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Identification of ecotropic proviral sequences in inbred mouse strains with a cloned subgenomic DNA fragment

(Southern blotting method/endogenous viruses/murine leukemia viruses/recombinant DNA)

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ABSTRACT **A specific probe for detecting ecotropic murine leukemia virus sequences was constructed by cloning a 500-base-pair DNA segment, corresponding to a portion of the *env* region of the AKR ecotropic virus, in a pBR322/*Escherichia coli* K-12 host/vector system. This probe was used to screen the cellular DNAs of six inbred strains of mice for the presence of ecotropic retroviral DNA sequences by the Southern blot hybridization procedure. Three copies of ecotropic viral DNA were detected in AKR/N (a high-ecotropic virus strain) and two were found in BALB/c (a low-ecotropic virus strain) DNAs. As expected, no sequences reactive with this probe were found in NFS mouse DNA (a virus-negative strain). However, cellular DNA sequences that reacted strongly with the ecotropic-specific DNA probe were detected in certain NZB, C57L, and 129 mice (all virus-negative strains). In contrast to the reactive sequences in AKR and BALB/c, the reactions were chiefly associated with *Eco*RI segments that were subgenomic in size.**

The chromosomal DNA of inbred and feral mice contains multiple copies of sequences reactive with murine leukemia virus (MuLV) nucleic acid probes (1–7). Among these sequences are complete, potentially infectious genomes of ecotropic and xenotropic viruses (8), the complete genetic information of amphotropic and additional xenotropic viruses that apparently cannot be expressed as infectious viruses in inbred mice (ref. 9; unpublished data), proviral DNA that is expressed only in the form of viral antigens (10–12), and probably many subgenomic viral DNA segments that are not expressed.

Because of their abundance in mouse chromosomal DNA and the extensive polynucleotide sequence homology between large portions of the genomes of the various classes of MuLV, little is known about the molecular organization of the endogenous proviral DNA sequences of the particular MuLV types. Although absorption of labeled MuLV cDNA with purified viral RNA from a heterologous MuLV decreases the crossreaction with endogenous sequences (6, 7), such probes still hybridize to a significant extent to related proviruses present in mouse DNA. Clearly, the preparation of nucleic probes that recognize type-specific regions of proviral DNA would offer great advantages.

The molecular cloning of AKR ecotropic MuLV (AKV) DNA (13) has made it possible to generate such a probe. We have subcloned, in *Escherichia coli* K-12 with a pBR322 vector, a portion of the AKV *env* gene region that is specific for ecotropic viruses in the operational sense that it does not react either with cellular DNA prepared from the prototype ecotropic-negative mouse strain NFS/N or with genomic DNA of a xenotropic MuLV.

We describe here the construction and characterization of the recombinant plasmid, illustrate the strategy developed for using the cloned DNA to identify endogenous ecotropic proviruses, and report the presence of a unique set of nucleotide sequences reactive with this probe in the DNA of several mouse strains that, like NFS/N, are biologically negative for ecotropic MuLV production.

MATERIALS AND METHODS

Mice. *AKR/N*, BALB/c, C57L, and NFS/N mice were obtained from the National Institutes of Health colony. NZB and 129 mice were obtained from The Jackson Laboratory.

DNA Extraction and Purification. Individual livers were removed from weanling mice, finely minced, suspended in 15 ml of 0.15 M NaCl/0.1 M EDTA/0.02 M Tris·HCl, pH 8.0 (NET buffer), and homogenized with three or four strokes in a tightly fitting Teflon homogenizer. The suspension was sedimented for 20 sec at 1500 rpm and 0°C in a swinging bucket rotor. The deeply pigmented supernatant was carefully decanted and the loosely packed pellet of liver nuclei was resuspended in NET buffer and centrifuged for 5 sec at 700 rpm and 0°C. The low-speed-centrifugation/resuspension of nuclei was repeated five or six times until the supernatant became clear. Nuclear pellets, suspended in NET buffer, were lysed in the presence of proteinase K (E. M. Biochemicals, Elmsford, NY) at 50 μg/ml and 1% $NaDodSO_4$ for 3 hr at 37°C. Samples were phenol-extracted, spooled on a glass rod after addition of 2 vol of cold ethanol, extensively dialyzed, and digested with pancreatic RNase (25 μg/ml). After a final phenol extraction, liver DNA preparations were dialyzed against 0.01 M Tris·HCl (pH 8.0).

Restriction Endonuclease Digestion, Gel Electrophoresis, and Hybridization. All restriction enzymes used in these studies were obtained from New England Biolabs except for *Eco*RI, which was purchased from Bethesda Research Laboratory (Rockville, MD). Completeness of digestion of cellular DNAs was monitored by adding ^{32}P-labeled human adenovirus type 5 DNA to an aliquot of the reaction mixture and evaluating its cleavage by gel electrophoresis and autoradiography. Restricted cellular DNA samples were electrophoresed in 0.6% horizontal agarose gels as described (14) and transferred to nitrocellulose sheets as outlined by Southern (15).

Recombinant plasmid DNA preparations were labeled by the nick-translation procedure (16) and had specific activities of $8–12 \times 10^7$ cpm/μg. Radiolabeled AKV cDNA was synthesized by using purified viral RNA and avian myeloblastosis virus (AMV) reverse transcriptase (17) and had a specific activity of $4–10 \times 10^8$ cpm/μg. Preincubation of 13×18 cm ni-

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Abbreviations: MuLV, murine leukemia virus; AKV, AKR ecotropic MuLV; AMV, avian myeloblastosis virus; kb, kilobase(s).

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trocellulose sheets was carried out as described (18) and the filters were hybridized in sealed plastic bags containing 10 ml of 3× NaCl/Cit, pH 7.4 (1× is 0.15 M NaCl/0.015 M sodium citrate), 10× Denhardt's solution (1× is 0.02% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone) (19), 0.1% $NaDodSO_4$, sheared salmon sperm DNA (50 μg/ml), and labeled DNA (3×10^6 cpm/sheet) at 60°C for 16–36 hr. Nitrocellulose membranes were washed as described (18), air dried, and exposed to preflashed Kodak XR-2 film.

Construction of Recombinant Plasmids. A recombinant Charon 4A phage containing an infectious AKV provirus (13) was generously supplied by Douglas Lowy. Subgenomic segments of this AKR ecotropic proviral DNA were cloned in pBR322 as described (20) and as outlined in Fig. 1. When it was necessary to generate molecules containing flush ends, restriction endonuclease-digested DNA samples were incubated with AMV reverse transcriptase in 50 mM Tris·HCl, pH 8.3/6 mM $MgCl_2$/40 mM KCl/containing 100 μg of bovine serum albumin per ml and each of the four deoxyribonucleotide triphosphates at 18 μM. Blunt-end ligations were carried out in 66 mM Tris·HCl, pH 7.6/66 mM $MgCl_2$/66 μM ATP/10 mM dithiothreitol containing 100 μg of albumin per ml and 0.5 unit of T4 ligase (Bethesda Research Laboratory). *E. coli* K-12 strain HB101 was transformed with recombinant plasmids, and ampicillin-resistant, tetracycline-sensitive bacteria were examined for the presence of specific AKR proviral DNA segments (see Fig. 1).

RESULTS

Construction of a Hybridization Probe Specific for Ecotropic MuLV. Many lines of evidence indicate that a major region of nonhomology among the different MuLVs is located in the portion of the viral genome encoding the envelope glycoprotein (*env* gene). The single region of heterology observed by electron microscopy after hybridization of AKV cDNA to MCF247 RNA mapped at 1.95–2.75 kilobases (kb) from the 3′ end of genome RNA (21). This result suggested that sequences located in this region of the AKR ecotropic MuLV distinguish this provirus from at least one MCF and, by implication, from its xenotropic MuLV parent. Thus, if isolated in pure form, this *env* segment of AKR had the potential of being a specific probe for the identification of ecotropic proviruses in mouse DNA.

Accordingly, we prepared a series of recombinant plasmids containing different portions of the envelope region of AKR MuLV. This was made possible by the kind gift from Douglas Lowy of the phage recombinant (λ AKR 623) that contained a complete infectious AKR proviral DNA (13). Our strategy was to subclone a portion of the AKR ecotropic provirus extending

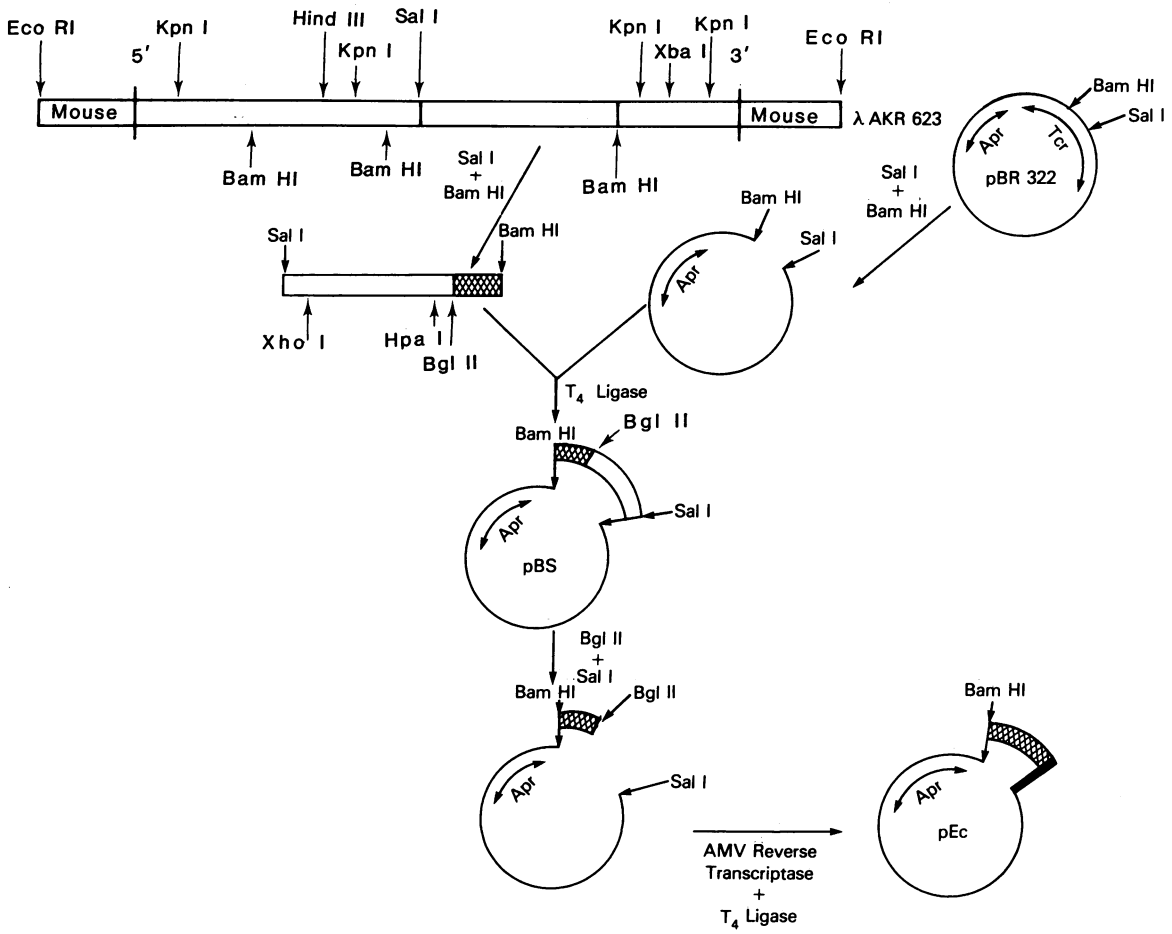

FIG. 1. Scheme for the construction and cloning of an ecotropic MuLV-specific DNA probe. λ AKR 623 (13) DNA, which contains a complete infectious AKR provirus as well as flanking mouse sequences, was mixed with pBR322, digested with *Bam*HI/*Sal* I, incubated with T4 ligase, and used to transform the HB101 derivative of *E. coli* K-12 as described (20). The DNA from ampicillin-resistant colonies was screened by hybridization to ^{32}P-labeled AKR cDNA to identify plasmids (pBS) carrying the 2.7-kb *Bam*HI/*Sal* I fragment. The pBS plasmid was then digested with *Bgl* II, *Hpa* I, or *Xho* I followed by cleavage with *Sal* I. (Only the *Bgl* II/*Sal* I digestion is shown in the figure.) Flush ends were then regenerated with AMV reverse transcriptase and blunt-end ligation was carried out. The locations of *Bam*HI (6), *Kpn* I (13), and *Xho* I (13) cleavage sites have been previously published; *Hin*dIII, *Sal* I, and *Xba* I sites have been mapped by D. L. Steffen (personal communication). The hatched region represents the 500-base-pair *Bam*HI/*Bgl* II DNA segment in pEc-B4 that specifically reacted with AKR but not NFS mouse DNA.

Table 1. Reactivity of recombinant plasmid DNAs with AKR or NFS mouse DNAs

AKV DNA segment present in plasmid	Map coordinates*	Hybridization to AKR DNA	Hybridization to NFS DNA
*Bam*HI/*Xho* I	1.85–4.35	+	+
*Bam*HI/*Hpa* I	1.85–2.55	+	+
*Bam*HI/*Bgl* II	1.85–2.35	+	−

Recombinant plasmids were constructed, nick translated, and used as probes in blot hybridization assays of *Bam*HI-digested AKR or NFS mouse DNA (see Fig. 2). +, Presence of a discrete DNA band(s); −, no reaction.

* In kb from the 3′ end of unintegrated linear AKV DNA.

leftward from the *Bam*HI site located 1.9 kb from the 3′ terminus (6) (Fig. 1). This segment would contain sequences that heteroduplex analyses indicated to be absent from MCF247 virus (21). Accordingly we constructed a recombinant plasmid containing the *Bam*HI/*Sal* I fragment, which maps between 1.8 and 4.5 kb from the 3′ terminus of unintegrated linear DNA (ref. 6; D. L. Steffen, personal communication). This plasmid (designated pBS) DNA was propagated in *E. coli* HB101 and its DNA then was digested with enzymes (*Bgl* II, *Hpa* I, or *Xho* I) that cut the AKV insert one or more times but do not cleave pBR322 DNA. The digested pBS DNA preparations were then cleaved with *Sal* I, generating a series of recombinant plasmids containing varying amounts of AKV proviral DNA extending from the *Bam*HI site toward the 5′ terminus. The linear molecules resulting from these treatments were incubated with AMV reverse transcriptase to regenerate flush ends, circularized by blunt-end ligation with T4 ligase, and propagated in *E. coli* HB101.

Ecotropic MuLVs have not been recovered from NFS (or NIH Swiss) mice, and NFS mouse DNA has been shown to lack a portion of AKV cDNA sequences that are present in strains that carry complete ecotropic provirus (1, 22). Therefore, the ability of DNA prepared from the three AKV *env* region clones to hybridize to AKR but not to NFS mouse DNA was used to select clone(s) that specifically recognize AKV-like endogenous ecotropic sequences. Only one of the three recombinants (pEc-B4), which was generated by the recircularization of *Bgl* II/*Sal* I-cleaved pBS plasmid DNA, showed such specificity (Table 1).

Further characterization of pEc-B4 DNA was achieved in the experiments shown in Fig. 2. *Bam*HI cleaves unintegrated linear AKR MuLV DNA at three sites (6), generating two internal restriction fragments (2.96 and 1.85 kb). These two fragments can be identified when ^{32}P-labeled AKV cDNA is hybridized to nitrocellulose membranes containing *Bam*HI-digested λ AKR 623 DNA (Fig. 2). (Two other bands were also present which contained viral as well as flanking mouse and λ phage sequences.) When AKR or NFS mouse DNA preparations were digested with *Bam*HI and hybridized to the AKR MuLV cDNA probe, multiple bands, previously observed by others (4–7) and presumably representing endogenous MuLV sequences, appeared on the autoradiogram. An entirely different result was obtained when the *Bam*HI-digested AKR or NFS mouse DNAs were hybridized to labeled pEc-B4 DNA. No DNA bands were visualized in the digested NFS mouse DNA preparation, and a single fragment, which comigrated with the 2.96-kb internal *Bam*HI viral DNA fragment of λ AKR 623, was present in the AKR mouse DNA preparation. A similar hybridization result was obtained with labeled pEc-B4 DNA and *Kpn* I-cleaved AKR and NFS mouse DNAs. A single 4.2-kb fragment, which comigrated with an authentic AKR MuLV cleavage product present in λ AKR 623 DNA, was detected in AKR mouse DNA but no bands were observed in *Kpn* I-digested NFS mouse DNA.

As a further test of the specificity of pEc-B4 DNA for reaction with MuLV ecotropic sequences, DNA was prepared from mink lung tissue culture cells chronically infected with AKR xenotropic or pseudotyped AKR ecotropic MuLVs (23) and digested with *Bam*HI. As was observed with AKR mouse DNA (Fig. 2), a single 2.96-kb fragment was present in digested DNA from mink cells infected with the ecotropic MuLV (Fig. 3, lanes 4 and 1), but the xenotropic MuLV-infected cells gave no band. A low level of background hybridization was seen with this DNA, but the same reactivity was seen with the uninfected mink cell DNA (Fig. 3, lanes 2 and 3).

Identification of Ecotropic MuLV DNA Sequences in Inbred Mice. DNAs from six strains of inbred mice (AKR/N, BALB/c, NFS, 129, C57L, and NZB/N), representing high-, low-, and non-virus-yielding mice with respect to ecotropic MuLV expression, were examined for the presence of sequences reactive with the pEc-B4 probe. Various restriction enzyme digestions were carried out with enzymes whose sites of

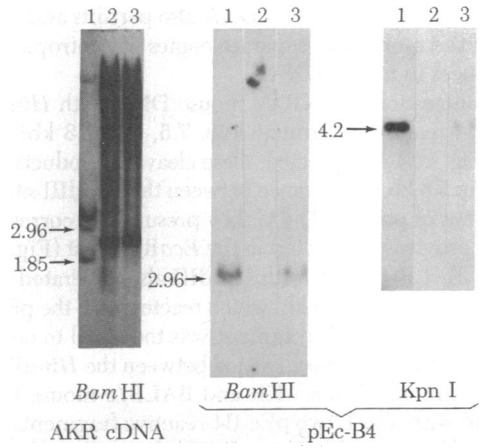

FIG. 2. Hybridization of labeled pEc-B4 DNA to AKR or NFS mouse DNAs. λ AKR 623 (13) DNA (0.1 ng) (lanes1) and NFS (lanes 2) and AKR (lanes 3) mouse DNAs (5 μg) were digested to completion with *Bam*HI or *Kpn* I, electrophoresed for 16 hr in a 0.6% agarose gel at 2 V/cm, transferred to nitrocellulose membranes (15), and hybridized to the probes indicated. *Hin*dIII fragments of wild-type λ DNA were used as size standards (shown in kb).

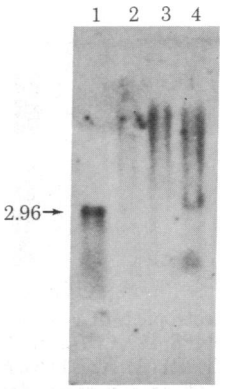

FIG. 3. Hybridization of labeled pEc-B4 DNA to infected or uninfected mink cell DNA. *Bam*HI-cleaved DNA (5 μg) was subjected to electrophoresis in a 0.6% agarose gel for 20 hr at 2 V/cm, transferred to nitrocellulose membranes (15), and hybridized with the pEc-B4 probe. The DNAs were from mink cell cultures that were uninfected (lane 2), infected with AKR xenotropic virus (lane 3), or infected with pseudotyped AKR ecotropic virus (23) (lane 4). Lane 1 contained 0.1 ng of *Bam*HI-digested λ AKR 623 DNA.

cleavage of the AKV provirus (Fig. 1) generated internal fragments or allowed examination of the *env* region of the provirus in combination with one or the other of both flanking cellular sequences. This approach was warranted by the observation that the restriction map of endogenous ecotropic proviruses is constant between highly diverse mice (D. L. Steffen and S. K. Chattopadhyay, personal communications; our unpublished observation).

*Eco*RI does not cleave within the AKV genome (6). Thus, the number of *Eco*RI restriction fragments from a cleaved mouse DNA preparation that react with the pEc-B4 probe should represent the minimal number of endogenous ecotropic genomes. Because the size of unintegrated AKR MuLV proviral DNA is approximately 8.8–9.0 kb (13), *Eco*RI cleavage products smaller than this which react with the pEc-B4 probe presumably represent partial copies of endogenous ecotropic viral DNA or related genomes that are differently organized from AKV. After digestion with *Eco*RI, three major bands (27, 24, and 14.5 kb) were visualized with AKR/N DNA (Fig. 4*A*); BALB/c mouse DNA showed one major band (23 kb) and two minor bands (7.8 and 7.5 kb). Again, NFS mouse DNA did not hybridize to labeled pEcB4 DNA (Fig. 4*A*).

Surprisingly, DNA from the three other ecotropic MuLV-negative mouse strains (129, C57L, and NZB) reacted strongly with this probe (Fig. 4*A*). The *Eco*RI fragments detected in 129 and C57L mice were 9.9, 7.4, 6.8, 4.4, and 3.7 kb; those in NZB mice were 9.9, 6.8, and 4.4 kb. Each of the three DNA preparations had its own characteristic *Eco*RI pattern although common fragments were present. The predominant 4.4-kb fragment present in NZB mouse DNA was barely detectable in 129 and C57L DNA. The prominent 3.7-kb band found in 129 DNA was barely visible in C57L DNA preparations and was absent in NZB DNA. Similarly, the major 7.4-kb *Eco*RI fragment present in both 129 and C57L DNAs was missing from NZB DNA. No bands were seen when C57L DNA was hybridized to labeled pBR322 plasmid DNA. It should be noted that other preparations of C57L, 129, and NZB/N DNAs did not hybridize to labeled pEc-B4 DNA. However, some of the bands detected in ecotropic-negative mice were also identified in some BALB/c, AKR/J, and AKV-2 congeneic mouse DNAs (unpublished data).

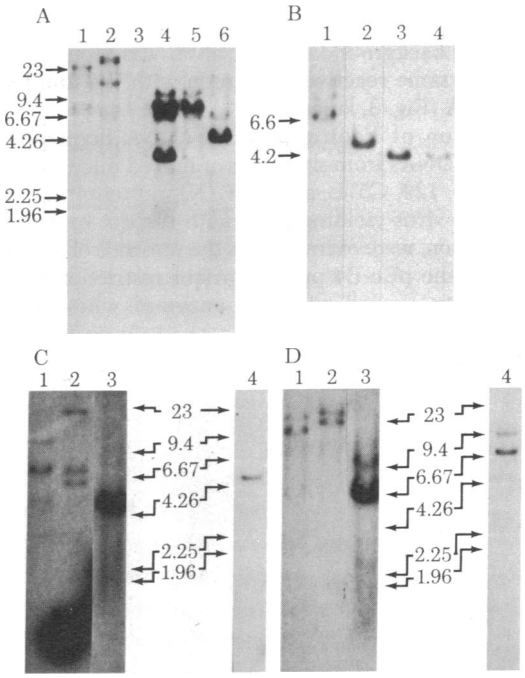

FIG. 4. Detection of ecotropic MuLV sequences in inbred mouse DNAs. BALB/c, AKR/N, NFS, 129, C57L, and NZB/N mouse DNAs (5 μg) were digested with *Eco*RI (*A*), *Kpn* I (*B*), *Hin*dIII (*C*), and *Xba* I (*D*), electrophoresed in 0.6% agarose gels as described in Fig. 2, and hybridized to ^{32}P-labeled pEc-B4 DNA after transfer to nitrocellulose membranes (15). The *Hin*dIII- and *Xba* I-digested C57L mouse DNA preparations were electrophoresed in a separate gel. Size markers (kb) were *Hin*dIII fragments of wild-type λ DNA. Lanes: (*A*) 1, BALB/c; 2, AKR/N; 3, NFS; 4, 129; 5, C57L; 6, NZB; (*B*) 1, C57L; 2, NZB/N; 3, AKR/N; 4, BALB/c; (*C* and *D*) 1, BALB/c; 2, AKR/N; 3, NZB/N; 4, C57L.

To evaluate the internal organization of endogenous ecotropic MuLV sequences, mouse DNAs were digested with *Kpn* I or *Bam*HI, enzymes that cleave the linear form of unintegrated AKR MuLV DNA four and three times, respectively (6, 13). The 4.2-kb *Kpn* I fragment which maps between 1.5 and 5.7 kb from the 3′ terminus (13) and the 2.96-kb internal *Bam*HI fragment (6) (Fig. 1) contain the sequences present in the pEc-B4 probe. After digestion of AKR or BALB/c DNAs with *Kpn* I, a single 4.2-kb fragment was detected (Fig. 4*B*). Similarly, a single 2.96-kb band was present in *Bam*HI-digested AKR (see Fig. 2) or BALB/c DNA preparations (data not shown). In contrast, hybridization of labeled pEc-B4 DNA to *Kpn* I-digested NZB DNA resulted in the appearance of a 4.8-kb fragment (Fig. 4*B*); *Kpn* I digestion of C57L DNA generated three cleavage products (6.7, 8.4, and 12 kb) which reacted with the pEc-B4 probe. In both cases the internal 4.2-kb *Kpn* fragment characteristic of endogenous ecotropic proviruses was not detected. Similarly, the internal 2.96-kb *Bam*HI fragment (see Fig. 1) of AKV DNA was absent from *Bam*HI-digested NZB and C57L DNAs (data not shown).

Restriction enzymes that cleave an endogenous ecotropic provirus a single time at a site to the right or the left of the region corresponding to pEc-B4 DNA can be used in conjunction with the pEc-B4 probe to characterize the cellular sequences flanking the endogenous ecotropic MuLV segments. *Hin*dIII and *Xba* I, which cleave AKV DNA a single time on either side of the segment containing pEcB4 sequences (D. L. Steffen, personal communication) (Fig. 1), were used for such analyses. Besides providing information about similarities or differences in the cellular sequences immediately adjoining endogenous MuLV DNA segments, cleavage with "one-cut" enzymes and hybridization to labeled pEc-B4 DNA also permits additional estimates of the number of separate copies of ecotropic viral DNA sequences in mouse DNA.

After the digestion of AKR/N mouse DNA with *Hin*dIII, three pEc-B4-reactive fragments (23, 7.5, and 6.3 kb) were identified (Fig. 4*C*). As expected, these cleavage products were all larger than 5.8 kb, the distance between the *Hin*dIII site and the 3′ terminus of proviral DNA; they presumably correspond to the three proviruses detected in the *Eco*RI digest (Fig. 4*A*). Digestion of BALB/c DNA with *Hin*dIII also generated three fragments (13.5, 7.5, and 5.0 kb) which reacted with the pEc-B4 probe (Fig. 4*C*). The 5-kb fragment was too small to contain all of the viral DNA sequences located between the *Hin*dIII site and the 3′ terminus. When AKR and BALB/c mouse DNAs were cleaved with *Xba* I, two pEc-B4-reactive fragments were detected in each preparation (35 and 25 kb for AKR, and 28 and 18 kb for BALB/c) (Fig. 4*D*).

*Hin*dIII digestion of NZB or C57L DNA resulted in a single 5-kb fragment that reacted with the pEc-B4 probe (Fig. 4*C*). *Xba* I cleavage of NZB or C57L DNA generated a prominent 6.6-kb as well as a 9.6-kb fragment in each case (Fig. 4*D*).

DISCUSSION

Using molecular cloning techniques, we have constructed a recombinant plasmid containing a portion of the *env* region of AKR MuLV which appears to specifically recognize ecotropic proviral genomes in preparations of mouse DNA. When used in blot hybridization assays the pEc-B4 probe reacted with only a few discrete cleavage products in each of the digested mouse DNA preparations examined. The numerous crossreacting cellular DNA bands recognized by other nucleic acid probes, which hamper the unambiguous identification of specific classes of endogenous proviruses, failed to hybridize to pEc-B4 DNA.

The evidence for the specificity of the pEc-B4 probe rests chiefly on two observations—i.e., the probe reacts with AKR and BALB/c but not with NFS cellular DNA, and it reacts with ecotropic AKV but not with a xenotropic MuLV of AKR origin. The negative result with NFS DNA further indicates that the pEc-B4 probe does not react with xenotropic viruses or with at least one amphotropic MuLV because NFS DNA is known to contain the full genomic information of these viruses (ref. 9; unpublished observation).

It should be stressed that the ecotropic specificity of the pEc-B4 probe is at present a relative term, defined in terms of the AKV-type proviruses. Whether it reacts with laboratory-derived ecotropic viruses such as the ecotropic components of Moloney and Friend virus populations has not been determined. Given the dynamic nature of type C viruses and the frequency of *env* gene recombinants, it seems possible that some viruses that are biologically different from AKV will be found to contain at least a portion of the pEc-B4-reactive sequences.

The data obtained with AKR/N and BALB/c DNA are in reasonable accord with the Mendelian genetic studies on these strains but indicate that there are somewhat more proviral copies than Mendelian virus-inducing loci. AKR carries two loci for induction of infectious ecotropic MuLV (8); three pEc-B4-reactive bands were seen with AKR/N DNA. The third band could represent the AKR locus that is detectable by its ability to induce antibody to MuLV (24), or it could indicate that one of the virus-inducing loci contains more than one provirus. BALB/c mice carry a single virus-inducing locus (25), on chromosome 5 (26); BALB/c DNA contained a single *Eco*RI band corresponding to an AKV-type provirus. However, *Eco*RI digestion of BALB/c DNA also generated several pEc-B4-reactive molecules of subgenomic size, suggesting that BALB/c also carries some type of ecotropic-related provirus that is not identical to AKV. The finding of two or three bands reactive with pEc-B4 in BALB/c DNA digested with *Xba* I or *Hin*dIII is in accord with this.

The technique of using selected enzymes to characterize proviruses in terms of their flanking cellular sequences provides a way to define each provirus by a set of unique molecular sizes; although unambiguous assignments may not be possible in mice carrying multiple proviral copies, the availability of congeneic mouse strains in which a single locus has been bred into the NFS genetic background will make this characterization feasible. Such characterization will be helpful for identifying allelic loci in different mice, for analyzing the genetic stability of endogenous proviruses, and for detecting newly inserted proviruses as in tumors (6, 27) or germ cells (28).

An unexpected finding in the present studies was the identification of pEc-B4-reactive sequences in some mice from three strains considered from biologic and biochemical studies to be negative for complete ecotropic genomes. The pEc-B4-reactive sequences in these three strains showed features markedly different from the proviruses in AKR and BALB/c but were surprisingly similar to one another. Most of the *Eco*RI DNA bands were shared among the three strains, but their relative intensities differed markedly. The overall intensity of the dominant band(s) in each strain was so marked that the band must be composed of a number of identical copies. These sequences may well represent tandem arrays of partial genomes. The *Hin*dIII and *Xba* I cleavage patterns were also strikingly similar in the different strains, further supporting the idea that the sequences are similarly organized. Why they are present in ecotropic-negative mice and not in AKR or BALB/c remains to be determined.

It must be noted that these pEc-B4-reactive sequences in the ecotropic-negative mice have not yet been proven to be integrated into chromosomal DNA; however, the possibility that the bands represent unintegrated proviruses seems remote, particularly in view of the low level of MuLV expression that is characteristic of strains C57L and 129.

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