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Genetic Analysis of Endogenous Xenotropic Murine Leukemia Viruses: Association with Two Common Mouse Mutations and the Viral Restriction Locus *Fv-1*

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We have defined 40 endogenous xenotropic virus (*Xmv*) loci from several common inbred strains of mice by examining provirus-cell DNA junction fragments in recombinant inbred mice. Some inbred strains carried unique proviruses, but most *Xmv* loci were present in several strains, indicating that many *Xmv* integration events preexisted modern inbreeding. It was also clear that most *Xmv* junction fragment variation between inbred strains resulted from independent integration events and not modification or restriction site polymorphism following integration. Chromosomal assignments were determined for 32 *Xmv* loci by comparing their recombinant inbred strain distribution patterns to those of known genetic markers. The *Xmv* loci were generally dispersed throughout the genome, but several chromosomal regions contained more than one provirus. Furthermore, several close genetic associations with cellular genes were discovered. Four *Xmv* loci were closely linked to *Fv-1*^{b}, a dominant viral resistance gene present in C57BL/6J, BALB/cJ, A/J, and several other strains. *Xmv-28* was closely linked to *rd* (retinal degeneration), and *Xmv-10* was closely linked to *a* (non-agouti), both of which are old mutations as inferred from their broad distribution in mice. We suggest that *Xmv* integration contributed to genetic diversity in the past and that much of this diversity exists today in common laboratory strains.

Endogenous retroviruses can be found in the germ lines of many vertebrates. These proviral elements are interspersed throughout the chromosomes of an individual and are typically heritable in a stable Mendelian fashion. Three of the four morphological groups of the family *Retroviridae* are represented as endogenous proviruses in common strains of laboratory mice: type A (intracisternal-A particles; 500 to 1,500 elements per haploid genome), type B (mouse mammary tumor virus; 0 to 8 elements), and type C (murine leukemia virus [MLV] related; 30 to 60 elements) (for reviews, see references 6 and 34). In addition, a sizable number of more distantly related proviruses reside in the mouse genome (GLN, VL30, and related elements; 10, 11). Given the great number of endogenous proviruses that have been acquired in mice, it is not surprising that some integration events resulted in specific genetic consequences for the host. Known instances include dominant resistance to viral infection by the *Fv-4* gene product (17, 38) and naturally occurring recessive mutations caused by proviral insertional inactivation of the cellular genes dilute (7, 12) and hairless (37). Since only a fraction of all endogenous proviruses has been well defined, it seems likely that further characterization will yield additional, possibly novel types of functional viral associations with cellular genes. With this in mind, our primary goal was to identify genetically endogenous type C murine proviruses of inbred mice in a comprehensive manner.

Endogenous type C murine proviruses are of biological interest because of their relationship to MLVs, which appear in high-leukemic strains of mice (6, 34). They have been divided into four subclasses based on the potential host range encoded in the *env* gene (35). Most contain either a polytropic or modified polytropic viral envelope. A smaller number have a xenotropic envelope, and a handful encode an ecotropic envelope (13, 22, 36). It is likely that one or a few members of the polytropic and xenotropic classes donate *env* and long terminal repeat (LTR) sequences, respectively, for the majority of leukemogenic mink cell focus-forming (MCF) recombinant viruses (9, 15, 26, 35; J. P. Stoye, C. Moroni, and J. M. Coffin, manuscript in preparation).

Endogenous nonecotropic MLVs have been extremely difficult to analyze genetically because of their numbers (30 to 60 per strain) and the lack of specific probes. While some loci were mapped using nonecotropic *env* probes (3, 40), previous studies did not allow identification of the majority of nonecotropic proviruses. Smaller cloned probes (22) and synthetic oligonucleotides (36, 37) discriminate between nonecotropic MLV subclasses, each reacting with a subset of fragments on Southern blots as compared with earlier probes. We have used the oligonucleotides to analyze nonecotropic MLVs, by examining provirus segregation in recombinant inbred (RI) mice.

RI mice are derived from random matings of second-generation progeny of two genetically distinct inbred strains and subsequent inbreeding by brother-sister mating for at least 20 generations. This protocol results in a unique, presumably random mixture of the genomes of the two progenitors for each RI strain. Each genetic marker that is polymorphic between the founder strains has a characteristic strain distribution pattern (SDP) in a set of RI strains (1, 39). Provirus-cell DNA junction fragments also segregate as discrete genetic units and can thus be identified by using RI strains. SDP analyses can be used further to assess linkage between two different markers, because linked genes tend to segregate together and have similar SDPs. They can also be used to determine probable gene order of closely linked loci and to estimate map distances between pairs of loci. Endog-

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enous type C proviruses should be valuable tools in RI genetics, providing a large, dispersed series of chromosomal markers that can be accessed by conventional molecular cloning procedures.

Described herein are 40 endogenous *Xmv* (xenotropic murine virus) loci (9) from A/J, AKR/J, BALB/cJ, C57BL/6J, C57L/J, C3H/HeJ, and DBA/2J inbred strains, as defined by junction fragment segregation in RI mice. In addition to providing chromosomal locations for most *Xmv* proviruses, these analyses revealed several novel, intriguing associations between *Xmv* proviruses and cellular genes. In future communications we will describe the MCF virus-related endogenous polytropic and modified polytropic loci, for which we propose the gene symbols *Pmv* and *Mpmv*, respectively (manuscripts in preparation).

MATERIALS AND METHODS

Mice. Most inbred and RI mice described herein were purchased from The Jackson Laboratory (Bar Harbor, Maine) between July 1987 and February 1988; DNA samples taken before 1984 were used for the BXD-31 and BXD-32 strains. Spleen DNAs from the CXB, AXB, BXA, LXB, SWXL, LXPL, and BXJ RI strains were kindly provided by N. Copeland and N. Jenkins (National Cancer Institute, Frederick Basic Research Facility, Frederick, Md.). Various other inbred strains were kindly provided by Paul Neumann (Children's Hospital, Boston, Mass.). The AXB and BXA sets were originally founded by, and are maintained in the laboratory of, M. Nesbitt (University of California at San Diego, La Jolla).

DNA preparation, electrophoresis, and Southern blotting. High-molecular-weight spleen or liver DNA was prepared as previously described with minor modifications (8, 36). Briefly, cells were lysed by using a solution of 10 mM Tris hydrochloride (pH 7.5), 5 mM EDTA, 5 mM NaCl plus 1% sodium dodecyl sulfate, and 100 μg of pronase per ml and incubated overnight at 37°C. Nucleic acids were then purified by several phenol-chloroform-isoamyl alcohol extractions, precipitated with ethanol, and suspended in 10 mM Tris hydrochloride (pH 7.5)–5 mM EDTA–5 mM NaCl. Cellular RNA was removed by digestion with pancreatic RNase A, and high-molecular-weight genomic DNA was precipitated in ethanol. DNA (8 or 10 μg) was digested with either *Eco*RI or *Pvu*II as recommended by the manufacturer (Boehringer Mannheim Biochemicals and New England BioLabs, Inc., products were used interchangeably) and fractionated by electrophoresis in 0.8% agarose-TBE (36) gels at 75 V for 21 h. Optimal fragment resolution was achieved when the 2.0-kilobase-pair *Hin*dIII-digested lambda DNA marker was run to approximately 21 cm from the origin on a 23-cm-long horizontal apparatus; no proviral fragments smaller than 1.8 kilobase pairs were observed previously. "Smiling" fragments (see Fig. 2) were avoided by not incorporating ethidium bromide into the gel, which was stained after electrophoresis (see Fig. 3). The gels were processed and transferred to nitrocellulose membranes (Schleicher & Schuell, Inc.) as previously described (8, 36, 37).

Oligonucleotide hybridizations. Oligonucleotide preparation, labeling, and genomic blot hybridizations were done as described by Stoye and Coffin (36) with slight modifications. Briefly, the *Xmv*-specific 28-mer oligonucleotides JS-6 and JS-10 were purified by high-performance liquid chromatography, mixed together (JS 6-10) in an equimolar ratio, 5′ end labeled with [^{32}P]ATP to an approximate specific activity of

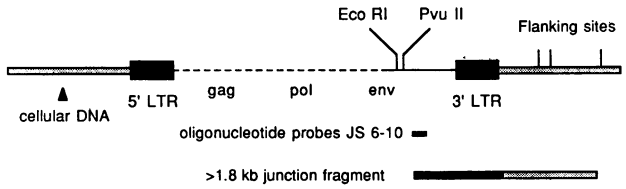


FIG. 1. A typical endogenous *Xmv* provirus. A composite restriction map is shown of three xenotropic proviruses cloned from HRS/J mice (35; unpublished results); it depicts provirus-cell DNA junction fragments. Restriction sites: E, *Eco*RI; P, *Pvu*II. ▬, LTRs; ——— and — — —, the viral genome; ▥, cellular DNA. Several other *Pvu*II restriction sites, but no *Eco*RI sites, are present in the proviral region depicted as a dashed line. kb, Kilobase pair.

10^9 cpm/μg, and separated from free [^{32}P]ATP by use of a C18 SEP-PAK syringe column (Waters Associates, Inc.). Filters were prehybridized for at least 2 h at 62°C in an appropriate volume of 6× NET (36)–10× Denhardt solution–0.5% Nonidet P-40–1% sodium dodecyl sulfate–100 μg of denatured salmon sperm DNA per ml plus 50 μg of yeast RNA per ml. Radiolabeled probe was added to a final concentration of 0.5×10^6 cpm/5 ml of hybridization buffer, and hybridization was allowed to proceed for 12 to 16 h at 62°C. Filters were washed four times in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.2% sodium dodecyl sulfate for 15 min at 25°C and then washed four times in the same solution at 62°C. Filters were dried and exposed to Kodak XAR-5 film at −70°C for 15 to 21 days using a Cronex Lightning-Plus intensifying screen (E. I. du Pont de Nemours & Co.).

Linkage analysis in RI strains. SDPs of provirus-cell DNA junction fragments segregating in RI strains were compared with those of known markers. Values for estimated recombination frequencies (r) were calculated by using the formula: $r = R/(4 - 6R)$, where R is the number of discordant strains per the number of total strains. The standard error (SE) of r was calculated by using the formula $SE = \{[r(1 + 2r)(1 + 6r)^2]/4n\}^{1/2}$, where n is the total number of strains analyzed; all values of r shown in Table 2 were significant. Upper and lower limits for r were calculated at the 95% confidence limits by the method of Silver (30).

RESULTS

We employed the synthetic oligonucleotide probes JS-6 and JS-10 (36) to identify endogenous *Xmv* loci because they afford good resolution of provirus-cell DNA junction fragments on Southern blots. This is due to the detection of viral fragments that are of minimal size, since the probes and restriction sites used are near the 3′ end of the proviral genome. The oligonucleotides JS-6 and JS-10, whose sequences were based on a cloned NZB xenotropic virus (21) and a cloned HRS xenotropic provirus (35), respectively, are specific for xenotropic *env* genes and do not react with polytropic or ecotropic sequences (36). Because the *Eco*RI and *Pvu*II sites are well conserved (35), JS 6-10 reacts with unique provirus-cell DNA junction fragments when hybridized to *Eco*RI- or *Pvu*II-digested DNA (Fig. 1).

Distribution of *Xmv* loci in several common strains of inbred mice. The molecular sizes of *Xmv Eco*RI junction fragments vary among inbred strains that are progenitors of RI mice (Fig. 2; 36). We studied this observation further by examining junction fragment segregation in RI mice. SDPs of segregating *Xmv* loci were obtained and matched to each other so that each locus was defined by both *Eco*RI and

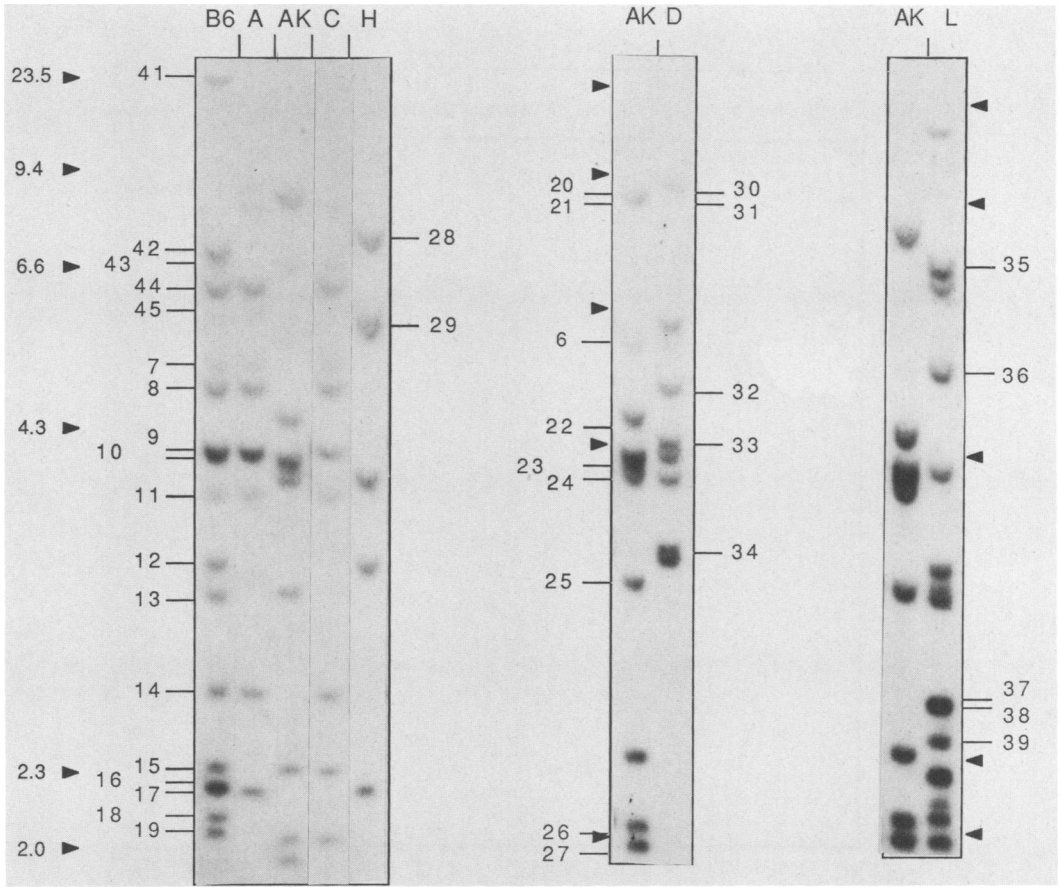


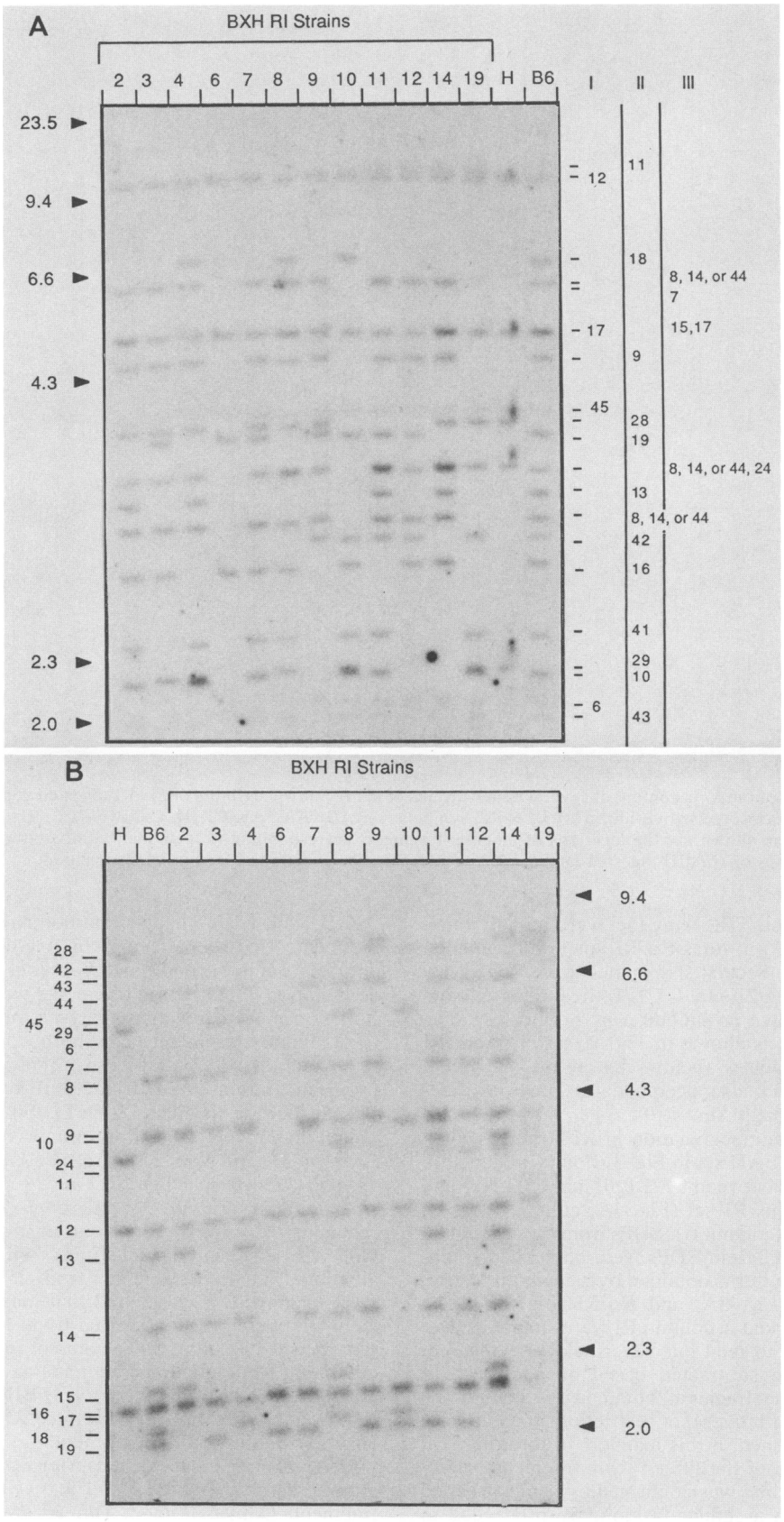
FIG. 2. A comparison of the *Xmv* content of several inbred strains of mice. Shown are blots of *Eco*RI-digested genomic DNA from inbred strains fractionated in agarose gels, hybridized to JS 6-10. Symbols: A, A/J; AK, AKR/J; B, C57BL/6J; C, BALB/cJ; D, DBA2/J; H, C3H/HeJ; L, C57L/J. Also shown are the locations of junction fragments corresponding to *Xmv* loci, numbered as described in the text. Arrowheads show positions of *Hin*dIII-digested lambda DNA molecular size standards (sizes in kilobase pairs).

*Pvu*II junction fragments. The *Xmv* loci were initially numbered *Xmv-1* through *Xmv-40* as *Eco*RI junction fragments, based on descending molecular size in the strains C57BL/6J, AKR/J, C3H/HeJ, DBA/2J, and C57L/J (the strains A/J and BALB/cJ appear to have no unique *Xmv* proviruses). The final *Xmv* numbering, as shown in Fig. 2, results from RI strain segregation analyses (below) based on our initial numbering scheme and also accomodates previously reserved loci *Xmv-1* through *Xmv-5* (9).

Xmv junction fragment segregation in RI strains is illustrated by the 12-strain BXH set in Fig. 3. Four viral junction fragments were shared between C57BL/6J and C3H/HeJ and did not segregate in this RI set (Fig. 3A, column I). Most proviruses segregated (column II). SDPs from these could be directly obtained; 11 different SDPs were immediately confirmed by comparing patterns obtained by using both restriction enzymes *Pvu*II (Fig. 3A) and *Eco*RI (Fig. 3B). The proviral fragments marked in column III were similar in size, making SDPs difficult to read but nevertheless segregated. Those SDPs could be subtracted based on a clear SDP reading of the junction fragment obtained for one of the proviruses analyzed with the other restriction enzyme. Five more SDPs were obtained in this manner. In toto, an SDP was obtained for each of the fifteen *Xmv* fragments segregating in the BXH set and was confirmed by using a second restriction enzyme. In a similar fashion, we proceeded to examine *Xmv* segregation in the BXD, AXB, CXB, AKXD, AKXL, and LXB RI sets (data not shown). Almost all *Xmv* fragments that were shared in one RI set segregated in another (Table 1), and most *Xmv* loci deemed identical by comigration of restriction fragments were confirmed as such in the ensuing segregation analyses, or by comigration of a third junction fragment, or by both.

A total of 40 *Xmv* loci were identified in the seven progenitor strains (*Eco*RI junction fragments are shown in Fig. 2; loci are listed in Table 2). Fifteen proviruses were unique to a given progenitor strain (*Xmv-20*, *Xmv-21*, *Xmv-22*, *Xmv-23*, and *Xmv-25* in *AKR/J*; *Xmv-30*, *Xmv-32*, *Xmv-33*, and *Xmv-34* in DBA/2J; *Xmv-28* in C3H/HeJ; *Xmv-35*, *Xmv-36*, *Xmv-37*, *Xmv-38*, and *Xmv-39* in C57L/J), and the remaining 25 were present in at least one other progenitor. Only *Xmv-6* appeared in all strains. When *Eco*RI, *Pvu*II, and additional junction fragments from 25 other inbred strains were examined, it was found that many apparently unique *Xmv* loci were actually carried by at least one other strain (unpublished results). Since some of these strains contained additional *Xmv* junction fragments and also served as progenitors for RI strains (e.g., NZB/BINJ, C58/J, 129/J, and SM/J), approximately 10 additional *Xmv* loci may be similarly defined in future experiments.

Heterogeneity in endogenous *Xmv* envelope sequences. The autoradiograms in Fig. 2 and 3 revealed that a subset of fragments (*Xmv-6*, *Xmv-7*, *Xmv-20*, *Xmv-31*, *Xmv-40*, *Xmv-43*, and *Xmv-45*) reacted poorly with the probe mixture. We


A
BXH RI Strains
2 3 4 6 7 8 9 10 11 12 14 19 H B6
I II III
23.5
9.4
6.6
4.3
2.3
2.0
12
17
45
6
11
18
8, 14, or 44
7
15,17
9
28
19
8, 14, or 44, 24
13
8, 14, or 44
42
16
41
29
10
43
B
BXH RI Strains
H B6 2 3 4 6 7 8 9 10 11 12 14 19
9.4
6.6
4.3
2.3
2.0
28
42
43
44
45
29
6
7
8
9
10
24
11
12
13
14
15
16
17
18
19

believe that *Xmv-43* corresponds to the inducible xenotropic provirus *Bxv-1*, since a *Bxv-1*-specific LTR probe (Stoye et al., in preparation) reacts with junction fragments identical in size to *Xmv-43* and cosegregates with *Xmv-43*. The poorly hybridizing fragments were probably related *Xmv* proviruses that were mismatched with the JS 6-10 probe. Fortuitously, this made it somewhat easier to match *Xmv* junction fragments in the RI strain analyses and also in other inbred strains. While there was no obvious correlation between variable *Xmv* hybridization and RI or inbred strain distribution, it is possible that the heterogeneity reflects mixed historical origins of *Xmv* loci not easily delineated by the known genealogy of the inbred strains. Sequence heterogeneity in *Xmv* proviruses has also been observed in the LTR, in internal restriction sites, and in the *Fv-1* determinant in the viral CA protein (4; unpublished results). We have found this type of nucleotide sequence heterogeneity to be unique to *Xmv* loci, since in similar studies of MCF-related endogenous MLVs neither *Pmv* nor *Mpmv* proviruses varied greatly in their hybridization to the corresponding oligonucleotide (unpublished results).

Linkage analyses. SDPs for all *Xmv* loci segregating in the RI strains (listed in Table 1) were compared with those of both published and unpublished loci previously typed in the RI sets. Many *Xmv* loci had SDPs that were identical or nearly identical to those of known loci. Thus, 32 of the 40 *Xmv* loci could be assigned to a chromosome based on linkage to a previously mapped locus. The nature of these assignments is presented in Table 2, as are values for recombination frequency (r) with lower and upper limits calculated at the 95% confidence level. Several linkage relationships will now be described.

***Xmv* proviruses linked to the *Fv-1*^{b} gene on chromosome 4.** *Xmv-8*, *Xmv-9*, *Xmv-14*, and *Xmv-44* had perfect concordance in 36 RI strains examined together and with the *Fv-1*^{b} viral resistance gene of C57BL/6J mice (Tables 1 and 2), and on the basis of the 95% confidence limits, they were less than 2.85 centimorgans (cM) from each other and from *Fv-1*. As judged by analysis of *Eco*RI, *Pvu*II, and additional 3′ junction fragments, each provirus was also present in BALB/cJ and A/J mice (Fig. 2) and in three other *Fv-1*^{b} inbred strains examined (I/LnJ, RIIIS/J, and FVB/N; data not shown) and was absent from all *Fv-1*^{n} and *Fv-1*^{nr} strains tested (data not shown). This additional concordance suggests that the loci are closer than 2.85 cM apart, given the additional generations of breeding of the above strains since their presumptive points of divergence.

Concordance between *Xmv-28* and the *rd* mutation. *Xmv-28*, which was unique to C3H/HeJ among the seven progenitor strains (Table 2), had the same SDP as the *rd* (retinal degeneration) mutation in the 12-strain BXH set (Table 1). We also examined *Xmv-28* fragments in the BXJ, LXPL, and SWXL RI sets and continued to find concordance of *Xmv-28* with *rd* in a total of 13 additional strains (Table 1). As judged by analysis of *Eco*RI, *Pvu*II, and additional junction fragments, *Xmv-28* was found to be present in all *rd* strains and absent from all *rd*$^+$ strains tested (unpublished results). The sum of RI strain analyses indicates that these two loci are less than 4.32 cM apart (Table 2); moreover, their concordance among the inbred strains suggests closer linkage.

***Xmv-10* is closely linked to the *a* mutation.** The mutation non-agouti, *a*, which is carried by C57BL/6J, AKR/J, DBA/2J, and C57L/J, and several other inbred strains (32), had concordance with *Xmv-10* in the BXH and CXB RI sets, as well as in the BXJ, SWXL, and LXPL RI sets (total of 33 strains; Table 1). *Xmv-10* was absent from inbred strains carrying other alleles at the agouti locus and was present in all *a* strains examined except SM/J and ST/bJ (unpublished results). The RI data indicate that *Xmv-10* is less than 3.14 cM from *a* (Table 2), and the concordance in most inbred strains suggests close linkage. However, the absence of *Xmv-10* from two non-agouti inbred strains may imply that it did not cause the mutation.

Y-linked *Xmv* loci. *Xmv-7* and *Xmv-11* were on the Y chromosome in strains A/J, BALB/cJ, C57BL/6J, and C57L/J as judged by analysis of *Eco*RI, *Pvu*II, and additional junction fragments of male and female mice (Table 2). Most other strains that carried *Xmv-7* also had *Xmv-11* (unpublished results). A third provirus, *Xmv-40*, was also male specific but hybridized very poorly to the probes used in this study and was therefore more difficult to characterize (data not shown). Neither *Xmv-7* nor *Xmv-11* had inbred strain distributions quite as broad as the *Mus musculus* subsp. *musculus*-specific Y chromosome repetitive sequence (2), and thus they probably entered the germ line after subspeciation. Either of these proviruses may correspond to the endogenous Y-linked MLV reported earlier (24), given the similarity in strain distribution.

Relationship of *Xmv* loci to previously defined nonecotropic MLVs. Our RI strain analyses were compared with earlier studies using less-specific nonecotropic probes to define endogenous MLVs in RI strains. Both the *env* loci (3) and the *XP* loci (40) are collectively termed *Xmmv*, for xenotropic MCF murine virus. Theoretically, the *Xmv*, *Pmv*, and *Mpmv* loci should encompass all nonecotropic MLVs that do not have deletions of the probe-reactive region (36). A direct comparison with previous studies is difficult owing to the different nature of the *env* probes and experimental approaches. Therefore, only RI set SDPs and inbred strain distributions could be compared to estimate identity between proviruses. We conclude that *Xmv-42* and *Xmmv-3*, *Xmv-16* and *Xmmv-2*, and *Xmv-34* and *Xmmv-1* are possibly identical by these criteria. *Xmv-32* had concordance with *Xmmv-61* in 50 RI strains but was present in the opposite inbred strain and may simply be closely linked (*Xmmv-61* was defined as a C57BL/6J fragment [40]). Likewise, it was not possible to affirm or deny identity between previously defined BALB/cJ loci *Xmv-1* through *Xmv-5*, identified using somatic cell hybrids (9), and certain *Xmv* loci defined here. Several other *Xmmv* loci had good matches with *Pmv* or

FIG. 3. Segregation of *Xmv* junction fragments in the BXH sets of RI strains. *Pvu*II-digested (A) and *Eco*RI-digested (B) genomic DNAs from males of BXH strains 2, 3, 4, 6, 7, 8, 9, 10 (founded by using a C3H/HeJ male and C57BL/6J female), 11, 12, 14, and 19 (constructed by the reciprocal cross) plus the two progenitor strains were fractionated on agarose gels, blotted, and hybridized to JS 6-10. Fragments corresponding to particular *Xmv* loci are numbered in both panels. Columns I, II, and III in panel A indicate nonsegregating, segregating, or comigrating *Xmv* fragments, respectively, as described in the text. *Pvu*II fragments corresponding to *Xmv-8*, *Xmv-14*, and *Xmv-44* were ambiguous because these loci cosegregated in all genetic crosses analyzed. The DNA sample from strain BXH-19 was only partially digested with *Eco*RI in panel B; this sample was re-analyzed on other occasions. Arrowheads show positions of *Hin*dIII-digested lambda DNA molecular size standards (sizes in kilobase pairs).

TABLE 1. RI SDPs of 36 *Xmv* loci[a]

Locus	RI Set	+ Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	I	II	III
Xmv-41	CXB	B	C	B		C	B	B	C	C																																
	BXH	B		B	H	B		H	B	B	H	B	B	H		H					B																					
	BXD	B	B	D			D	B		D	D		D	B	B	B	B	B		B	B	B	B	D	B	D	D		D	D	B	D	B	D					A	A	X	E	s
	AXB	B	A	A	B	B	B	B	A	B	B	B	B	A	B	B				B		A	B	A	B	A	B															
	BXA	B	A	A		A		B	B	B	B	B	A	B	A	B			A	A	A	B		A	B	A	A															
	AKXL	L						L	L	L	A			L	L	L		L	L		L		L			L	L			A	A								A	A			
Xmv-42	CXB	B	B	B		B	B	B	C	C																																
	BXH	B		H	H	H		H	H	H	B	B	B	B		H					B																					
	BXD	B	B	D			D	B		B	D		B	D	B	D	D	D		B	D	D	D	D	B	B	B		B	B	D	B	B	D									
	AXB	B	B	B	A	B	A	A	A	A	B	B	A	A	A	B				A		A	B	A	A	A	B															
	BXA	B	B	B		B		B	A	A	B	A	A	B	B	B			A	A	B	A		A	B	A	B															
	AKXL	L					A	A	A	A	L			L	L	A		A	A		A		L			L	L			L	L								A	A			
Xmv-43	BXH	B		B	B	B		H	B	B	H	B	B	H		H					B																					
	AXB	B	B	A	B	B	B	B	A	B	B	B	A	B	B					B		B	B	A	A	A	B															
	BXA	B	B	A		A		B	B	B	B	B	A	B	B	B				A	A	B																					
Xmv-44	BXH	B		B	B	B		H	B	B	B	H	B	B		B					H																					
	BXD	B	D	B			D	B		D	B		B	D	D	D	B	D		D	B	B	D	B	B	B	B		D	D	B	D	B	D									
Xmv-45	CXB	B	B	C		B	C	C	B	C																																
	BXD	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							X	u	P
	AKXL	L						L	L	L	A			L	A	A		L	A		L		A			A	A			A	L								L	A	X	u	P
Xmv-8	BXH	B		B	B	B		H	B	B	B	H	B	B		B					H																					
	BXD	B	D	B			D	B		D	B		B	D	D	D	B	D		D	B	B	D	B	B	B	B		D	D	B	D	B	D									
Xmv-9	BXH	B		B	B	B		H	B	B	B	H	B	B		B					H																					
	BXD	B	D	B			D	B		D	B		B	D	D	D	B	D		D	B	B	D	B	B	B	B		D	D	B	D	B	D									
Xmv-10	CXB	B	C	C		C	B	B	B	B																																
	BXH	B		B	H	B		H	B	H	H	B	B	H		H					B																			X	s	P
	LXPL	L	L	P	L	P		L																																X		P
	SWXL	L				S			L					L		L	L	S	L																					X		P
	BXJ	B	B	B																																				X		P
Xmv-12	CXB	B	B	C		B	B	B	C	C																																
	AXB	B	B	B	A	A	B	B	A	A	A	A	B	A	B	A	A				A		A	B	B	A	B	B														
	BXA	B	B	B		A		B	B	A	A	B	B	A	B	A			A	A	B	A		B	A	B	B															
	AKXD	D	A	A	D			A	A	A	A	A	D	A	D	D	D	A		A		D	A	A	A	A	A	D	A	D												
	AKXL	L					A	L	L	L	A			L	A	L		L	A		L		L			L	L				A	A								L	A			
Xmv-13	CXB	B	B	C		C	C	C	B	C																																
	BXH	B		B	H	B		H	H	H	H	H	B	H		B					H																					
	BXD	B	D	D			B	D		B	D		D	D	D	D	D	D		B	B	D	D	B	D	D	D		D	D	B	D	B	D									
	AXB	B	A	B	A	A	B	A	B	A	A	B	A	B	A	A					A		A	B	B	B	A	A														
	BXA	B	B	B		A		B	B	A	A	*	B	A	B	B			B	A	B	B		B	B	B	B															
	AKXL	L					A	A	L	L	L			A	L	L		A	A		A		L			A	L				L	L								L	A			
Xmv-14	BXH	B		B	B	B		H	B	B	B	H	B	B		B					H																					
	BXD	B	D	B			D	B		D	B		B	D	D	D	B	D		D	B	B	D	B	B	B	B		D	D	B	D	B	D									
Xmv-15	BXH	B		B	H	H		H	H	B	H	H	H	H		B					H																					
	BXD	B	D	D			D	B		D	D		B	D	B	D	B	D		D	D	B	D	B	D	B	D		B	B	D	D	B	D							X	E	s
	AXB	B	A	A	B	A	B	B	A	A	B	B	A	B	B	B				A		A	B	A	B	B	B														X	E	s
	BXA	B	A	A		B		A	A	A	A	B	B	A	A	B			A	A	A	B		B	A	A	A														X	E	s
	AKXD	AK	D	D	D			D	D	D	A	A	D	A	D	D	D	A		D		D	D	D	A	D	D	A	D	A											X	E	s
	AKXL	AK					L	L	A	A	A			A	A	L		L	A		L		L			A	L				L	L								A	A	X	E	s
Xmv-16	CXB	B	C	C		C	B	B	B	C																																
	BXH	B		B	B	H		B	B	B	H	B	H	B		B					H																					X	s	P
	BXD	B	B	D			B	D		D	D		B	D	B	D	B	D		D	D	B	D	B	D	D	D		B	D	D	D	B	D							X	s	P
	AXB	B	A	A	B	B	B	B	A	B	B	A	B	B	B					A		A	B	A	B	A	A														X	s	P
	BXA	B	A	A		B		A	A	A	A	B	B	A	A	B			A	A	A	B		B	A	A	B														X	s	P
	AKXL	L						L	L	A	L			L	L	L		L	A		L		L			A	L				L	L								L	A	X	s	P
Xmv-17	CXB	B	B	C		B	C	C	B	C																																
	BXD	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							X	s	s
	AKXL	L					L	L	L	L	A			L	A	A		L	A		L		A			A	A			A	L								L	A	X	s	s

[a] Shown are SDPs from several RI sets for most *Xmv* loci. The column labeled RI Set shows the cross used (e.g., BXD stands for the RI set derived from the cross C57BL/6J × DBA/2J), and the Strain column indicates the progenitor strain carrying the provirus. Columns 1 to 38 indicate the RI strain number (CXB RI strains D, E, G, H, I, J, and K are denoted by numbers 1, 2, 4, 5, 6, 7, and 8, respectively). Rows designate the RI sets. Letters in the body of the table are used as generic symbols for the inheritance of alleles of *Xmv* loci in individual RI strains. *Xmv*-positive strains are indicated in boldface type; an asterisk shows when the RI strain was probably still segregating at the marker locus. Inbred strain symbols are as described in the legend to Fig. 2 (except A is used for both A/J and AKR/J in the body of the table). Additional symbols: SW, SWR/J; PL, PL/J; J, SJL/J. An X appears in column I if the SDP could not be obtained directly with both restriction enzymes. Columns II and III qualify these cases for *Eco*RI or *Pvu*II fragments, respectively. s, SDP obtained by subtraction from a comigrating proviral fragment; u, SDP readable with only limited confidence; E and P, SDP directly readable with *Eco*RI or *Pvu*II, respectively.

TABLE 1—*Continued.*

Locus	RI Set	+ Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	I	II	III
Xmv-18	CXB	B	C	B		B	C	B	B	C																																	
	BXH	B		H	H	B		H	H	B	H	B	H	H		H					H																						
	BXD	B	D	B			D	D		D	B		D	B	D	B	B	B		B	D	B	B	B	D	B	B		D	B	B	B	B	D					A	L			
	AXB	B	B	B	A	B	A	A	B	A	A	B	B	A	B	B				B		B		B	A	B	A																
	BXA	B	B	B		B		B	A	B	A	A	B	A	A	B			B	A	A	A		A	B	B	A																
	AKXL	L					L	L	L	L	L			L	L	L		L	L		L		A			L	L			A	A								A	L			
Xmv-19	CXB	B	C	C		B	B	C	C	C																															X	s	P
	BXH	B		H	B	H		B	B	H	B	B	B	B		H					H																						
	BXD	B	B	D			B	D		D	D		D	D	B	D	D	D		D	D	B	B	B	B	B	B		B	B	D	B	D	D								
	AXB	B	B	A	B	A	B	A	A	B	A	B	B	B	B	B				B		B	A	A	A	B	B																
	BXA	B	B	B		B		A	A	A	B	A	B	B	A	A			A	A	B	A		A	A	A	A																
	AKXL	L						A	A	L	L			A	A	L		L	L		L		A			L	L			L	L								L	A	X	s	P
Xmv-20	AKXD	AK						A	A			D			D	A	D	A		A		A	D	D		D	A	D											X	u	u
	AKXL	AK					A	A	A	A	L			L	L	A		A	A		A		L			L	L			A	A								A	A	X	u	u
Xmv-21	AKXD	AK	A	D	A			D	D	A	D	D	A	D	A	A	A	D		A		D	A	D	D	A	D	A	A	D												
	AKXL	AK					A	A	L	L	A			A	L	L		L	A		L		L			A	A			L	A								L	A			
Xmv-22	AKXD	AK	D	A	A			D	A	A	D	A	D	D	A	D	A	A		D		D	A	D	D	D	D	D	D	A												
	AKXL	AK					L	A	L	L	L			L	A	L		L	A		L		L			L	A			L	L								A	L			
Xmv-23	AKXD	AK	D	A	A			D	A	A	D	A	D	D	A	D	A	A		D		D	A	D	D	D	D	D	D	A										X	u	P
	AKXL	AK					L	A	L	L	L			L	A	L		L	A		L		L			L	A			L	L								A	L	X	E	s
Xmv-24	AKXL	AK					A	L	L	L	L			L	L	L		L	A		L		L			A	L			L	L								L	L	X	E	s
	BXD	D	D	B			D	B		D	D		B	B	D	B	D	B		D	D	B	B	B	D	B	D		D	B	D	B	D	D						X	E	s
	BXH	H		B	B	B		B	B	H	B	B	H	B		H					H																						
Xmv-25	AKXD	AK	D	D	A			A	D	A	D	A	A	A	D	D	D	A		A		D	D	D	A	A	D	A	D	A												
	AKXL	AK						L	L	A	L			L	A	L		L	A		L		L			A	L			L	A								L	A	X	s	P
Xmv-26	CXB	C	C	C		B	C	C	C	C																																	
	AKXD	AK	A	D	D			A	D	A	A	A	A	D	A	D	D	A		A		A	A	A	A	D	D	D	A	D												
	AKXL	AK						L	L	L	A			L	A	L		L	A		L		L			A	A			A	A								L	A	X	s	P
Xmv-27	AKXD	AK	A	D	A			D	D	D	D	D	A	A	D	A	D	A		D		D	A	D	A	A	D	D	D	A										X	E	s
Xmv-28	BXH	H		H	H	H		B	H	H	H	B	B	B		H					H																					
	LXPL	PL	L	P	P	L		P																																		
	SWXL	SW				L			S					S		L	S	S	S																								
	BXJ	J	J	B																																						
Xmv-29	BXH	H		B	H	H		B	B	H	B	H	B	B		B					H																				X	E	s
	BXD	D	B	D			B	D		B	D		B	D	B	B	D	D		D	B	D	B	D	D	D	B		B	B	B	B	D	D						X	E	s
	AKXD	D	D	D	A			A	A	A	D	A	D	D	A	D	A	A		D		D	A	D	D	D	D	D	D	A										X	E	s
Xmv-30	AKXD	D	A	D	D			A	D	D	D	A	D	D	A	A	A	A		A		A	A	A	D	A	D	A	D	D												
	BXD	D	B	D			D	D		B	B		B	D	B	D	B	B		B	B	B	B	B	D	B	B	D		B	D	B	B	D	D							
Xmv-31	CXB	C	B	C		B	B	B	C	C																																	
	BXD	D	B	D			B	D		B	B		B	B	B	B	D	D		D	B	B	D	B	D	D	D		D	D	B	B	B	D						X	u	s
	AKXD	D	A	D	A			A	A	D	D	A	A	A	D	A	A	A		A		A	D	A	D	D	A	A	D	D										X	u	u
Xmv-32	AKXD	D	A	D	A			D	D	A	A	D	A	D	A	A	A	D		A		D	A	A	A	A	D	A	A	A												
	BXD	D	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						X	s	P
Xmv-33	AKXD	D	A	A	D			A	D	D	A	A	D	D	A	A	A	A		A		A	A	A	A	A	D	A	D	D												
	BXD	D	B	D			B	B		B	B		B	D	B	D	D	B		B	B	B	B	D	B	B	D		B	B	B	B	D	D								
Xmv-34	AKXD	D	D	D	D			A	A	A	D	A	A	A	D	A	D	D		D		A	A	A	A	A	A	A	A	A												
	BXD	D	D	D			D	B		B	B		D	D	B	B	B	D		B	D	D	B	D	B	B	B		D	D	B	B	D	D						X	E	s
Xmv-35	AKXL	L					A	L	L	L	A			A	L	L		A	L		A		L			L	A			A	A								A	A	X	E	s
Xmv-36	AKXL	L					L	L	L	A	L			L	A	L		L	L		L		A			A	L			A	A								L	A			
Xmv-37	AKXL	L					A	A	L	L	L			L	L	A		L	A		L		A			L	L			L	A								A	A	X	s	s
Xmv-38	AKXL	L					A	L	L	A	L			L	A	A		L	A		L		A			L	A			L	L								A	A	X	s	s
Xmv-39	AKXL	L					L	A	A	L	L			L	L	L		L	A		A		A			L	A			A	A								A	A			

Mpmv loci, suggesting possible identity (manuscripts in preparation), and in future communications we will describe attempts to reconcile our results with those reported previously. Nevertheless, utilization of our xenotropic virus-specific oligonucleotide probes provides a convenient method for following the segregation of the majority of xenotropic proviruses present in common inbred strains of mice.

TABLE 2. Linkage data of *Xmv* loci and RI progenitor strain distribution[a]

Chromosome	Locus	Fragment size (kb) ^ Eco RI	Pvu II	Progenitor Strain	Linked markers Locus	"allele"	Gene Order	Discordant/ Total RI Strains	r	95% confidence limits of r (x100)
1					*Pep-3*	b	1	2 / 18	0.0333	0.35 - 18.1
	Xmv-21	8.5	13.5	AK	*Sas-1*	a	2	2 / 24	0.0238	0.26 - 11.34
					Ly-22	b	3	4 / 24	0.0556	1.28 - 21.27
					Xmv-32	-	4	4 / 24	0.0556	1.28 - 21.27
	Xmv-32	4.35	2.55	D	*Ly-22*	a		0 / 50	0.0000	0 - 1.99
					Xmmv-61	-		0 / 50	0.0000	0 - 1.99
	Xmv-43	6.7*	1.9*	B,AK,C,D,L	*Apoa-2*		1d	0 / 12	0.0000	0 - 10.97
					Ly-17	b	2	0 / 12	0.0000	0 - 10.97
	Xmv-41	24	2.4	B,L	*Ly-9*	b	1	2 / 64	0.0082	0.1 - 3.24
					Spna-1	b	2	6 / 106	0.0155	0.54 - 3.7
2	*Xmv-10*	3.8	2.2	B,A,AK,D,L	*a*	*a*		0 / 33	0.0000	0 - 3.14
4	*Xmv-8*	4.4	5.7,3.31,3	B,A,C	*Fv-1*	b		0 / 36	0.0000	0 - 2.85
	Xmv-14	2.55	"	"						
	Xmv-44	6	"	"						
	Xmv-9	3.9	4.3	"						
5	*Xmv-45*	5.5*	3.8*	B,A,H,L	*Xmv-17*	+	1	0 / 50	0.0000	0 - 1.99
					Pmv-40	-	1	4 / 26	0.0500	1.17 - 18.28
	Xmv-17	2.25	4.6	B,A,H,L	*Xmv-45*	+	2	0 / 50	0.0000	0 - 1.99
					Mpmv-23	-	3	0 / 26	0.0000	0 - 4.13
					Xmv-34	-	4	1 / 26	0.0102	0.03 - 6.96
					Mpmv-13	+	5	1 / 18	0.0152	0.04 - 11.55
					En-2		6	0 / 7	0.0000	0 - 26.56
					Rmcf	r	1	4 / 24	0.0556	1.28 - 21.27
	Xmv-34	3.2	2.31	D	*Xmv-17, -45*	-	1	1 / 26	0.0102	0.03 - 6.96
					Mpmv-23	+	2	4 / 50	0.0227	0.56 - 6.58
	Xmv-28	7.1	3.7	H	*rd*	*rd*		0 / 25	0.0000	0 - 4.32
7	*Xmv-30*	8.7	7.6	D	*Tam-1*	b		0 / 49	0.0000	0 - 2.03
					Xmv-30	+	1	7 / 50	0.0443	1.59 - 11.16
					Mtv-1	+	2	2 / 50	0.0106	0.12 - 3.17
	Xmv-33	4	2.05	D	*Mpmv-1*	-	2p	0 / 26	0.0000	0 - 4.13
8	*Xmv-26*	2.05	4	AK,C	*Defcr*			4 / 24	0.0556	1.28 - 21.27
					Xmv-12	-		13 / 48	0.1140	4.96 - 28.11
					Gr-1	a	1	0 / 7	0.0000	0 - 26.56
					Xmv-26	-	1	13 / 48	0.1140	4.96 - 28.11
	Xmv-12	3.15	9.5	B,D,H,L	*Nat-1*		2	5 / 35	0.0455	1.3 - 13.85
9					*Lap-1*	a	1	7 / 60	0.0354	1.3 - 8.53
	Xmv-16	2.3	2.7	B,L	*Xmmv-2*	+	2	1 / 26	0.0102	0.03 - 6.96
					Apoa-1	b	3	8 / 81	0.0290	1.17 - 6.42
					P450-3		4	4 / 30	0.0417	1 - 14.24
					Ncam	b	4	7 / 43	0.0539	1.9 - 14.23
					Xmv-15	+, -	5	20 / 96	0.0757	4.12 - 13.91
					Xmv-16	-	1	2 / 17	0.0357	0.37 - 20.09
	Xmv-25	3.05	2.6	AK	*Thy-1*	a	2	0 / 40	0.0000	0 - 2.54
					Apoa-1	a	2	0 / 23	0.0000	0 - 4.76
					Xmv-16	+, -	1	20 / 96	0.0757	4.12 - 13.91
					Apoa-1	a, b	2	17 / 97	0.0595	3.13 - 11.04
	Xmv-15	2.35	4.55	B,AK,C	*P450-3*		3	3 / 30	0.0294	0.54 - 11.02
					Ncam	a, b	3	10 / 60	0.0556	2.37 - 12.46
					d	+	4	11 / 50	0.0821	3.49 - 19.52

TABLE 2—*Continued.*

Chromosome	Locus	Fragment size (kb) ^ Eco RI	Pvu II	Progenitor Strain	Linked markers Locus	"allele"	Gene Order	Discordant/ Total RI Strains	r	95% confidence limits of r (x100)
11					*Evi-2*	a	1	5 / 32	0.0510	1.43 - 16.13
	Xmv-20	8.7*	2.25*	AK	*Hox-2*	b	2d	3 / 32	0.0273	0.51 - 10.01
					Xmv-42	-	3	2 / 18	0.0333	0.35 - 18.1
					Emv-14	-	1	3 / 18	0.0556	0.95 - 27.34
					Xmv-20	-	2	2 / 18	0.0333	0.35 - 18.1
					Hox-2		3	8 / 59	0.0426	1.66 - 9.99
					Mpmv-4	+	3	4 / 76	0.0143	0.37 - 4.01
	Xmv-42	6.9	2.9	B,L	*Xmmv-3*	+	4	0 / 26	0.0000	0 - 4.13
13	*Xmv-27*	1.9	4.7	AK,L	*Tcrg*			2 / 24	0.0238	0.26 - 11.34
	Xmv-13	3	3.15	B,L	*Rn7s-13*		1p	7 / 58	0.0368	1.35 - 8.95
					Lth-1	o	2	19 / 66	0.1267	6.31 - 27.05
					As-1	b	3	29 / 109	0.1107	6.78 - 20.31
14	*Xmv-19*	2.1	3.6	B,L	*Tcra*	b		0 / 93	0.0000	0 - 1.03
15	*Xmv-37*	2.5	3.55	L	*C6*			4 / 28	0.0455	1.07 - 16.02
16	*Xmv-35*	7.5	3.35	L	*D21S52*			1 / 18	0.0152	0.04 - 11.35
					Pmv-14	-		1 / 18	0.0152	0.04 - 11.35
17	*Xmv-36*	4.81	2.35	L	*Hba-ps4*	b	1	0 / 18	0.0000	0 - 6.42
					Pim-1	b	2	4 / 25	0.0526	1.22 - 19.66
19	*Xmv-18*	2.15	6.6	B,L	*P450-2c*			16 / 62	0.1053	5.06 - 22.78
Y	*Xmv-7*	4.6*	5.71*	B,A,C,L						
	Xmv-11	3.5	11	B,A,C,L						
	Xmv-40	4.5*	7.8*	B,A,C,D,H						
unassigned	*Xmv-22*	4.4	1.85, 2.45	AK	*Xmv-23*	+		0 / 18	0.0000	0 - 6.42
	Xmv-23	3.85	1.85, 2.45	AK	*Xmv-22*	+		0 / 18	0.0000	0 - 6.42
	Xmv-29	4.8	2.26	H,D	*Xmv-22, -23*	+		2 / 24	0.0238	0.04 - 11.55
	Xmv-24	3.7	3.3	AK,D,H	*Xmmv-7, -54*	+		2 / 26	0.0217	0.24 - 10.08
	Xmv-31	8*	2.3*	D,A,C						
	Xmv-38	2.45	3.5	L						
	Xmv-39	2.4	8.8	L	*Pca-1*	b		3 / 21	0.0455	0.8 - 19.97
	Xmv-6	4.75*	2*	all						

[a] A total of 32 *Xmv* loci were assigned to chromosomes by comparing their SDPs in RI strains (see also Tables 1; some not shown) with those of known loci. Manuscripts are in preparation for *Pmv* and *Mpmv* loci. Most marker locus SDPs will be found in chapter 18 of the forthcoming second edition of *Genetic Variants and Strains of the Laboratory Mouse*, M. F. Lyon and A. G. Searle (ed.). Data for chromosome 16 loci *Sod-1*, *App*, *D21S16*, and *D21S52* are given in reference 5. Data for chromosome 8 locus *Nat-1* is given in a recent paper (19a). Data for *Defcr*, a presence-versus-absence restriction fragment length polymorphism of the mouse cryptdin cDNA probe, was kindly provided by Andre Ouellette, Shriners Burns Institute, Boston, Mass. Listed by chromosomes are *Xmv* loci, including 3' junction fragment sizes and RI progenitor strain distribution, as well as closely linked marker loci *r* (estimated recombination frequency), plus upper and lower limits of *r* (×100) using 95% confidence levels (30). For convenience, the alleles of certain markers segregating with *Xmv* loci are shown, when this information was available (also see symbols below). *Xmv* loci are listed in their probable order on the chromosome; top and bottom represent proximal and distal, respectively. The probable gene order of marker loci is indicated by numbers 1 (most proximal) through 5 (most distal), and the order of the *Xmv* locus relative to its closest markers, when available, is shown by the suffix p (proximal) or d (distal) or by relative positioning in the rows of the table. Boldface marker loci are anchor loci, whose positions have been firmly established by conventional crosses. Symbols: *, *Xmv* loci that hybridized poorly to the probe, as described in the text; ^, junction fragment sizes were estimated to 0.1 kilobase pair, but the 0.01 decimal place was used to formally distinguish comigrating fragments (it is suggested that several progenitor strain DNAs be used as standard when analyzing unknown samples, since molecular sizes can appear to vary from gel to gel); +, proviral marker locus that segregates with the *Xmv* locus; −, proviral marker whose absence segregates with the *Xmv* locus.

DISCUSSION

Recombinant inbred (RI) mice provide a powerful system for mapping genetic differences between inbred strains and are ideal tools for studying interspersed gene families, such as endogenous proviruses. Once a set of RI strains has been constructed and characterized with respect to numerous marker loci, a great deal of linkage information can be gained rapidly by comparing the SDP for an unmapped locus with those of previously mapped loci. Because RI strains are inbred, they are particularly advantageous for mapping loci which are either present or absent, such as endogenous MLV proviruses. Thus, the restriction fragments of both progenitor strains can be scored unambiguously in the RI strains without recourse to judgments about band intensities. However, RI strain mapping does have several drawbacks that must be considered in their use. First, since the number of RI strains in a given set is generally small and because of the effect of RI strain formation on map expansion, usually only close linkages (<10 cM) are detectable in RI analysis. In addition, because of the low a priori probability that any two markers are closely linked, it is necessary to adopt a rather stringent significance level when analyzing RI data (31). In the present study, these limitations were partially overcome by analyzing several RI sets with the same markers. The facts that C57BL/6J, AKR/J, and DBA/2J were progenitors for more than one RI set and that many *Xmv* proviruses were shared among inbred strains, helped us in this respect. Furthermore, as the number of RI markers

increases, so does the likelihood of having SDPs characteristic of a particular region of the genome. Mapping of the endogenous *Xmv*, *Pmv*, and *Mpmv* loci (manuscripts in preparation for the proposed *Pmv* and *Mpmv* loci) has alone added more than 100 new RI strain markers, many of which are cross-referenced between RI sets.

Applying class-specific oligonucleotide probes to RI strain analysis allowed us to identify genetically endogenous nonecotropic MLVs of inbred mice in a comprehensive manner. Each of the oligonucleotides reacted with a manageable number of provirus-cell junction fragments, and they recognized fragments of a broad molecular weight range when used in conjunction with restriction enzymes that cleave near the 3′ end of the proviral DNA. An additional, unexpected bonus of the *Xmv* probes was the ability to hybridize differentially to *env* genes that were diverged slightly from those xenotropic sequences on which the synthesis of the oligonucleotide probes was based. The overall validity of these methods is evident from the results; a comprehensive inbred strain distribution was obtained for most *Xmv* loci, and several novel, intriguing linkage relationships were discovered.

The utility of RI strains for mapping and linkage analysis of endogenous proviruses requires that the proviruses be reasonably stable during the inbreeding process. Although endogenous proviruses may be gained and lost more rapidly than other DNAs, in greater than 7,000 generations of RI strain breeding only one potential new *Xmv* insertion was observed—in an AKXD-2 RI mouse (data not shown). In addition, of the proviruses common to both progenitors of an RI cross (approximately 30% of all proviruses), only one potential excision event was observed (*Xmv-9* was present in A/J and C57BL/6J and missing from RI strain AXB-7 [data not shown]). We therefore conclude that *Xmv* proviruses may still be acquired but at a slow rate. Deletion events leading to loss of the probe-reactive region may also occur, but they appear to be rare.

It is clear from our analyses that most *Xmv* polymorphism among the RI progenitor strains was due to independent insertion events and not to restriction fragment modification following integration. This conclusion was based on the fact that no two proviruses segregated with complementarity in any given RI cross. For example, if C57BL/6J and DBA/2J proviral fragments were actually alleles of one another created by restriction site polymorphism in one strain subsequent to insertion, they would have complementary SDPs in the BXD RI set. This phenomenon was not observed among *Xmv* loci. The existence of *Xmv* insertion site polymorphism in inbred mice does present an obvious paradox, because these animals, unlike some wild mice, do not express cell surface receptors for xenotropic virus (27). Any or all of the following models might explain this. (i) Mice actually do have functional xenotropic receptors that exhibit a very narrow tissue distribution and/or limited expression. (ii) *Xmv* proviruses are acquired by pseudotyping or intracellular transposition within the germ line. (iii) *Xmv* proviruses found today descend from mice that did encode functional xenotropic receptors. The fact that many endogenous *Xmv* loci were shared among inbred strains (Table 2 and unpublished results) proves that they were present prior to inbreeding. Furthermore, given that some contemporary wild mice populations do have a receptor for xenotropic virus (16), it is likely that this receptor was segregating in populations that were ancestors for inbred strains—thus allowing for ongoing insertions—but may not have been fixed in the group of immediate founders. It is nevertheless possible that either of the first two explanations contribute to a slow acquisition of endogenous *Xmv* proviruses in inbred mice today.

Our data hint that the immediate gene pool for inbred strains was actually quite limited. Only 15 of 40 *Xmv* loci were unique to an RI progenitor strain, and many of these could be found in other inbred strains. Nevertheless, considerable *Xmv* polymorphism must have existed within that pool. Given the history of inbred mice, this may not be surprising. Most laboratory strains are highly inbred, but represent a very recent (circa 1900) fixation of genes from mildly inbred and outbred mice carried by mouse suppliers, along with the introduction of genes from wild mice. Such historically recent events would have resulted in independent assortment of proviruses from strains that already differed in *Xmv* content. Furthermore, the antecedents of these immediate inbred strain founders are known to have been crossed with each other and Japanese mice during the latter few centuries in America and Europe (20). (One can easily imagine that, in Europe at least, this genetic mixing had been occurring for a millenium.) In addition, while MCF-like (i.e., *Pmv* or *Mpmv*) proviruses prevail in European wild mice and *Xmv* proviruses are found mainly in Asia, both can be found in mice isolated from hybrid zones (18). Thus, some of the more distant ancestors of inbred strains may have actually been natural genetic hybrids. If such interbreeding populations differed in host factors affecting virus resistance (including cell surface receptors), xenotropic viruses would have been continually provided with new environments in which to proliferate. It is likely that these older events contributed to the *Xmv* polymorphism and heterogeneity that preexisted modern inbreeding.

As expected, the *Xmv* loci are dispersed throughout the genome. Because as many as 20 loci can be detected with one probe, they may be very useful in mapping new genes and mouse mutations, not only in the RI sets but also in other genetic crosses involving common inbred strains. Nevertheless, regions of chromosomes 1, 4, 5, and Y contained several *Xmv* loci. Whether this clustering is significant remains to be seen. Contrary to previous reports on MLV and related sequences, we cannot conclude that endogenous *Pmv*, *Mpmv*, or *Xmv* loci are preferentially linked to either Ly antigens (3, 40) or histocompatibility antigens (29). Perhaps the large numbers of both endogenous viral sequences and immunodominant antigens explain the apparent linkage in those studies. While congenic strains may provide supportive evidence of close linkage, it should be considered that markers as distant as 10 cM from the selected locus may often remain linked, even in a large number of backcross generations (1). Moreover, the criteria for interpreting close linkage using RI strains may be questionable unless a large number of strains can be examined. It is therefore judicious to be conservative in examining linkage in RI strains by (i) estimating confidence limits for the recombination frequency (*r*, Table 2; 30) and (ii) examining linkage relationships in several, large RI sets when possible. Further analysis of close linkage should be pursued by other means, such as classical genetic crosses and/or a broader analysis of available inbred strains, if applicable.

The type of linkage relationships we initially sought were of *Xmv* proviruses causing known mouse mutations via insertional inactivation, as proven for an ecotropic provirus, *Emv-3* (7), and a polytropic provirus (37). Proviruses that potentially cause mutations would not segregate at all from the genetic marker in question, even in a large number of RI strains, and are most likely to be associated with recessive

mutations. While some proviruses had perfect concordance with enzyme markers (e.g., *Tam-1*) or restriction fragment length polymorphisms in structural genes (e.g., *Tcra*, a T-cell receptor gene), these relationships probably reflect close linkage rather than functional association. However, the relationships of *Xmv-10* and *Xmv-28* with the mutations *a* and *rd*, respectively, fit most of the criteria for mutagenic events. It remains to be shown whether either provirus caused the respective mutation, but concordance in other inbred and congenic strains carrying *a* or *rd* is supportive of the hypothesis (unpublished results). While *Xmv-10* was found to be absent from two non-agouti strains (SM/J and ST/bJ), it is conceivable that the *a* allele carried by these strains is of a different genetic origin than in most non-agouti strains, especially since *a* is an easily recognizable mutation which may have been isolated more than once. It is alternatively possible that *Xmv-10* was originally present in these strains but has since been lost by a deletion not resulting in phenotypic reversion. We will further examine relationships of *Xmv* proviruses with both *rd* and *a* by analysis of flanking sequence and a search for revertants, which have in the past provided evidence for causal relationships between insertion elements and mouse mutations (7, 37).

Perhaps the most intriguing linkage relationship discovered was that of *Xmv-8*, *Xmv-9*, *Xmv-14*, and *Xmv-44* with the *Fv-1*^{b} gene (Tables 1 and 2; unpublished results). While this relationship may again suggest a functional tie between an *Xmv* provirus and a cellular gene, the properties of *Fv-1* restriction confound our attempts to propose a sound model for this striking linkage data. The *b* allele of *Fv-1* restricts replication of N-tropic viruses, whereas the *n* allele similarly affects B-tropic viruses. This restriction is not absolute, however, and may be overcome by using a higher multiplicity of virus (19, 25). It has long been known that the *Fv-1* viral determinant maps to a stretch in the CA region of *gag* (23, 28), and it has been proposed that *Fv-1* affects viral DNA formation after synthesis of linear DNA but prior to integration (14, 41). Given our linkage data, it is provocative to speculate that *Fv-1*^{b} might be the consequence of a defective viral gene product, encoded by one or all of the *Fv-1*^{b}-linked *Xmv* loci, which interferes with an early step in replication (perhaps by competing for a necessary cellular factor) but can be inhibited by more input virus. Such a model does not easily account for the reciprocity of *Fv-1* (i.e., *Fv-1*^{n} restriction of B-tropic viruses), especially since no strains carrying the *Fv-1*^{n} allele contain these proviruses. Alternatively, it is possible that an *Xmv* provirus was selected via insertional activation to enhance expression of the endogenous *Fv-1*^{b} gene in certain tissues. In this context it is noteworthy that the LTR of an ancient type C provirus has been shown to confer tissue-specific regulation to a cellular gene (33). Regardless, a functional model involving the *Fv-1*^{b}-linked proviruses might involve the existence of four copies. While they appear not to be tandemly arrayed (RI strain AXB-7 was missing *Xmv-9* but neither *Xmv-8*, *Xmv-14*, or *Xmv-44* fragments were altered; data not shown), any amplification event must have occurred within a limited region surrounding the provirus because each provirus is present on a unique restriction fragment. We are currently analyzing DNA flanking these proviruses to further explore this curious phenomenon.

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