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Localization of the Amphotropic Murine Leukemia Virus Receptor Gene to the Pericentromeric Region of Human Chromosome 8

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The host range of retroviruses is determined primarily by the presence of specific receptors on target cells which are recognized by the retroviral envelope glycoprotein. Somatic cell hybrids have been used to determine the chromosomal locations of several retroviral receptors in mice prior to their molecular cloning. Here we report that by using human-Chinese hamster somatic cell hybrids and a retroviral vector, we have mapped the receptor for the amphotropic murine leukemia virus to the pericentromeric region of human chromosome 8.

Binding of the envelope glycoprotein to a specific cell surface receptor is the first event in the retroviral infection process. The presence of this receptor is the major determinant of the tropism of a given virus. Amphotropic murine leukemia virus (MuLV) infects cells from many tissues in a wide range of mammalian species, including mice, rats, dogs, and humans (31), indicating the broad distribution of the receptor for this virus. Interestingly, hamster cells are resistant to infection by amphotropic, ecotropic, and xenotropic MuLV (5, 31). The resistance of these cells to infection by these viruses is due to the absence of functional receptors on the surfaces of the cells (3, 14, 24). On the basis of this observation, somatic cell hybrids between hamster and mouse cells have been used to localize the receptor genes for the murine ecotropic virus to mouse chromosome 5 (5, 15, 16, 24, 26), amphotropic virus to mouse chromosome 8 (5), xenotropic and mink cell focus-forming viruses to mouse chromosome 1 (13, 14), and mouse mammary tumor virus to mouse chromosome 16 (6).

To determine the chromosomal localization of the human amphotropic receptor gene, a well-characterized set of human-Chinese hamster hybrids (2, 4, 7, 9–11, 17) were tested for susceptibility to infection with an amphotropic retroviral vector carrying the *neo* selectable marker. Cell culture conditions, virus preparation, and infection conditions were, in general, as described previously (21). Helper virus-free vector preparations were generated by using PA317 retrovirus packaging cells (19, 20) containing the LN vector (22). This amphotropic vector does not infect the parental CHO-K1 cell line (<1 CFU/ml of virus), but the LN vector produced by a gibbon ape leukemia virus-based packaging cell line, PG13, is capable of infecting CHO-K1 cells (21). Since the only difference between virions produced by these two packaging cell lines is in the envelope protein (the *gag* and most of the *pol* proteins in both PA317 and PG13 cell lines are derived from Moloney MuLV), the restriction to infection must be at the level of interaction of the amphotropic envelope with its cellular receptor.

Hybrid cell lines were seeded at 10^5 per 10-cm dish on day 1 and infected with 0.1 ml of amphotropic *neo*-virus from PA317/LN cells (22) on day 2. On day 3, the cells were trypsinized and divided 9:10 or 1:10 into selective medium containing G418 (0.5 mg of active drug per ml). Drug-resistant colonies were stained and counted after 7 to 10 days of selection. Hybrids were scored as infectible when 0.1 ml of the amphotropic vector induced at least 500 G418-resistant colonies and were scored negative when no colonies were induced by 0.1 ml of virus. Under these criteria, only hybrid cells containing human chromosome 8 were susceptible to infection (Table 1). The fact that hybrid 706-CL17, which retained only human chromosome 8, was almost as susceptible to infection by the amphotropic vector ($\sim 2.5 \times 10^4$ CFU/ml) as were human HeLa cells under the same conditions of infection (10^5 CFU/ml) indicates that no other human chromosome was required.

Besides chromosome 8, no other human chromosome conferred susceptibility to infection in the CHO hybrids (Table 1), with the possible exception of chromosome 3 in hybrids 50-3 and 314-2 that were partially susceptible to infection by the amphotropic LN vector (10^3 and 1.5×10^3 CFU/ml, respectively). Although no evidence for the presence of fragments of chromosome 8 in the hybrids containing chromosome 3 was detected by using the human chromosome 8-specific markers glutathione reductase (GSR) and *mos* oncogene homolog probes, either in the hybrids or in G418-resistant cells derived by infection of these hybrids with amphotropic LN vector, we cannot rule out the presence of a small fragment of chromosome 8 in these hybrids that could confer susceptibility to amphotropic vectors. It is also formally possible that a gene for a low-affinity amphotropic retrovirus receptor is carried on human chromosome 3.

To more closely map the position of the amphotropic receptor on human chromosome 8, human-hamster hybrids containing fragments of human chromosome 8 were tested for susceptibility to infection by the amphotropic virus. Hybrids R30-5B and R30-2A contain a complete human chromosome 14 and either the 8p11→qter or q13→8qter portion of human chromosome 8, respectively (Fig. 1). Hybrid 229-3A contains the 8pter→q11 portion of human chromosome 8 and other human chromosomes (Fig. 1). Only the R30-5B and 229-3A hybrids were susceptible to infection by the amphotropic retrovirus. Because these two positive hybrids share only the regions close to the centromere on chromosome 8, the amphotropic receptor maps to human chromosome 8p11→q11.

The CHO-K1 cells used in these studies are resistant to

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TABLE 1. Susceptibility of hybrids to amphotropic vector infection

Hybrid	Infectivity[a]	Human chromosome present[b]																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
706-B8	+	—	—	—	4	—	—	7	8	—	—	—	12	13	14	15	16	—	—	—	—	21	—	—	—
706-D5	−	—	2	—	—	—	—	—	—	—	—	—	—	—	—	15	—	—	—	—	20	—	—	—	—
706-D1R	+	—	2	—	4	—	—	—	8	—	—	—	12	13	—	—	16	17	18	—	—	—	22	—	—
50-3	+/−	—	—	3	—	—	6	—	—	—	—	11	12	—	14	—	—	—	18	—	—	21	—	X	—
314-2	+/−	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
78-1	+	1	—	—	—	—	6	7	8	—	10	11	12	—	—	—	—	—	18	—	—	21	22	X	—
976-17B	−	1	—	—	4	5	6	—	—	9	—	11	—	13	14	15	—	—	18	—	20	21	22	—	—
476-8A	+	—	—	—	4	5	—	—	8	9	—	—	12	—	—	—	—	17	—	—	—	21	22	X	—
640-12	−	—	—	—	—	5	—	—	—	9	—	—	12	—	—	—	—	—	—	—	—	—	—	—	—
826-23A3	+	—	—	—	—	5	6	—	8	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
826-26A	−	—	—	—	—	5	6	7	—	—	—	11	12	—	—	—	—	—	—	19	—	21	—	—	—
879-1B	−	—	—	—	—	5	6	—	—	9	—	—	12	—	—	—	—	—	—	19	—	21	—	—	—
762-8A	−	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	Y
640-51	−	—	—	—	—	—	—	—	—	9	—	—	—	—	—	—	—	—	—	—	—	—	22	—	—
225-3A	−	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	—	—	—	—	—	—	—	—
129-18A	−	—	—	3q	—	—	—	—	—	9q	—	—	—	—	—	—	—	17	—	19	—	21	—	—	—
30-5E	+	—	—	—	—	—	—	—	8	—	—	—	—	—	14	—	—	—	—	—	—	—	—	—	—
706-CL17	+	—	—	—	—	—	—	—	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
225-2A-R2A	+	—	—	—	—	—	—	—	8	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] +, ≥5 × 10^3 CFU/ml; +/−, 1 × 10^3 to 1.5 × 10^3 CFU/ml; −, <10 CFU/ml (no colonies).
[b] Numbers indicate whole chromosome; q indicates that only the long arm of the chromosome specified is present.

infection by ecotropic, amphotropic, and xenotropic retrovirus vectors (Table 2). However, these vectors are capable of infecting NIH 3T3 cells (ecotropic), HeLa and NIH 3T3 cells (amphotropic), and HeLa cells (xenotropic) (Table 2). On the other hand, CHO-K1 cells are susceptible to infection by a gibbon ape leukemia virus-based retroviral vector (Table 2) (21). This vector shares Moloney MuLV core particles with the ecotropic, amphotropic, and xenotropic vectors (21). The fact that all four vectors share the same transcriptional unit and core proteins and differ only in their envelope proteins suggests that the lack of functional receptors is the cause of the resistance to infection seen in CHO cells. Although we have mapped the amphotropic receptor to a relatively small portion of human chromosome 8, this region contains many genes. Therefore, it is possible that multiple human genes are required for infection. To determine whether a single gene encoding a murine retrovirus receptor was sufficient to render CHO cells susceptible to infection, an expression plasmid (pJET) encoding the ecotropic MuLV receptor *Rec-1* (1) was introduced into CHO-K1 cells by transient transfection as described previously (22). Two days after transfection with the ecotropic receptor gene, the CHO-K1 cells were readily infected by an ecotropic retrovirus vector yet were resistant to infection by both amphotropic and a xenotropic virus vectors (Table 2). These results confirm that the resistance of CHO cells to ecotropic MuLV infection is due only to the lack of a functional receptor on the cell surface.

To rule out the possibility that human chromosome 8 might render CHO cells susceptible to infection by means

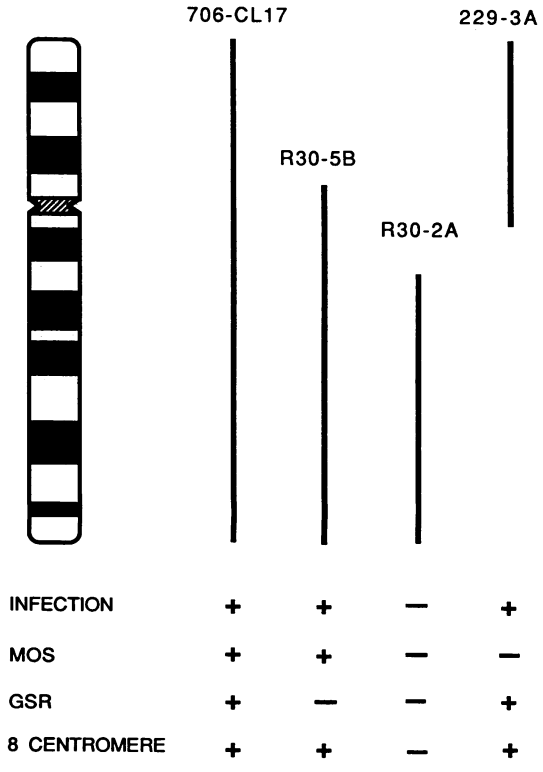

FIG. 1. Regional localization of the amphotropic MuLV receptor on human chromosome 8. CHO-K1–human somatic cell hybrids containing the different portions of human chromosome 8 (solid lines) were infected with the amphotropic retroviral vector. Susceptibility (+) and resistance (−) to infection as well as the presence (+) and absence (−) of human chromosome 8-specific markers are indicated at the bottom of each line. The locations of the break points were determined by cytogenetic analysis. The presence of the chromosome 8 centromere was determined by in situ fluorescence by using a chromosome 8 biotinylated alphoid sequence (Oncor, Gaithersburg, Md.). The presence of Mos sequences was determined by Southern blot analysis with a ^{32}P-labeled DNA probe essentially as described previously (2, 29). Human GSR was determined as described previously (9). *mos* and GSR are on human chromosome 8 (8q11-q12 and 8p21.1, respectively). The amphotropic receptor gene is located between them close to the centromere.

TABLE 2. Susceptibility of CHO cells to infection by murine and gibbon ape retroviral vectors

Virus producer line[a]	Infectivity of target cells[b]				
	NIH 3T3	HeLa	CHO-K1	CHO + *Rec-1*[c]	706-CL17
PE501/LN	+	−	−	+	−
PA317/LN	+	+	−	−	+
PX325/LN	−	+	−	−	−
PG13/LN	−	+	+	+	+

[a] PE501, PA317, and PG13, which are ecotropic, amphotropic, and gibbon ape retrovirus packaging cells, respectively, have been described previously (20–22). The xenotropic retrovirus packaging cell line PX325 (12) contains a defective helper virus genome that is identical to that used to make the PA317 amphotropic packaging cells, except that the amphotropic *env* region was replaced with that of the NZB xenotropic virus (25). All packaging cells contain and produce the retrovirus vector LN that expresses the *neo* gene.

[b] Target cells (1×10^5 to 5×10^5) were plated in 10-cm dishes 1 day before infection, and G418 was added the day after infection. Infection with virus (0.1 to 1 ml) from the different producer lines was scored as positive (+) when $\geq 10^3$ G418-resistant colonies per ml of added virus were obtained. Cell lines were scored negative (−) when no G418-resistant colonies were induced by 1 ml of virus.

[c] CHO-K1 cells transfected with the ecotropic receptor (CHO + *Rec-1*) were infected 2 days after transfection.

independent of specific receptor-envelope interactions, the 706-CL17 hybrid containing chromosome 8 was also tested for its susceptibility to infection by the xenotropic vector. No G418-resistant colonies were obtained after infection of 706-CL17 cells with the xenotropic vector, whereas HeLa cells infected in parallel were readily susceptible to infection by this virus (Table 2). Therefore, there is no correlation between chromosome 8 and susceptibility to infection by the xenotropic virus. These results strongly suggest that the specific interaction between the amphotropic envelope protein and the amphotropic receptor on the cell surface confers susceptibility to amphotropic infection in these hybrid cells. On the basis of our results, we conclude that the 8p11→q11 region of human chromosome 8 contains the human high-affinity receptor gene for amphotropic MuLV.

The possibility remains that the region of human chromosome 8 which confers susceptibility to amphotropic infection encodes a modification activity or an accessory molecule that is necessary for infection but is not present in CHO-K1 cells. However, the facts that CHO cells are resistant to infection by amphotropic, ecotropic, and xenotropic viruses and that this segment of human chromosome 8 or the ecotropic receptor gene confers susceptibility only to amphotropic or ecotropic infection, respectively, would imply multiple mechanisms of resistance for each of these viruses. The ecotropic receptor gene product is a transmembrane protein and no modifying enzyme activity has been associated with it, yet it is all that is required for susceptibility to infection. Therefore, it is unlikely that the gene found in human chromosome 8 that allows infection by amphotropic virus encodes a modification activity that is responsible for the susceptibility to infection.

Comparative analysis of human and mouse chromosome 8 indicates that both include some similar genes which encode glutathione reductase, tissue plasminogen activator, and DNA polymerase-beta (18, 30). However, homologous loci on human chromosome 8 have also been reported to be on mouse chromosomes 3 and 14 (23). Reciprocally, a significant portion of mouse chromosome 8 corresponds to a portion of human chromosome 16. The region of synteny between human chromosome 8 and mouse chromosome 8 could extend to the location of the gene for the human amphotropic receptor. It is therefore possible that the amphotropic receptor found on human chromosome 8 is a functional homolog of the murine receptor gene found on mouse chromosome 8.

The receptor genes for several other retroviruses have been mapped to specific human chromosomes. The receptor gene for human T-cell leukemia viruses types 1 and 2 is on human chromosome 17 cen-qter (27). The receptor for the human immunodeficiency virus, CD4, is on human chromosome 12 (8). The receptor for type D simian retroviruses is on human chromosome 19 (28). The amphotropic receptor is the only receptor gene assigned to human chromosome 8 thus far; therefore, the receptor used by the amphotropic retrovirus is not shared by any of the retroviruses whose receptors have been mapped to other chromosomes. Our interest will now focus on the biochemical characterization and molecular cloning of this receptor.

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