

## Internal Organization of Endogenous Proviral DNAs of Xenotropic Murine Leukemia Viruses

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The internal organization of endogenous xenotropic murine leukemia virus proviruses was determined in a series of blot hybridization experiments in which DNA from several different inbred mouse strains, digested with restriction enzymes known to cleave xenotropic proviral DNAs at least twice, was annealed to generalized murine leukemia virus or xenotropic *env*-specific DNA probes. Comigrating bands of variable intensity which hybridized to the xenotropic *env* probe were identified in all inbred mouse DNA preparations. At least seven classes of endogenous xenotropic proviral DNA with respect to *SacI* cleavage maps were detected in mouse DNA. Two of the seven classes were indistinguishable from proviruses associated with known infectious xenotropic murine leukemia viruses. These results are consistent with the existence of related but organizationally distinct families of endogenous xenotropic proviral DNA that are present in different relative abundances in mouse genomic DNA.

The genome of *Mus musculus* contains many copies of murine leukemia virus (MuLV)-related sequences (7, 19, 20), but little is yet known of their organization. Ecotropic MuLV genomes, which constitute no more than a small fraction of these sequences, are known to be present either as full-length copies indistinguishable from integrated proviruses formed after exogenous infection or as deletions thereof (16, 19). The ecotropic proviral DNAs are known to be integrated at multiple sites in the host cell genome (3, 6, 16), and in high-virus mice, they can be reinserted at new sites in the germ line DNA (17). Little is known of endogenous xenotropic virus genomes except that results from liquid hybridization studies show that they appear to be more abundant than ecotropic genomes (2, 7).

We have recently isolated, by molecular cloning, a 500-base-pair (bp) segment of the envelope (*env*) gene of a xenotropic MuLV that serves as a type-specific DNA probe which does not cross-react with ecotropic proviral DNA (1). We have used this probe to analyze the DNA of various strains of mice for the copy number, organization, and polymorphism of the endogenous xenotropic genomes. This paper describes the analyses done with restriction enzymes that cleave at multiple sites within the proviral DNA of infectious xenotropic virus isolates and their use in the characterization of endogenous xeno-

tropic proviruses. A subsequent paper will describe comparable studies using no-cut and one-cut enzymes that would be expected to generate fragments containing flanking nonviral cell DNA.

### MATERIALS AND METHODS

**Mice.** BALB/c, C57L, and NFS mice were supplied by the National Institutes of Health Small Animal Production Facility, Bethesda, Md. C3H/FgLw mice were obtained from Lloyd Law, National Institutes of Health, in 1979. NZB/J and AKR/J mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. F/St mice were obtained from H. C. Morse, National Institutes of Health. *Mus molossinus* mice (colony III) were kindly provided by Thomas Roderick, the Jackson Laboratory. NFS mice congenic for the *Akv-2* locus (NFS.*Akv-2*) were bred in our laboratory.

**Purification and restriction endonuclease digestion of genomic DNA.** High-molecular-weight DNA from mouse liver was purified as previously described (3). *EcoRI* and *SacI* restriction enzymes were purchased from New England Biolabs, Beverly, Mass., and *BglII* was obtained from Bethesda Research Laboratories, Rockville, Md. All enzymes were used as recommended by the suppliers. Completeness of digestion was verified by adding lambda DNA to a sample of each reaction mixture and comparing its cleavage pattern by gel electrophoresis with the known cleavage pattern for each restriction enzyme. Restricted DNA was electrophoresed through 0.8% agarose slab gels as previously described (3, 9).

**MuLV DNA probes.** The molecular cloning and characterization of a 6.8-kilobase (kb) generalized MuLV DNA probe (MuLV<sub>gen</sub>) which contains a 1.8-kb

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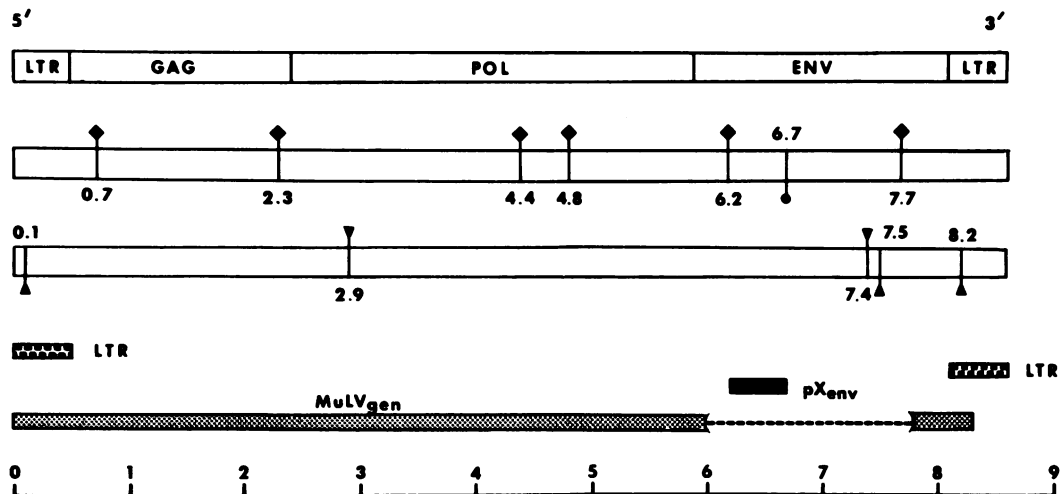


FIG. 1. Restriction map of NFS-Th-1 xenotropic proviral DNA and regions of reactivity with the pMuLV<sub>gen</sub>, pX<sub>env</sub>, and LTR DNA probes. The scale at the bottom is in kb. Symbols: ◆, *Bgl*III; ●, *Eco*RI; ▼, *Sac*I; △, *Pst*I.

deletion of viral envelope sequences (Fig. 1) have been previously described (10, 14). We have also reported the construction of a recombinant plasmid containing a 500-bp DNA segment (pX<sub>env</sub>), derived from the *env* region of cloned NFS-Th-1 xenotropic proviral DNA (Fig. 1), that specifically hybridizes to xenotropic and mink cell focus-forming (MCF) proviruses and not to ecotropic proviral DNAs (1). A 600-bp *Kpn*I fragment consisting of a complete copy of the long terminal repeat (LTR) was isolated from a recombinant plasmid (pHaSV) containing Harvey sarcoma virus proviral DNA (14).

Recombinant plasmid DNA or DNA fragments isolated by preparative agarose gel electrophoresis were labeled by nick translation (13) and had specific activities of  $8 \times 10^7$  to  $13 \times 10^7$  cpm/ $\mu$ g.

**Nucleic acid hybridization.** After electrophoresis through agarose gels, restricted DNA was transferred to nitrocellulose membranes as described by Southern (18). Nitrocellulose filters were then hybridized to various <sup>32</sup>P-labeled DNA probes as described by Israel et al. (9) and modified by Buckler et al. (1). A recombinant plasmid (pX<sub>env</sub><sup>5'-6.7</sup>) (1) which contained a DNA segment extending 6.7 kb from the 5' terminus of NFS-Th-1 proviral DNA and was cleaved with *Bgl*III plus *Eco*RI served as an internal hybridization marker in some experiments.

## RESULTS

**Strategy for characterizing the organization of xenotropic proviruses present in inbred mice.** The recombinant plasmid pX<sub>env</sub> contains a 500-bp DNA segment derived from the *env* region of cloned xenotropic proviral DNA (1). This segment, a *Bgl*III-*Eco*RI fragment which maps 6.2 to 6.7 kb from the 5' terminus of the NFS-Th-1 xenotropic provirus (Fig. 1), hybridizes to both  $\alpha$ - and  $\beta$ -class (2) xenotropic and several differ-

ent MCF proviral DNAs; no annealing to ecotropic proviruses was observed (1).

The internal organization of endogenous xenotropic proviral DNAs present in the mouse genome was examined by hybridizing labeled pX<sub>env</sub> DNA to preparations of mouse liver DNA previously digested with restriction enzymes that are known to cleave more than once within expressed xenotropic proviruses; these include *Pst*I (1, 5), *Sac*I (1, 5), and *Bgl*III (1). The only DNA hybridizing to the pX<sub>env</sub> probe after digestion with these enzymes is internal proviral DNA fragments containing xenotropic envelope sequences. Other proviral DNA cleavage products that contain *gag* or *pol* but not the *env* regions of endogenous xenotropic proviruses do not anneal to the xenotropic envelope-specific probe.

Since the *Eco*RI site at 6.7 kb is highly conserved among xenotropic and MCF proviral DNAs (1, 5, 8, 15) and is located at the 3' end of the cloned xenotropic envelope segment (Fig. 1), *Eco*RI can be used in combination with *Sac*I or *Bgl*III to more finely map internal restriction enzyme sites in endogenous xenotropic proviruses.

Numerous studies have pointed to the presence of multiple copies of MuLV-related proviruses in normal mouse DNA (6, 7, 19, 20). Digestion of cellular DNA with multi-cut restriction enzymes such as *Sac*I and *Bgl*III, with or without *Eco*RI, provides a way of recruiting subgenomic fragments into discrete bands which represent related classes of endogenous proviruses.

**Digestion of mouse DNA with *Pst*I and hybrid-**

ization to the xenotropic MuLV envelope-specific probe. It has been previously shown that complete ecotropic and xenotropic proviral DNAs contain a *Pst*I site in the U<sub>3</sub> portion of each of the LTR segments (1, 5, 6). In addition to these two sites, several xenotropic proviruses have a *Pst*I site mapping 7.4 kb from the 5' terminus, and some contain additional *Pst*I sites located elsewhere in the proviral DNA (1, 5). When BALB/c, AKR/J, NFS.Akv-2, and C57L mouse DNAs were cleaved with *Pst*I and analyzed by the Southern blot hybridization technique with labeled pX<sub>env</sub> DNA, two prominent bands (7.4 and 3.3 kb) were visualized in all four preparations (Fig. 2). A 2.6-kb band was also present in all four DNAs but was quite faint in NFS.Akv-2 DNA compared with the other three preparations. Though not clearly seen in all lanes in Fig. 2, 5.2- and 4.4-kb *Pst*I cleavage products of varying intensity were also present. The 2.6-kb fragment, which is less prominent in NFS.Akv-2 DNA (Fig. 2, lane c), was undetectable in DNA prepared from 129/J mice (data not shown).

The prominent 7.4-kb *Pst*I cleavage product visualized in Fig. 2 is identical in size to the *Pst*I fragment derived from the  $\beta$  class of xenotropic proviruses (1, 2, 5), whereas the minor 4.4- and 5.2-kb fragments are identical in size to those previously reported for  $\alpha$ -class BALB-IU-2 and AKR-Th-6 xenotropic proviruses, respectively (1, 5). Thus, most mouse strains appear to contain copies of both  $\alpha$ - and  $\beta$ -class xenotropic proviral DNAs as defined by *Pst*I digestion, whether they are expressed or not.

**Digestion of mouse liver DNA with *Sac*I or *Sac*I plus *Eco*RI.** In the experiments described in this and the subsequent sections, restricted mouse DNA was hybridized to labeled pMuLV<sub>gen</sub> and pX<sub>env</sub> DNAs. Figure 3A shows the results of a blot hybridization experiment in which DNAs from eight inbred mouse strains were digested with *Sac*I, electrophoresed, blotted, and annealed to the generalized MuLV probe. Each DNA preparation contained prominent 4.5-, 3.7-, 2.2-, and 0.8-kb reactive *Sac*I cleavage products. Reactive 6.4- and 1.4-kb bands were also present but varied markedly in intensity between strains.

When the same *Sac*I-digested mouse liver DNAs were tested with the xenotropic *env*-specific probe, two strongly reactive cleavage products (4.5 and 3.7 kb) were visualized in all preparations (Fig. 3B). A common 2.3-kb band of varying intensity was also present, being particularly prominent in Molossinus, AKR/J, and NZB/J mice (Fig. 3B, lanes b, f, and h, respectively). A less prominent 6.4-kb fragment was also visualized in all eight mouse DNAs after longer exposure of the autoradiogram shown in Fig. 3B (data not shown).

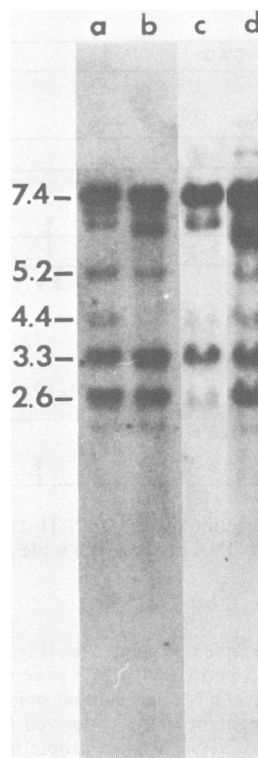
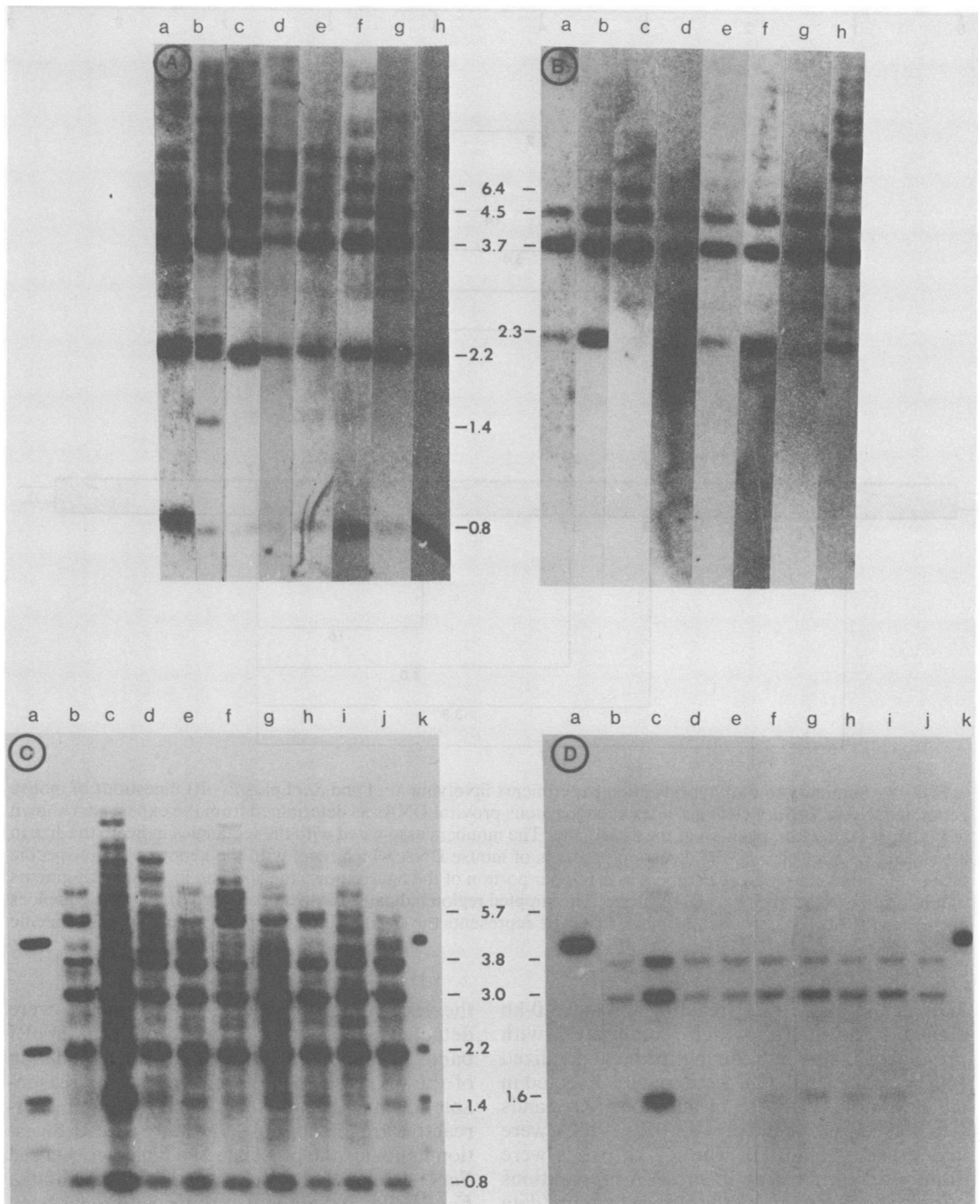


FIG. 2. Hybridization of the xenotropic *env* probe to *Pst*I-digested mouse DNA. Five-microgram samples of BALB/c (lane a), AKR/J (lane b), NFS.Akv-2 (lane c), and C57L (lane d) mouse liver DNAs were digested with *Pst*I, electrophoresed in different lanes of a horizontal agarose gel, transferred to a nitrocellulose membrane, and hybridized to the <sup>32</sup>P-labeled pX<sub>env</sub> DNA probe as described in the text. The numbers indicate the size in kb of pX<sub>env</sub>-reactive DNA digestion products calculated by comparison with known fragments in a marker mixture of  $\lambda$  DNA cleaved with *Hind*III or *Sma*I and  $\phi$ X174 replicative-form DNA cleaved with *Hpa*II or *Hae*III.

The 4.5- and 3.7-kb bands which hybridized to labeled pX<sub>env</sub> DNA corresponded to two of the prominent *Sac*I cleavage products annealing to the generalized MuLV probe (Fig. 3A and B).

To more precisely map the *Sac*I sites in different classes of reactive endogenous proviruses, the cellular DNAs were sequentially cleaved with *Sac*I and *Eco*RI and annealed to labeled DNA probes. Figure 3C shows the results of a blot hybridization of nine mouse liver DNAs digested with *Sac*I plus *Eco*RI and hybridized to <sup>32</sup>P-labeled pMuLV<sub>gen</sub>. Four prominent comigrating fragments (3.8, 3.0, 2.2, and 0.8 kb) as well as a pair of bands (1.4 and 1.6 kb) of varying intensity were present in all nine preparations. In a companion gel, the same DNAs were annealed to the xenotropic *env*-specific probe (Fig.



**FIG. 3.** Reactivity of *SacI*- or *SacI* plus *EcoRI*-digested mouse DNA with the  $MuLV_{gen}$  or  $pX_{env}$  DNA probes. Mouse liver DNAs (5  $\mu$ g per lane) were cleaved with *SacI* (A and B) or *SacI* plus *EcoRI* (C and D), electrophoresed through an agarose horizontal slab gel, and transferred to nitrocellulose filters. Membranes shown in (A) and (C) were hybridized with the  $pMuLV_{gen}$  DNA probe, whereas membranes shown in (B) and (D) were hybridized with the  $pX_{env}$  DNA probe. Mouse DNAs shown in (A) and (B) are: BALB/c (lane a), *M. molossinus* (lane b), NFS.Akv-2 (lane c), C3H/FgLw (lane d), C57L (lane e), AKR/J (lane f), NFS (lane g), and NZB/J (lane h). The DNAs shown in (C) and (D) are: BALB/c (lane b), *M. molossinus* (lane c), F/St (lane d), NFS.Akv-2 (lane e), C3H/FgLw (lane f), C57L (lane g), AKR/J (lane h), NZB/N (lane i), and NFS (lane j). An internal hybridization marker mixture consisting of *BglIII* plus *EcoRI*-digested  $pX_{env}$  DNA (1) and containing fragments 4.4, 2.2, 1.6, 1.4, and 1.0 kb in size was electrophoresed in (B, lanes a and k). Only the 4.4- and 0.5-kb (run-off gel) fragments hybridized to the  $pX_{env}$  probe (1) (D, lanes a and k).

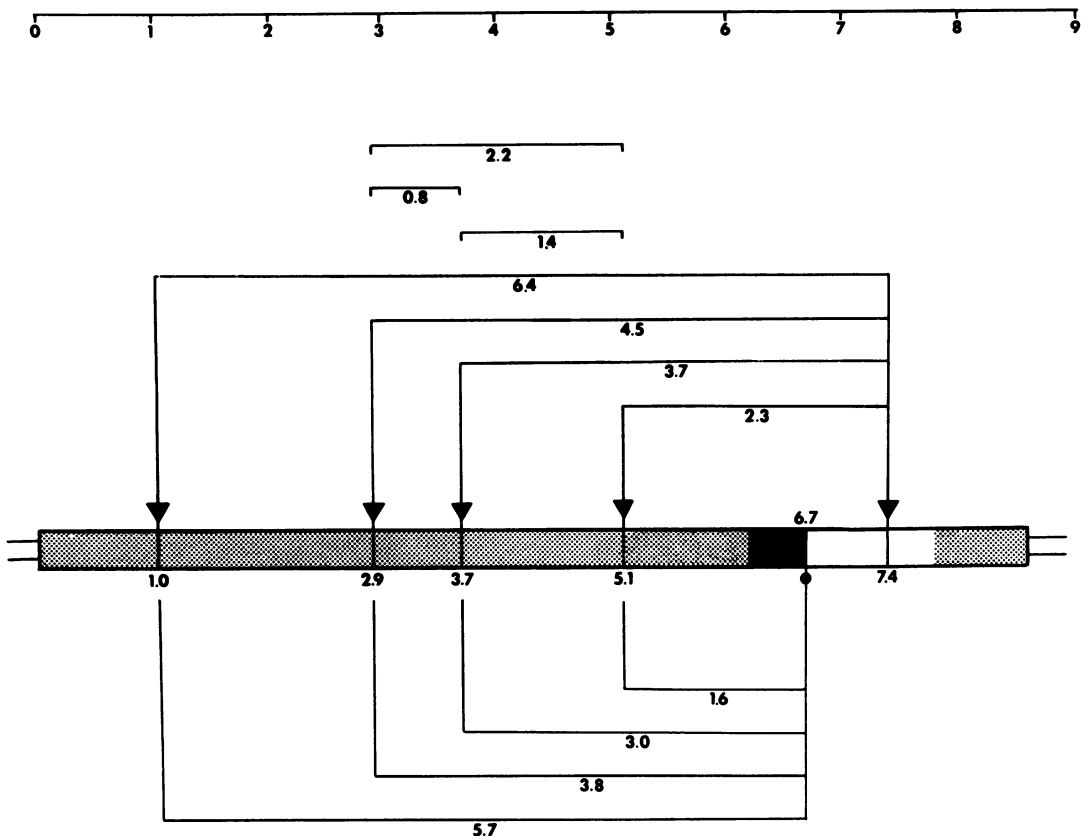


FIG. 4. Summary of blot hybridization experiments involving *SacI* and *SacI* plus *EcoRI* digestions of mouse genomic DNAs. ▼, *SacI* cleavage sites in endogenous proviral DNAs as determined from the experiment shown in Fig. 3; ● (at 6.7 kb), position of the *EcoRI* site. The numbers associated with the rectangles indicate the size in kb of *SacI* or *SacI* plus *EcoRI* digestion products of mouse DNA which react with the xenotropic *env*-specific probe; the bracketed triad of numbers in the upper portion of the figure shows the size and location of fragments that hybridize to the MuLV<sub>gen</sub> DNA probe. The stippled region indicates the location of proviral DNA sequences present in the MuLV<sub>gen</sub> probe; the black rectangle represents the size and location of the xenotropic *env*-specific probe.

3D). The strongly pX<sub>env</sub>-reacting 3.8- and 3.0-kb cleavage products, which comigrated with prominent bands hybridizing to the generalized MuLV probe (Fig. 3C), were readily detected in all nine mouse DNAs. In addition, two bands (1.6 and 5.7 kb) reactive with pX<sub>env</sub> DNA were also present (Fig. 3D). The 5.7-kb bands were faint but could be seen in all DNA preparations after longer exposure of the autoradiogram (data not shown).

Analysis of the blot hybridization experiments shown in Fig. 3B and D revealed that *EcoRI* digestion of mouse liver DNAs previously cleaved with *SacI* resulted in a 0.7-kb reduction in the size of several pX<sub>env</sub>-reactive bands. The prominent 4.5- and 3.7-kb *SacI* cleavage products shown in Fig. 3B became the 3.8- and 3.0-kb fragments shown in Fig. 3D. Similarly, the fainter 2.3- and 6.4-kb *SacI* bands hybridizing to

the xenotropic *env*-specific probe (Fig. 3B) were detected as 1.6- and 5.7-kb *SacI* plus *EcoRI* bands in Fig. 3D. From the known map location of the pX<sub>env</sub> segment (Fig. 1), it seems reasonable to infer that the 0.7-kb shortening of *SacI*-reactive fragments observed after *EcoRI* digestion indicates the presence of a highly conserved *SacI* site located 700 bp to the 3' side of the *EcoRI* site. A *SacI* site at this position has been mapped in all xenotropic proviral DNAs examined (5, 8). The xenotropic *env*-reactive 5.7-, 3.8-, 3.0-, and 1.6-kb *SacI* plus *EcoRI* fragments (Fig. 3D) locate the position of other *SacI* sites (with respect to the *EcoRI* site at 6.7 kb) in endogenous xenotropic proviruses. These data have been used to formulate the *SacI* and *EcoRI* maps of endogenous xenotropic proviral DNAs shown in Fig. 4. The highly conserved *SacI* site located within 0.7 kb of the *EcoRI* site

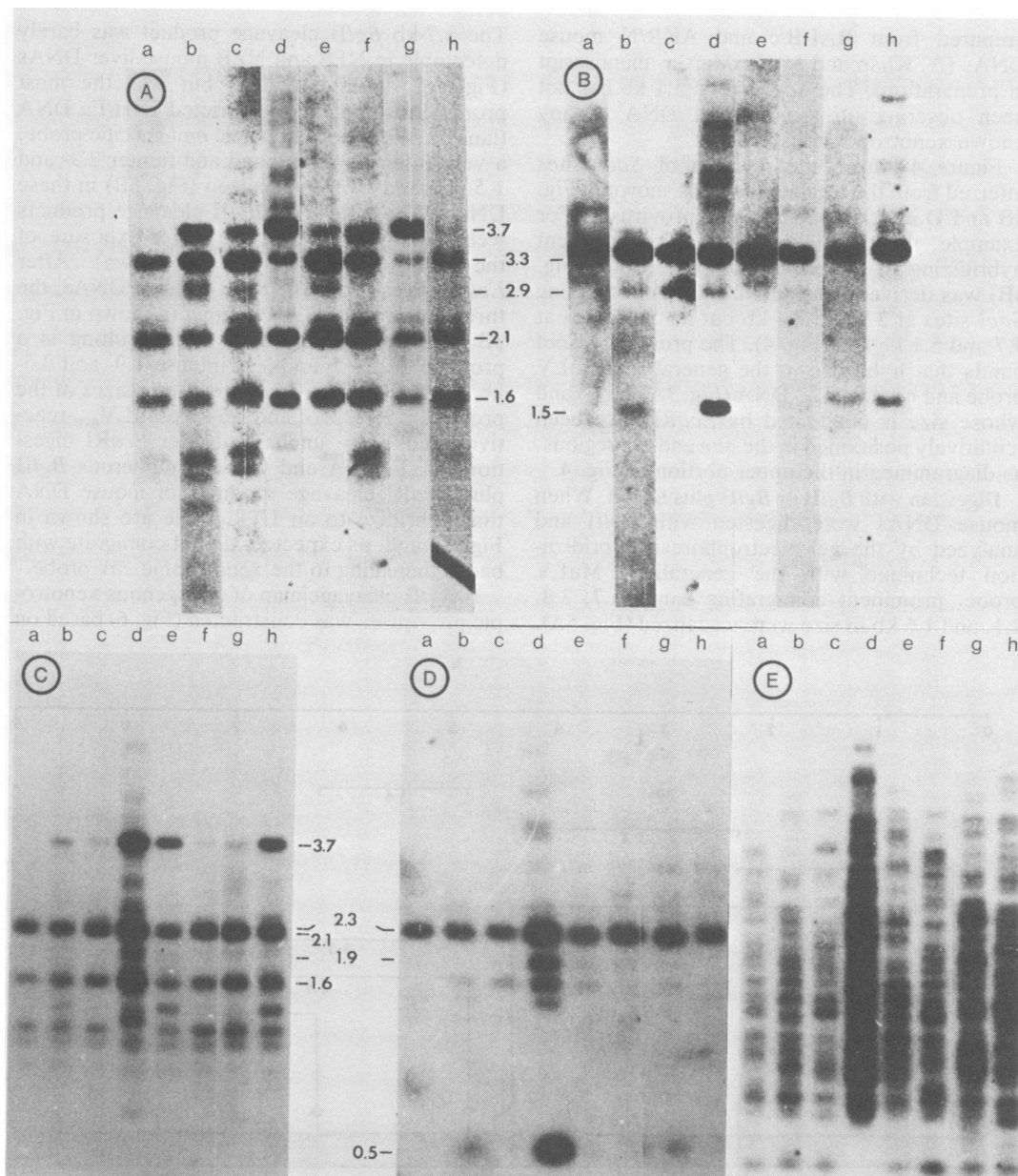


FIG. 5. Hybridization of *Bgl*II- or *Bgl*II plus *Eco*RI-digested mouse DNA with MuLV<sub>gen</sub>, pX<sub>env</sub>, or LTR DNA probes. Mouse liver DNAs (5 µg per lane) were cleaved with *Bgl*II (A and B) or *Bgl*II plus *Eco*RI (C-E), electrophoresed through agarose gels, and transferred to nitrocellulose membranes. Membranes were hybridized to the MuLV<sub>gen</sub> (A and C), xenotropic *env* (B and D), or LTR (E) DNA probes. Mouse DNAs shown in (A) and (B) are: NFS (lane a), AKR/J (lane b), BALB/c (lane c), *M. molossinus* (lane d), NFS.Akv-2 (lane e), C57L (lane f), C3H/FgLw (lane g), and NZB/J (lane h). In C-E, the DNAs examined were: NFS (lane a), AKR/J (lane b), BALB/c (lane c), *M. molossinus* (lane d), F/St (lane e), NFS.Akv-2 (lane f), C57L (lane g), and C3H/FgLw (lane h).

positioned 7.4 kb from the 5' terminus. The *Sac*I sites mapping at 2.9 and 3.7 kb, which would generate the prominent pX<sub>env</sub>-reactive 4.5- and 3.7-kb fragments, respectively, in Fig. 3B, have also been detected in several xenotropic proviral

DNAs (5, 8). Although the *Sac*I site located at 1.0 kb has not been found in the proviral DNAs of known infectious xenotropic proviruses, a site in this position has been identified in several clones of endogenous MuLV-related proviruses

prepared from BALB/c and AKR/N mouse DNAs (A. Khan and M. A. Martin, manuscript in preparation). The *SacI* site at 5.1 kb has not been observed in the proviral DNA of any known xenotropic virus.

Figure 4 shows the position of *SacI* sites inferred from the fragment lengths shown in Fig. 3B and D in related xenotropic proviruses. For example, the prominent 4.5-kb *SacI* fragment hybridizing to the xenotropic *env* probe (Fig. 3B) was derived from proviral DNA containing *SacI* sites at 2.9 and 7.4 kb but no *SacI* sites at 3.7 and 5.1 kb (see Fig. 4). The prominent *SacI* bands that hybridize to the generalized MuLV probe and not to pX<sub>env</sub> DNA (Fig. 3A and C) and whose size is unaffected by *EcoRI* have been tentatively positioned in the *gag* and *pol* regions, as diagrammed in the upper portion of Fig. 4.

**Digestion with *BglII* or *BglII* plus *EcoRI*.** When mouse DNAs were digested with *BglII* and analyzed by the gel electrophoresis-hybridization technique with the generalized MuLV probe, prominent comigrating bands 3.7, 3.3, 2.1, and 1.6 kb in size were visualized (Fig. 5A).

The 3.7-kb *BglII* cleavage product was barely detectable in NFS and NZB mouse liver DNAs (Fig. 5A, lanes a and h) but was the most prominent fragment in restricted C3H/Fg DNA (lane g). With the xenotropic *env*-specific probe, a very intense 3.3-kb band and fainter 2.9- and 1.5-kb bands were visualized (Fig. 5B) in these DNAs. The latter two *BglII* cleavage products were readily visualized on longer exposure of the autoradiogram (data not shown). After *EcoRI* digestion of the *BglII*-cleaved DNAs, the three comigrating *BglII* fragments shown in Fig. 5B were reduced 1 kb in size, resulting in a prominent 2.3-kb and less intense 1.9- and 0.5-kb bands (Fig. 5D). In contrast, the sizes of the prominent 3.7-, 2.1-, and 1.6-kb pMuLV<sub>gen</sub>-reactive bands were unchanged after *EcoRI* digestion (cf. Fig. 5A and C). The numerous *BglII* plus *EcoRI* cleavage products of mouse DNA that hybridize to an LTR probe are shown in Fig. 5E and, as expected, do not comigrate with bands annealing to the xenotropic *env* probe.

A *BglII* cleavage map of endogenous xenotropic proviruses was constructed (Fig. 6) based on

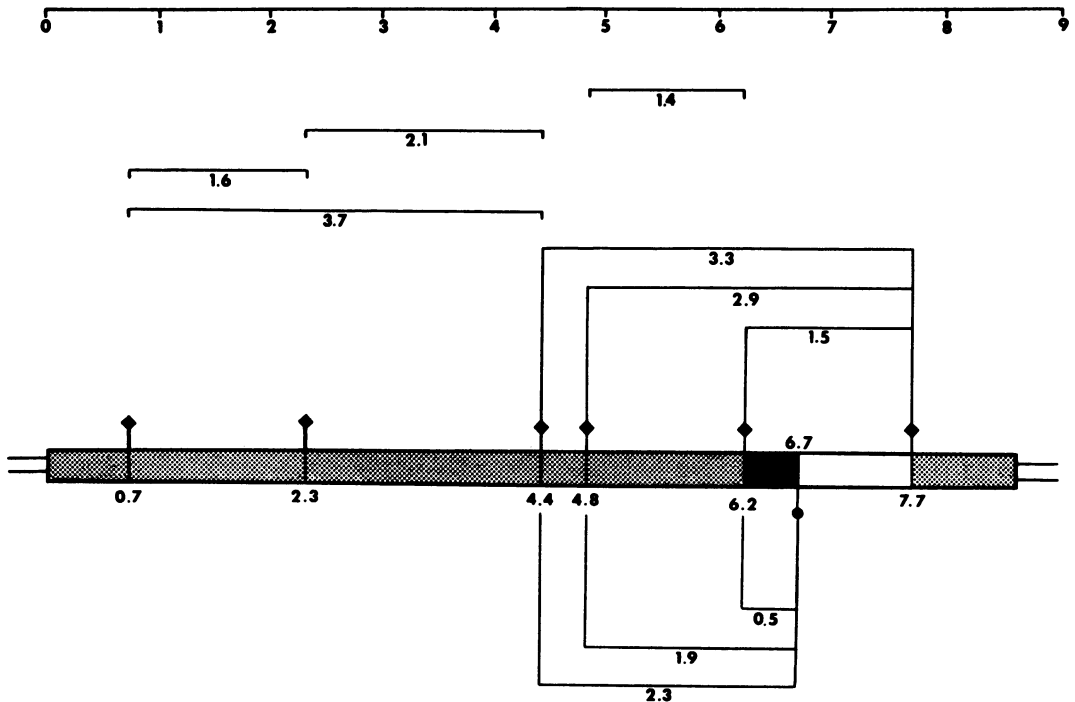


FIG. 6. Summary of blot hybridization experiments involving *BglII* and *BglII* plus *EcoRI* digestions of mouse genomic DNAs. ♦, *BglII* cleavage sites in NFS-Th-1 xenotropic proviral DNA (1) previously shown in Fig. 1; ● (at 6.7 kb), position of the *EcoRI* site. The numbers within the rectangles indicate the size, in kb, of *BglII* or *BglII* plus *EcoRI* digestion products of mouse DNA which react with the xenotropic *env*-specific probe; the bracketed numbers in the upper portion of the figure show the size and location of fragments that hybridize to the MuLV<sub>gen</sub> DNA probe. The stippled region indicates the location of proviral DNA sequences present in the MuLV<sub>gen</sub> DNA probe; the black rectangle represents the size and location of the xenotropic *env*-specific probe.

the results presented in Fig. 5. A highly conserved *Bgl*II site mapping within 1.0 kb of the *Eco*RI site has been positioned at 7.7 kb, a location identical to that present in the provirus of NFS-Th-1 xenotropic MuLV (Fig. 1 and reference 1). The *Bgl*II sites identified in the endogenous xenotropic proviruses are all present in NFS-Th-1 xenotropic proviral DNA (Fig. 6). However, the most abundant endogenous xenotropic provirus present in mouse chromosomal DNA contains *Bgl*II sites at 4.4 and 7.7 kb but no sites at 4.8 or 6.2 kb (see Fig. 6).

The positions of *Bgl*II cleavage sites in proviral DNA segments that hybridize to the generalized MuLV probe and not to the xenotropic *env*-specific probe are also shown in Fig. 6. It is assumed that many of these pMuLV<sub>gen</sub>-reactive *Bgl*II cleavage fragments, mapping to the *gag* and *pol* regions, were derived from proviral DNA containing xenotropic *env*-specific determinants.

#### DISCUSSION

In this paper, we have utilized both a generalized MuLV and a xenotropic *env*-specific DNA probe to assess the internal organization of endogenous retroviral DNA segments present in several different inbred strains of mice. When mouse chromosomal DNAs digested with restriction enzymes known to cleave at multiple sites within xenotropic proviral DNAs were hybridized to the generalized MuLV probe, a series of comigrating, strongly reacting bands were visualized in all mouse DNAs examined, along with numerous, faint, noncomigrating cleavage products (Fig. 3A and C, and 5A and C). The comigrating, intensely reacting bands undoubtedly reflect the recruitment of subgenomic segments of closely related proviruses that are present in multiple copies in the mouse genome. Strikingly, the hybridization experiments with labeled xenotropic *env*-specific DNA demonstrated the presence of several bands common to all mouse DNAs examined which comigrated with some of the same prominent bands detected with the generalized MuLV probe. Although it is risky to derive quantitative information from blot hybridization experiments of mammalian genomic DNAs, it is tempting to speculate that a majority of the endogenous MuLV proviruses exist as multicopy families of detectably different yet closely related proviral DNAs that contain xenotropic *env*-specific sequences. This is in contrast to ecotropic proviruses that are generally present in small copy numbers (3, 6).

When the internal organization of the endogenous xenotropic proviral DNAs was compared with the proviral DNA restriction maps of xenotropic MuLV isolates, interesting correlations

were observed. In the case of *Sac*I, the existence of at least seven different endogenous proviruses containing xenotropic *env*-specific sequences could be inferred (Fig. 7). Based on the results of *Sac*I and *Sac*I plus *Eco*RI digestion of mouse DNAs (Fig. 3), the *Sac*I site located at 7.4 kb and present in the proviral DNAs of all xenotropic MuLVs examined to date (5, 8) is also conserved in multicopy families of endogenous xenotropic proviruses. The family of endogenous xenotropic proviruses containing *Sac*I sites at 7.4 and 5.1 kb (proviral DNAs A through C; Fig. 7) has at least three members, differing by the presence or absence of *Sac*I sites at 3.7 and 2.9 kb. The provirus of the infectious NZB-IU-6 xenotropic MuLV has *Sac*I cleavage sites identical to those shown for proviral DNA E in Fig. 7 (5), as does the xenotropic-related AKR 40 virus. Similarly, the *Sac*I restriction maps of the NFS, BALB-2, Kyushu, AKR 6, and C58 xenotropic proviruses resemble proviral DNA F in Fig. 7 (1, 5). *Sac*I sites detected at 1.0 and 5.1 kb and present in endogenous proviruses A through C and G (Fig. 7) have not been detected in the proviral DNAs of infectious xenotropic MuLVs. In this regard, several endogenous MuLV-related DNA segments molecularly cloned from BALB/c and AKR/J mouse DNAs contain the *Sac*I site at 1.0 kb (A. Khan and M. A. Martin, submitted for publication). It should be noted that the labeled xenotropic *env*-specific DNA used in these experiments is similar to an MCF xenotropic *env* probe recently used to detect MCF-like *env* sequences in AKR mouse DNA (4). Consequently, some of the proviral DNA restriction maps shown in Fig. 7 could represent endogenous proviruses containing *env* segments that are incorporated into MCF viruses rather than being potentially inducible xenotropic MuLVs.

Throughout these studies, we have endeavored to correlate the internal organization of endogenous retroviral DNA with that of proviruses of known infectious MuLVs. The results of solution hybridization experiments (7) as well as blot hybridization assays in which the xenotropic *env* probe was employed in conjunction with restriction enzymes that do not cleave within xenotropic proviral DNA (M. D. Hoggan et al., manuscript in preparation) all point to the presence of numerous copies of endogenous xenotropic proviruses in mouse DNA. The data presented in this paper indicate that the many copies of xenotropic proviral DNA fall into closely related yet detectably different families. It is very likely that a majority of the endogenous xenotropic proviruses detected in mouse DNA are never expressed and only a few represent potentially inducible and fully infectious retroviruses.



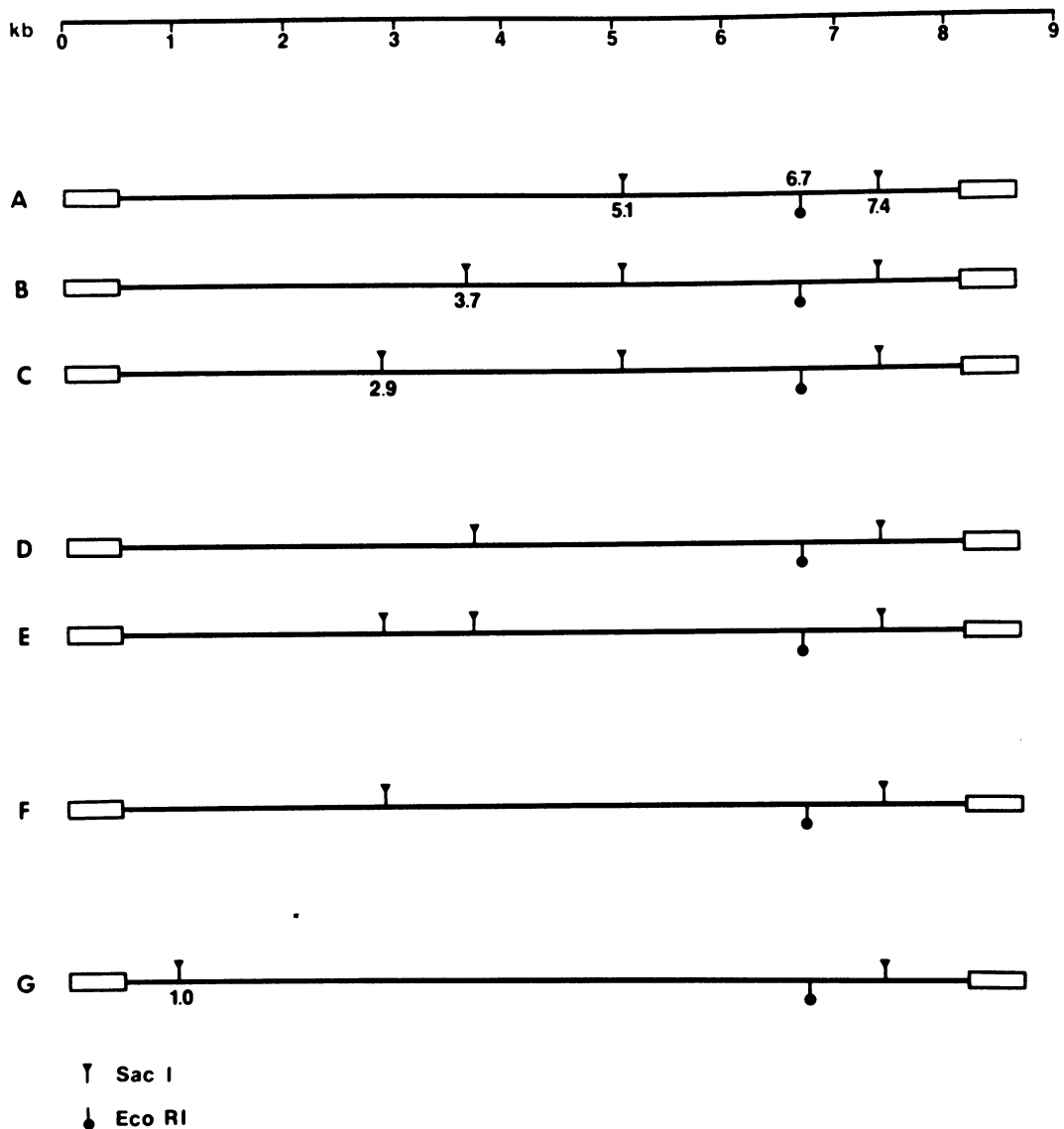


FIG. 7. Cleavage maps of endogenous proviral DNAs containing xenotropic *env* sequences predicted from *SacI* and *SacI* plus *EcoRI* digestions of mouse DNA. Symbols: ▼, *SacI*; ●, *EcoRI*.

Although the experiments described do not assist the investigator in predicting which xenotropic proviruses will be expressed and which will not, they do indicate that the relative abundance of a particular family of proviral DNA does not correlate with the recovery of its corresponding infectious virus. This is best illustrated by comparing the *PstI* cleavage patterns of the endogenous xenotropic proviruses present in AKR/J, BALB/c, and NFS congenic mice with the *PstI* digests of the proviral DNAs associated with the infectious xenotropic MuLVs isolated

from these three strains (1). *PstI* cleavage of the NFS-Th-1 xenotropic provirus generates a single 7.4-kb  $pX_{env}$ -reactive fragment that comigrates with the prominent *PstI* digestion product detected in the four DNAs shown in Fig. 2 (1, 5). On the other hand, the proviruses of the xenotropic MuLVs recovered from AKR/J and BALB/c mice contain 5.2- and 4.4-kb *PstI* segments, respectively, each of which corresponds to a faint band visualized in the autoradiogram shown in Fig. 2 (1, 5). Assuming that band intensity correlates with provirus copy number,

the proviral DNA families expressed in AKR and BALB/c mice are significantly less abundant than the type isolated from NFS animals.

This same lack of correlation between the copy number of a particular class of endogenous xenotropic proviral DNA and the type of xenotropic MuLV expressed was also observed in the experiment involving *Bgl*III digestion. Cleavage of NFS-Th-1 xenotropic proviral DNA with *Bgl*III generates seven restriction fragments, one of which (1.5 kb) reacts with the pX<sub>env</sub> DNA probe (Fig. 1). All mice contain many copies of an endogenous xenotropic provirus which generates a 3.3-kb fragment after digestion with *Bgl*III (Fig. 5B). Only a few copies of an endogenous provirus containing *Bgl*III restriction sites at 6.2 and 7.7 kb (which would generate the 1.5-kb fragment characteristic of the NFS-Th-1 xenotropic provirus) were detected in the mouse DNAs examined.

Inducible loci for xenotropic MuLVs have been mapped to a chromosome 1 locus, *Bxv-1*, in five mouse strains, including AKR and BALB/c (11). As mentioned above, the proviral DNAs of the AKR and BALB/c xenotropic MuLVs can be readily distinguished from one another on the basis of characteristic *Pst*I restriction maps (1, 5); low-copy-number endogenous proviruses with *Pst*I cleavage maps typical of these two xenotropic proviruses have been identified in most mouse DNAs tested (Fig. 2). Assuming that AKR and BALB/c xenotropic MuLVs arise as direct transcription products of endogenous proviral DNAs, the results of genetic mapping experiments coupled with the blot hybridization studies of mouse genomic DNA reported here point to the presence of at least two different xenotropic proviruses at the *Bxv-1* locus in different mouse strains. The *Bxv-1* locus may therefore contain a cluster of related xenotropic MuLV DNA segments that are differentially expressed in various mouse strains and may resemble other repeated DNA segments, such as the globin genes, in organization (12). Blot hybridization experiments are currently in progress in which the xenotropic *env*-specific probe and DNA prepared from mouse-Chinese hamster somatic cell hybrids are being used to further map the chromosomal location of the different classes of endogenous xenotropic proviral DNA.

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