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Endogenous Xenotropic Murine Leukemia Virus-Related Sequences Map to Chromosomal Regions Encoding Mouse Lymphocyte Antigens

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DNAs of all inbred mouse strains contain multiple copies (18 to 28 copies per haploid mouse genome) of endogenous xenotropic murine leukemia virus-related sequences detectable by Southern analysis with a xenotropic murine leukemia virus *env* gene-specific probe. After *Pvu*II digestion, we identified a subset of xenotropic murine leukemia virus-related sequences that are well resolved by agarose gel electrophoresis and can be mapped to specific chromosomes by using recombinant inbred mouse strains. Interestingly, three of six xenotropic proviral loci that we mapped were integrated near genes encoding mouse lymphocyte antigens (Ly-m22, chromosome 1; Ly-m6, chromosome 2; and Ly-m10, chromosome 19) and a fourth xenotropic proviral locus mapped near a gene on chromosome 4 that has a major influence on xenotropic virus cell surface antigen levels. These studies indicate that xenotropic proviral loci are located on many different mouse chromosomes and may be useful markers for molecularly cloning and characterizing regions of the mouse genome important in lymphocyte development.

Inbred mouse strains carry multiple copies of endogenous retrovirus-related sequences (for a review, see reference 10) composing as much as 0.05% of the total mouse genome and 3% of the Y chromosome (6, 27). These endogenous murine leukemia viruses (MuLVs) are integrated in many different chromosomal sites (11, 19, 22, 23) and were presumably acquired by germline infection. Endogenous MuLVs can be grouped into three classes based upon their host range: ecotropic MuLVs, which infect murine cells; xenotropic MuLVs, which infect cells of heterologous species; and mink cell focus-forming (MCF) viruses, which infect both murine and heterologous cells. Nucleic acid hybridization studies suggest that MCF viruses are *env* (envelope) gene recombinants between ecotropic and xenotropic MuLVs (9, 13, 29, 37). *env* gene sequences homologous to those carried by MCF viruses have also been identified in uninfected mouse cell DNA, indicating they are present in the mouse germline and may represent the progenitor of the MCF virus *env* gene (7, 8, 21).

Because retroviruses can integrate at many different sites in host chromosomes, they have the capacity to act as insertional mutagens either by interrupting normal gene expression or by activating expression of cellular sequences flanking proviral integration sites. In fact, mutating retroviral DNA integrations of both types have been shown to occur in vivo (16, 18, 26, 38) and have been implicated as the causative agent of two coat color mutations of mice (11, 19). In view of these results, we were interested in determining the chromosomal locations of endogenous murine retrovirus-related sequences with the goal of identifying mutations that are caused by viral integrations in inbred mouse strains. If found, the viral sequences associated with these mutations would serve as useful markers to clone and characterize both mutant and wild-type alleles at the molecular level. These studies also would have the added advantage of placing several new molecular markers on the mouse linkage map which, in turn, will be useful for cloning and characterizing other linked genes within the mouse genome.

Recently, Buckler et al. (5) have identified a 500-base-pair *Eco*RI-*Bgl*II fragment from the NFS-Th-1 xenotropic virus *env* gene that is a useful probe for mapping the chromosomal locations of both xenotropic and MCF proviral DNAs. This probe, designated pX_{env}, hybridizes to α and β xenotropic proviral DNA as well as MCF proviral DNA but not to ecotropic proviral DNA. pX_{env} has been shown by Hoggan et al. (17) to hybridize to 18 to 28 different *Hin*dIII or *Eco*RI restriction enzyme fragments in all mouse DNAs. Furthermore, each inbred strain exhibited unique characteristic pX_{env} reactive fragments that were stable during inbreeding and were useful for showing genealogical relatedness among the inbred strains. However, because of the large number of fragments carried by each inbred strain, it was difficult to determine their chromosomal location.

In the experiments described here, we have identified an enzyme, *Pvu*II, that produces a limited subset of pX_{env}-positive fragments that are characteristic of each inbred mouse strain. These fragments are well resolved by agarose gel electrophoresis and can be mapped by standard genetic techniques. Six of these fragments were mapped to defined chromosomal regions which, in three cases, were located near genes encoding mouse lymphocyte antigens. Although these proviruses were not associated with any of the known mouse mutations, they may be useful molecular markers for cloning and characterizing regions of the mouse genome important in lymphocyte development.

MATERIALS AND METHODS

Isolation of high-molecular-weight DNA from mouse spleens. High-molecular-weight DNA was prepared from frozen mouse spleens as previously described (20).

Restriction enzyme analysis, electrophoresis, DNA transfers, and hybridization. DNAs (15 μg/lane) were digested at

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37°C overnight with *Pvu*II (Bethesda Research Laboratories), electrophoresed through 0.6% agarose gels, and transferred to nitrocellulose as previously described (20). Filters were hybridized, washed, and autoradiographed as previously described (20) with the following modification. Of the pX_{env} plasmid DNA (kindly provided by M. A. Martin, National Institutes of Health), 2 μg was labeled to greater than 10^9 cpm/μg with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham Corp.) and the T4 DNA polymerase labeling system (available from Bethesda Research Laboratories). Filters were hybridized with 2.5×10^6 cpm of ^{32}P-labeled pX_{env} plasmid DNA per ml in a volume of 8 to 20 ml.

RESULTS

Distribution of pX_{env}-positive *Pvu*II restriction enzyme fragments in DNAs of 59 inbred strains and substrains of mice. To facilitate the mapping of pX_{env}-positive proviral loci in inbred mouse strains, we initially screened representative inbred mouse DNAs with several different restriction enzymes in an attempt to identify an enzyme that produced a less complex pattern of pX_{env} fragments than observed by Hoggan et al. (17) using *Hin*dIII or *Eco*RI. Preliminary analysis of five different restriction enzymes, including *Bam*HI, *Pvu*II, *Bgl*II, *Kpn*II, and *Xba*I, showed that *Pvu*II was such an enzyme (not shown). In each of 59 inbred strains and substrains analyzed, most of the hybridized probe localized in only three intensely hybridizing bands of 2.8, 1.3, and 1.0 kilobases (kb) (Fig. 1; NZB/Icr not shown). Each of these bands was present in every strain tested, with the exception of the 1.3-kb band, which was not detected in ST/bJ, SWR/J, CBA/CaJ, CBA/H-T6J, and CBA/N DNAs. The intensity of hybridization of these bands indicates that each band is composed of multiple pX_{env}-positive fragments. The intensity of hybridization of these three bands also varied from strain to strain, indicating that there are quantitative differences in the number of pX_{env}-positive 2.8-, 1.3-, and 1.0-kb *Pvu*II fragments in different inbred strains. The remaining label was distributed among a relatively small but well-resolved subset of low-intensity bands that varied in size and number from strain to strain. At least 26 such bands were identified, which have been ordered by size and compiled into a strain distribution pattern (Table 1). These bands will be referred to as XP fragments where X denotes that the fragment was detected with the pX_{env} probe and P indicates that the fragment was generated by *Pvu*II digestion. This designation is followed by a number ranging from 1 to 29 (including the three intensely hybridizing bands), increasing inversely with respect to the molecular weight of the XP fragment. In compiling the results shown in Table 1, similar-sized XP fragments that were carried by different inbred strains and substrains were assigned as a single XP fragment. However, it is likely that some of these XP fragments will represent different proviral loci that, by chance, happened to produce the same sized XP fragments.

At present, we do not know which XP fragments represent xenotropic or MCF proviral DNA sequences. In contrast to the highly related nature of the ecotropic proviruses, each xenotropic and MCF virus so far characterized has a unique restriction enzyme pattern (7, 8). In general, however, *Pvu*II digestion of xenotropic or MCF proviral DNA produces an internal viral DNA fragment of either 0.9 or 1.2 kb detectable with the pX_{env} probe. Interestingly, two of the three intensely hybridizing XP fragments listed in Table 1, XP-28 and XP-29, were of similar sizes, indicating these XP fragments may represent the internal viral DNA fragments commonly seen in xenotropic and MCF proviral DNAs. The origin of the other XP fragments is unknown. They may represent proviruses not previously characterized in replication-competent xenotropic or MCF proviruses or they may represent defective proviruses.

Most of the XP fragments shown in Fig. 1 were smaller than genome size (8.8 kb), while fragments larger than genome size were relatively rare and present in only a few strains. These larger fragments, XP-1 through XP-6, probably represent cell-virus junction fragments. The nature of the smaller XP fragments remains to be determined.

Two XP fragments, XP-19 (3.7 kb) and XP-20 (3.6 kb), were similar in size and difficult to resolve in this analysis. Therefore, the assignment of these two XP fragments is at present only tentative. In addition, XP-23 varied in intensity between different strains and substrains and within a single strain in different experiments. This fragment has also been tentatively assigned from the data shown in Fig. 1, but, due to its variable nature, has not been further analyzed and is not included in the strain comparisons described in subsequent sections.

As seen in Fig. 1 and Table 1, closely related substrains had identical or nearly identical XP patterns. Two notable exceptions to this rule were CBA/J and C57BL/KsJ, two substrains whose genetic origins are unclear. The CBA/J and CBA/Ca substrains diverged at about their F_{30} generation of inbreeding. However, CBA/J mice, but not CBA/CaJ mice, are believed to have subsequently been outcrossed to a C3H substrain because CBA/J mice carry several genetic markers characteristic of C3H mice (28). Interestingly, the XP strain distribution patterns of CBA/J and C3H mice are identical (Table 1). The genetic origin of the C57BL/KsJ substrain is also unclear as is its relationship to the other C57BL substrains. In fact, the XP distribution pattern of C57BL/KsJ is more similar to two unrelated substrains, DBA and AKR, than to other C57BL substrains.

A number of closely related strains with common ancestry also exhibited XP pattern homologies. The most extensive set of related strains listed in Table 1 are those derived from the Bagg albino, including BALB/c, A, C3H, and CBA (30). With the exception of XP-24, the A substrains have XP patterns identical to those of the BALB/c substrains. Except for XP-19, the C3H and A substrains are identical. The CBA substrains, with the exception of CBA/J, are also C3H-like, except they carry an additional XP fragment, XP-6, and are negative for XP-28. CBA/N is additionally distinct among the CBA substrains in that it has XP-7.

Three other strains listed in Table 1 are also derived from crosses in which one of the progenitors was a BALB/c substrain. These include the HTG/GoSfSn, SEC/1ReJ, and HRS/J substrains (30). HTG/GoSfSn and SEC/1ReJ mice have XP patterns which are almost identical to BALB/c, whereas HRS/J differs from BALB/c at XP-24.

XP homologies were also detected between other related strains. C57L/J and C57BR/cdJ are both brown subline descendents from a mating of female 57 and male 52 from the Lathrop stock (30) and are unique among the C57 strains in being XP-1 positive. The only other XP-1-positive strain is C58/J, which is also related to C57L/J and C57BR/cdJ (30). BDP/J was derived from an outcross of P/J (30) and both strains have identical XP distribution patterns. Furthermore, both strains are related to DBA, which is again seen in their XP strain distribution patterns.

The XP patterns of many other strains can be assembled from the XP patterns of two known ancestors. These include strains such as LT/Sv, which is a recombinant inbred strain

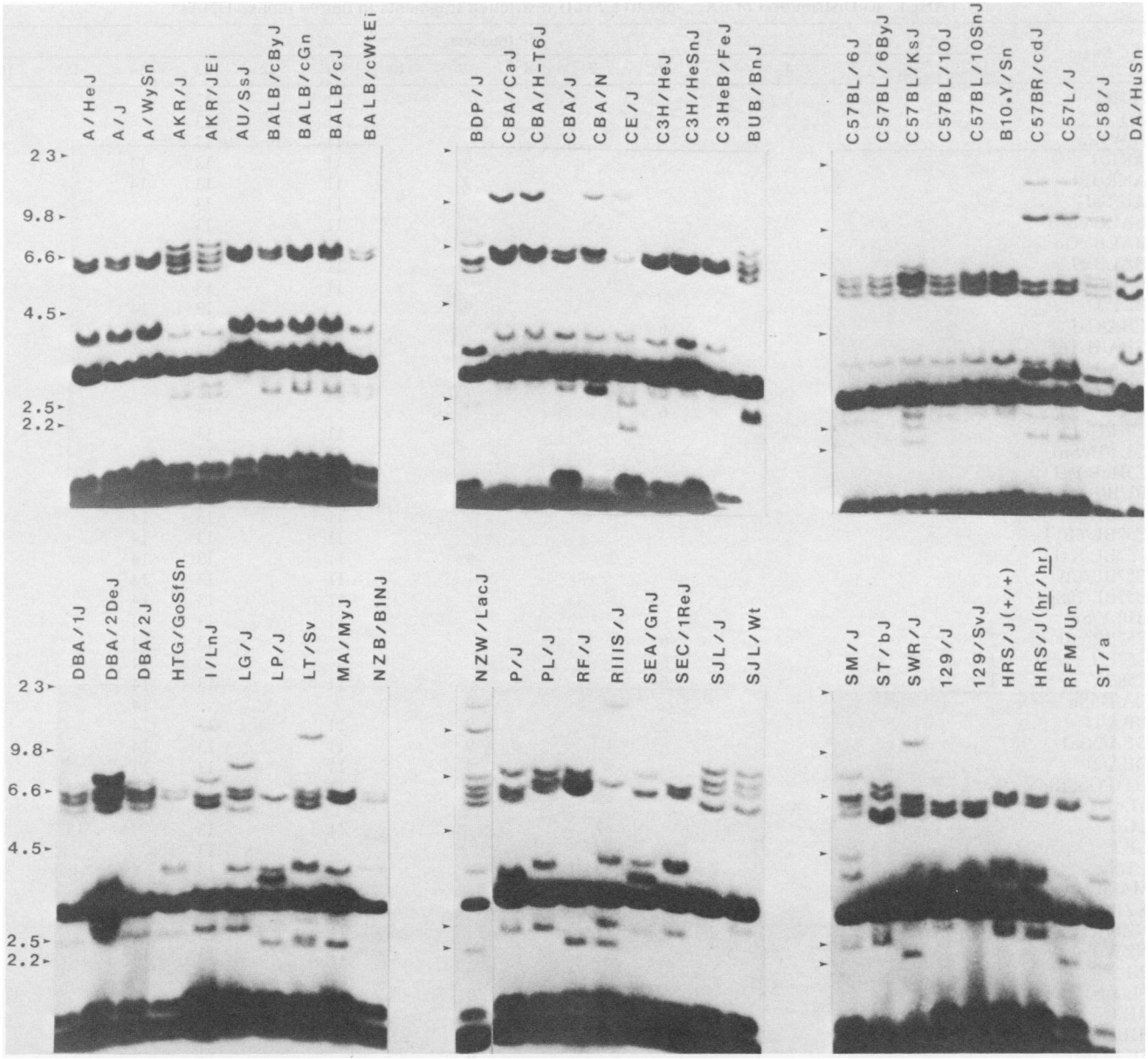

FIG. 1. Characterization of the endogenous xenotropic and MCF MuLV-related sequences in DNA of 58 inbred mouse strains. High-molecular-weight DNAs (15 μg per lane) prepared from spleens were digested to completion with *Pvu*II, electrophoresed through 0.6% agarose gels, Southern blotted, and hybridized with a ^{32}P-labeled pX_{env} probe. The molecular weight of ^{32}P-labeled *Hin*dIII-digested λ DNA fragments electrophoresed in parallel lanes of the same gels are indicated by the arrows. No differences in the XP patterns of HRS/J mice homozygous for the wild-type (+) or hairless (*hr*) mutant allele were detected.

derived from BALB/c and a C58 substrain carrying the light mutant allele at the brown locus (30), and SEA/GnJ, which is a recombinant inbred strain derived from BALB/c and P/J.

XP strain distribution patterns of 116 RI mouse strains. We next analyzed the XP strain distribution patterns of 116 recombinant inbred (RI) strains derived from crosses of AKR/J × DBA/2J, AKR/J × C57L/J, C57BL/6J × DBA/2J, C57BL/6J × C3H/HeJ, BALB/cByJ × C57BL/6ByJ, NZB/BlNJ × 129/J, NZB/Icr × C58/J, and SWR/J × C57L/J mice, designated AKXD, AKXL, BXD, BXH, CXB, NX129, NX8, and SWXL, respectively. Of the XP bands listed in Table 1, 17 were represented in one or more of the RI series. As expected, the high-copy-number bands (XP-22, XP-28, and XP-29) were present in all of the RI strain DNAs analyzed, with the exception of XP-28, which was not detected in NX8-9 and SWXL-14 DNA (not shown). Again, as in the standard inbred strains, the intensities of hybridization of XP-22, XP-28, and XP-29 fluctuated noticeably from strain to strain.

The RI strain distribution patterns for the other 14 XP bands are summarized in Table 2. Those bands that were positive in one progenitor of an RI series but negative in the other had segregation ratios between 0.31 and 0.69 (Table 2), values within the range expected for the segregation of a single proviral locus. In some cases, both progenitors of an RI series appeared to carry the same XP fragment. If this

TABLE 1. Distribution of pX_{env}-positive *Pvu*II restriction fragments in inbred mouse DNAs[a]

Strain	XP fragment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A/HeJ											11		13			
A/J											11		13			
A/WySn											11		13			
AKR/J									9		11		13	14		
AKR/JEi									9		11		13	14		
AU/SsJ											11		13			
BALB/cByJ											11		13			
BALB/cGn											11		13			
BALB/cJ											11		13			
BALB/cWtEi											11		13			
BDP/J									9				13	14		
CBA/CaJ						6					11		13			
CBA/H-T6J						6					11		13			
CBA/J											11		13			
CBA/N						6	7				11		13			
CE/J						6							13			
C3H/HeJ											11		13			
C3H/HeSnJ											11		13			
C3HeB/FeJ											11		13			
BUB/BnJ									9		11		13	14		
C57BL/6J											11		13	14		
C57BL/6ByJ											11		13	14		
C57BL/KsJ									9		11		13	14		
C57BL/10J											11		13	14		
C57BL/10SnJ											11		13	14		
B10.Y/Sn											11		13	14		
C57BR/cdJ	1					6							13	14		
C57L/J	1					6							13	14		
C58/J	1					6					11		13	14		
DA/HuSn									9					14		
DBA/1J									9		11		13	14		
DBA/2DeJ									9		11		13	14		
DBA/2J									9		11		13	14		
HTG/GoSfSn											11		13			
I/LnJ				4					9				13	14		
LG/J							7				11		13		15	
LP/J													13			
LT/Sv						6					11		13	14		
MA/MyJ											11		13			
NZB/BlNJ											11		13			
NZB/Icr							7				11		13			
NZW/LacJ			3		5				9		11		13	14		
P/J									9				13	14		
PL/J									9		11		13			
RF/J									9		11		13			
RIIIS/J		2									11					
SEA/GnJ									9				13			
SEC/1ReJ											11		13			
SJL/J								8		10		12		14		
SJL/Wt								8		10		12		14		
SM/J							7				11		13	14		
ST/bJ									9		11			14		
SWR/J					5						11		13	14		
129/J													13	14		
129/SvJ													13	14		
HRS/J+/+											11		13			
HRS/J-*hr*/*hr*											11		13			
RFM/Un										10		12	13			
ST/a											11			14		
Size (kb)	16.9	15.4	14.6	13.5	10.4	10.2	7.3	7.3	6.6	6.5	6.3	6.2	6.0	5.6	5.5	5.3

were the case, then all members of the RI series should be positive for the XP fragment. If, however, they are not identical (located at the same chromosomal position), but represented two different chromosomal sites (loci), then one-fourth of the RI strain members should carry both, one-fourth should carry neither, and one-half should carry one or the other. A pattern of the last type was observed at position XP-9 in AKXD RI mice. In all other cases, an XP band that

TABLE 1—*Continued*

XP fragment													Alloantigens			Gpd-1
17	18	19	20	21	22	23	24	25	26	27	28	29	Ly-m10	Ly-m6	Ly-m22	
		19	20		22						28	29				*b*
		19	20		22						28	29	2	1	2	
		19	20		22						28	29				
			20		22	23	24				28	29	2	2	2	*b*
			20		22	23	24				28	29				
		19	20		22						28	29			1	*b*
		19	20		22		24				28	29				
		19	20		22		24				28	29				
		19	20		22		24				28	29	2	1	2	*b*
			20		22	23	24				28	29				
				21	22		24				28	29			1	*a*
			20		22							29				*b*
			20		22							29				
			20		22	23					28	29	1	1	2	*b*
			20		22	23						29				
			20		22		24			27	28	29	2	1	2	*a*
			20		22	23					28	29	2	1	2	*b*
			20		22						28	29				
			20		22						28	29				*b*
					22		24				28	29			1	*b*
			20		22	23					28	29	2	2	2	*a*
			20		22						28	29				
			20		22	23	24		26		28	29			2	*a*
			20		22	23					28	29			2	*a*
			20		22	23					28	29				
			20		22	23					28	29				
			20	21	22	23		25			28	29	1	2	2	*a*
			20	21	22	23		25			28	29	1	2	2	*a*
			20	21	22	23		25	26		28	29	2	2	2	*a*
		19			22	23					28	29				
					22		24				28	29	1	2	1	*a*
					22		24				28	29				
					22		24				28	29	1	2	1	*b*
			20		22		24				28	29				
					22		24				28	29			1	*b*
			20		22		24				28	29			2	*b*
			20	21	22					27	28	29			2	*b*
		19	20		22	23		25	26		28	29				
			20		22	23					28	29	2	2	2	
17		19			22		24		26		28	29	2	1	1	*b*
17		19			22		24		26		28	29				
			20		22				26		28	29				
				21	22		24				28	29			1	*a*
			20		22		24				28	29	2		2	*a*
					22				26		28	29		2	1	*a*
		19			22	23			26		28	29			1	*b*
			20	21	22		24				28	29			1	*a*
		19	20		22		24				28	29			2	*b*
					22						28	29	2	2	1	*b*
					22	23					28	29				
	18	19			22		24				28	29			1	*b*
					22	23	24					29			1	*b*
					22	23			26			29		2	1	*b*
			20	21	22	23					28	29	2	2	2	*a*
			20	21	22						28	29				
		19	20		22	23					28	29	2		2	*a*
		19	20		22	23					28	29				
					22	23			26		28	29				
		19			22				26		28	29				
4.6	4.4	3.7	3.6	3.3	2.8	2.4	2.3	2.1	2.0	1.9	1.3	1.0				

[a] A total of 29 endogenous xenotropic and MCF virus-related *Pvu*II restriction enzyme fragments, designated XP, detected in 59 inbred mouse strain DNAs, have been ordered according to size (in kb) and the fragments carried by each inbred strain or substrain summarized. The strain distribution patterns for Ly-m10 (31), Ly-m6 (31), and Ly-m22 (32) lymphocyte alloantigens as well as the glucose-6-phosphate dehydrogenase (*Gpd-1*) alleles (1) are included for comparison.

TABLE 2. RI strain distribution patterns of pX_{env}-positive *Pvu*II restriction enzyme fragments[a]

XP	NX8 RI strains													
	3	4	5	6	9	13A	13B	15	16	17	18	19	20	R
1	N	N	N	N	N	N	N	N	8	8	N	8	8	0.69
6	8	8	N	N	N	8	8	N	8	N	8	8	N	0.46
7	N	N	8	8	N	N	N	8	8	8	8	8	N	0.46
11	+	+	+	+	+	+	+	+	+	+	+	+	+	−
13	+	+	+	+	+	+	+	+	+	+	+	+	+	−
14	8	8	8	8	N	N	8	8	8	8	8	N	N	0.31
17	N	8	8	8	N	N	N	N	N	8	N	N	N	0.69
19	N	8	N	8	N	N	N	N	8	N	N	N	8	0.69
20	N	8	8	8	N	8	8	N	8	N	N	N	8	0.46
21	8	8	8	8	N	8	8	8	N	N	N	8	N	0.38
24	N	8	8	N	N	8	8	N	8	N	8	8	N	0.46
25	8	8	N	8	8	N	N	N	8	N	8	8	8	0.38
26	+	+	+	+	+	+	+	+	+	+	+	+	+	−

XP	NX129 RI strains						
	1	2	5	7	10	12	R
11	N	1	N	1	1	N	0.50
13	+	+	+	+	+	+	−
14	1	1	N	N	1	N	0.50
17	1	N	1	N	N	1	0.50
19	N	1	N	1	N	N	0.67
20	N	1	N	1	N	N	0.67
21	1	N	N	N	N	1	0.67
24	N	1	N	1	1	1	0.33
26	1	1	1	N	N	N	0.50

XP	AKXL RI strains																		
	5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38	R
1	A	A	A	L	A	A	L	A	L	L	L	L	A	A	A	A	A	L	0.61
6	L	L	L	L	L	A	A	A	L	L	A	L	A	A	L	L	L	L	0.33
9	A	L	A	A	A	L	L	L	L	A	L	L	A	A	A	L	A	A	0.56
11	A	A	L	A	L	A	A	L	A	L	A	L	L	L	L	A	L	A	0.50
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
21	L	A	A	L	A	A	L	L	L	A	A	A	L	L	L	L	A	A	0.50
24	A	L	A	L	L	A	A	A	L	L	L	L	A	L	L	L	A	A	0.44
25	L	A	A	L	L	L	A	L	A	L	A	A	A	L	L	L	A	A	0.50

XP	AKXD RI strains																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28	R
9	−	+	+	+	+	+	−	+	+	+	++	+	+	+	+	+	+	++	−	−	+	++	+	−	−	+	++	−
11[b]	D	D	D	D	A	A	D	A	D	A	A	A	D	A	D	A	A	D	A	A	D	A	A	A	A	A	A	0.63
13[b]	D	D	D	D	D	D	A	D	D	A	A	D	A	D	A	A	A	A	D	D	A	D	A	A	A	D	A	0.48
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
20	A	D	A	D	D	D	D	A	A	D	A	D	A	A	A	D	D	A	D	A	A	A	A	D	A	A	A	0.59
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−

XP	SWXL RI strains							
	4	7	12	14	15	16	17	R
1	L	L	S	S	S	S	L	0.57
5/6	++	++	−	++	+	+	+	−
11	L	S	L	S	L	L	S	0.43
13	+	+	+	+	+	+	+	−
14	+	+	+	+	+	+	+	−
20	L	S	L	S	S	S	L	0.57
21	L	S	S	L	S	L	S	0.57
25	S	S	L	L	L	L	S	0.43
26	L	S	S	S	S	L	S	0.71

XP	CXB RI strains							
	D	E	G	H	I	J	K	R
11	+	+	+	+	+	+	+	−
13	+	+	+	+	+	+	+	−
14	B	B	C	C	B	C	B	0.57
19	C	B	C	C	B	B	C	0.43
20	+	+	+	+	+	+	+	−
24	B	C	B	B	C	B	C	0.57

XP	BXH RI strains												
	2	3	4	6	7	8	9	10	11	12	14	19	R
11	+	+	+	+	+	+	+	+	+	+	+	+	−
13	+	+	+	+	+	+	+	+	+	+	+	+	−
14	H	H	B	B	H	H	H	B	B	B	B	H	0.50
20	+	+	+	+	+	+	+	+	+	+	+	+	−

XP	BXD RI strains																										
	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32	R
9	D	D	D	B	B	D	B	D	D	B	D	B	D	D	D	B	B	D	B	D	D	D	D	B	D	D	0.35
11[b]	D	D	D	B	B	B	D	D	B	B	B	D	B	D	D	B	B	B	B	B	D	D	B	B	D	D	0.54
13[b]	D	B	D	B	D	D	B	B	D	D	D	B	D	D	B	B	B	D	D	D	D	B	D	B	D	B	0.46
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
20	B	B	D	B	B	D	D	B	D	B	D	B	B	B	B	B	D	D	D	D	D	D	D	D	B	D	0.46
24	B	B	D	B	B	B	D	B	B	B	D	B	B	B	B	D	D	B	B	D	B	D	B	D	B	B	0.69

[a] The XP fragment distribution patterns in the NX8, NX129, AKXL, AKXD, BXD, SWXL, CXB, and BXH, RI strains (for crosses, see text) are summarized. A plus (+) sign denotes that both progenitor strains carried the same size XP fragment. In most cases, these fragments represented the same proviral locus because they did not segregate in the RI strain members. However, XP-9 (AKXD) segregated as two distinct loci and was typed based upon its hybridization intensity. XP-5 and XP-6 (SWXL) were of similar sizes and could not be definitively assigned in this analysis. The segregation ratio (R) of each XP fragment was determined by dividing the number of RI strains carrying the female progenitor allele by the total number of RI strains analyzed.

[b] XP-11 and XP-13 in the AKXD and BXD RI strains were typed according to their relative hybridization intensities.

was present in both progenitors of an RI strain family segregated as a single proviral locus (Table 2).

In DBA/2J DNA, XP-11 and XP-13 had higher relative hybridization intensities than in other strains such as AKR/J and C57BL/6J. Consequently, in AKXD and BXD RI mice it was possible to derive a segregation pattern for XP-11 and XP-13 based upon their hybridization intensities. No XP-11- or XP-13-negative strains were observed in the 53 AKXD and BXD RI strains analyzed, suggesting that the proviral loci represented by XP-11 and XP-13 in AKR/J and C57BL/6J DNAs are also present in DBA/2J DNA. We could not, however, determine from the current data whether the proviral DNA sequences present in DBA/2J DNA that account for the increased hybridization intensities of XP-11 and XP-13 are linked to the commonly shared XP-11 and XP-13 proviral loci.

A third instance of elevated band intensity was seen in C57L/J and C58/J DNAs. Both of these related strains showed elevated XP-21 hybridization intensities relative to the other single-copy bands within these same strains or relative to XP-21 in 129/J DNA. Nonetheless, segregation ratios of 0.50, 0.38, and 0.57 for XP-21 in the AKXL, NX8, and SWXL RI strains, respectively, indicated that XP-21 segregates as a single locus of uniform elevated hybridization intensity. These observations suggest that XP-21 is composed of two or more proviral genomes that are closely linked and segregate as a single Mendelian unit. As shown in the following section, XP-21 carried by C57L/J and C58/J mice mapped to the same chromosomal position as XP-21 in 129/J mice, so that all of these proviral sequences were associated with a single proviral locus.

Mapping of XP fragments to defined chromosomal regions of the mouse genome. Three XP fragments, XP-1, XP-14, and XP-20 segregated concordantly with mouse lymphocyte alloantigens Ly-m10.1, Ly-m6.2, and LY-m22.2 (Table 3). The loci coding for these antigens are on chromosomes 19 (31), 2 (24, 31), and 1 (32), respectively.

XP-20 segregated concordantly with the lymphocyte alloantigen Ly-m22.2 in 65 RI strains, including 27 AKXD, 26 BXD, 5 NX129 (NX129-2 was not analyzed), and 7 SWXL RI strains. Of the RI strains, 32 were XP-20 positive and Ly-m22.2 positive, and 33 strains were XP-20 negative and Ly-m22.2 negative (designated Ly-m22.1 positive). Thus, in a total of 65 RI strains, there were no recombinants between XP-20 and the lymphocyte alloantigen Ly-m22.2. Of the inbred strains (Table 1) typed for XP-20, 34 also have been typed for Ly-m22.2. With two exceptions, AU/SsJ and SEA/GnJ, XP-20 was again concordant with Ly-m22.2.

Similarly, XP-14 was concordant with the lymphocyte alloantigen Ly-m6.2 on chromosome 2 in all but one RI strain analyzed. Of 25 BXH, CXB, and NX129 RI strains typed, 13 were XP-14 positive, Ly-m6.2 positive, 11 were XP-14 negative, Ly-m6.2 negative (designated Ly-m6.1 positive), and one was XP-14 negative, Ly-m6.2 positive. The single negative association occurred in the BXH-2 RI strain, a strain that is unique in the RI strains typed because throughout life they spontaneously produce a B-ecotropic virus (B-ecotropic viruses are thought to be recombinant viruses derived from N-ecotropic and xenotropic viruses) (4, 14). Of the 18 inbred strains (Table 1) typed for both XP-14 and Ly-m6.2, 10 are XP-14 positive and Ly-m6.2 positive, and 6 are XP-14 negative and Ly-m6.2 negative. The two discordant strains identified, MA/MyJ and RF/J, are XP-14 negative and Ly-m6.2 positive.

The strain distribution pattern of XP-1 in the 17 AKXL (AKXL-12 was not analyzed) RI strains showed that this fragment is linked to the locus encoding the lymphocyte alloantigen Ly-m10.1 on chromosome 19. No recombinants were found between these two loci in all 17 AKXL RI strains analyzed. Seven of the strains were XP-1 positive and Ly-

TABLE 3. Summary of the chromosomal location of endogenous xenotropic proviral DNA sequences detected by *Pvu*II digestion and hybridization with a pX_{emv} Probe

Xenotropic proviral locus[a]	Associated gene	Chromosomal location	RI series typed	No. of recombinants/ total tested	Map distances (cM)[b]
Xp-1	*Ly-m10*	19	AKXL	0/17	<5.3
Xp-9	*Igh-C*	12	AKXL	1/17	1.6 ± 1.7
Xp-11	*Rmcf*	5	AKXD	2/27	2.1 ± 1.6
Xp-14	*Ly-m6*	2	BXH CXB NX129	1/25	1.1 ± 1.1
Xp-20	*Ly-m22*	1	AKXD BXD NX129 SWXL	0/65	<1.2
Xp-21	*Gpd-1*	4	AKXL NX129 NX8 SWXL	2/43	1.3 ± 0.9

[a] The six XP fragments mapped to defined chromosomal locations based upon their segregation in the RI series, as described in Table 2, have been given *Xp* locus designations. *X* denotes the hybridization probe (pX_{emv} probe), and *p* denotes the restriction enzyme (*Pvu*II) used in the identification of the XP fragments. The number of the locus designates the XP fragment associated with it.

[b] Estimates of map distances in cM and their standard errors are calculated by methods that relate the observed frequency of recombinants among RI strains to the recombination frequency. In cases in which no recombinant was detected, the 95% upper confidence limits of map distances are calculated. The equations used in these calculations have been described by Taylor (33).

m10.1 positive, and 10 were XP-1 negative and Ly-m10.1 negative (designated Ly-m10.2 positive). Of 18 inbred strains (Table 1) typed for XP-1 and Ly-m10.1, two strains are XP-1 positive, Ly-m10.1 positive and 12 strains are XP-1 negative, Ly-m10.1 negative. Of the four discordant strains identified, three strains are XP-1 negative, Ly-m10.1 positive and one strain is XP-1 positive, Ly-m10.1 negative.

XP-21 segregated concordantly in 41 of 43 RI strains analyzed with the glucose-6-phosphate dehydrogenase-1 allele, *Gpd-1*^{a}, on chromosome 4. Of the 43 AKXL, NX129 (NX129-2 was not analyzed), SWXL, and NX8 RI strains analyzed, only the two strains NX8-3 and NX8-19 were discordant (XP-21 positive and *Gpd-1*^{b}) (12). Of the 7 inbred strains (Table 1) that are XP-21 positive, all but LP/J carried the *Gpd-1*^{a} allele. However, among the XP-21 negative strains, both the *Gpd-1*^{a} and *Gpd-1*^{b} alleles are widely represented.

The doubly intense XP-11 band seen in DBA/2J DNA mapped near a locus on chromosome 5 that affects susceptibility or resistance to infection by an exogenous MCF retrovirus (15). DBA/2J mice carry the resistant allele (*Rmcf*^{r}), whereas AKR/J mice carry the susceptible allele (*Rmcf*^{s}). Only two AKXD strains, AKXD-5 and AKXD-25, of the 27 AKXD RI strains analyzed were recombinants between XP-11 (as scored by relative hybridization intensity) and the *Rmcf*^{r} allele. Both of these RI strains appeared to carry the singly intense XP-11 band of AKR/2J yet were *Rmcf*^{r}.

XP-9 was mapped in 17 AKXL RI strains (AKXL-7 was not analyzed) to the constant region of the immunoglobulin heavy chain locus, *Igh-C*, on chromosome 12 (34). Only one discordant strain, AKXL-12, was identified. As was noted previously (Table 2), the XP-9 fragment in DBA/2J is not linked to the AKR/J XP-9. Not surprisingly, the XP-9 fragment of DBA/2J showed no linkage with *Igh-C* in the BXD RI strains.

A summary of the linkages found between the XP bands and their associated gene markers is given in Table 3. Where an XP band was shown to segregate as a single locus and was mapped to a specific chromosome, we have designated that particular XP fragment as an *Xp* locus (*Xp* refers to endogenous xenotropic virus-related locus identified by *Pvu*II digestion and hybridization with the pX_{env} probe). Individual *Xp* loci are identified by a numerical designation based upon the XP band they are associated with. This nomenclature is consistent with that used previously for the endogenous avian leukosis viruses (2), mouse mammary tumor viruses (36), and ecotropic MuLVs (20).

DISCUSSION

We have used a hybridization probe, designated pX_{env}, specific for xenotropic and MCF proviruses to study the organization and distribution of these sequences in chromosomes of inbred mouse strains. To date, we have identified at least 29 distinct pX_{env}-positive restriction enzyme fragments resulting from *Pvu*II digestion (designated XP fragments) in DNAs of 59 different inbred mouse strains and substrains. Most of these XP fragments appeared to represent single proviral loci based upon their segregation patterns in recombinant inbred mice. As yet, we do not know whether these fragments represent xenotropic or MCF proviral DNA sequences, as the pX_{env} probe does not discriminate between these two classes of viruses. We also are unable to determine whether the XP fragments represent internal or cell-virus junction fragments. However, the presence of common proviruses shared by many related strains and substrains of mice indicates that most of the proviral sequences were acquired before the establishment of inbred mouse strains and that these viral integrations are relatively stable.

Of 14 low-copy number XP fragments that were represented in one or more of the RI series screened, 6 were mapped to defined chromosomal locations based upon their segregation patterns within the various RI series analyzed. These six proviral loci mapped to six different chromosomes (chromosomes 1, 2, 4, 5, 12, and 19) and in three cases were found to be closely associated with genes encoding the mouse lymphocyte cell surface antigens; Ly-m6, Ly-m10, and Ly-m22. These Ly antigens were identified and mapped by Tada et al. (31, 32) using monoclonal antibodies. Interestingly, the genes controlling the expression of murine cell surface antigens are not randomly scattered throughout the genome, but instead occur in gene-clusters, e.g., (i) *Lyt-1/Ly-m10* on chromosome 19, (ii) *Mls/LyM/Ly-m20/Ly-m22* on chromosome 1, (iii) *Ly*-m6/*Ly-m11/H-3* on chromosome 2, and (iv) *Ly-ml9/Lyb-2* on chromosome 4 (31). The presence of these gene-clusters may reflect a higher order of organization of genes that are functionally closely related and their molecular analysis may give important insights into the functioning of genes involved in lymphocyte development.

The close linkage of three of six proviral loci to loci encoding Ly antigens suggests that these endogenous proviruses have some function in normal lymphocyte development or, in fact, encode these particular Ly antigens. At present, it is not possible to determine whether either of these alternatives is correct. However, since retroviral *env* gene products are often expressed on the cell surface (25), it is not unreasonable to conclude that in some cases, lymphocyte cell surface antigens are encoded by endogenous retrovirus-related sequences. Importantly, in all three cases in which we found an association of an Ly antigen with xenotropic virus-related sequences, it was the allele encoding the lymphocyte antigen that was virus associated. In addition, only 1 discordant RI strain out of 107 RI strains analyzed was found between an Ly antigen and its associated proviral genome indicating that these loci are closely associated. The one discordant strain, BXH-2, which is XP-14 negative and Ly-m6 positive, is unique among the RI strains analyzed in that throughout life it produces a B-ecotropic MuLV (3). As B-ecotropic viruses are thought to be recombinants between N-ecotropic and xenotropic viruses (4, 14), it is possible that the Ly-m6 monoclonal antibody recognizes determinants on the BXH-2 B-ecotropic virus. If this were the case, then BXH-2 mice would be Ly-m6 positive, although the antigen was not encoded by the common Ly-m6 locus. It is this property of retroviruses, their ability to encode the same antigen but be integrated at many different chromosomal sites, that complicates this analysis. It is also conceivable that the Ly antigens are encoded by cellular DNA sequences flanking proviral DNA integration sites, the expression of which are somehow affected by viral DNA integration.

Of the 70 inbred strains and substrains that were typed for either Ly-m6, Ly-m10, or Ly-m22, 62 were concordant for their associated proviral loci. Of the eight discordant strains identified, three were virus positive and Ly antigen negative, and five were virus negative and Ly antigen positive. This is again inconsistent with the hypothesis that Ly antigens are virally encoded. However, these inconsistencies could also be explained by: (i) related Ly antigens are encoded by more than one proviral locus, (ii) some proviral loci have suffered mutations that prevent their expression,

(iii) some strains carry regulatory sequences that affect provirus expression, or (iv) the presence of different proviral loci which produce the same sized XP fragments.

A fourth XP fragment, XP-21, mapped near *Gpd-1* (1.3 ± 0.9 centimorgans [cM] on chromosome 4. In turn, *Gpd-1* is located within 1 cM of *Fv-1* (Friend virus restriction locus) (35), a gene affecting susceptibility or resistance of cells to ecotropic virus infection, as well as a gene that has a major influence on xenotropic virus cell surface antigen levels (25). The xenotropic MuLV cell surface antigen is a virus-related antigen(s) expressed on the lymphocyte cell surface of all inbred mice. However, the levels of xenotropic MuLV cell surface antigen vary from strain to strain, and the gene that primarily affects these levels has been mapped to *Fv-1* or a closely linked gene (25). Therefore, four of six XP fragments that we mapped were found to be closely associated with genes that affect lymphocyte cell surface antigens. Interestingly, a fifth proviral locus, *Xp-11*, maps near a gene affecting susceptibility or resistance to MCF virus infection. Whether *Xp-11* directly affects MCF virus susceptibility is not yet clear. By using these proviral DNA sequences as molecular markers for use in cloning experiments, it may be possible to elucidate the functions of these and other proviral genes within the mouse genome.

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