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Nonecotropic Murine Leukemia Viruses in BALB/c and NFS/N Mice: Characterization of the BALB/c *Bxv-1* Provirus and the Single NFS Endogenous Xenotrope

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We used hybridization probes that react specifically with xenotropic and mink cell focus-forming virus envelope sequences to characterize the nonecotropic proviruses of BALB/c and NFS/N mice. Analysis of somatic cell hybrids with different BALB/c chromosomes showed that the 9 xenotropic and more than 20 MCF virus-related proviral sequences in this mouse were present on more than nine BALB/c chromosomes. Multiple copies were found on chromosomes 1, 4, 7, 12, and probably 11, and the copies found on a single chromosome were not identical by restriction enzyme mapping. We also identified and characterized the proviral sequences that give rise to infectious xenotropic virus in both BALB/c and NFS/N mice. BALB/c contains the major locus for induction of infectious virus in inbred mice, *Bxv-1*, which is on chromosome 1. We showed that this locus contains a single xenotropic provirus on an 18-kilobase *Hin*dIII fragment. Restriction enzyme analysis of a hybrid cell DNA that contains only the *Bxv-1* xenotropic provirus showed that the *Bxv-1* provirus contains restriction enzyme sites characteristic of the infectious virus induced from BALB/c fibroblasts. The *Bxv-1* provirus and its flanking sequences also contain the same restriction sites as the provirus thought to contribute U3 long terminal repeat sequences to leukemogenic (class I) AKR MCF viruses. Analysis of cell hybrids made with the nonvirus-inducible strain NFS/N showed that the single xenotropic virus *env* gene of NFS mice, here termed *Nfxv-1*, is not on chromosome 1. Unlike that of *Bxv-1*, the restriction map of *Nfxv-1* does not resemble that of any known infectious xenotropic virus including xenotropic viruses isolated from NFS mice. These data suggest that *Bxv-1*, but not *Nfxv-1*, is a full-length xenotropic provirus that can be transcribed directly to produce infectious virus.

Genetic determinants of murine leukemia viruses (MuLVs) are inherited as chromosomal genes in mice. The efficiency of production of infectious virus differs sharply among mice, and genetic studies have used these differences to identify specific chromosomal loci for induction of infectious virus. Multiple inbred mouse strains, including BALB/c, AKR, C57BL, C57L, C58, and possibly F/ST, carry a single genetic locus for xenotropic virus induction, termed *Bxv-1*, which has been mapped to chromosome 1 (14, 18). *Bxv-1* can be induced to produce infectious virus in fibroblast cultures by halogenated pyrimidines (1) or in spleen cell suspensions by the mitogen lipopolysaccharide (7). Although *Bxv-1* is the most commonly found induction locus in inbred mice, some strains contain other xenotropic virus induction genes. MA/My contains *Bxv-1* together with a second unlinked locus (12). NZB contains two induction loci nonallelic with *Bxv-1* (6), and the Japanese mouse, *Mus musculus molossinus*, carries three or four induction loci, none of which show linkage to markers on chromosome 1 (12). Finally, it has been possible to isolate infectious xenotropic virus from mice which do not contain classical induction loci. Fibroblasts and spleen cells from NFS mice do not produce xenotropic virus after induction, but occasionally virus can be isolated from thymuses of young NFS mice (4; J. Hartley, personal communication).

Whereas many of the expressed ecotropic MuLVs have been cloned and characterized, biochemical and genetic analysis of endogenous nonecotropic MuLVs has been largely limited to characterization of cloned endogenous sequences and chromosomal mapping of a few envelope-related genes (2, 10, 17, 21). Comparisons of inbred mice by Southern blot analysis have not identified the expressed xenotropic MuLVs because, in contrast to the ecotropic MuLVs, more than 25 germ line copies of nonecotropic sequences are present in the DNAs of all mouse strains, including those which are induction negative (9). Furthermore, until recently, hybridization probes were not available that could distinguish xenotropic from mink cell focus-forming (MCF) virus-related envelope genes (19). In this study, we made use of Chinese hamster × mouse somatic cell hybrids and specific hybridization probes to study the chromosomal distribution of sequences related to nonecotropic MuLVs. We also identified and characterized the specific provirus associated with the *Bxv-1* induction locus and the single detectable xenotropic MuLV *env* sequence in NFS mice.

MATERIALS AND METHODS

Cells. Somatic cell hybrids were generated by fusion of Chinese hamster E36 cells with peritoneal cells of BALB/c or NFS. *Akv-2* mice. The isolation and characterization of these hybrids have been described previously (13, 15). These hybrids retain a full complement of hamster chromosomes and different subsets of mouse chromosomes. Mouse chromosomes were identified by Giemsa-trypsin banding followed by staining with Hoechst 33258. Hybrids were also typed for various marker loci on 17 chromosomes. Xenotropic virus induction from the (*Bxv-1*) locus was scored in all BALB/c hybrid cells after treatment with 5-iododeoxyuridine as described previously (14). Hybrids

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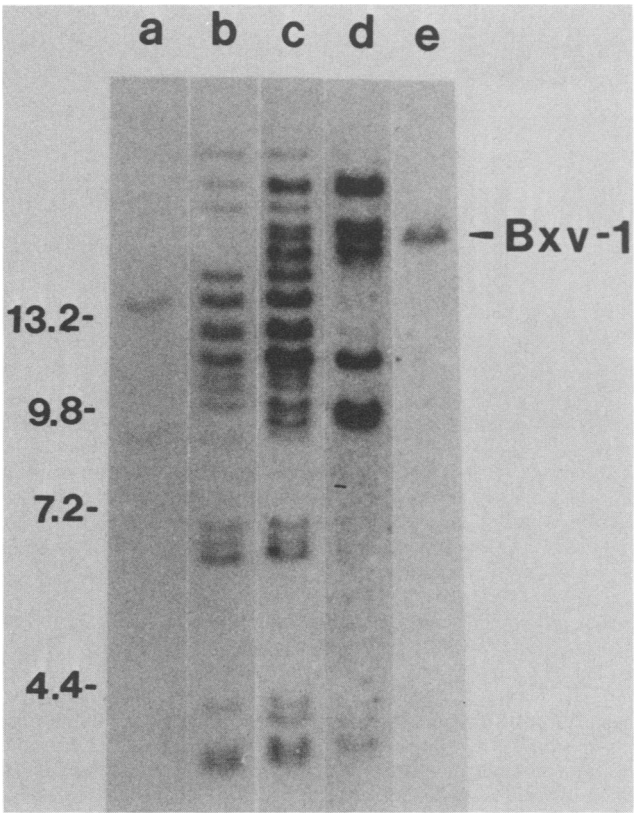

FIG. 1. Reactivity of pX_{env} and the xenotropic and MCF virus-specific hybridization probes with *Hin*dIII-digested DNAs of BALB/c liver and BALB/c mouse × Chinese hamster somatic cell hybrids. Marker fragment sizes (10^3) are indicated to the left. Lanes: a, hybrid HM26, MCF virus *env* probe; b, BALB/c, MCF virus *env* probe; c, BALB/c, pX_{env} probe; d, BALB/c, xenotropic virus *env* probe; e, HM26, xenotropic virus *env* probe. Lanes a, b, and c were from duplicate membranes produced by bidirectional blotting from the same gel. Lanes d and e were from a duplicate gel.

were also typed for the chromosome 1 locus *Rmc-1* (susceptibility to exogenous MCF virus) (11).

Several subclones of primary hybrid lines were specifically isolated to facilitate identification of MuLV-related sequences on chromosome 1. Two BALB/c hybrids and one NFS hybrid which contained a number of mouse chromosomes, including chromosome 1, were maintained in culture for several weeks to generate a chromosomally heterogeneous cell population. Subclones were then prepared by plating single cells in microtiter dishes. Two subclones of each primary hybrid were selected for further characterization such that one of each pair retained chromosome 1 and one did not.

One pair of subclones was isolated after five such rounds of serial subcloning. After each round, 5 to 10 subclones were typed for isozyme phenotypes. The subclone containing *Pep-3*, a chromosome 1 marker, and the fewest number of additional mouse isozymes was selected for further subcloning. In this way, one clone was isolated which lost all mouse isozyme markers except *Pep-3*. Karyotypic analysis of this subclone, HM26, failed to reveal any intact mouse chromosomes although it must have retained a segment of chromosome 1 since it expressed three marker loci on this chromosome—*Pep-3*, *Rmc-1*, and *Bxv-1*. HM26 also contained a part of chromosome 15 because of the presence of mouse c-*myc* and c-*sis* oncogene sequences (5, 16). A sister subclone, HM25, lacked the chromosome 1 markers, contained the chromosome 15 markers, and also contained chromosome 17.

DNA isolation and agarose gel electrophoresis. High-molecular-weight DNA was extracted from liver and cell cultures as previously described (8). Restriction endonucleases were obtained from Bethesda Research Laboratories, Bethesda, Md., or from New England BioLabs, Beverly, Mass. Restriction fragments were electrophoresed on 0.4% agarose gels for 40 h at 24 V and transferred to nitrocellulose membranes as previously described (9), except that duplicate membranes were prepared from each gel by bidirectional blotting.

Preparation of DNA probes and nucleic acid hybridization. The construction, characterization, and use of the probe pX_{env} has been previously detailed (3). This probe represents a 500-base-pair fragment of the envelope gene of the NFS-Th-1 xenotropic virus. It hybridizes with viruses of both xenotropic and MCF virus host range classes. Two additional single-stranded probes of about 100 base pairs were prepared from analogous regions of MCF247 and from NZB-IU-6 (19). These probes are type specific in their hybridization to MCF and xenotropic MuLVs.

Hybridization was carried out in 50% formamide–3 × SSC (1 × SCC is 0.15 M NaCl plus 0.015 M sodium citrate) for 18 h at 42°C. This was followed by four, 5-min washes (0.1 × SSC, 0.1% sodium dodecyl sulfate at room temperature) followed by two to four 15-min washes at 50°C. Membranes were dried and then exposed to Kodak X-AR film.

RESULTS

Analysis of BALB/c hybrid cells and identification of *Bxv-1*. Proviruses that contain xenotropic or MCF virus-related *env* sequences either have no *Hin*dIII sites or may have a single *Hin*dIII site at 5.0 kilobases (kb) (4, 10). *Hin*dIII-digested genomic DNAs from inbred mice contain more than 25 fragments which hybridize with the generalized envelope probe for nonecotropic MuLVs, pX_{env} (9). BALB/c DNA contains 9 xenotropic *env*-reactive fragments and about 20 fragments related to the MCF virus *env* sequence (Fig. 1).

To describe the chromosomal distribution of these proviral genes and identify the sequences associated with the *Bxv-1* induction locus, we analyzed a series of hamster × mouse somatic cell hybrids with different complements of BALB mouse chromosomes. Chinese hamster DNA has no sequences which cross-hybridize to the three envelope probes.

In attempting to correlate specific fragments with specific mouse chromosomes, it became obvious that it would not be possible to establish a definitive chromosomal assignment for each of the fragments for three reasons. (i) Not all mouse chromosomes were adequately represented in this panel of BALB/c hybrids. For example, none of these hybrids retained mouse chromosome 11, and mouse chromosomes 9 and 10 were both present in hybrid BM20, but both were absent from all other hybrids. (ii) The smaller fragments were difficult to resolve. (iii) Many of the *Hin*dIII *env*-reactive bands were produced by the comigration of fragments generated from different proviruses. Some, like the bands at 3.5 and 12.3 kb, could be resolved into two separate bands in occasional blots of BALB/c DNA, but it was generally not possible to determine which of these was present in hybrid cells. In other cases, comigrating frag-

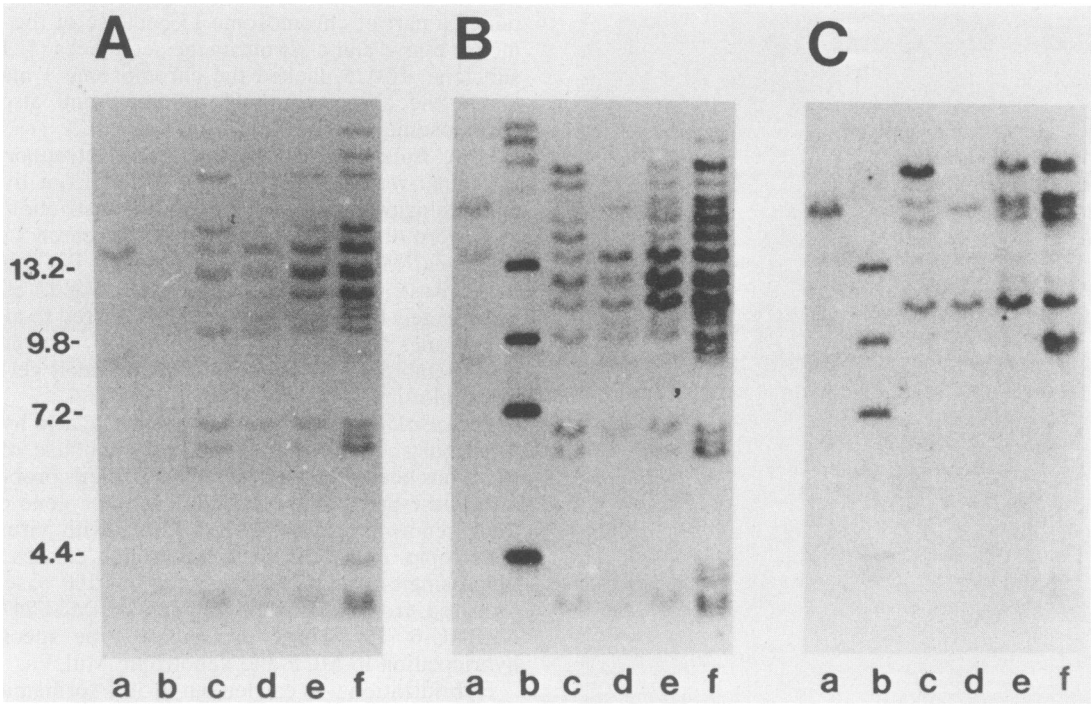

FIG. 2. Detection of various MCF and xenotropic virus *env*-related genes in *Hin*dIII-digested somatic cell hybrid DNAs. The filters were hybridized to MCF virus *env*-specific (A), pX_{env} (B), and xenotropic virus *env*-specific (C) hybridization probes. Filters in panels A and B were produced by bidirectional blotting of the same gel, and the panel C filter was from a second gel. The molecular weight markers (10^3) included cleavage products of NFS-Th-1 xenotropic MuLV cloned in pBR322, which hybridized with the xenotropic virus *env*-specific and pX_{env} probes but not with the MCF MuLV *env*-specific probe. Lanes: a, hybrid HM26; b, markers; c, HM61; d, HM59; e, HM62; f, BALB/c.

ments at 22.9 and 11.3 kb could be resolved into two separate bands by hybridization with MCF or xenotropic virus *env* probes.

Among the 22 hybrids examined, one clone lacked any pX_{env}-reactive fragments. This clone, HM25, contained only chromosome 17 and a fragment of mouse chromosome 15, indicating that these segments of the BALB/c mouse genome lack sequences related to the envelope region of the xenotropic and MCF virus genome.

The other 21 clones contained different subsets of the BALB/c *env* genes, allowing us to assign specific viral fragments to mouse chromosomes (Fig. 2; Table 1). Several fragments (10.8, 9.9, 9.4, 6.6, and 4.2) were present in both male and female BALB/c DNAs but absent from all of the hybrids. Since the only chromosome absent from all of these hybrids is chromosome 11, it is assumed that these fragments result from proviral genes on this chromosome. Other proviral sequences could be mapped by their presence or absence in the different hybrids. For example, the 27.5-kb MCF virus-related fragment was present in two hybrids and absent in 20. The two hybrids containing this sequence were also the only two hybrids which contained chromosome 5. Since no other chromosome showed any correlation, this proviral sequence was assigned to chromosome 5.

It was particularly important to identify the *env* sequences on chromosome 1 since this chromosome contains the *Bxv-1* induction locus. Previous studies showed that the presence of chromosome 1 in hybrid cells is necessary and sufficient for the virus inducibility phenotype, indicating that the proviral genes expressed as a virus are on this chromosome (14). To identify all of the proviruses on chromosome 1, we examined the full set of BALB/c hybrids together with three additional pairs of sister subclones which were karyotypically similar except for the presence of chromosome 1 in one member of each pair. Use of the generalized *env* probe showed that chromosome 1 contains four proviral *env*-reactive genes which produce *Hin*dIII fragments of 18.1, 13.7, 10.4, and 8.9 kb. The 13.7-kb fragment is actually a doublet produced by proviral genes on chromosomes 1 and 12 since five hybrids lacking chromosomes 1 contain this fragment and chromosome 12 and three hybrids lack this fragment and both chromosomes. The four proviruses on chromosome 1 are not closely linked since hybrid HM26 contains only the centromeric end of chromosome 1 and this hybrid lacks the 10.4-kb fragment but contains the 18.1-, 13.7-, and 8.9-kb fragments (Fig. 2).

Use of the xenotropic and MCF virus-specific *env* probes revealed that only one of the four *env* genes on chromosome 1, the 18.1-kb fragment, is reactive with the xenotropic virus-specific probe (Fig. 1, lane e). The smaller fragments react with the MCF virus-specific probe. These data indicate that the *Bxv-1* induction locus corresponds to the 18.1-kb xenotropic provirus sequence. Digestion of HM26 DNA with other enzymes revealed that the *Bxv-1* envelope is present on a 9.5-kb *Eco*RI fragment and a 6.8-kb *Xba*I fragment.

Comparable analyses were done for each of the *env*-reactive fragments that could be unequivocally identified in the hybrid lines. In this way, many of the proviral genes contained in fragments large enough to contain a full-length virus could be mapped to specific chromosomes. These are summarized in Table 1. These data also show that multiple copies of xenotropic or MCF MuLV*env* genes are present on several mouse chromosomes. In addition to chromosome 1, other chromosomes carry more than one viral gene. Two xenotrope-related genes were mapped to chromosome 4,

TABLE 1. Correlation between specific mouse chromosomes and the nonecotropic virus *env* sequence in Chinese hamster × mouse somatic cell hybrids

Fragment size (kb)	*env* reactivity		Chromosome no.	No. of hybrids scored (no. with *env*/no. with chromosome)[a]				Locus designation
	Xenotropic virus	MCF virus		+/+	−/−	+/−	−/+	
27.5		×	5	3	16	0	0	*Mmv-1*
22.9	×							
22.9		×	3	7	10	0	0	*Mmv-2*
20.8		×	7	10	6	0	0	*Mmv-3*
18.8	×		4	7	10	0	0	*Xmv-1*
18.1	×		1	11	10	0	0	*Bxv-1*
16.7	×		4	7	10	0	0	*Xmv-2*
15.1		×	7	10	6	0	0	*Mmv-4*
13.7		×	1	9	3	5	0[b]	*Mmv-5*
13.7		×	12	5	3	0	0	*Mmv-6*
12.3		×	12	8	4	2	0[c]	*Mmv-7*
12.3		×						
11.3	×		16	5	8	0	0	*Xmv-3*
11.3		×						
10.8		×	11	0	12	0	0	*Mmv-8*
10.4		×	1	8	12	0	1[d]	*Mmv-9*
9.9	×		11	0	12	0	0	*Xmv-4*
9.9		×						
9.4	×		11	0	15	0	0	*Xmv-5*
8.9		×	1	9	11	0	0	*Mmv-10*
6.9		×	2	5	6	0	0	*Mmv-7*[e]
6.6		×	11	0	16	0	0	*Mmv-11*
6.3		×	3	8	9	0	0	*Mmv-12*
4.2		×	11	0	14	0	0	*Mmv-13*
3.9	×							
3.7	×							
3.7		×						
3.5		×						
3.5		×						

[a] For all chromosomal assignments, correlations with the other 19 mouse chromosomes produced at least two discordancies. The numbers of hybrids given for each fragment differ since each fragment could not be unequivocally identified in each DNA.

[b] The 13.7-kb band is a doublet. One fragment was mapped to chromosome 1 since it is present in hybrids carrying only this chromosome. The other is on chromosome 12 since three lines lacking this fragment lack both 1 and 12 and five lines containing the fragment lack chromosome 1 but contain 12. One of these five lines contains only chromosome 12.

[c] Two proviruses produce 12.3-kb *Hin*dIII fragments. One of these is on chromosome 12. Four hybrids lacking the fragment lack 12. The two discrepancies must represent hybrids that contain the second provirus.

[d] This hybrid contains a fragment of chromosome 1.

[e] This corresponds to a previously mapped sequence (17).

two MCF virus-related genes were mapped to chromosome 7, and two MCF virus-related genes were mapped to chromosome 12. This analysis could not determine whether these syntenic genes were closely linked although, as indicated above, the four copies on chromosome 1 were not present in a single cluster.

Differences among syntenic proviral sequences. Various restriction enzymes cleave retroviral genomes at multiple sites, producing internal fragments. Not all infectious viruses contain the same sites, and differences have also been observed among endogenous MuLVs (4, 8). Therefore, to compare the syntenic sequences on chromosomes 1, 4, 7, and 12, we digested DNAs from hybrids containing only the proviral sequences on these chromosomes with *Pst*I, *Bgl*II, *Kpn*I, and *Sac*I. Data for *Bgl*II and *Sac*I are shown in Fig. 3. The fragments generated by these enzymes are consistent with the fragment sizes produced from various infectious viruses. However, proviral sequences found on the same chromosomes are clearly not identical. For example, whereas the hybrid which contains only the two chromosome 7 proviruses contains only one *Bgl*II fragment, indicating that the two proviral genes on chromosome 7 both contain the same internal *Bgl*II sites (Fig. 3A, lane c), use of *Pst*I, *Kpn*I (data not shown), and *Sac*I (Fig. 3B, land d) produced two fragments. Similarly, the two proviral genes on chromosomes 12 and 4 and the three on chromosome 1 produced two and three fragments, respectively, after digestion with three of the four enzymes used.

Internal structure of *Bxv-1*. Because infectious xenotropic viruses vary in their physical maps (4), we characterized the proviral sequences at this induction locus. Since the *Bxv-1* provirus was the sole BALB/c xenotropic *env*-related sequence in hybrid HM26, it was possible to use this hybrid to generate a restriction enzyme map of the region surrounding the *env* sequence. The resulting map of the *Bxv-1* provirus was compared with restriction maps of various xenotropic MuLVs isolated from mice known to contain *Bxv-1* (Fig. 4). The *Bxv-1* endogenous sequence closely resembles BALB-IU-2, C58-L1, and AKR-6, but differs from AKR-40.

Xenotropic virus sequences in NFS/N mice. NFS/N contains a single xenotropic virus *env* gene (19), here termed *Nfxv-1*, and the NFS.*Akv-2* congenic strain contains this provirus together with one additional xenotropic virus *env* gene, presumably derived from its AKR parent (Fig. 5). Cells from neither NFS nor congenic mice could be induced to produce xenotropic virus.

We analyzed a small set of somatic cell hybrids derived from NFS.*Akv-2* mice to determine whether xenotropic

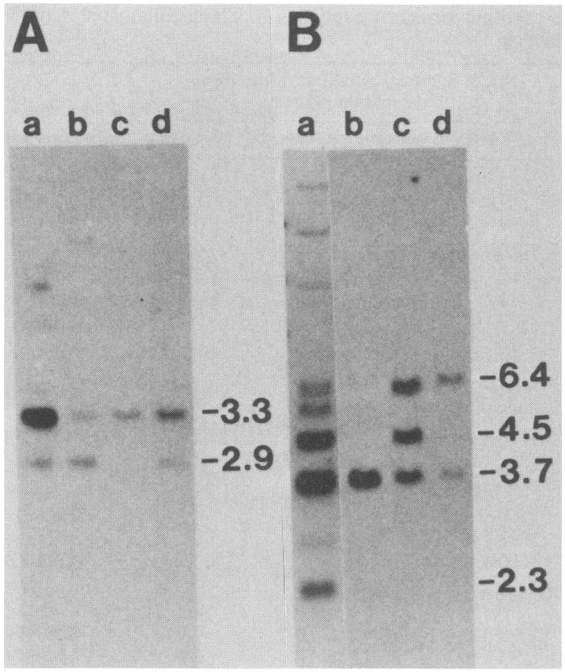

FIG. 3. Reactivity of *Bgl*II (A)- and *Sac*I (B)-digested mouse and hybrid cell DNAs with the pX_{env} probe. The hybrid DNAs contained only single chromosomes bearing pX_{env}-related sequences. Panel A (lanes): a, BALB/c; b, hybrid HM26 (chromosome 1); c, HM12 (chromosome 7); d, HM20 (chromosome 12). Panel B (lanes): a, BALB/c; b, HM20; c, HM26; d, HM12. The number to the right of each panel indicates molecular weights (10^3).

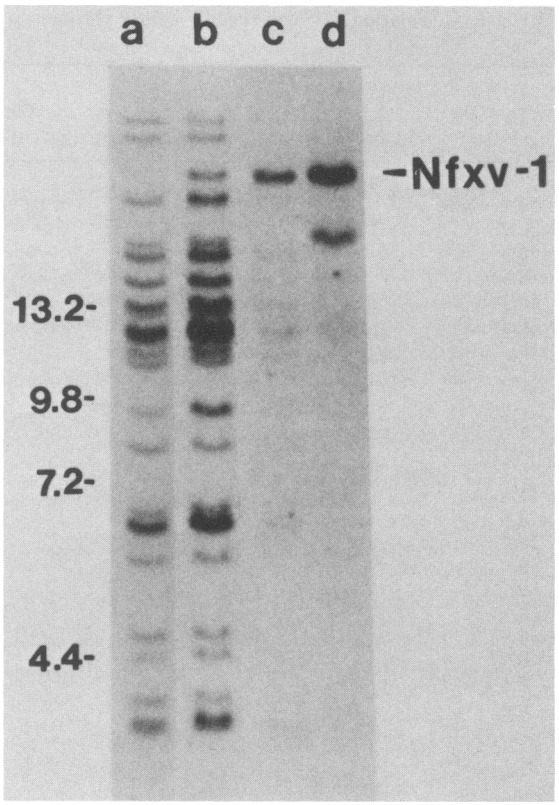

FIG. 5. Reactivity of pX_{env} and the xenotropic and MCF virus-specific hybridization probes with *Hin*dIII-digested DNAs of NFS/N and NFS.*Akv-2* mice. Fragment sizes (10^3) are indicated to the left. Lanes: a, NFS/N; MCF virus *env* probe; b, NFS/N, pX_{env}; c, NFS/N, xenotropic virus *env* probe; d, NFS.*Akv-2*, xenotropic virus *env* probe.

virus *env* sequences are present on chromosome 1 in these mice. Five of the seven hybrids tested retained chromosome 1, but only two of these contained *Nfxv-1*, and all seven hybrids retained the AKR xenotropic virus *env* sequence. Therefore, neither the xenotropic virus *env* gene inherited from AKR mice nor the *Nfxv-1* sequence was present on chromosome 1. Presumably, the AKR *env* gene is linked to *Akv-2* on chromosome 16.

Using single and double digests of NFS mouse DNA, we produced a restriction map of *Nfxv-1* (Fig. 6). This map is substantially different from the infectious virus isolated from this mouse, particularly in the region analogous to the *pol* gene. *Nfxv-1* is also substantially different from all known infectious xenotropic viruses. These data, together with the

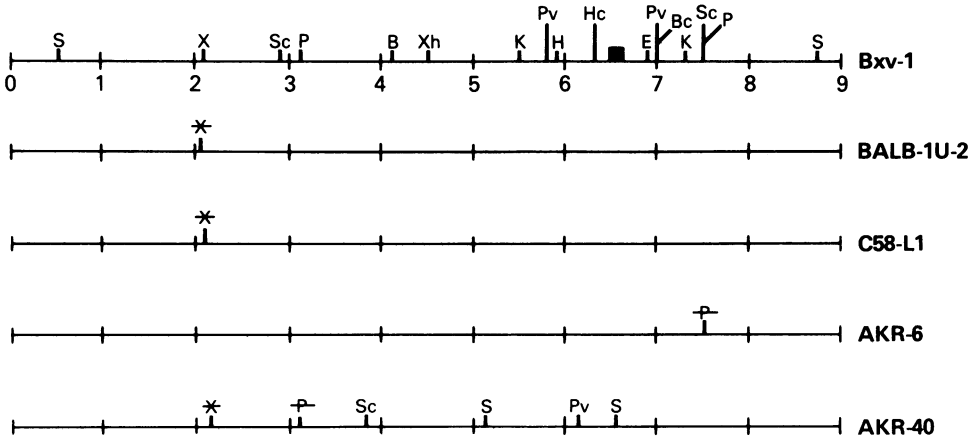

FIG. 4. Comparison of restriction endonuclease maps of *Bxv-1* and infectious xenotropic MuLVs isolated from mouse strains known to contain *Bxv-1*. Restriction maps of BALB-IU-2, C58-L1, AKR-6, and AKR-40 were taken from reference 4. Sites present in these four viruses were identical to those in *Bxv-1* except where indicated. Additional sites are indicated by the appropriate symbols, and the absence of sites is indicated by symbols marked with a slash. Symbols: S, *Sma*I; X, *Xba*I; Sc, *Sac*I; P, *Pst*I; B, *Bam*HI; Xh, *Xho*I; K, *Kpn*I; Pv, *Pvu*II; H, *Hpa*I; Hc, *Hin*cII; E, *Eco*RI; Bc, *Bcl*I. The solid square indicates the proviral location of sequences corresponding to the hybridization probe.

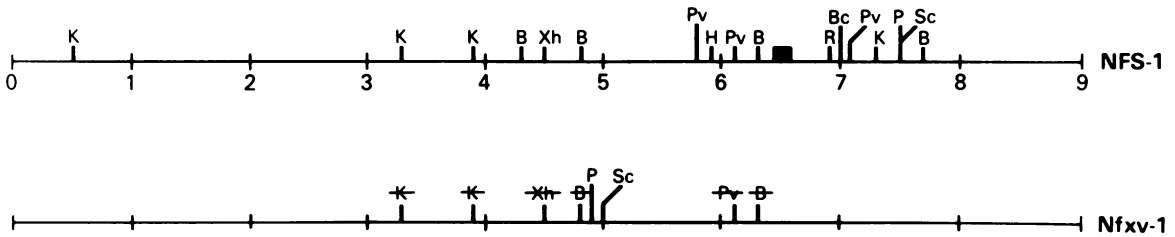

FIG. 6. Comparison of restriction endonuclease maps of *Nfxv-1* and the infectious virus NFS-Th-1 (NFS-1). The NFS-Th-1 restriction map was taken from references 4 and 10. Sites indicated in NFS-Th-1 are present in *Nfxv-1* except where indicated. Additional sites are indicated by appropriate symbols, and the absence of sites is indicated by symbols marked with a slash. Symbols: K, *Kpn*I; B, *Bam*HI; Xh, *Xho*I; Pv, *Pvu*I; P, *Pst*I; Sc, *Sac*I; R, *Eco*RI, B, *Bgl*II; H, *Hpa*I. The solid square represents sequences corresponding to the hybridization probe.

fact that the virus is not a classical induction locus, suggest that *Nfxv-1* is not an intact xenotropic provirus.

DISCUSSION

This analysis of the chromosomal distribution of the nonecotropic virus envelope genes confirmed that retroviral sequences are present on multiple mouse chromosomes (2, 17, 21). In fact, one of the *env* genes identified here (designated *Mmv-71* in Table 1) is apparently equivalent to the *Hin*dIII spleen focus-forming virus-reactive fragment also mapped to chromosome 2 by Meruelo and colleagues (17). This study differs from the previous works, however, in that use of hybrid cells and low-percentage agarose gels to resolve large restriction fragments made it possible to map a greater percentage of the sequences present in a single strain. In fact, we mapped most of the endogenous fragments large enough to contain full-length proviruses.

The derivation of specific hybridization probes made it possible to identify and characterize the proviral genes associated with xenotropic virus expression in BALB/c and NFS/N mice. The characterization of these retroviral genes emphasizes that the molecular mechanisms responsible for virus production in these mice are different. NFS/N mice have been classified as low-virus-mice since it is not possible to induce virus from cultured NFS/N fibroblasts or spleen suspensions. However, viruses with a xenotropic host range have been isolated by cocultivating large numbers of thymus cells with mink lung cells (4; J. Hartley, personal communication). The data shown here indicate that the NFS/N endogenous *env* sequence does not resemble the infectious virus isolated from these mice. We conclude, therefore, that this viral sequence, *Nfxv-1*, most likely represents an envelope gene not associated with an intact xenotropic provirus, but the virus produced by these mice is the result of recombinational events involving the *Nfxv-1 env* sequences.

In contrast to NFS, cells from mice containing *Bxv-1* are readily induced to produce virus, and a number of virus isolates with a xenotropic host range have been obtained from different *Bxv-1*-positive strains. Several of these, namely BALB-IU-2, C58-L1, AKR-6, and AKR-40, have been compared by restriction enzyme mapping (4). Our data indicate that *Bxv-1* resembles all but AKR-40. This difference cannot be simply explained by polymorphism at the AKR *Bxv-1* locus since the two AKR isolates also differ from one another. The best explanation for these differences is that AKR-40 is a recombinant rather than a primary product of the *Bxv-1* locus. AKR-40 was isolated from the thymus of a 6-month-old mouse, and recombinant viruses are readily isolated from this tissue.

Whether the *Bxv-1* locus has an important role in the biology of mice has never been established. However, it should be noted that Quint and colleagues (20) used the U3 segment of a leukemogenic AKR MCF virus to show that the provirus associated with this long terminal repeat was present in only one copy in most inbred mice (20). It is probable that this copy is in fact the *Bxv-1* provirus for several reasons. (i) Only strains carrying *Bxv-1* (14) have these U3 sequences (20); (ii) the internal restriction maps of *Bxv-1* and the U3-containing provirus coincide; and (iii) enzymes such as *Hin*dIII, *Xba*I, and *Eco*RI, which produce cell-virus junction fragments, produce same-sized fragments from *Bxv-1* and the U3-containing provirus, indicating that the flanking sequences are also the same. Therefore, *Bxv-1* may make an important contribution to the generation of recombinant leukemogenic (class I) MCF viruses in AKR mice.

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