$, as evidenced by the presence of both pelletable $p30^{CA}$ and $gp70^{SU}$. Since M.10 seems to produce virions containing correctly processed Env proteins, the defect in the virus seems to result from a functional defect in the chimeric gp70.

The defectiveness of the M.10 chimeric $gp70^{SU}$ is surprising since, as shown above, the M.A chimeric $gp70^{SU}$ is fully functional. This difference must be due to sequence differences in the carboxy halves of 10A1 $gp70^{SU}$ and amphotropic $gp70^{SU}$. We attempted to identify the region responsible for the defectiveness of M.10 by making exchanges within the carboxy half of $gp70^{SU}$. By use of conserved *Eco*RI and *Bgl*I sites, the hypervariable region and its immediate 3′ flanking sequences of *env* were alternately exchanged between 10A1 and amphotropic 4070A to produce M.A-10 and M.10-A (Fig. 1). Neither of these chimeras gave rise to infectious viruses, although cells containing them produced virus particles detectable by immunoprecipitation with anti-$p30^{CA}$ and anti-$gp70^{SU}$ antisera (data not shown). A similar set of constructions between 10A1 and amphotropic MuLV that yielded 10.A-10 and A.10-A were fully functional (data not shown), showing that the A-10 and 10-A chimeric *Eco*RI-*Bgl*I C-terminal regions are not intrinsically defective. We conclude that the MCF N-terminal region cannot combine with either the A-10 or 10-A C-terminal region to form a functional $gp70^{SU}$.

The 10.M chimera exhibited a complex interference pattern (Table 1; Fig. 2). The MuLV containing the 10.M chimeric $gp70^{SU}$ had amphotropic receptor specificity, as 10.M interfered with HaSV(ampho) and cells infected with amphotropic MuLV interfered with HaSV(10.M) superinfection. However, in addition to this functional amphotropic receptor specificity, 10.M interfered with Ha(Mo-MCF) and Ha(10A1). Taken together, these results indicate that the 10.M chimera has one functional receptor specificity, i.e., amphotropic; in addition, it can bind to both the MCF and 10A1 receptors but cannot use them to initiate infection.

DISCUSSION

In this study, we have analyzed the receptor specificities of a series of chimeric $gp70^{SU}$s. The chimeras were designed to separate two variable regions of $gp70^{SU}$ in order to determine which, if either, of these regions determines receptor specificity.

Our results, summarized in Fig. 2, revealed the existence of a highly complex series of interactions between different domains of gp70. Thus, in only two of the six chimeras could receptor specificity be attributed simply to a single region of the molecule. The properties of the chimeras and their implications for the origin of the different receptor specificities are discussed below.

Two chimeras were found to be able to infect cells via one receptor but to also interfere with infection at one or more additional receptors. One way to explain this observation is to postulate a second function for $gp70^{SU}$, in addition to receptor binding. According to this hypothesis, these chimeras would be unable to carry out this second function after binding the receptor in question. This idea is analogous to that used to explain similar results with TM protein mutants by Delwart and Panganiban (4) and Granowitz et al. (10): in the case of these mutants, it seems likely that gp70 can bind to the receptor but that the mutant TM is defective. An alternate hypothesis assumes that in interference with superinfection, the receptor is prevented from reaching the cell surface as a result of an encounter with a newly synthesized Env molecule within the cell. According to this hypothesis, one or both of these molecules is in an immature state at the time of this encounter. The chimeric Env would then be capable of interacting with the receptor intracellularly, although the mature chimeric Env on a virion would be incapable of binding the corresponding mature receptor at the cell surface.

Determinants for amphotropic receptor specificity. Our analysis shows clearly that most of the chimeras have amphotropic receptor specificity, since three of the five functional chimeras (A.10, A.M, and 10.M) can use only the amphotropic receptor to infect NIH 3T3 cells (Fig. 2). Both chimeric Envs containing the amino half of amphotropic $gp70^{SU}$ had amphotropic receptor specificity. Therefore the amphotropic receptor specificity apparently maps to sequences within the amino half of $gp70^{SU}$. This region contains two variable loops of sequence which are either absent or different in the xenotropic and MCF-polytropic $gp70^{SU}$ sequences (15); further studies are required to define the participation of these sequences in receptor specificity. Both A.M and 10.M have additional attributes which will be discussed later.

Determinants for 10A1 receptor specificity. The interference properties of 10A1 (18) suggest that its Env is able to

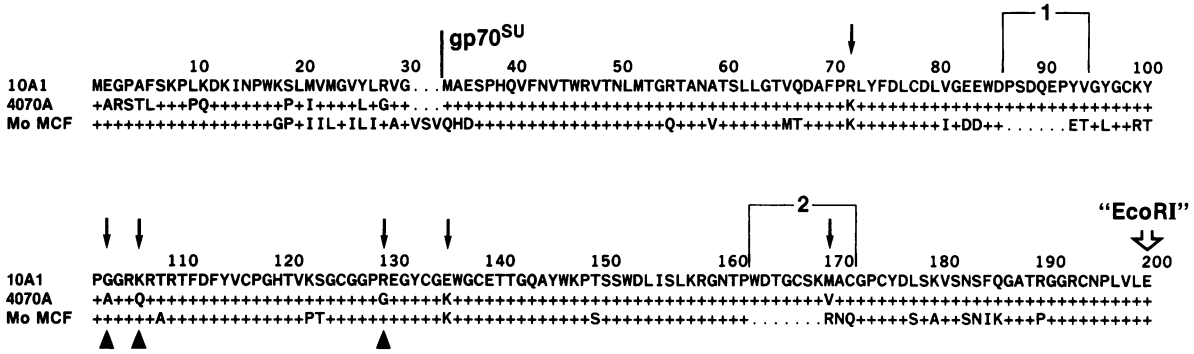

FIG. 4. Protein sequence comparison of the amino-halves of gp70^{SU}s. 10A1 (15), amphotropic 4070A (15), and Mo-MCF (1) sequences are aligned. Amphotropic and Mo-MCF sequence identities with 10A1 are denoted by +, while sequence differences are displayed by single-letter amino acid code. Periods indicate spaces inserted for maximizing alignment. The start of mature gp70^{SU} is indicated with a bar, and the two amphotropic insertions are indicated by numbered brackets. Arrows highlight sequence differences between 10A1 and amphotropic 4070A MuLV; triangles indicate the sequence differences from 4070A that are shared by 10A1 and Mo-MCF.

recognize both the unique 10A1 receptor and the amphotropic receptor. In our present analysis, we have not identified any chimeras which interact with the 10A1 receptor but not the amphotropic receptor. However, the results showed that 10A1 receptor specificity can apparently originate in two distinct ways.

First, in the chimeras between amphotropic and 10A1 sequences, 10A1 receptor specificity is clearly determined by the N-terminal portion of 10A1 gp70^{SU} in the 10.A chimera (Fig. 2). Our previous sequence analysis (15) has shown 10A1 MuLV to be an in vivo recombinant containing mostly amphotropic *env* sequences except for a hypervariable region (in the C-terminal region of 10A1 gp70^{SU}) consisting of endogenous MCF-related polytropic *env* sequences. This observation led us to propose that 10A1 receptor specificity may arise by a combinatorial mechanism, i.e., as a result of interactions between two different regions, neither of which is from a virus with 10A1 receptor specificity (15). It is clear that this is not the case, as 10.A displays 10A1 receptor specificity whereas A.10 does not. Rather, in comparing 10A1 and amphotropic sequences of the mature gp70^{SU} molecule up to the *Eco*RI restriction site (amino acid 200) used to join the chimeras, we find six amino acid differences (Fig. 4). Since 10.A recognizes the 10A1 receptor whereas amphotropic MuLV (A.A) does not, one or more of these six amino acid differences is clearly responsible for 10A1 receptor specificity in this combination. We are currently examining the contributions of these amino acids to 10A1 receptor specificity by site-directed mutagenesis.

While this mapping of 10A1 receptor specificity is rather straightforward, another chimera that displays this same specificity is more difficult to interpret. The M.A combination is made up of Mo-MCF N-terminal and amphotropic C-terminal sequences; neither of these MuLVs can use the 10A1 receptor, yet when these components are joined together, the resulting chimera displays 10A1 receptor specificity (Fig. 2). To our knowledge, this is the first clear case of a combinatorial interaction between two portions of gp70^{SU} that produces a third, distinct receptor specificity.

It is interesting to compare these results with previous observations on avian retroviruses. Like MuLVs, these viruses are polymorphic with respect to receptor specificity, and their gp85^{SU} molecules have discrete regions of sequence variation (2). Chimeras between different avian subgroups were extensively investigated by Dorner et al. (6, 7). They found that certain gp85^{SU} chimeras exhibited the receptor specificity of both parental viruses. This additive receptor specificity should be distinguished from the combinatorial or interactive receptor specificity that we observed with M.A MuLV.

What is responsible for the 10A1 receptor specificity of M.A? As described above, M.A and 10.A clearly exhibit 10A1 receptor specificity whereas amphotropic MuLV (A.A) does not. One inference from these data might be that both MCF and 10A1 N termini can determine 10A1 receptor specificity. Therefore, the presence of common amino acids in MCF and 10A1 N termini that are different from those in the amphotropic N-terminus sequence are provocative candidates for 10A1 receptor specificity. In this 170-amino-acid region of mature gp70^{SU}, there are three amino acids in common between 10A1 and Mo-MCF that are not shared with amphotropic MuLV (Fig. 4). Thus, it is possible that one or more of these three amino acid differences determines 10A1 receptor specificity when combined with the amphotropic, but not with the MCF, carboxy half of gp70^{SU}. The mutagenesis experiments outlined above should clarify this possibility also.

Determinants for MCF receptor specificity. The most complex interactions that we observed were those which might determine MCF receptor specificity. Consideration of three facts seems relevant to an understanding of these interactions.

First, it is notable that no chimera was able to infect cells by using the MCF receptor. This finding implies that both N and C termini of the MCF gp70^{SU} contribute to its specificity for the MCF receptor, i.e., that MCF receptor specificity is generated by interaction between these domains.

Second, both of the chimeras containing the MCF C terminus, i.e., A.M and 10.M, are apparently able to bind the MCF receptor, although they are unable to use it for infection. This observation suggests that the MCF C terminus contains information for MCF receptor binding.

Third, it was noted above that the properties of the 10.A chimera suggest that specificity for the 10A1 receptor is carried in the 10A1 N terminus. However, the 10.M chimera, while it is able to bind the 10A1 receptor, cannot use it for infection. This difference between 10.A and 10.M suggests the possibility that the MCF C terminus contains sequences that can mask or suppress functional 10A1 receptor specificity in 10.M.

As noted above, the 10A1 receptor specificity of the M.A

chimera (resembling that of the 10.A chimera) suggests that the MCF N terminus may actually participate in binding to the 10A1 receptor. However, in this case, why does the MCF Env (M.M) itself not possess 10A1 receptor specificity? One possibility is that the MCF C terminus masks 10A1 specificity in this case, as postulated above to explain the properties of 10.M.

Defectiveness of the M.10 chimera. The only chimera that did not form a functional *env* gene was the M.10 combination. M.10 MuLV cannot infect mouse, CHO, rat, or mink cells. Since the M.10 Env is incorporated into virions (Fig. 3), the M.10 envelope is presumably on the plasma membrane of the transfected CHO cells bearing this chimeric MuLV. This M.10 $gp70^{SU}$ is apparently nonfunctional with respect to receptor binding as well as receptor utilization, since NIH 3T3 cells transfected with M.10 show no superinfection interference. Thus, M.10 appears to be defective in $gp70^{SU}$-mediated receptor binding. (Of course, the M.10 chimera also contains two other gene fusions: between Mo-MuLV and Mo-MCF at the *SalI* site in the coding region for reverse transcriptase, and between 10A1 and Mo-MuLV at the *ClaI* site in the TM gene. However, these fusions yield fully functional products and are not responsible for the defectiveness of M.10, since they are also present in the infectious genomes M.A and A.10, respectively.)

It was surprising that the M.10 chimera was defective whereas the M.A chimera was not. This difference must reflect a difference between the 10A1 and amphotropic carboxy halves. We attempted to localize the 10A1 sequences responsible for the defectiveness of M.10 by reciprocally exchanging portions of amphotropic and 10A1 carboxy halves (Fig. 1); however, neither of the resulting chimeras (M.10-A and M.A-10) was functional (data not shown). Thus, one possibility is that both the hypervariable region and C-terminal region of 10A1 Env contain sequences which are independently incompatible with the Mo-MCF N-terminal sequences. However, while incompatibility between these sequences is one explanation, there is another possible mechanism for the lack of M.10 $gp70^{SU}$ function. From our chimeric results, it seems possible that the Mo-MCF amino half, unlike the 10A1 or amphotropic amino half, does not contain determinants for receptor binding. In that case, it might be that the 10A1 C-terminal region (unlike the amphotropic C-terminal region) does not have enough information to interact with the N-terminal determinants of MCF $gp70^{SU}$ and bind a receptor.

Conclusions. Our analysis of receptor specificity using chimeric *env*s has revealed a complex pattern of receptor specificities, with some unexpected results (Fig. 2). They can be briefly summarized as follows. The amphotropic amino half of $gp70^{SU}$ is always associated with amphotropic receptor specificity and appears to be responsible for this receptor specificity. The 10A1 amino half is also correlated with 10A1 receptor specificity; however, when it is combined with the Mo-MCF carboxy half, the resulting 10.M chimera can only bind to, not infect via, the 10A1 receptor. The Mo-MCF amino half of $gp70^{SU}$ was not clearly associated with a receptor specificity. The carboxy halves of $gp70^{SU}$s did not have a clear association with a receptor specificity except for the Mo-MCF carboxy half of $gp70^{SU}$. This region of Mo-MCF is consistently associated with MCF receptor binding, though it requires the corresponding Mo-MCF N-terminal portion for full function in infection. From these observations, it appears that receptor specificity can be formed in two ways: by determinants in the N-terminal portion of $gp70^{SU}$ or by combinatorial interactions between two regions. Further studies are in progress to better describe PRM receptor specificity.

After this work was submitted for publication, Battini et al. (1a) published a report describing properties of a similar set of chimeras. The results of the two studies are generally consistent except that the chimera corresponding to our M.A chimera was defective in the experiments of Battini et al., whereas M.A was fully infectious in our hands. One possible explanation for this discrepancy is that different MCF isolates were used as parents of the chimeras constructed in the two laboratories.

ACKNOWLEDGMENTS

We thank Marguerite Vogt for the Mo-MCF clone; Donald Blair, James Cunningham, Hung Fan, and Nancy Rice for helpful discussions and comments on the manuscript; and Carol Shawver for preparation of the manuscript.

This research was sponsored by the National Cancer Institute under contract N01-CO-74101 with ABL.

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