JOURNAL OF VIROLOGY, Sept. 1982, p. 943–951
0022-538X/82/090943-09$02.00/0
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Vol. 43, No. 3

# Molecular Cloning of a Highly Leukemogenic, Ecotropic Retrovirus from an AKR Mouse

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Received 12 February 1982/Accepted 7 June 1982

SL3-3 is a leukemogenic, ecotropic retrovirus produced by a T-cell line derived from a spontaneous lymphoma of an AKR mouse. We have isolated a molecular clone of its DNA provirus from infected NIH 3T3 fibroblasts. Cloned proviral DNA produced infectious virus upon transfection onto NIH 3T3 cells. Virus derived by transfection induced lymphomas at high frequency in AKR/J, C3H(f)/Bi, CBA/J, and NFS/N mice. Heteroduplex and RNase $T_1$ fingerprinting analyses showed that the genomes of SL3-3 and the non-leukemogenic virus, Akv, contain no major substitutions relative to one another and differ by only a few base changes. These results unambiguously show that SL3-3 is a highly leukemogenic virus and that major rearrangements of the genome relative to Akv are not required for virulence.

Retroviruses isolated from AKR mice differ in the ability to induce lymphomas. The focus of our studies with these viruses has been to determine what features of the leukemogenic viruses are responsible for their oncogenic potential. The approach used has been to compare the genomes and gene products of the leukemogenic isolates with those of the non-leukemogenic virus, Akv, since some of the differences relative to Akv must account for the difference in virulence.

RNase $T_1$ fingerprinting studies have shown that the leukemogenic virus that most closely resembles Akv is SL3-3 (30). Differences between the genomes of these viruses are apparently limited to a few changes, some of which are single-base changes (30). SL3-3 is one of two leukemogenic, ecotropic viruses produced by a T-cell line, SL3, derived from a spontaneous lymphoma of an AKR mouse (15, 26, 30). It was isolated by infection of NIH 3T3 fibroblasts with supernatant fluid from SL3 cells, followed by two successive rounds of endpoint dilution on NIH 3T3 cells. The RNase $T_1$ fingerprint of the SL3-3 genome has a pattern characteristic of a single retrovirus genome (30). However, the possibility remained that the leukemogenic activity of the virus might be due to contamination by another viral variant present in an amount too small to be detected by fingerprinting.

To determine whether the SL3-3 component is itself leukemogenic, we constructed a molecular clone of the SL3-3 provirus from infected NIH 3T3 cells. Virus derived by transfection of NIH 3T3 fibroblasts with cloned DNA was tested for leukemogenic activity.

## MATERIALS AND METHODS

**Materials.** Restriction enzymes were from New England Biolabs, Bethesda Research Labs, or Boehringer Mannheim Corp. T4 DNA ligase and *Escherichia coli* DNA polymerase I were from New England Biolabs. NDase I and bacterial alkaline phosphatase were from Worthington Diagnostics. Radiolabeled nucleotides were from New England Nuclear Corp. T4 polynucleotide kinase was from PL Biochemicals.

**Viruses, cells, and mice.** NIH 3T3 fibroblasts were grown in Dulbecco modified Eagle medium containing 10% calf serum. Isolation of the SL3-3 virus has been previously described (30).

*E. coli* strains LE392, HB101, and MBM7014 were grown in YT or LB medium. The λ cloning vector, Charon 4, was from a Charon library supplied by F. Blattner and colleagues (2). All strains of *E. coli* used for the in vitro assembly of phages containing chimeric DNA were from the kit accompanying the Charon library. λAKR-623 was provided by D. Lowy (21). Isolation of the molecular clone of Akv in pBR322, pAKR59, has been previously described (19).

AKR/J and CBA/J mice were obtained from Jackson Laboratories. NFS/N mice were obtained from the Veterinary Resources Branch, National Institutes of Health. Foster-nursed C3H/Bi mice were obtained from the University of Minnesota mouse colony. Breeding pairs of each strain of mice were maintained in our laboratory by brother-sister mating. Mice used in these experiments were weaned at about 21 days of age. Males and females from each litter were maintained in separate cages.

**Construction of recombinants.** A 500-μg amount of

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DNA isolated from SL3-3-infected NIH 3T3 cells was digested with 1,000 60-min units of *Eco*RI for 2 h at 37°C. DNA was then phenol-chloroform extracted, ethanol precipitated, suspended in 500 μl of TE (10 mM Tris-hydrochloride [pH 7.4], 1 mM disodium EDTA), heated to 68°C for 5 min, layered on an 11-ml, 10 to 25% [wt/vol] sucrose gradient in buffer A (40 mM Tris-hydrochloride [pH 8.0], 20 mM sodium acetate, 18 mM NaCl, 2 mM disodium EDTA), and centrifuged at 26,000 rpm for 20 h in a Beckman SW41 rotor. Fractions were collected, and a sample of each was run on an agarose gel in buffer A with *Hin*dIII-digested λ DNA as a size standard. Fractions sedimenting at greater than 10 kilobase pairs (kbp) were pooled and concentrated by ethanol precipitation.

*Eco*RI-digested Charon 4 arms were prepared by sucrose gradient centrifugation as described previously (23). A 2-μg amount of *Eco*RI arms was ligated to 1 μg of pooled SL3-3/NIH insert DNA with $10^2$ U (New England Biolabs; *Hin*dIII units) of T4 DNA ligase in a volume of 8 μl. Ligated DNA was assembled into phages by using the protocol which accompanies the Charon library. Approximately $7 \times 10^5$ recombinant phages were plated in YT containing 0.6% agarose and grown overnight at a density of $5 \times 10^4$ per 500 $cm^2$ on a 1.5% agar YT plate. Phages were transferred to nitrocellulose, lysed in NaOH, and neutralized as previously described (1).

Phages were screened for the presence of sequences homologous to an Akv probe by using a subclone of the Akv provirus in pBR322, pAKR59 (19). A 1-μg amount of pAKR59 DNA was nick translated by using 50 μCi of each $\alpha$-$^{32}P$ to a specific activity of approximately $2 \times 10^8$ dpm/μg. Nitrocellulose filters were prehybridized for 5 h at 65°C in buffer B (3× SSC [1× SSC = 0.15 M NaCl plus 0.015 M sodium citrate], 0.08% bovine serum albumin, 0.08% polyvinylpyrrolidone, 0.08% Ficoll, 0.5% sodium dodecyl sulfate, 0.1% sodium pyrophosphate) with 20 μg of *E. coli* DNA per ml and then hybridized for 24 h with a $10^7$-dpm denatured probe in buffer B. After hybridization, filters were washed with 2× SSC–0.2% sodium dodecyl sulfate for 1 h at 50°C, followed by two washes at 50°C for 1 h in 0.1× SSC. Filters were then dried and autoradiographed.

Plaques which hybridized relatively darkly were picked and eluted in 500 μl. A 10-μl amount each of $10^{-2}$, $10^{-3}$, $10^{-4}$, and $10^{-5}$ dilutions was replated in 0.3 ml of 0.6% agarose in YT. Plaques were rescreened with the Akv probe. Hybridizing plaques were picked from the lowest density plating that gave a positive signal and rescreened through three additional rounds of plaque purification.

All procedures were performed according to the National Institutes of Health guidelines for recombinant DNA research at the P1 EK1 level.

**Transfection.** Transfections on NIH 3T3 fibroblasts were performed as described previously (10). A 20-μg amount of DNA was diluted to 0.5 ml with HBS (140 mM NaCl, 5 mM KCl, 0.7 mM $Na_2HPO_4$, 0.1% glucose, 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid]-NaOH [pH 7.05]). For transfections of cloned DNA, 1 μg of DNA isolated from a phage lysate was mixed with 19 μg of salmon sperm DNA. HBS-diluted DNA was precipitated with 1/10 volume of 1.25 M $CaCl_2$ for 10 to 20 min at room temperature and added to a 30-$cm^2$ plate seeded with $5 \times 10^5$ NIH 3T3 cells the previous day. After 3 to 4 h, the medium was removed from the cells, the cells were washed once with medium, and 1 ml of 15% glycerol in HBS was added for 4 min at room temperature. The glycerol-HBS was then removed, the cells were washed with medium, and the cultures were maintained in 5 ml of medium. Cells were trypsinized and passaged 1:10 every 3rd day. Production of infectious virus was assayed by using a standard reverse transcriptase assay. A 1-ml amount of supernatant fluid was centrifuged for 30 min at 9,000 × *g* in a Brinkman Eppendorf centrifuge. The supernatant was removed, and the pellet was suspended and assayed for reverse transcriptase activity by measuring incorporation of [$^3$H]TTP with a polyriboadenylate:oligodeoxythymidylate template primer (35).

**Heteroduplex analysis.** DNAs from λSL3-95 and λAKR-623 were mixed, denatured, annealed, and spread for electron microscopy as previously described (36). Measurements of DNA length were made by using a Summa Graphics bit pad connected to an Apple II computer with a program for electron microscopic measurements written by A. Zelenetz (personal communication). Measurements were made from 15× photographic enlargements of 5,000× magnified electron micrographs. The measurements reported represent the average of measurements from 15 separate heteroduplexes.

**RNase $T_1$ fingerprinting.** Fingerprinting was performed by techniques similar to those described previously (31). Briefly, infected NIH 3T3 fibroblasts derived by transfection of cloned DNA were grown for 9 days in 20 850-$cm^2$ roller bottles, each containing 20 ml of culture fluid. The medium was changed twice per day. Virus-containing culture fluid was immediately chilled on ice, centrifuged at 2,000 rpm in an IEC 276 rotor to pellet cells and debris, and stored on ice. Virus was pelleted by centrifugation at 17,000 rpm in a Beckman type 19 rotor for 3 h at 4°C and suspended in TNE (100 mM NaCl, 10 mM Tris-hydrochloride [pH 7.4], 1 mM EDTA). A 6-ml amount of suspended virus was further purified on an 11-ml step gradient, consisting of 2 ml of 45% sucrose and 3 ml of 25% sucrose in TNE, by centrifugation for 12 h at 32,000 rpm in a Beckman SW41 rotor at 4°C. Virus banded at the 25 to 45% sucrose interphase and was subsequently stored frozen at −70°C. Virus was always frozen within 72 h of harvest.

Viral particles were lysed with sodium dodecyl sulfate and were then proteinase K digested as previously described (31). Viral RNA was isolated by phenol-chloroform extraction, and polyadenylated RNA was selected on oligodeoxythymidylic acid-cellulose. 70S RNA was isolated by sucrose gradient fractionation, and polyadenylated 70S RNA was again selected by oligodeoxythymidylic acid-cellulose chromatography. The RNA was then digested with RNase $T_1$ and bacterial alkaline phosphatase, 5′ end labeled with [$\gamma$-$^{32}P$]ATP and T4 polynucleotide kinase, electrophoresed in two-dimensional gels, and autoradiographed as previously described (31).

**Leukemogenicity testing.** A 0.1-ml amount of supernatant fluid from infected NIH 3T3 fibroblasts was injected intraperitoneally into newborn mice. Mice were injected before they were 72 h old, usually before they were 24 h old. They were weaned at 21 days of age and monitored daily for disease. Moribund mice

were sacrificed and necropsied to determine whether they were lymphomatous.

## RESULTS

**Cloning strategy.** The approach used to molecularly clone the integrated provirus of SL3-3 was similar to that used to clone the provirus of Akv (21). DNA isolated from SL3-3-infected NIH 3T3 fibroblasts (SL3-3/NIH DNA) was digested with a restriction enzyme that did not cleave the provirus and inserted into the λ vector, Charon 4. Phages were plated, and the resulting plaques were screened for hybridization to an Akv probe.

***Eco*RI does not cleave the SL3-3 provirus.** The restriction enzyme *Eco*RI does not cleave the provirus of Akv (21). Fingerprint data show that the genome of SL3-3 is very similar to that of Akv (30). To determine whether *Eco*RI could also be used to construct recombinants of the SL3-3 provirus, SL3-3/NIH DNA was digested exhaustively with *Eco*RI and tested for infectivity in a transfection assay. *Eco*RI digestion did not reduce the infectivity relative to undigested DNA, indicating that the provirus is not cleaved by this enzyme (data not shown).

**Isolation of a molecular clone of SL3-3.** To facilitate isolation of infectious clones, *Eco*RI-digested SL3-3/NIH DNA was enriched for fragments larger than about 10 kbp by size fractionation on a surcrose gradient. Enriched DNA was ligated to the *Eco*RI arms of the cloning vector, Charon 4. Phages containing SL3-3/NIH DNA were plated and screened by hybridization for the presence of sequences homologous to Akv proviral DNA. The radiolabeled probe used was a nick-translated pBR322 subclone of the Akv provirus, pAKR59 (19). Plaques that gave a relatively dark signal on autoradiography were picked, replated at low density, and rescreened with the Akv probe through four rounds of plaque purification. DNA from one of the recombinant phages was infectious in a transfection assay. This clone was designated λSL3-95. All subsequent studies reported here were performed with this clone.

**Characterization of the cloned virus.** Genomic RNA isolated from the virus derived by transfection of λSL3-95 on NIH 3T3 cells was characterized by RNase $T_1$ fingerprinting (Fig. 1A). The oligonucleotide pattern is the same as that reported previously for the biologically isolated SL3-3 (30). Thus, to the limit of resolution of this technique, the genomes of the molecular clone and the biological isolate of SL3-3 are identical.

Comparison of the genome encoded in λSL3-95 with that of Akv by RNase $T_1$ fingerprinting shows that the two viruses are closely related. The fingerprint of Akv virus derived by transfection of NIH 3T3 cells with the molecular clone of its provirus, λAKR-623 (21), is shown in Fig. 1B. This oligonucleotide pattern is the same as that reported previously for biological isolates of Akv (31). A schematic comparison of the two fingerprints is presented in Fig. 1C. Most of the oligonucleotides are common to both viruses. Five are unique to SL3-3, whereas four are found only in Akv. The numbering system for oligonucleotides used here is the same as that used previously (30, 31). Approximate locations within the genome of the oligonucleotides unique to either of the viruses are summarized in Fig. 2 (30, 31). Within the *gag* and *pol* genes, oligonucleotides 329, 379, and 398 are unique to SL3-3, whereas oligonucleotides 23B, 29, 58, and 99A are unique to Akv. Oligonucleotides 29 and 329 are related by a single base change (see Fig. 2 legend). Assignments of positions for the oligonucleotides within the *gag* and *pol* genes are only approximate.

Oligonucleotides 208B and 36B, which are unique to SL3-3, are located near the 3′ end of the genome (Fig. 2). Both are related to sequences in Akv by single base changes, and their precise positions have been defined (19, 30). Their presence has been correlated with the leukemogenic capability of several viruses from AKR mice (3, 29, 30). Sequences in the corresponding region of avian leukosis virus may be associated with the oncogenic potential of this virus (33, 37).

**Heteroduplex analysis.** RNase $T_1$ fingerprinting resolves about 15% of the viral genome into individual oligonucleotides. Although the oligonucleotides are randomly distributed along the genome, regions between them cannot be investigated by this technique. To search for possible deletions, insertions, or rearrangements, heteroduplexes were formed between the proviral DNAs of SL3-3 and Akv. DNA isolated from SL3-95 was annealed to DNA from λAKR-623 and spread for electron microscopy (Fig. 3). Each of these clones consists of a proviral genome, flanked on either side by the mouse cellular sequences into which the virus integrated, and the λ vector arms. The DNAs from the two clones are homologous in three regions (Fig. 3A). One homologous region is comprised of the proviral DNAs; the arms of the vectors comprise the other two. NIH 3T3 sequences flanking the proviruses are not complementary and do not hybridize.

The two proviruses are annealed for a region of approximately 8.7 kbp, and contain no substitutions relative to one another detectable by this technique. They are arranged in the same 5′ to 3′ orientation relative to the large and small λ arms. Based on the nonhomology of the flanking sequences, they are integrated into different sites in the mouse genome. The arrangement of

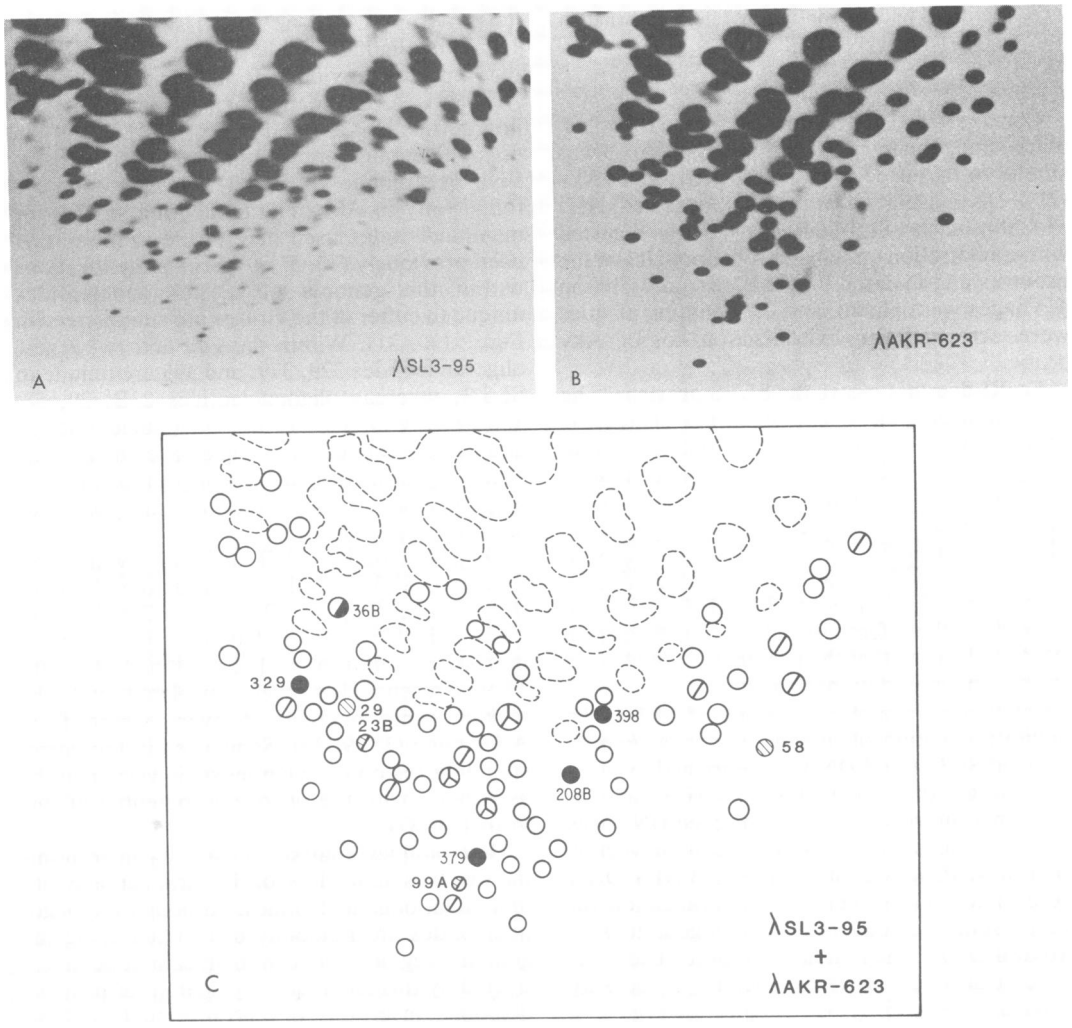



FIG. 1. RNase $T_1$ fingerprint analysis of the RNA genomes encoded by λSL3-95 and λAKR-623. Virus was harvested from cultures of infected NIH 3T3 cells derived by transfection of phage DNA. Polyadenylated 70S RNA was isolated, digested with RNase $T_1$, 5′ end labeled with [γ-$^{32}$P]ATP and T4 polynucleotide kinase, and electrophoresed in two dimensions as previously described (31). Autoradiographs of viral RNA from λSL3-95 (A) and λAKR-623 (B) and a schematic representation comparing the two fingerprints (C) are shown. The numbering system used here is the same as that used previously (30). ○, Oligonucleotides shared between the two viruses; ●, oligonucleotides unique to SL3-3; ⊘, oligonucleotides unique to Akv.

the sequences in λSL3-95 is summarized in Fig. 3B.

Together, heteroduplex analysis and RNase $T_1$ fingerprinting show that the genomes of SL3-3 and Akv are very similar. No large substitutions can be detected between the two viruses, and the changes in nucleotide sequence are slight. At least three of the differences detected in the fingerprint analysis can be accounted for by single base changes.

**Leukemogenicity of SL3-3.** To test whether the virus encoded by the cloned provirus is leukemogenic, virus derived by transfection of λSL3-95 onto NIH 3T3 cells was injected into newborn mice of several inbred strains. The capacity of the virus to accelerate the onset of disease in AKR/J mice and to induce disease in C3H(f)/Bi, CBA/J, and NFS/N mice, strains which have a low spontaneous incidence of lymphomas, was measured (Table 1). The virus accelerated the onset of disease in 100% of recipient AKR/J mice. Lymphomas were also generated in 100% of recipient C3H(f)/Bi and CBA/J mice used in this study. The average latent period for disease varied slightly among different strains, but, except for five C3H and two NFS animals, it

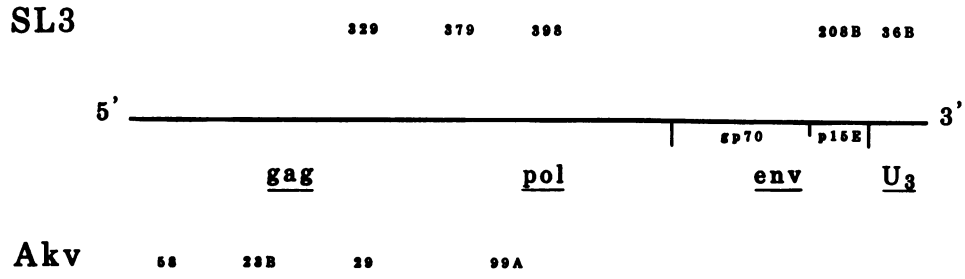



FIG. 2. Map showing the locations of the RNase $T_1$-resistant oligonucleotides unique to SL3-3 and Akv. A linear representation of the organization of the viral genome is presented. Oligonucleotides shown above the line are unique to SL3-3, and those below the line are unique to Akv. The positions of the oligonucleotides show their approximate location within the genome. Positions of oligonucleotides within the *gag-pol* portion of the genome can only be approximated. The order presented here is arbitrary, except that oligonucleotides 58 and 23B are located 5' to the others (Crowther, unpublished data). Localization of oligonucleotide 208B in the p15E coding region and localization of oligonucleotide 36B in the $U_3$ noncoding region have been discussed previously (19, 30). Oligonucleotides which are shared between the two viruses (approximately 90% of the resolvable oligonucleotides) are not shown. Oligonucleotide 29 in Akv and oligonucleotide 329 in SL3-3 are related by a single base change. Their sequences are as follows:

29 5' CAACCUCCACCCCUG 3'
329 5' CAACCUCCACCCCG 3'

ranged from as early as 63 days to no later than 122 days.

To confirm that the λAKR-623 clone encoded non-leukemogenic Akv, virus derived by transfection of cloned Akv DNA onto NIH 3T3 cells was also injected into newborn AKR/J and C3H(f)/Bi mice (Table 1). Cloned Akv did not accelerate the onset of disease in AKR/J mice, nor did it induce disease in C3H(f)/Bi mice.

Lymphoma induction by the molecularly cloned isolate of SL3-3 was compared with that of the uncloned, biological isolate derived by endpoint dilution (Table 1). The leukemogenic potentials of both isolates are very similar. The uncloned isolate induced disease in 100% of recipient AKR/J and C3H(f)/Bi mice. It also induced disease in NFS/N and CBA/J mice. The latent periods for disease were comparable for the cloned and uncloned isolates.

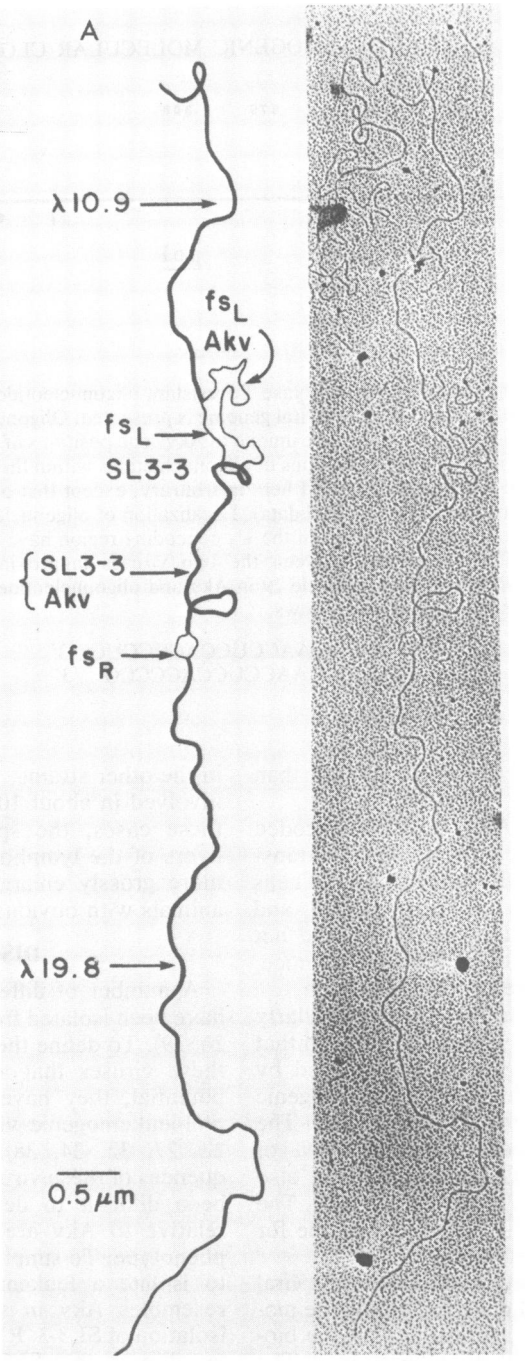
No obvious enrichment or dilution of the viral leukemogenic potential occurred during the molecular cloning process, indicating that the biological isolate was free of any other viruses. The only notable difference between the two isolates was the higher incidence of lymphoma induction by the cloned isolate in CBA/J mice. It is not clear why this difference was observed.

Each mouse in this study was necropsied to investigate the nature of its disease. In every case, the gross pathology was typical of lymphoma, including enlargement of the thymus, spleen, mesenteric lymph nodes, various peripheral lymph nodes, liver, or kidneys. The organs involved varied among different strains. In AKR/J mice, the thymus was always involved; in the other strains, the thymus was not greatly involved in about 10 to 20% of the animals. In these cases, the spleens and sometimes the livers of the lymphomatous animals were even more grossly enlarged than was the case for animals with obvious thymic disease.

## DISCUSSION

A number of different leukemogenic viruses have been isolated from AKR mice (4, 9, 13, 14, 26, 29). To define the regions of the genomes of these viruses that account for the oncogenic potential, they have been compared with the non-leukemogenic virus Akv (5–8, 11, 12, 17, 22, 27, 32, 34, 38). However, since the sequences of these viruses vary extensively, it has been difficult to determine which differences relative to Akv are essential for the virulent phenotype. To simplify this problem, we sought to isolate a leukemogenic virus that closely resembles Akv in structure. This led to the isolation of SL3-3. RNase $T_1$ fingerprinting studies showed that SL3-3 more closely resembles Akv than any other leukemogenic virus isolated from AKR mice (30). The observations reported here eliminate the possibility that a minor, unrecognized component in the SL3-3 viral stocks is responsible for the oncogenic activity. To our knowledge, SL3-3 virus is the most potent virus yet isolated from AKR mice in terms of host range, incidence, and latent period of disease.

**Role of SL3-3 in leukemogenesis.** Heteroduplex and RNase $T_1$ fingerprint analyses demonstrated that the virus encoded by λSL3-95 contains no large substitutions relative to Akv. Instead,


A
λ10.9
fs_L
Akv
fs_L
SL3-3
SL3-3
Akv
fs_R
λ19.8
0.5 μm

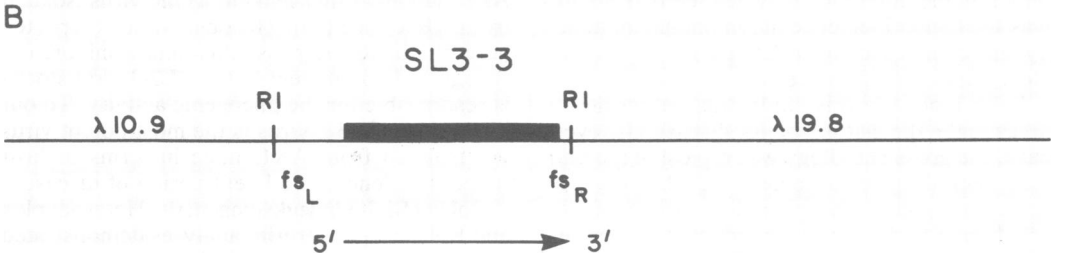

B
SL3-3
λ10.9
RI
RI
λ19.8
fs_L
fs_R
5′
3′

TABLE 1. Leukemogenicity of molecularly cloned and uncloned viruses[a]

| Mouse strain | Source of virus | No. of mice | | Incidence of disease (%) | Mean latent period (days) | Earliest-latest latent period (days) |
|---|---|---|---|---|---|---|
| | | Inoculated | Diseased | | | |
| AKR/J | λSL3-95 | 15 | 15[b] | 100 | 77 | 63–88 |
| AKR/J | SL3-3 uncloned | 9 | 9 | 100 | 79 | 61–91 |
| C3H(f)/Bi | λSL3-95 | 21 | 21 | 100 | 110 | 66–219 |
| C3H(f)/Bi | SL3-3 uncloned | 9 | 9 | 100 | 112 | 73–117 |
| NFS/N | λSL3-95 | 20 | 13 | 65 | 101 | 63–142 |
| NFS/N | SL3-3 uncloned | 5 | 3 | 60 | 131 | 58–176 |
| CBA/J | λSL3-95 | 6 | 6 | 100 | 103 | 84–120 |
| CBA/J | SL3-3 uncloned | 9 | 2 | 20 | 100 | 90–109 |
| AKR/J | λAKR-623 | 5 | 0[b] | | | |
| C3H(f)/Bi | λAKR-623 | 7 | 0 | | | |

[a] A 0.1-ml amount of supernatant containing approximately $10^5$ IU of virus from infected NIH 3T3 cells was injected intraperitoneally into newborn mice of the strain indicated. (Viral titer was determined by endpoint dilution on NIH 3T3 fibroblasts.) Mice were injected before they were 72 h old. Lethargic mice were sacrificed and necropsied. All mice scored as positive for disease had a gross pathology typical of lymphomas. Data from uncloned SL3-3 are presented.

[b] AKR mice which survived for 200 days were scored as negative for virally induced disease. The longest an AKR mouse has survived after inoculation with SL3-3 is 91 days. Clearly, virally induced and spontaneous lymphomas in AKR mice can be distinguished on the basis of the latent period.

SL3-3 differs from Akv by only minimal changes, several of which, at least, are single base changes. Any hypothesis for the mechanism of oncogenesis by this class of viruses must account for how such a limited number of changes can cause such a large difference in leukemogenic potential.

Two general possibilities may explain the difference in leukemogenic potential. One possibility is that a viral protein is altered in SL3-3 relative to Akv such that it can perform an oncogenic function. Viral proteins may have a direct role in oncogenesis similar to that of the glycoprotein of spleen focus-forming virus (20). Differences in viral proteins may allow the leukemogenic virus to be recognized by or to replicate in the appropriate target cell (18, 24).

The second possibility is that the virus may act indirectly by activating a cellular transforming gene product. SL3-3 may differ from Akv in sequences which have a noncoding function such that the ability of the virus to induce expression of a cellular transforming gene is altered (16, 25, 28, 33). It is possible that SL3-3 is not the ultimate leukemogenic component, but that it induces the formation of a recombinant genome which encodes leukemogenic determinants. In this case, an explanation of the difference between Akv and SL3-3 would have to account for the highly elevated ability of the latter to form leukemogenic recombinants.

Isolation of a molecular clone of SL3-3 provides the means to investigate these possibilities. Construction of site-specific recombinants

FIG. 3. Heteroduplex between DNAs from λSL3-95 and λAKR-623. DNAs isolated from phages were mixed, denatured, neutralized, reannealed, and spread for electron microscopy as described by Tiemeier et al. (36). (A) Heteroduplex formed between the two DNAs. Five distinct regions are apparent. From top to bottom, these are (i) the homology between the 10.9-kbp λ arms, (ii) a region of nonhomology consisting of flanking cellular sequences, (iii) the homologous region between the SL3-3 and Akv proviruses in the 5′ to 3′ orientation, (iv) a second, shorter region of nonhomology consisting of cellular flanking sequences, and (v) the homology between the 19.8-kbp λ arms. Determination of the 5′ to 3′ orientation of the provirus is based on the observation that it is arranged in the same orientation relative to the short and long λ arms as the provirus in λAKR-623, whose orientation has previously been determined (21). Size measurements from 15 heteroduplexes show that the homology between the two proviruses extends for approximately 8.7 kbp. Measurements for the 5′ flanking sequences ($fs_L$) are 6.9 and 2.1 kilobases (kb). Comparison with measurements reported by Lowy et al. (21) for λAKR-623 shows that the 6.9-kb sequence is from the Akv clone. Therefore, the 2.1-kb sequence is from λSL3-95. Measurements for the 3′ flanking sequences ($fs_R$) are 1.0 and 0.6 kb. It is not clear which clone contains each of these sequences. (B) Simplified schematic representation of the sequences in λSL3-95.

between SL3-3 and Akv should elucidate which sequences are responsible for the oncogenic potential of SL3-3. Nucleotide sequencing of λSL3-95 should allow deduction of precisely which viral proteins or functions are altered in the leukemogenic recombinants.

## ACKNOWLEDGMENTS

We thank Berthe Willumsen, Donald Tenney, Finn Pedersen, Andy Zelenetz, and Ira Fox for helpful discussions. We thank Doug Lowy for the λAKR-623 clone. We thank Martha Mann and colleagues for assistance with the mouse colony.

This work was supported by National Cancer Institute grant CA 19341. J.L. is the recipient of Public Health Service postdoctoral fellowship CA06389 from the National Institutes of Health. W.H. is the recipient of an American Cancer Society Faculty Research Award.

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