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Characterization of an Infective Molecular Clone of the B-Tropic, Ecotropic BL/Ka(B) Murine Retrovirus Genome

JUNGSUH P. KIM, HENRY S. KAPLAN,* AND KIRK E. FRY

Cancer Biology Research Laboratory, Department of Radiology, Stanford University Medical Center, Stanford, California 94305

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Using molecular cloning techniques, we amplified the unintegrated, linear proviral DNA of the BL/Ka(B) virus, a non-leukemogenic retrovirus of mouse strain C57BL/Ka. Two independent clones in lambda phage vector 607 and one subclone in pBR322 were infective when transfected into mouse fibroblasts. Analysis of the progeny virus revealed biological properties and a restriction map identical to those of the parental viral shock. Comparison of the restriction map with the maps of other ecotropic murine viruses reveals many similarities. Particularly interesting is the comparison of the N-tropic Akv virus and the B-tropic BL/Ka(B) virus. The long terminal repeats of the two viruses are virtually identical, as are 22 of 23 restriction sites located outside of the region which spans from 1.8 to 3.8 kilobases from the left end of the genome. Within this region, however, only three of nine sites examined are shared. This suggests that the BL/Ka(B) virus was derived from an endogenous N-tropic virus closely related to Akv by recombinational events which altered the sequence in the last half of the *gag* gene and the first third of the *pol* gene. This change is probably responsible for the observed difference in the *Fv-1* tropism of the two viruses.

Murine leukemia viruses are endogenous to mouse strain C57BL, as well as to other inbred strains of mice. These viruses have been divided into several classes based upon their host range. Ecotropic viruses are those that can infect only cells of the species of origin; xenotropic viruses are those that can infect only cells of species other than the species of origin; and dual-tropic (MCF) viruses are those that can infect both types of cells. Murine ecotropic viruses have been further subdivided into those that replicate preferentially in cells derived from mice of *Fv-1*^{bb} genotype (B-tropic) or those derived from mice of *Fv-1*^{nn} genotype (N-tropic) (39). C57BL/Ka mice harbor at least four different classes of retroviruses, the biological attributes of which have been extensively studied (27). Three of these have been shown to be devoid of thymotropic or lymphomagenic activity and to replicate well on fibroblastic cells of the appropriate tropism. Of these viruses, BL/Ka(B) is a B-tropic ecotrope; BL/Ka(N) is an N-tropic ecotrope; and BL/Ka(X) is a xenotrope (27). The fourth class, unlike the others, replicates in vivo in lymphoid cells within the thymus and is highly oncogenic, inducing neoplastic transformation in susceptible target cells of the thymus, bone marrow, spleen, and fetal liver (9, 10). This class of virus, generically termed radiation leukemia virus (RadLV), replicates well in vitro only in nonproducer, T-lymphoma-derived cell lines (10, 28).

We are making a detailed study of the genomes of these four classes of viruses to determine whether there are distinctive genomic regions which can be consistently associated with thymotropism and lymphomagenicity. To do this, we have chosen to amplify representative genomes of each of these four classes of virus by molecular cloning techniques. These clones will then be tested for infectivity, restriction mapped, and partially sequenced. This paper reports the molecular cloning and mapping of the BL/Ka(B) virus genome.

MATERIALS AND METHODS

Cells and viruses. BL/Ka(B) virus was originally isolated from C57BL/Ka embryo fibroblast cultures infected with RadLV (27). SC-1 cells were originally obtained from J. W. Hartley and W. P. Rowe (National Institutes of Health, Bethesda, Md.) (19). The SC-1 [BL/Ka(B)] cell line was derived by infecting SC-1 cells at the limiting dilution with the BL/Ka(B) virus isolate (12). The BL-5 cell line is an established line derived from C57BL/Ka mouse embryo fibroblasts (13, 27, 30). BL/VL$_3$ is a lymphoma cell line derived from a RadLV-induced thymic lymphoma of a C57BL/Ka mouse (28) and produces RadLV/VL$_3$ virus (10). BL/RL$_{12}$-NP is a nonproducer lymphoma cell line derived from a lymphoma induced by fractionated whole body X-irradiation of a C57BL/Ka mouse (28).

The mink lung (ML) cell line was provided by A. J. Hackett (then at the Naval Biomedical Research Laboratory, Oakland, Calif.).

Virus-specific DNA probes. pJS-2 is a pBR322 vector plasmid containing 3.1 kilobases (kb) of murine retrovirus-specific DNA sequences derived from cDNA synthesized in detergent-solubilized RadLV/VL_3 virions (34, 44, 45). Comparison of its restriction map with that of other murine retroviruses demonstrates that it was derived from the left one third of the viral genome and contains the left long terminal repeat (LTR) and most of the *gag* gene. We expected this probe to cross-hybridize with the BL/Ka(B) virus since murine retrovirus *gag* genes are highly conserved. Preliminary experiments with unintegrated proviral BL/Ka(B) DNA indicated that this was the case. Later experiments were carried out with probes synthesized from the cloned BL/Ka(B) proviral DNA.

Blot hybridization analysis. DNA samples were electrophoresed horizontally in 0.7 to 1.0% agarose gels (Bio-Rad Laboratories), using 50 mM Tris-borate (pH 8.3)–1.0 mM EDTA, stained with 1 μg of ethidium bromide per ml, and nicked by UV irradiation. After denaturation, the DNA molecules were transferred to nitrocellulose sheets (BA85; Schleicher and Schuell) overnight (52). Bound DNA was detected by hybridization with ^{32}P-labeled probe under conditions of 50% formamide–0.75 M NaCl–0.075 M sodium citrate (5× SSC) for 24 to 48 h at 42°C. The nitrocellulose was then washed in 50% formamide–5× SSC and finally in 5× SSC until all the unbound probe was removed. Radioactivity was detected by using autoradiography with intensifying screens, XAR-5 film (Eastman Kodak), and exposure at −70°C.

Preparation of BL/Ka(B) proviral DNA. Nonproducer SC-1 cells were seeded at 8.5 × 10^5 cells per 150-cm^2 dish. Three days later, producer SC-1 [BL/Ka(B)] cells were cocultivated with these SC-1 cells at a ratio of 1:5 in Eagle minimal essential medium (MEM), supplemented with 10% fetal calf serum. At 24 h after the initiation of the cocultivation, unintegrated proviral DNA was harvested by the procedure described by Hirt (21), extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol (50:48:2, vol/vol/vol) and precipitated with ethanol. The precipitate was redissolved in 0.01 M Tris-hydrochloride (pH 7.8)–0.001 M EDTA (TE) and digested with RNase A (Worthington Diagnostics) at 50 μg/ml for 1 h at 37°C. Proteinase K (Beckman Instruments, Inc.) was then added at 50 μg/ml, and digestion was continued for another hour. This solution was extracted with phenol-chloroform-isoamyl alcohol as above and ethanol precipitated. Digestion products were removed by chromatography through a Bio-Gel A-15m (Bio-Rad) column equilibrated with TE. The fractions containing the excluded DNA were located by UV absorption and pooled.

Molecular cloning of BL/Ka(B) proviral DNA. The linear, unintegrated proviral DNA was cloned by the direct addition of *Eco*RI linkers, using the methods described by Scott et al. (47). A 100-μg amount of DNA purified as above was layered onto a 15 to 30% linear glycerol gradient containing 0.1 M NaCl, 0.05 M Tris (pH 7.4), and 0.001 M EDTA and centrifuged at 37,000 rpm in a Beckman SW-40 rotor for 18 h at 20°C. Fractions containing viral DNA were identified by blot hybridization, using probe made from plasmid pJS-2 (LTR-*gag* probe). These fractions were about 40-fold enriched for proviral DNA. The DNA in these fractions was methylated with *Eco*RI methylase to block the generation of additional clonable fragments when the *Eco*RI linkers were cut from the ends of the proviral DNA. Methylation was carried out under conditions suggested by the manufacturer (Bethesda Research Laboratories). The methylated DNA was then phenol extracted and concentrated by ethanol precipitation. *Eco*RI linkers were added by using T_4 polynucleotide ligase in 66 mM Tris-chloride (pH 7.6)–10 mM $MgCl_2$–1.0 mM ATP–10 mM dithiothreitol for 14 h at 12°C. The *Eco*RI linkers had previously been phosphorylated, using T_4 polynucleotide kinase (BRL). The completeness of ligation was monitored by the formation of multimeric concatemers of the *Eco*RI linkers (33).

*Eco*RI sites were generated at the ends of the proviral DNA by digestion with *Eco*RI nuclease. This DNA was then ligated to *Eco*RI-digested lambda 607 DNA (37) at 4°C for 14 h as described above. After in vitro packaging (5, 15, 23), the resulting phage preparation was plated on *Escherichia coli* strain DP50 SupF at a density of 25,000 phage per square plate (23 by 23 cm; Irvine Scientific). The ratio of clear recombinant to turbid nonrecombinant phage was about 1:30. Plaques were screened by the procedures described by Benton and Davis (3), using nick-translated probe made from pJS-2. Phage containing retroviral DNA sequences were cloned and purified, and the DNA was mapped by restriction nuclease digestion (8).

Subcloning of the retroviral sequences. The DNA from one of the phages, which contained a complete copy of the retroviral DNA (designated lambda B1), was treated with *Eco*RI nuclease. The retroviral DNA was purified by velocity sedimentation and then ligated to the DNA of pKH47, a plasmid derived from pBR322 by the insertion of approximately 100 base pairs (bp) of a polydeoxyadenylate:polydeoxythymidylate duplex segment into its *Pvu*II site (20), which had been treated with *Eco*RI nuclease and bacterial alkaline phosphatase. *E. coli* C600 was transfected with the mixture, and seven resulting tetracycline-resistant clones were analyzed. The plasmid DNA from these clones was purified (4) and analyzed by restriction digestion. Five of the clones contained a complete copy of the proviral DNA. During mass cultivation of these clones, a recombinational event occurred between the two direct repeats of the proviral DNA (36), leading to the deletion of all but one LTR in about 90% of the plasmids.

Subsequently, we used this mixture of both deleted and undeleted plasmids and transformed *E. coli* HB101, a *recA* strain. Two types of transformed clones were obtained. One class (pB2) contained recombinant plasmids with a single retroviral LTR sequence, and the other class (pB1) contained a complete retroviral sequence. These two classes of plasmids were stable during mass cultivation.

Sequencing the LTR. The nucleotide sequence of the LTR contained in subclone pB2 was determined by the method of Maxam and Gilbert (35). pB2 DNA was digested in separate experiments with *Eco*RI, *Kpn*I, or *Bst*NI, phosphorylated, and redigested with *Kpn*I in the first case and *Eco*RI in the second and third cases.

Infectivity testing of the cloned DNA. BL-5 or SC-1

TABLE 1. Transfection of various target cells with cloned DNAs and lambda 607 DNA

DNA used	Amt (μg)	Reverse transcriptase activity of target cell culture fluids[a]		
		SC-1	BL-5	ML
Lambda B1 or lambda B2	10	+	+	–[b]
	1	+	ND[c]	
	0.1	+	ND	
	0.01	–	ND	
	0.001	–	ND	
pB1	1	+	ND	
Lambda 607	10	ND	–	
	1	–	ND	
pJS-2	5	ND	–	

[a] Assayed 7 to 10 days after transfection; considered positive (+) if the resuspension of the pellet from a 1.4-ml culture with reaction mixture incorporated more than 5,000 cpm (0.28 pmol) of dTMP per 60 min of incubation at 30°C.

[b] None; assayed up to 6 weeks after transfection.

[c] ND, Not determined.

cells were grown to 4×10^5 cells per 60-mm dish in MEM supplemented with 10% fetal calf serum and antibiotics. Transfection with cloned DNA (Table 1) was carried out by the calcium phosphate precipitation technique (18, 38). At 7 or 10 days after transfection, reverse transcriptase activity present in the supernatant was determined, using polyriboadenylate and oligodeoxythymidylate (11, 13–18) as template and primer (26). The host range of the progeny virus was determined by the in vivo immunofluorescence assay (11). The progeny virus was prepared as above, and its restriction nuclease map was determined and compared with that of the parental virus.

RESULTS

Characterization of cloned and cellular proviral DNA. BL/Ka(B) unintegrated retroviral DNA was analyzed by electrophoresis and blot hybridization after digestion with either *Eco*RI or *Hin*dIII. As expected, the pJS-2 probe (LTR-*gag* sequences) detected retroviral DNA sequences (Fig. 1). The two faint bands migrating faster than the predominant linear band at 8.6-kb probably represent closed circles containing one or two copies of the LTR sequence (17, 48). Since neither *Eco*RI nor *Hin*dIII digestion altered the migration of the circular or linear retroviral sequences, we conclude that the proviral DNA contains no sites for either of these enzymes.

Digestion of the unintegrated proviral DNA with *Bam*HI produced two fragments (3.6 and 1.8 kb), which were detected by the LTR-*gag* probe. By examining the extent of the hybridization of these bands to the probe, we were able to assign these fragments to the left and right ends of the genome as follows. The 3.6-kb fragment must lie at the left, and the 1.8-kb fragment must lie at the right of the genome, because the 3.6-kb fragment hybridizes more intensely to the LTR-*gag* probe than does the 1.8-kb fragment.

*Kpn*I digestion produced three bands (0.4, 2.2, and 1.4 kb) detectable with the same probe. Similarly, we oriented the 0.4-kb fragment to the U_3 region of the left LTR, the 2.2-kb fragment to the U_5-*gag* region, and the 1.4-kb fragment to the *env*-U_3 region on the basis of their hybridization intensities.

The two independent recombinant lambda DNA molecules (lambda B1 and lambda B2) were digested with *Eco*RI, and an 8.6-kb retroviral sequence was released. Based on additional restriction analysis, the retroviral sequence in each recombinant was found to be identical,

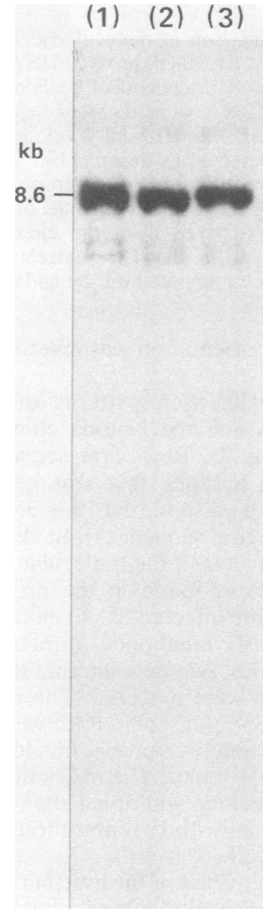

FIG. 1. Detection of BL/Ka(B) proviral DNA in Hirt extracts of newly infected SC-1 cells. BL/Ka(B) proviral DNA was electrophoresed on a 0.7% agarose gel without any restriction endonuclease digestion (lane 1) and after digestion with *Eco*RI (lane 2) or *Hin*dIII (lane 3). The DNA was detected by blot hybridization, using a probe synthesized from pJS-2.

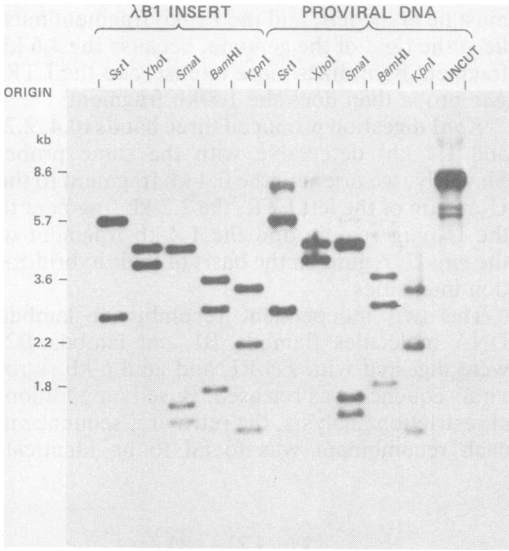

FIG. 2. Comparison of molecularly cloned and in vivo unintegrated BL/Ka(B) proviral DNAs by restriction analysis. *Eco*RI fragments of lambda B1 and BL/Ka(B) proviral DNA were digested separately with *Sst*I, *Xho*I, *Sma*I, *Bam*HI, and *Kpn*I. Retroviral sequences were detected as described in the legend to Fig. 1, using probe synthesized from pB1. Faint, slowly migrating bands observed in the proviral tracks are presumably derived from the circular proviral molecules present in the Hirt extract. Fragments smaller than 0.6 kb migrated off the end of the gel.

except that its orientation was inverted (data not shown).

Restriction digestion patterns of BL/Ka(B) proviral DNA and the lambda clone B1 were compared (Fig. 2). Retroviral sequences were detected with a probe that was generated by nick-translating plasmid pB1 (this probe detects all of the retroviral sequences). In all digestions, the restriction sites in the molecular clone were identical to those found in the proviral DNA isolated from the infected SC-1 cells. In addition to the previously mentioned *Bam*HI fragments of 3.6 and 1.8 kb, two new internal fragments of 2.8 and 0.4 kb were detected. Three new *Kpn*I fragments of 3.4, 0.4, and 0.6 kb were detected with the representative probe, in addition to the three previously found. The restriction map was expanded to include additional sites by simultaneous digestions with two and three restriction enzymes (Fig. 3).

Nucleotide sequence of the junction between the vector and retroviral sequences. Figure 4 shows the autoradiogram of a DNA sequence gel which contains sequences of pB2 representing the left and right ends of the cloned retrovirus. There is an inverted sequence of 13 bases, AATGAAAGACCCC, at each end of the LTR, as has been found in other retroviruses (7, 14, 51, 53, 54). Sequence studies (14, 22, 32, 51) of other molecular clones of integrated retroviruses have suggested that the integration event occurs precisely between the second A and the next T at both ends of the LTR. Cloning by the direct addition of *Eco*RI linkers preserved the two A bases at each end of the virus, as demonstrated by the sequence determination. The complete sequence of the LTR, 529 bp in length, was determined (Fig. 5). It contains all of the features of other murine retrovirus LTRs previously reported in the literature (14, 49, 54, 56). The LTR of BL/Ka(B) contains no direct repeats as have been found in the LTRs of Akv or the murine sarcoma virus (14, 40).

Infectivity testing of the cloned retroviral genome. To study the structural and functional relationships of DNA, it is imperative to test the biological expression of the genetic material. This requires that the cloned retroviral DNA be infective when introduced into the appropriate mouse cells. Table 1 summarizes our transfection results using SC-1, BL-5, and ML cells with various types and amounts of DNA molecules. Lambda B1 and B2 undigested molecules were infective in both mouse cell lines but not in the ML line. The efficiency of transfection of the BL-5 cell line was reduced twofold when the lambda B1 molecules were digested with *Eco*RI before transfection. Control transfections with lambda 607 DNA were negative for retrovirus production. Plasmid subclone pB1 also proved to be infective in SC-1 cells by transfection and in BL/RL_{12}-NP cells by the protoplast fusion procedure (data not shown). As expected, plasmid pJS-2, which contains only 3.1 kb of the left-hand portion of the viral genome, was not infective in BL-5 cells.

To show that the progeny viruses were derived from the exogenously added DNA and not from the induction of an endogenous virus, we analyzed both the host range of the progeny virus and the restriction map of its proviral DNA. The host range of the progeny virus was identical to that of the parental BL/Ka(B). It infected SC-1, BL-5, and BL/RL_{12}-NP cells with one-hit kinetics and NIH/3T3 cells with two-hit kinetics but failed to infect ML cells. Virus produced by transfected SC-1 cells was injected intrathymically into C57BL/Ka mice. After 1 and 4 weeks, the thymuses were removed and tested for the expression of murine retroviral antigens by an indirect immunofluorescence assay (11). No thymotropic activity could be detected, again mimicking the parental virus, which does not infect the thymus in vivo. Proviral DNA derived from the progeny virus of the transfected SC-1 cells was analyzed by restriction endonuclease digestion. This DNA was digested in separate reactions with *Kpn*I,

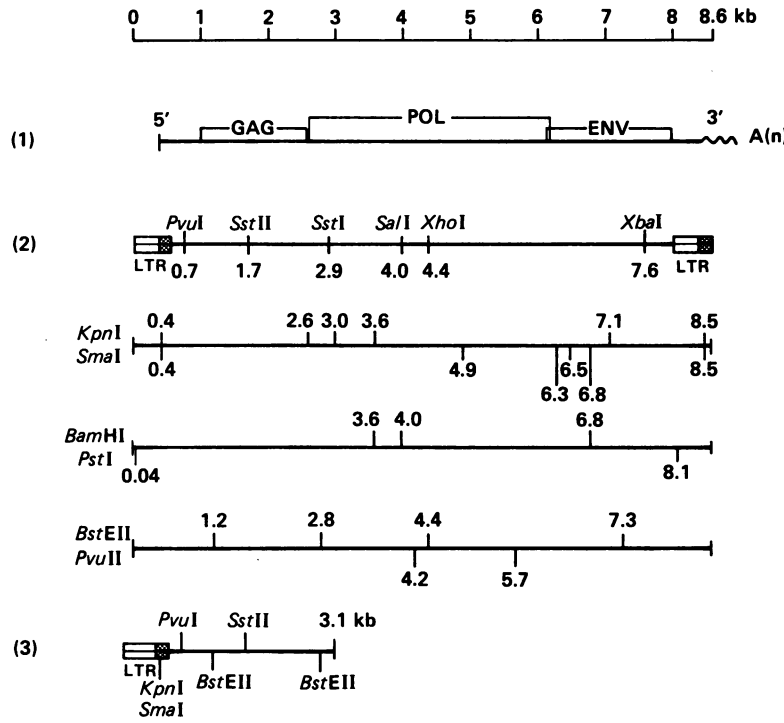

FIG. 3. Restriction endonuclease map of BL/Ka(B) proviral DNA. (1) Structure of BL/Ka(B) viral RNA. The *gag*, *pol*, and *env* genes were mapped on the viral genome as explained in the text. (2) Restriction endonuclease map of lambda B1 retroviral DNA. No sites were found for *BclI*, *ClaI*, *Eco*RI, or *Hin*dIII. "LTR" represents the U_3, R, and U_5 regions found in all retroviruses. (3) Restriction endonuclease map of pJS-2 retroviral DNA. The map was aligned with that of lambda B1 retroviral DNA.

*Bam*HI, *Sma*I, *Sal*I, and *Xho*I. In all cases, the restriction pattern, as analyzed by blot hybridization, was identical to that obtained with the parental proviral DNA.

DISCUSSION

We undertook the cloning of the BL/Ka(B) virus as an initial step in characterizing the role played by murine retroviruses in the induction of T cell thymic lymphomas in X-irradiated mice. Leukemogenic viruses can be isolated from most, but not all, primary radiation-induced thymic lymphomas in X-irradiated mice (27–29). We wish to determine which genetic determinants of the leukemogenic retrovirus isolates are important for the induction of these neoplasms. The first step will be to determine the genomic differences between the leukemogenic isolates and the non-leukemogenic viruses, such as BL/Ka(B). To do this, we chose to use recombinant DNA techniques to clone prototypic examples of the retroviruses known to be present in mouse strain C57BL/Ka.

To demonstrate biological potential, the cloned retroviral DNA must be infectious upon reintroduction into appropriate cells. The clones of the BL/Ka(B) virus genome were infectious, and the progeny viruses were tested and shown to have a host range identical to that of the parental virus used to generate the cloned proviral DNA.

Three forms of unintegrated retroviral DNA are present in cells shortly after infection with retrovirus (57). It is not known which of these forms is productively integrated into the host genome. Normal integration occurs between the second A and third T from each end of all unintegrated proviral DNAs thus far examined. Presumably, some enzyme recognizes this sequence and carries out the integration process. The addition of *Eco*RI linkers to the ends of the linear, unintegrated viral DNA has not prevented its integration and expression. It has not been determined whether, after transfection with the cloned retroviral DNA, integration occurs as it does during natural infection.

Restriction endonuclease maps of the other prototypic C57BL/Ka virus isolates have been generated, using probes synthesized from the molecular clones described in this paper (R. A. Grymes, M. L. Scott, J. P. Kim, K. E. Fry, and H. S. Kaplan, manuscript in preparation). With this information, molecular clones of these viruses have been constructed and are being tested for infectivity and host range.

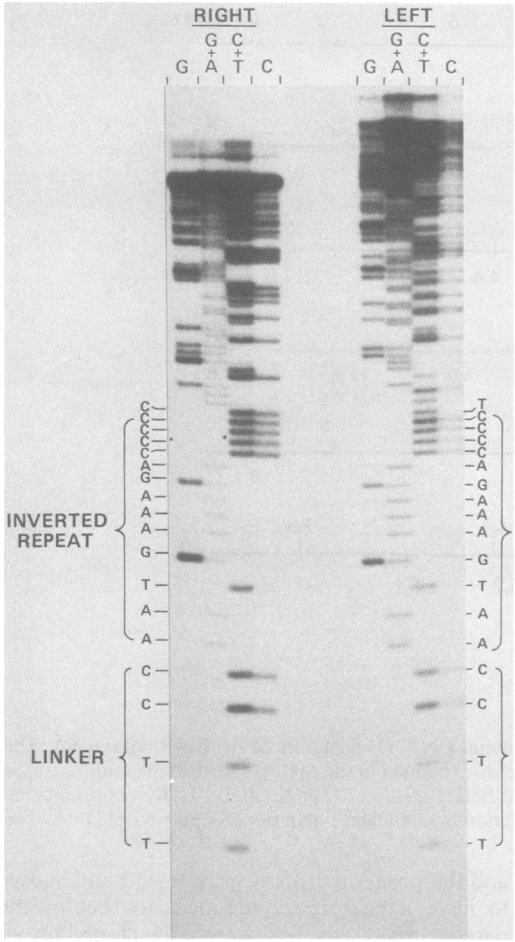

FIG. 4. DNA sequences of the left and right ends of BL/Ka(B) DNA. Plasmid pB2 DNA was treated with *Eco*RI, and the 5′ ends were labeled with $^{32}PO_4$. Subsequently, the DNA was digested with *Kpn*I, and two fragments (417 and 112 bp) were purified. The sequence of these fragments was determined by specific chemical modification and partial cleavage (35).

The genomes of many murine retroviruses have been characterized by restriction endonucleases and nucleotide sequencing techniques (6, 7, 40–42, 50, 55). The genome of the BL/Ka(B) virus conserves many of the restriction endonuclease sites found in other ecotropic murine retroviruses. Based on the location of these sites and the previously cited work, we mapped the approximate locations of the *gag*, *pol*, and *env* genes on the BL/Ka(B) virus genome (Fig. 3).

Mice of the AKR strain spontaneously express a high level of a non-leukemogenic, ecotropic retrovirus (Akv), which is N tropic (39). This virus has been extensively studied by others (6, 40). We have made an extensive comparison between the Akv virus and BL/Ka(B) virus with regard to the sequence of the LTRs and shared restriction sites. Murine retrovirus LTR sequences have been extensively examined (14, 49, 54, 56). Comparison of the LTR sequences of Moloney murine leukemia virus, murine sarcoma virus, and Akv virus has revealed numerous differences among the various viruses, especially in the U_3 region. In addition to point mutations and small deletions, duplications have been noted of 58 to 99 bp (56). In the case of the AKR-614 sequence that we used for comparison, this duplication apparently arose during the passage of the virus before molecular cloning because unpassaged virus directly from AKR mice does not contain this duplication (40). Figure 5 shows the comparison of the BL/Ka(B) virus LTR sequence with that of the AKR-614 from which we deleted one of two direct repeats of 99 bp. Except for three point mutations and one 2-bp deletion, the sequences are identical. This suggests that the BL/Ka(B) virus was derived from the ecotropic, N-tropic endogenous retrovirus which inhabits many strains of mice. Comparison of the Akv restriction endonuclease map with that of the B-tropic BL/Ka(B) virus reveals that all of the sites (Fig. 6) from the 5′ ends of the genomes to 1.7 kb and from 4.0 kb to the 3′ ends of the genomes are conserved, with the single exception of a *Cla*I site at 5.6 kb, which is missing in the BL/Ka(B) genome. The region from 1.8 to 3.8 kb, however, appears to be highly divergent. Akv contains a *Bam*HI site at 1.8 kb, an *Hin*cII site at 2.0 kb, an *Hin*dIII site at 3.0 kb, and a *Sac*I (*Sst*I) site at 3.8 kb which are absent from BL/Ka(B). Conversely, BL/Ka(B) contains unique sites for *Kpn*I between 2.6 and 3.6 kb, for *Sst*I (*Sac*I) at 2.9 kb, and for *Bst*EII at 2.8 kb. These differences in the last half of the *gag* gene and the first third of the *pol* gene easily allow one to distinguish the BL/Ka(B) ecotropic virus from the Akv ecotropic virus of the AKR strain of mice. Since Akv is of a different *Fv-1* tropism and tropism is thought to be determined by the *gag* gene, some of these differences might be tropism related (16, 43, 46).

It has been suggested, on the basis of immunological comparisons, that the B-tropic viruses are the result of a recombinational event between an endogenous xenotropic virus and an N-tropic, ecotropic virus (1, 2). C57BL-derived N-tropic viruses usually contained AKR murine leukemia virus-like p12, p30, and gp71, whereas the B-tropic viruses, including BL/Ka(B), contained xenotropic-like p12, non-AKR murine leukemia virus-like p30 (not xenotropic), and ecotropic gp71. These results suggested that a single recombinational event had occurred in the p30 coding region of the genome to produce a virus with a xenotropic left end and an ecotropic right end. Our data suggest a different model.

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IVR------- -->
AATGAAAGAC CCCTTCATAA GGCTTAGCCA GCTAACTGCA GTAACGCCAT 50
AATGAAAGAC CCCTTCATAA GGCTTAGCCA GCTAACTGCA GTAACGCCAT 50

*
CTTGCAAGGC ATGGGAAAAT ACCAGAGCTG ATGTTCTCAG AAAAACAAGA 100
TTTGCAAGGC ATGGGAAAAT ACCAGAGCTG ATGTTCTCAG AAAAACAAGA 100


ACAAGGAAGT ACAGAGAGGC TGGAAAGTAC CGGGACTAGG GCCAAACAGG 150
ACAAGGAAGT acagacaggc tggaaagtac cgggactagg gccaaacagg 150


ATATCTGTGG TCAAGCACTA GGGCCCCGGC CCAGGGCCAA GAACAGATGG 200
atatctgtgg tcaagcacta gggccccggc ccagggccaa gaacagatgg 200

      ><
TCCCCAGAAA TAGCTAAAAC AACAACAGTT TCAAGAGACC CAGAAACTGT 250
tccccagaaA TAGCTAAAAC AACAAGAGTT TCAAGAGACC CAGAAACTGT 349

CTCAAGGTTC CCCAGATGAC CGGGGATCAA CCCCAAGCCT CATTTAAACT 300
CTCAAGGTTC CCCAGATGAC CGGGGATCAA CCCCAAGCCT CATTTAAACT 399

  +++++
AACCAATCAG CTCGCTTCTC GCTTCTGTAC CCGCGCTTAT TGCTGCCCAG 350
AACCAATCAG CTCGCTTCTC GCTTCTGTAC CCGCGCTTAT TGCTGCCCAG 449

   #######        *       **          mRNA- ---------->
CTCTATAAAA AGGGTAAAAA CCCCACACAC TCGGCGCGCC AGTCCTCCGA 400
CTCTATAAAA AGGGTAAGAA CCCC  ACAC TCGGCGCGCC AGTCCTCCGA 497

*                                  ======
CAGACTGAGT CGCCCGGGTA CCCGTGTATC CAATAAAGCC TTTTGCTGTT 450
TAGACTGAGT CGCCCGGGTA CCCGTGTATC CAATAAAGCC TTTTGCTGTT 547

 ==
GCATCCGAAT CGTGGTCTCG CTGATCCTTG GGAGGGTCTC CTCAGAGTGA 500
GCATCCGAAT CGTGGTCTCG CTGATCCTTG GGAGGGTCTC CTCAGAGTGA 597

             *
TTGACTGCCC AGCTTGGGGG TCTTTCATT 529
TTGACTGCCC AGCCTGGGGG TCTTTCATT 626
               <--- ------IVR
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FIG. 5. Comparison of the LTR sequence of BL/Ka(B) virus with that of Akv virus. Comparison of the LTR sequence from BL/Ka(B) (upper sequence) with that of Akv virus (lower sequence) as derived from the recombinant clone AKR-614 (56) is shown. The lower case letters in the Akv sequence denote the sequence which is repeated twice in the AKR-614 clone. Numbering of the Akv sequence corresponds to the numbering that would result if the 99-bp repeat were inserted at the point denoted by the "><" symbol. Control signals are denoted as follows: IVR, inverted repeat; "*," point mutations or deletions; "+++++," "CAT" box; "####," "TATA" box; "=====," sequence specifying polyadenylation; and "mRNA------," site of initiation of viral RNA synthesis.

BL/Ka(B) probably arose by a recombinational event between an ecotropic, N-tropic virus highly related to Akv in such a way that the region from about 1.8 to 3.8 kb was replaced with sequences derived from another endogenous virus, probably a xenotropic-related sequence since the *Sac*I (*Sst*I) site at 2.9 kb is highly conserved in xenotropic viruses.

The role that endogenous, ecotropic retroviruses play in the induction of thymic lymphomas after the irradiation of C57BL/Ka mice is not clear. Transplantation experiments have been carried out which show that the target T cells, which eventually become the tumor cells, need not be irradiated (24). This suggests that a virus may be induced in irradiated cells and that this

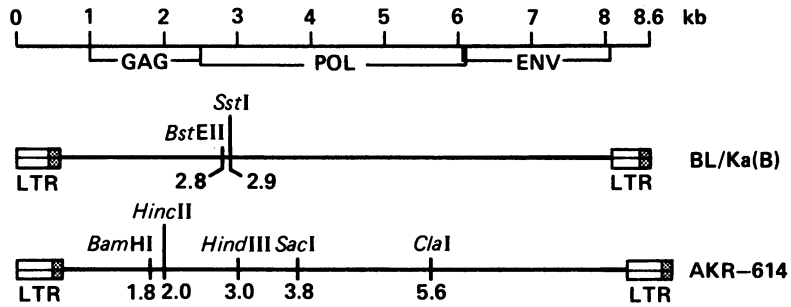

FIG. 6. Differences in the restriction endonuclease maps of the BL/Ka(B) and the AKR-614 proviral DNAs. Only restriction sites known to be unique to each DNA are shown on these maps. The restriction sites of the AKR-614 DNAs were obtained from published papers (7, 25, 31, 40).

virus may then secondarily infect unirradiated T cells and cause a change which leads to the frank expression of the lymphoma. It is not clear how this infection induces the transformation, which viruses or viral sequences are involved, or why the latent period is so long. Using the molecular probes that we have characterized here, we are examining DNA rearrangements in virus- and radiation-induced lymphomas to determine whether specific rearrangements can be correlated with the induction of such lymphomas.

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