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Comparison of the Restriction Endonuclease Maps of Unintegrated Proviral DNAs from Four Xenotropic Murine Leukemia Viruses

DAVID R. JOSEPH

Robert V. Slater Laboratory, Papanicolaou Cancer Research Institute, Miami, Florida 33136

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From purified linear and superhelical DNAs, the restriction endonuclease maps of four xenotropic murine leukemia virus DNAs from NFS, NZB, BALB/c, and AKR mice were determined with ten restriction endonucleases. Each xenotropic proviral DNA was found to have a unique restriction endonuclease map, with differences in the *gag*, *pol*, *env*, and terminal repeated sequence regions. However, type-specific *Sac*I and *Eco*RI sites in the *env* region were identical in all four xenotropic murine leukemia virus DNAs and were not found in ecotropic murine leukemia virus DNA. Comparison of the xenotropic murine leukemia virus DNA maps with maps of ecotropic murine leukemia virus DNA showed that the *pol* and terminal repeated sequence regions were highly conserved. Other similarities in ecotropic and some xenotropic viral DNAs suggest common origins.

Endogenous murine leukemia viruses (MuLV's) can be divided into two classes with respect to host range (1). The ecotropic MuLV infects and replicates in mouse cells but not in heterologous cells. Conversely, the xenotropic MuLV's (MuX's) are not infectious for most mouse cell lines but can replicate well in cells from other species (18). Integrated proviral DNAs of xenotropic viruses are present in the genomes of inbred mouse strains and wild mice, whereas only some strains have complete ecotropic MuLV proviral DNA (1, 6, 15). Although the molecular structures of these endogenous proviruses are not well characterized, recent studies have revealed the basic organization of the unintegrated proviruses and integrated proviral DNA acquired by infection (8, 22, 28, 31). These studies suggest that MuLV proviral DNA is remarkably similar to the structures of bacterial and eucaryotic transposable elements (29).

The endogenous xenotropic viruses are of particular interest because of their putative parental role in the formation of recombinant viruses, which include Friend spleen focus-forming virus, the mink cell focus-inducing (MCF) viruses (9, 11, 24, 30), and the B-tropic MuLV's (2, 14). All of these viruses have an altered host range and appear to have been formed by the recombination of ecotropic and xenotropic MuLV genomes (2, 9, 30). The MCF-type viruses, which include recombinants of Moloney MuLV (10), appear to be recombinants in the *env* gene region of the viral genome. Because their appearance precedes development of leukemia and because injection of MCF MuLV into newborn AKR mice will accelerate leukemia development, it has been proposed that these recombinants may be intermediates in spontaneous viral-mediated AKR leukemia (7, 11, 21).

In view of the potential roles of MuX's in viral recombination and leukemogenesis and their possible relation to endogenous eucaryotic transposable elements, I initiated an investigation into the nature of MuX proviral DNA. Hirt DNA from infected cells was subjected to horizontal agarose gel electrophoresis and analyzed for unintegrated viral DNA by Southern blotting (26) and hybridization with a virus-specific [^{32}P]cDNA probe (16). Fragment sizes were determined by comparison with *Hin*dIII-digested lambda [^{32}P]DNA which was run in parallel lanes. Hirt DNA from NIH 3T3 cells infected with an ecotropic AKR MuLV and Hirt DNA from mink cells infected with NFS, NZB, BALB/c, or AKR xenotropic virus yielded the expected (16, 23, 25, 34) 9-kilobase pair (kbp) linear form and two superhelical forms, which migrated as 4.5- to 5.0-kbp linear DNA. For the detailed restriction endonuclease analyses, I purified the linear and superhelical DNAs from agarose gels. Restriction endonuclease treatments of the faster-migrating forms have confirmed the assumption that these two forms were superhelical with one or two tandem copies of the terminal repeated sequence (TRS), because one-cut enzymes converted them to 9.0- and 8.5-kbp DNA, whereas enzymes which cleave only in the TRS yielded 8.5- and 0.6-kbp

DNAs. Hirt DNA from uninfected cells or virus-producing cells contained no detectable unintegrated proviral DNA.

To compare the ecotropic MuLV and MuX proviral DNAs and to identify the xenotropic viral DNA regions which correspond to the 3′ and 5′ ends of the viral RNA, I analyzed an AKR ecotropic MuLV proviral DNA. Purified linear or superhelical DNA was digested with each restriction enzyme, and the DNA fragments were analyzed by agarose gel electrophoresis, Southern blotting, and hybridization with AKR ecotropic MuLV [^{32}P]cDNA (16). The location of each restriction endonuclease site was identified by double digests, and the 3′ terminus was identified with a 3′-specific cDNA probe (33). The linear map (Fig. 1) which was generated is in agreement with previously published maps of ecotropic MuLV DNA (19, 22, 27, 34).

Because of the difficulty in obtaining xenotropic viral RNA, I also used the ecotropic MuLV cDNA probe for analyses of MuX DNAs. Experiments with the MuX [^{32}P]cDNA probe on restriction digests of AKR MuX proviral DNA revealed no differences in the bands of hybridization, as compared with the ecotropic MuLV probe (data not shown). However, small fragments from the 3′ end of the genome did not hybridize as intensely with ecotropic MuLV cDNA. Restriction endonuclease analysis of AKR MuX unintegrated linear and superhelical proviral DNAs revealed that this provirus contained apparently the same restriction sites for *Sal*I and *Xho*I as the ecotropic provirus (Fig. 1). *Xho*I or *Sal*I converted the MuX superhelical DNAs to 8.5- and 9.0-kbp fragments (Fig. 2B, lines h and j). Figure 2A (lane h) demonstrates the *Sal*I cleavage products of linear DNA, and the results from *Xho*I and *Sal*I double digests are summarized in Table 1. In contrast, the cleavage sites of the other restriction enzymes were clearly different from the sites of ecotropic MuLV DNA.

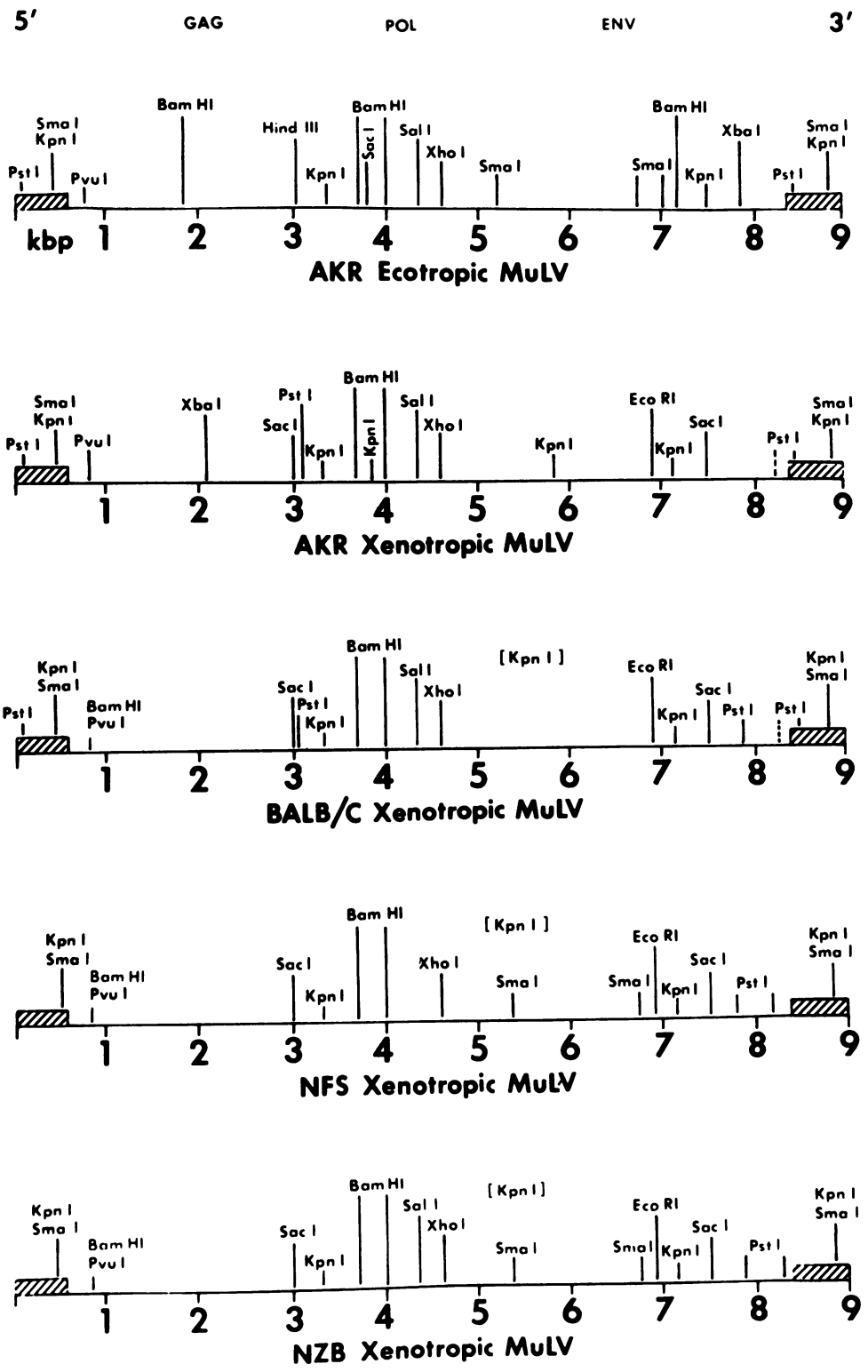
Digestion of purified AKR MuX linear DNA with *Bam*HI generated two detectable fragments of 5.0 and 3.7 kbp (Fig. 2A, lane a; Table 1). Double digests with *Bam*HI and *Xho*I or *Sal*I (Fig. 2A, lanes c and f) revealed that the large *Bam*HI fragment contained these restriction sites at 0.6 and 0.3 kbp, respectively, from a *Bam*HI site, which was located at 4.0 kbp on the AKR MuX linear DNA map (Fig. 1). Because cleavage of purified superhelical DNA with *Bam*HI resulted in identifiable fragments of 8.1 and 8.7 kbp (Fig. 2B, lane d), the two *Bam*HI sites were determined to be separated by 0.3 or 0.4 kbp. Thus, the second *Bam*HI site was at about 3.7 kbp (Fig. 1).

Interestingly, the order of the *Xho*I, *Sal*I, and *Bam*HI sites in the center of AKR xenotropic viral DNA appeared to be identical to the sites in the ecotropic MuLV genome. Based on this homology, I identified the 3′ and 5′ termini of the xenotropic viral DNA (Fig. 1).

Digestion of AKR MuX linear proviral DNA with *Eco*RI generated two fragments of 6.9 and 2.0 kbp (Table 1). Double digests with *Bam*HI and *Eco*RI revealed that *Eco*RI cleaved the large (5-kbp) *Bam*HI fragment to yield 2.8- and 2.0-kbp species (Table 1), whereas *Eco*RI treatment of superhelical DNA resulted in 9.0- and 8.5-kbp DNAs (Fig. 2B, lane e). These data indicate that the single *Eco*RI site was at about 7.0 kbp on the DNA map (Fig. 1). *Xba*I also cleaved the linear provirus at one site to yield 6.8- and 2.1-kbp fragments (Table 1). Double digests with *Bam*HI located the site at 2.1 kbp on the restriction endonuclease map (Fig. 1).

Cleavage of the MuX linear provirus with *Sac*I yielded three fragments of 4.5, 3.0, and 1.5 kbp. *Bam*HI, *Xho*I, *Sal*I, and *Eco*RI cleaved the large *Sac*I fragment (Table 1). In addition, *Xba*I cleaved the 3.0-kbp *Sac*I fragment to yield a 2.2-kbp DNA species. These data are consistent with *Sac*I sites at 3.0 and 7.5 kbp on the linear map (Fig. 1).

FIG. 1. *Restriction endonuclease maps of MuLV unintegrated linear DNA. All virus isolates were from Janet Hartley's laboratory (National Institutes of Health). The NFS (NFS 1) and AKR xenotropic (AKR 6) viruses were isolated by cocultivation of thymus cells and mink lung cells (20). The NZB xenotropic virus was from a NZB mouse embryo and was isolated by IdU treatment and cocultivation (20). The BALB/c xenotropic virus was induced with IdU from BALB/c fibroblasts. All xenotropic viruses were propagated on mink lung cells in Dulbecco modified Eagle medium with 10% fetal calf serum. The AKR ecotropic MuLV (AKR 2a), which was isolated from the thymus of a 2-month-old AKR mouse by cocultivation with SC-I cells (20), was propagated on NIH 3T3 cells in Eagle minimal essential medium with 10% fetal calf serum. The cleavage sites for each restriction enzyme were determined by double digests, as described in the text. The size of the restriction fragments from AKR MuX DNA is described in Table 1. The maps are oriented with respect to the 3′ and 5′ ends of the RNA; the striped area at each end of the DNA represents the TRS. The approximate locations of the gag, pol, and env genes (31) are shown at the top of the figure. The KpnI cleavage sites in AKR MuX DNA were determined from a molecular clone of superhelical DNA. In BALB/c, NZB, and NFS viral DNAs, at least two KpnI sites existed between 3.3 and 7.2 kbp that have not been mapped. Dashed lines represent possible sites.*


5′
GAG
POL
ENV
3′
Pst I
Sma I
Kpn I
Pvu I
Bam HI
Hind III
Kpn I
Bam HI
Sac I
Sal I
Xho I
Sma I
Bam HI
Sma I
Kpn I
Xba I
Sma I
Kpn I
Pst I
kbp 1 2 3 4 5 6 7 8 9
AKR Ecotropic MuLV
Sma I
Kpn I
Pst I
Pvu I
Xba I
Pst I
Sac I
Kpn I
Bam HI
Kpn I
Sal I
Xho I
Kpn I
Eco RI
Kpn I
Sac I
Pst I
Sma I
Kpn I
1 2 3 4 5 6 7 8 9
AKR Xenotropic MuLV
Kpn I
Sma I
Pst I
Bam HI
Pvu I
Bam HI
Sac I
Pst I
Kpn I
Sal I
Xho I
[Kpn I]
Eco RI
Kpn I
Sac I
Pst I
Pst I
Kpn I
Sma I
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BALB/C Xenotropic MuLV
Kpn I
Sma I
Bam HI
Pvu I
Bam HI
Sac I
Kpn I
Xho I
Sma I
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Sma I
Kpn I
Sac I
Pst I
Kpn I
Sma I
1 2 3 4 5 6 7 8 9
NFS Xenotropic MuLV
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Sma I
Bam HI
Pvu I
Bam HI
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NZB Xenotropic MuLV

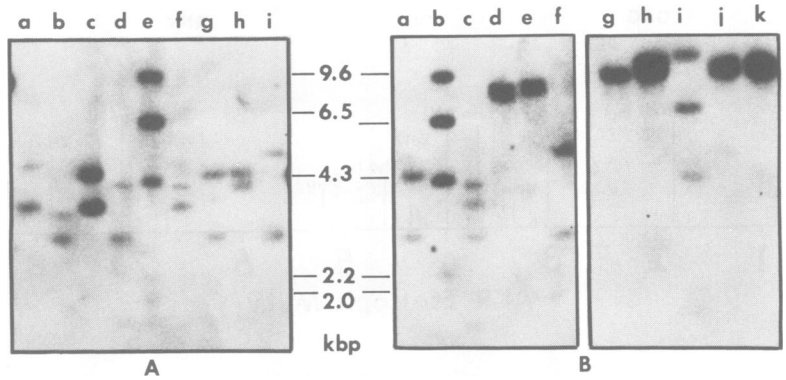

FIG. 2. *Restriction endonuclease digestion of AKR MuX linear (A) and superhelical (B) DNAs. Unintegrated proviral DNA was prepared by infection of cells in culture by cocultivation of MuLV-producing cells and homologous uninfected cells (16). Unintegrated MuLV proviral DNAs were purified from Hirt supernatant fractions (13) after infection, and the linear and superhelical proviral DNAs were purified from total Hirt DNA on Low-Melting Point Agarose gels (Bethesda Research Laboratories, Inc., Rockville, Md.). Linear or superhelical DNA was electrophoresed on 0.7% horizontal agarose slab gels (16), with and without restriction endonuclease treatment. Lambda DNA (0.25 μg) was included in each digest. After electrophoresis, the gel was stained with ethidium bromide and photographed. Restriction fragments of lambda DNA were analyzed to determine if digestion was complete. DNA was then transferred to nitrocellulose paper and hybridized with AKR ecotropic MuLV [^{32}P]cDNA (16, 32). After being washed (32), the membranes were dried and exposed at −80°C to Kodak X-Omat RP film with Du Pont Lightning Plus intensifying screens. A, Linear DNA; lanes are as follows: a, BamHI; b, BamHI and SacI; c, BamHI and SalI; d, BamHI and PstI; e, [^{32}P]DNA marker; f, BamHI and XhoI; g, SacI; h, SalI; i, PstI. B, Superhelical DNA; lanes are as follows: a, SacI and PstI; b, [^{32}P]DNA marker; c, SacI and XhoI; d, BamHI; e, EcoRI; f, PstI; g, SmaI; h, SalI; i, [^{32}P]DNA marker; j, XhoI; k, XbaI.*

*Pst*I cleavage of linear DNA yielded three fragments of 5.2, 3.0, and 0.5 kbp (Fig. 2A, lane i). Double digests with *Bam*HI and *Pst*I revealed that *Pst*I cleaved 0.7 kbp from the small *Bam*HI fragment and 0.7 kbp from the large *Bam*HI fragment (Fig. 2A, lane d). Because *Pst*I cleaved a 0.1- or 0.2-kbp piece from the large *Sac*I fragment, one *Pst*I site must have been nearly coincident with the *Sac*I site at 3.2 kbp, and two other *Pst*I sites must have been at 0.1 or 0.2 and apparently 8.3 kbp on the linear map. Also, because superhelical DNA yielded major *Pst*I fragments of the same size, there appeared to be *Pst*I sites in the TRS. These sites have been recently mapped with a cloned AKR MuX DNA provirus, as described in the legend to Fig. 1 (D. R. Joseph, manuscript in preparation). However, I was not able to confirm the presence of a *Pst*I site at 8.3 kbp with cloned DNA.

Further restriction endonuclease analysis of AKR MuX linear DNA also revealed *Sma*I sites in the TRS. Treatment of linear or superhelical DNA with *Sma*I generated a fragment of 8.5 kbp (Fig. 2b, lane g), whereas *Sma*I cleaved 0.5 kbp from the small *Bam*HI fragment (Table 1). These data indicate *Sma*I sites in the TRS at 0.5 and 8.9 on the linear map. *Hin*dIII was found not to cleave the superhelical or linear unintegrated provirus.

To further compare the various types of xenotropic viruses, I also mapped the proviral DNA of an iododeoxyuridine (IdU)-induced BALB/c virus. Restriction endonuclease analysis revealed that the restriction map is very similar to the AKR MuX provirus map (Fig. 1). Like AKR MuX DNA, BALB/c MuX DNA contained the apparently MuX-specific *Sac*I sites and the single *Eco*RI site which were not seen in ecotropic MuLV DNA. One obvious difference between the two proviruses was the banding pattern that was generated by *Bam*HI, which yielded 5.0- and 3.1-kbp fragments from BALB/c viral DNA. As with AKR MuX DNA, double digests with *Sal*I, *Xho*I, and *Eco*RI identified a *Bam*HI site at 4.0 kbp, whereas *Sac*I cleaved 0.8 kbp DNA from the 3.1-kbp *Bam*HI fragment. The additional finding that superhelical DNAs were converted to 5.8- and 5.2-kbp DNA species placed another *Bam*HI site at 0.8 kbp. The observation that partial digests of linear DNA with *Bam*HI generated an additional 5.3-kbp fragment indicated a third *Bam*HI site at 3.7 kbp (Fig. 1).

Another obvious difference between the two xenotropic viral DNAs was the presence of a *Pst*I site at 7.8 kbp. From BALB/c DNA, *Pst*I generated 2.9- and 4.8-kbp fragments with visible partial digest fragments of 5.5 and 7.8 kbp. Because *Eco*RI cleaved a 1.0-kbp DNA piece from the 4.8-kbp *Pst*I fragment, a *Pst*I site was determined to exist at 7.8 kbp. As with linear

DNA, *Pst*I generated 4.7- and 3.0-kbp DNA fragments from superhelical DNA. This finding indicates the presence of a *Pst*I site near the 5′ terminus of linear DNA; therefore, *Pst*I sites existed in the TRS. I have not yet ruled out the possibility of another *Pst*I site between 7.8 and 8.5 kbp. Also, unlike the case with AKR MuX DNA, no *Xba*I site existed, as *Xba*I did not cleave the linear or superhelical DNA or either large *Bam*HI fragment.

Restriction endonuclease mapping of proviral DNA from NZB and NFS MuX revealed that both of these DNAs had nearly identical restriction endonuclease maps (Fig. 1). As with BALB/c MuX DNA, both of these proviruses had the characteristic *Sac*I and *Eco*RI sites and no *Xba*I site (Fig. 3, lanes c and d). However, both of these viral DNAs had clear differences with BALB/c and AKR DNAs in the *Sma*I and *Pst*I maps. As with BALB/c MuX DNA, *Pst*I generated a 3.7-kbp fragment from the NZB or NFS MuX large *Bam*HI fragment; however, unlike BALB/c DNA, *Pst*I generated two fragments of 8.0 and 8.5 kbp from superhelical DNA. Also, *Pst*I appeared not to cleave the 3.0-kbp *Sac*I fragment. These findings suggest that no *Pst*I sites exist in the TRS of NZB or NFS DNA, but there were sites at 7.8 and 8.3 kbp (Fig. 1).

TABLE 1. *Sizes of AKR MuX unintegrated proviral DNA fragments from restriction endonuclease digests*[a]

Enzyme(s)	Linear fragments (kbp)	Major superhelical fragments (kbp)
*Xho*I	4.6, 4.4	9.0, 8.5
*Sal*I	4.6, 4.4	9.0, 8.5
*Bam*HI	5.0, 3.7, 0.3[b]	8.7, 8.1
*Eco*RI	6.9, 2.0	9.0, 8.5
*Xba*I	6.8, 2.1	
*Pst*I	5.3, 3.0, 0.5, 0.3[b], 0.1[b]	5.3, 3.0, 0.6
*Pvu*I	8.2, 0.8	
*Sma*I	8.5, 0.5, 0.1[b]	8.5, 0.6
*Sac*I	4.5, 3.0, 1.5	4.5, 4.5, 4.0
*Sal*I + *Xho*I	4.4, 4.3, 0.3[b]	
*Bam*HI + *Sac*I	3.6, 3.0, 1.5, 0.7[b], 0.3[b]	4.5, 4.0, 3.5
*Bam*HI + *Xho*I	4.4, 3.7, 0.6[b], 0.3[b]	8.1, 7.5
*Bam*HI + *Eco*RI	3.8, 2.9, 2.0, 0.3[b]	5.8, 5.2
*Bam*HI + *Sal*I	4.7, 3.7, 0.3[b], 0.3[b]	8.4, 7.8
*Bam*HI + *Xba*I	5.0, 2.0, 1.5, 0.3[b]	
*Bam*HI + *Pst*I	4.4, 3.0, 0.7, 0.5, 0.3[b], 0.1[b]	
*Bam*HI + *Pvu*I	5.0, 2.7, 0.8, 0.3[b]	
*Bam*HI + *Sma*I	4.8, 3.2, 0.5, 0.3[b], 0.1[b]	
*Sac*I + *Xba*I	4.5, 2.2, 1.5, 0.8	
*Sac*I + *Xho*I	3.0, 3.0, 1.5, 1.5	
*Sac*I + *Sal*I	3.5, 3.0, 1.5, 1.4	
*Sac*I + *Eco*RI	3.8, 3.0, 1.5, 0.6[b]	

[a] Fragment sizes were estimated by determination of the migration distance after electrophoresis with [^{32}P]DNA markers.

[b] Predicted fragments not visible by autoradiography.

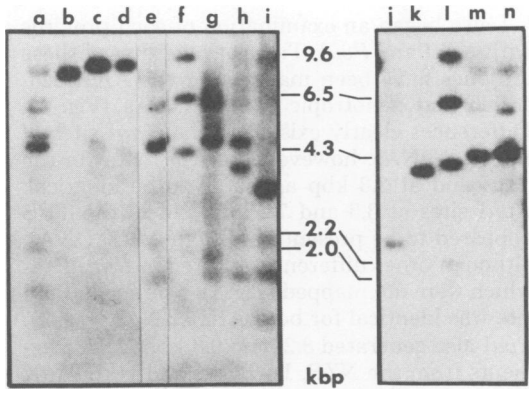

FIG. 3. *Digests of NFS, NZB, and BALB/c MuX DNAs with SmaI. Methods were as described in the legend to Fig. 2. NFS DNA: Lane a, SmaI (partial digest). BALB/c DNA: Lane b, SmaI. NFS DNA: Lane c, XbaI. NZB DNA; lanes are as follows: d, XbaI; e, SmaI; f, [^{32}P]DNA marker; g, SmaI and EcoI; h, SmaI and SalI (partial digest); i, SmaI and BamHI; j, SmaI and SacI; k, SmaI and XhoI; l, [^{32}P]DNA marker; m, SmaI. NFS DNA: Lane n, SmaI.*

Digestion of NZB or NFS linear DNA with *Sma*I generated visible fragments of 4.7, 2.1, and 1.6 kbp. I consistently found that a partial fragment (6.3 kbp) was present, even though the adenovirus or lambda DNA appeared to be completely cleaved (Fig. 3, lane e). Based on the finding that *Bam*HI, *Sal*I, *Sac*I, and *Xho*I converted the large *Sma*I fragment of NZB DNA to 1.2-, 3.7-, 2.4-, and 3.9-kbp fragments, respectively, *Sma*I sites were placed at 0.5 and 5.3 kbp. Further analyses of the 2.1-kbp *Sma*I fragment revealed that *Eco*RI converted it to a 1.9-kbp fragment which was 0.1 kbp smaller than the 2.0-kbp *Eco*RI fragment. Thus, *Sma*I sites also existed at 6.8 and 8.9 kbp (Fig. 1). Confirming the interpretation that *Sma*I sites exist in the TRS, *Sma*I generated from superhelical DNAs major fragments of the same size as those generated from linear DNA. Moreover, the predicted large partial fragments of linear DNA (5.3, 6.3, 6.8 kbp) could be seen in partial *Sma*I digests (Fig. 3, lane a).

In comparing NFS and NZB proviral DNAs, I was unable to cleave NFS linear or superhelical DNA or various fragments with *Sal*I. Therefore, NFS viral DNA was the only murine viral DNA I examined that did not contain the highly conserved *Sal*I site at 4.3 kbp.

I also began an examination of each provirus with *Kpn*I and *Pvu*I. The cleavage sites of these enzymes have been mapped for the AKR ecotropic and xenotropic MuLV DNAs (Fig. 1). Differences clearly existed in the *Kpn*I sites of the two DNAs; however, the *Kpn*I sites in the TRS and at 3.3 kbp appeared to be identical. *Kpn*I sites at 3.3 and 7.2 kbp and in the TRS appeared to be present in all four MuX DNAs, although other differences existed in *Kpn*I sites which were not mapped (Fig. 1). The single *Pvu*I site was identical for both AKR DNAs (Fig. 1). *Pvu*I also generated 8.2- and 0.8-kbp DNA fragments from the NZB, BALB/c, and NFS MuX DNAs, but these sites have not yet been confirmed by double digests.

In this study, I mapped the xenotropic proviral DNAs of viruses from four mouse strains and one ecotropic MuLV DNA with ten restriction endonucleases. My restriction endonuclease map of an AKR ecotropic MuLV unintegrated provirus agrees with previously published maps of other virus isolates of the unintegrated and integrated forms of this provirus (19, 22, 27, 34). Comparison of the restriction endonuclease maps of the AKR ecotropic MuLV provirus with MuX DNAs revealed only two regions of obvious homology. One region is in the center of the genome and appears to be in the *pol* region. The other highly conserved region is in the TRS, where *Sma*I and *Kpn*I sites existed in the AKR ecotropic MuLV and all four MuX proviruses. In addition, AKR ecotropic MuLV DNA shared a *Pst*I site in the TRS with AKR and BALB/c xenotropic viral DNAs. Based on these regions of homology, I identified the regions of the xenotropic proviruses which corresponded to the 3′ and 5′ termini of the viral RNA genome (Fig. 1).

It is of particular interest that, as with AKR ecotropic MuLV DNA, NZB and NFS viral DNAs and not BALB/c viral DNA contained *Sma*I sites at 5.3 and 6.8 kbp on the linear map. The fact that the 3′ terminal fragment of ecotropic MuLV DNA was 0.2 or 0.3 kbp smaller than this fragment from NZB-NFS viral DNA suggests that the site at 6.8 and not at 7.0 kbp is homologous with xenotropic viral DNA. It seems unlikely that the similarity in the maps is fortuitous. The findings of Chan et al. (4) support the interpretation that NZB viral DNA contains AKR ecotropic MuLV-specific regions in the envelope gene. With an AKR ecotropic MuLV-specific probe (*env* gene DNA fragment), they found DNA sequences in NZB mouse DNA that hybridize with [^{32}P]DNA. Since NZB mouse DNA does not contain the total ecotropic MuLV provirus, my results may explain their findings. These similarities may reflect on the origins of the various viruses, which could have been formed before integration into the germ line by recombination of common progenitor viral genomes.

Restriction endonuclease analyses of xenotropic proviral DNAs revealed that each provirus has a unique map, but with regions of homology. Each provirus contains what appears to be type-specific (i.e., MuX-specific) *Eco*RI (7.0 kbp) and *Sac*I (3.0 and 7.5 kbp) sites not present in N- or B-tropic ecotropic MuLV DNAs (4, 5, 22, 23, 27), which are highly conserved in various mouse strains. It is of special interest that two of these highly conserved sites are in the *env* region of the viral genome, whose gene product (glycoprotein 71) confers the xenotropic host-range property. It also appears that the *Kpn*I site at 7.2 kbp represents a type-specific site, but these data do not definitively discriminate it from the ecotropic DNA site at 7.4 kbp.

RNA and protein analyses have revealed at least two classes of xenotropic viruses. The NZB and NIH (NFS) viruses (class III) can be easily distinguished from the IdU-induced viruses (class II) such as BALB virus-2 by type-specific (glycoprotein 71) radioimmune assays (1, 12), and DNA-RNA hybridization (3). Thus, it was not surprising that comparison of NZB and NFS MuX DNAs with proviral DNA of an IdU-induced BALB/c MuX DNA showed numerous differences in restriction endonuclease sites. Unlike NZB and NFS MuX DNAs, BALB/c viral DNA contained no internal *Sma*I sites and did contain *Pst*I sites in the TRS. The *Sma*I site next to the *Eco*RI site in the *env* region, which was not found in the other xenotropic viral DNAs, appears to represent a specific type of MuX *env* gene product. Because the endogenous proviruses of the IdU-inducible xenotropic viruses from five mouse strains map to the same location near *Dip-1* on chromosome 1 (17), one would expect that these viruses from all strains would have a similar structure. Examination of a thymus-derived AKR MuX revealed another type of proviral DNA. Because the number of xenotropic viruses present in each mouse strain is not known, the differences in AKR viral DNA and the NZB-NFS viral DNAs may represent species-specific differences in the proviruses, or the various types of xenotropic viruses may be present in all or some mouse strains.

The results of this study will be useful for future studies on the organization of the numerous endogenous viral-related sequences in mouse DNA. For example, type-specific DNA probes can be developed to identify the endogenous xenotropic viral genomes. These restriction endonuclease maps will also be useful for charac-

terization of recombinant MuLV's, such as the MCF MuLV, which appear to be formed by recombination with MuX.

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