Structure of Retroviral RNAs Produced by Cell Lines Derived from Spontaneous Lymphomas of AKR Mice

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The retrovirus expression of eight independent lymphoid cell lines derived from spontaneous thymomas of AKR mice was investigated. The RNase T₁ fingerprints of viral 70S RNA produced by these cell lines were compared with genome structures of the non-leukemogenic Akv virus and with two types of cloned leukemogenic viruses derived from one of the thymoma cell lines. Viral RNAs from three cell lines, SL3, 4, and 7, were indistinguishable from one another. The fingerprint patterns indicated that these cell lines produce equal amounts of two prototype, leukomogenic SL viruses that were previously isolated from the SL3 cell line. Viral RNA produced by the SL1 and SL2 cell lines contained similar components, but at a different ratio. Two other cell lines (SL5 and SL11) produced viral RNAs that resemble those of AKR mink cell focus-forming viruses. One additional line, SL9, produced viral RNA of a novel structure. The complex pattern of viral RNA expression observed for these lymphoid cell lines can be interpreted in terms of recombination among three types of endogenous viral sequences: the Akv virus, a xenotropic virus, and an SL (for spontaneous leukemia) virus.

The AKR strain of mice was inbred for high incidence of spontaneous thymic lymphoma (4, 10). The high incidence of spontaneous lymphomas has been related to the presence of complete endogenous type C retroviruses in the germ line of these mice (2, 3, 8, 17). One such virus is the Akv virus, an ecotropic, N-tropic virus that is expressed in the animals from early in life (20). Although genetic loci (*Akv-1* and *Akv-2*) that encode the Akv provirus are dominant determinants for disease development (22), the virus itself does not accelerate the onset of lymphoma when injected into newborn AKR mice, nor does it induce disease in mice of low-leukemia-incidence strains (7, 11). A search for other viral agents relevant to the lymphoma development has identified a polymorphic group of viruses in the lymphoid tissues of late preleukemic or leukemic mice (5-7, 12, 21, 25).

Recently, we isolated two types of replication-competent viruses that induce rapid onset of thymic lymphoma (60 to 80 days) upon injection into newborn AKR and C3H/f mice (14). The viruses were cloned from a mixture of viruses produced by a lymphoid cell line, SL3, derived from a spontaneous tumor of an AKR mouse (7,

12). Studies of the structure of the genome of these viruses, called SL3-1 and SL3-2, demonstrated that both types differ from the Akv virus near the 3' end of the genome. A striking observation was that the genome of a cloned leukemogenic Gross A virus (Gross A/NIH) also differs from the Akv virus in this region of the genome and that some of the same non-Akv sequences present in Gross A/NIH were found in the viruses of SL3 origin (1, 14). The leukemogenic Gross A/NIH and SL3-1 viruses also differ from Akv virus in other regions of the genome. However, except for the 3' region, the non-Akv sequences of Gross A/NIH and the SL3 isolates differ from one another. This observation raises the possibility that the 3' region of the viral genomes encodes determinants of leukemogenic potency.

What is the origin of the changes in sequence in the 3' region? The differences in sequence between the Akv virus and the Gross A/NIH and SL3 viruses in this region could be due to simple mutations generated during the replication of the viruses. The difference in sequence is characterized by changes in two marker oligonucleotides, 36B and 208B, that are related to sequences in the Akv genome by one and two base changes, respectively (14). However, the observation that

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identical changes in sequence were present in leukemogenic viruses of AKR origin that have different passage histories raised the possibility that the 3' sequence of these viruses represented expression of an endogenous locus. The observation that the genome of the AKR mouse contains numerous proviral DNA sequences homologous to the Akv genomic RNA lends substance to this possibility (2, 8). We reasoned that if the changes observed in the 3' region of the virus did represent expression of an endogenous locus, then we might observe similar sequences in the RNA produced by other cell lines derived from spontaneous AKR thymomas. Here we report that five of eight lymphoid cell lines we examined expressed viral sequences identical to those present in the Gross A/NIH and SL3 viruses. We present evidence that expression of these sequences is linked to the expression of 5' genomic sequences that differ slightly from those of the Akv virus. Virion RNAs of structure similar to that of the AKR mink cell focus-forming (MCF) viruses were also produced by two of the SL cell lines that were examined. Another SL cell line produced a virus of a novel structure. A hypothesis for the origin of the polymorphic group of AKR viruses is presented.

MATERIALS AND METHODS

Viruses and cell lines. Virus-producing thymocyte and lymphocyte cell lines isolated from AKR mice with spontaneous disease were maintained as suspension cultures in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 μ g of streptomycin sulfate per ml, and 100 U of penicillin G per ml. Cell lines SL1, 2, 3, 4, 5, and 11 were established in the laboratory of E. F. Hays; SL7 and 9 were established in the laboratory of R. C. Nowinski (7, 12). The SL9 and 11 cell lines have not been described previously.

Isolation of virus RNA. Virus-producing cell cultures were harvested once or twice daily. The cell debris was removed by low-speed centrifugation, and the virus was pelleted from the clarified supernatant by centrifugation at 17,000 rpm at 4°C for 3 h in a Beckman type 19 rotor. Viral 70S RNA was purified as described previously (15, 16). Briefly, virus was homogenized in a few milliliters of cold 50 mM Tris-hydrochloride (pH 7.5)–0.1 M NaCl–1 mM EDTA (TNE), and was banded through a linear 25 to 45% (wt/vol) gradient of sucrose in TNE. Viral proteins were disrupted with 1% (wt/vol) sodium dodecyl sulfate (SDS) and digested with proteinase K. The viral RNA was isolated by velocity sedimentation through a linear 15 to 30% (wt/vol) sucrose gradient containing 0.1% (wt/vol) SDS. Isolated RNA was further purified and concentrated by oligodeoxythymidylate-cellulose chromatography followed by ethanol precipitation. Purified RNA was stored frozen in 5 to 10 μ l of 20 mM Tris-hydrochloride (pH 7.5)–2 mM EDTA.

RNAse T₂ fingerprints and oligonucleotide sequencing. Viral RNA was digested with RNAse T₁, 5' end labeled with ³²P, and subjected to two-dimensional polyacrylamide gel electrophoresis as described previ-

ously (15, 16). The resulting pattern of RNAse T₁-resistant oligonucleotides (the RNAse T₁ fingerprint) was visualized by autoradiography. Quantitation of the radioactivity in oligonucleotides was done by Cherenkov counting of oligonucleotides excised from the gel. Oligonucleotides for sequence analysis were excised from the gel, eluted from the gel pieces, ethanol precipitated, and suspended in 5 to 10 μ l of 20 mM Tris-hydrochloride (pH 7.5)–2 mM EDTA. The base sequences of the oligonucleotides were determined by partial enzymatic digestions with RNAse A, RNAse U₂, and RNAse from *Physarum polycephalum* as described previously (16). The 5'-terminal nucleotide was determined by complete enzymatic digestion with RNAse A, RNAse U₂, or RNAse T₂ (1).

Ordering of oligonucleotides along the viral RNA molecules. The approximate order of RNAse T₁-resistant oligonucleotides along the viral genome was determined as described previously (14, 15). Briefly, 50 to 100 μ g of viral RNA was diluted with 1 ml of 5 mM EDTA, pH 7.0, heat denatured for 5 min at 90°C, cooled rapidly to 4°C by the addition of 1 ml of chilled 4 $\times$ SSC (SSC: 0.15 M NaCl plus 0.015 M sodium citrate), and passed through an oligodeoxythymidylate-cellulose column to select for fragments of RNA retaining the 3' polyadenylate tail. These genomic and subgenomic fragments were size selected by velocity sedimentation at 37,000 rpm for 2 h at 20°C in an SW41 rotor through a 15 to 30% (wt/vol) linear sucrose gradient in TNE containing 0.2% (wt/vol) SDS. Fractions representative of different size classes of RNA fragments were taken from the gradient, purified by oligodeoxythymidylate-cellulose chromatography, and ethanol precipitated. RNA from each size class was subjected to RNAse T₁ digestion and fingerprinting.

RESULTS

The intent of this study was to determine the extent of diversity of structure of the RNA of virus produced by tumor cell lines derived from spontaneous tumors of AKR mice. Eight such lines, designated SL to denote their derivation from spontaneous lymphomas, were available for this study. Each cell line was developed from an independent tumor. The origins of SL1 to 7 are described in Materials and Methods. As a class, the SL viruses differ from the Akv virus in several respects. Viruses from some of the lines (SL1, 2, 3, 4, and 7) accelerate the onset of disease in AKR mice. These viruses form few, if any, plaques in the XC assay. To analyze the structure of the SL viruses, 70S RNA was prepared from cell-free supernatant of cultures of the SL cell lines. The fingerprints of the viral RNAs were compared with fingerprints of several other viruses of AKR origin that we and others have studied previously (9, 13–15, 18, 19).

Since many of the cell lines were found to produce viruses in small quantities (0.02 to 2 μ g of 70S RNA per liter of culture fluid), methods for analysis of small quantities of RNA by RNAse T₁ fingerprinting were used (15, 16).

After 70S RNA from purified viruses had been digested to completion by RNase T₁, the cleavage products were labeled with ³²P at their 5' termini. The oligonucleotide mixtures were fractionated by two-dimensional gel electrophoresis. A high-resolution electrophoresis system that permitted the separation of approximately 15% of the viral genome into discrete oligonucleotides was used.

Based on the analysis of the fingerprints of RNase T₁-resistant oligonucleotides, we divided the SL viruses into four classes.

SL viruses that very closely resemble the SL3 virus. The RNase T₁ fingerprints of viral RNAs from the different cell lines, SL3, 4, and 7, were indistinguishable. It can be seen in Fig. 1 that the patterns were similar with respect to both the positions and the relative intensities of the oligonucleotides. All oligonucleotides of these RNAs comigrated when mixtures of RNase T₁-digested, ³²P-labeled RNA were electrophoresed on the same gel. The comigration of two oligonucleotides indicates that they are identical with respect to chain length and base composition, but not necessarily with respect to nucleotide sequence. The extent of similarity of the RNase T₁ fingerprints from these three cell lines strongly suggests that the comigrating oligonucleotides are in fact identical, and we conclude that the viral RNAs of the separate cell lines are identical or very closely related. This pattern was reproducible in several experiments in which RNA derived from separate stocks of these cell lines was used. Moreover, the fingerprint of the viral RNA produced by a subclone of the SL3 cell line, SL3 Cl-1, derived from a single SL3 cell was identical to that of SL3 virus (unpublished data).

A schematic interpretation of the fingerprints of RNA of these viruses is presented in Fig. 2. This interpretation is based upon our detailed studies of the viruses produced by the SL3 cell line (14).

Several features of the fingerprints are noteworthy. First, two oligonucleotides, 208B and 36B, characteristic of the 3' region of the leukemogenic Gross A/NIH and cloned SL3 viruses, are present in the fingerprints of SL3, 4, and 7. The region of the fingerprint corresponding to oligonucleotide 36 is composed of two oligonucleotides, 36A and 36B. The presence of 36B in these viruses was confirmed by digestion of the mixture of oligonucleotides with RNase A, which yields two oligonucleotides characteristic of 36A and 36B that can be distinguished from each other by electrophoresis on DEAE paper (1). The identity of oligonucleotide 208B was confirmed by sequence determination.

The second observation of note is that the relative intensity of the unique oligonucleotides

FIG. 1. RNase T₁ fingerprints of viral RNA from the SL3 (A), SL4 (B), and SL7 (C) cell lines.

was not constant. The difference in intensity reveals heterogeneity of the viral RNAs, as the method used yields uniform labeling of oligonucleotides present in unimolar amounts (15). A detailed analysis of the unique oligonucleotides of the SL3 virus revealed that they could be divided into two groups, those present in unimolar ratios and those present in half-molar ratios. This observation suggested that the SL3, 4, and 7 cell lines produce a mixture of two types of viruses that are identical over most regions of the genome but are different in others. In the

FIG. 2. Schematic interpretation of a mixture of Akv and SL3 viral RNA. Open circles represent oligonucleotides shared with the Akv virus; solid circles represent oligonucleotides unique to the SL viruses; cross-hatched circles represent oligonucleotides unique to the Akv virus. The oligonucleotide above and slightly to the left of 2 is not shown on the schematic diagram, as it is an incompletely digested oligonucleotide that has a hairpin structure (15).

case of the SL3 virus, this possibility was confirmed by the isolation of two types of cloned, replication-competent viruses, SL3-1 and SL3-2 (14).

The order of the unique oligonucleotides along the genomes of the SL3 cloned viruses was determined and is presented in Fig. 3. The oligonucleotide numbers of the Akv genome are omitted in Fig. 3 for easier reading of the figure. The SL3-1 and SL3-2 viruses are identical except in the region that codes for the gp70 envelope glycoprotein. The gp70 region of the SL3-1 virus contains a substitution of xenotropic virus-related sequences in this region (14). The oligonucleotides that are present in less than molar amounts in the fingerprints of the SL3, 4, and 7 viral RNAs are derived from the gp70 region of the genome. In fact, a fingerprint of an equimolar mixture of genomic RNAs of the two cloned SL3 viruses yields the same pattern as that of the SL3, 4, and 7 viruses. We conclude that these cell lines produce equal amounts of two

types of virus which differ in the gp70 region of their genomes.

Finally, we noted several changes in the 5' region of the genomes of the SL3, 4, and 7 viral RNAs. Akv oligonucleotides 58, 23B, and 99A are missing from the fingerprints of SL3, 4, and 7. These oligonucleotides are present in the Akv virus isolated from normal thymuses of mice from the same colony which gave rise to the SL3 and SL4 cell lines. Also, oligonucleotide 379, absent from Akv, is present in unimolar amounts in the fingerprints of the SL3, 4, and 7 viral RNAs. Thus, in these cell lines, the expression of the 3'-region oligonucleotides 208B and 36B appears to be linked to the expression of a variant *gag-pol* region. We conclude that these SL cell lines produce equal amounts of two viruses which share identical *gag-pol* and extreme 3' terminal sequences which are closely related but not identical to the Akv virus. The SL3, 4, and 7 cell lines produce virus capable of accelerating leukemia in AKR mice (11) and, in

FIG. 3. Oligonucleotide maps of viruses derived from SL cell lines. The upper panel represents oligonucleotides present in Akv which are absent from other viruses. The lower panel represents oligonucleotides present in other viruses which are absent from Akv. Numbers in brackets represent weakly labeled oligonucleotides.

the case of the SL3 line, of inducing leukemia in C3H mice (14).

SL viruses that are similar to the SL3 viruses. The RNase T₁ fingerprints of virion RNAs from the SL1 and SL2 cell lines are very similar to each other. The RNase T₁ fingerprint of SL2 RNA and a schematic diagram of the fingerprints are shown in Fig. 4. The pattern of oligonucleotides in the fingerprints of SL1 and SL2 viral RNA is identical to that of SL3, 4, and 7. However, the labeling intensity of the oligonucleotides is different. Notably, non-Akv oligonucleotides which map in the gp70 region are present in much less than unimolar amounts, whereas those which comigrate with oligonucleotides found in Akv are present at or near unimolarity. As with SL3, 4, and 7, the non-Akv oligonucleotides 208B, 36B, and 379 are present in unimolar amounts, and Akv oligonucleotides 58, 99A, and 23B are absent. We conclude that the SL1 and SL2 cell lines produce a mixture of the same two viruses produced by the SL3, 4, and 7 lines, but in different amounts. Both the

SL1 and SL2 cell lines produce virus capable of accelerating leukemia in AKR mice (11).

SL viruses that resemble MCF viruses of AKR origin. The fingerprints of two of the SL viruses, SL5 and SL11, are markedly different from those of the SL3-like viruses. The intensity of the unique oligonucleotides in the fingerprints of these viral RNAs is uniform and does not display the heterogeneity characteristic of the SL3-like viral RNAs. Moreover, the fingerprint contains fewer oligonucleotides than do those of the SL3-like viruses. These observations suggest that each cell line produces only one major viral RNA species. The map order of the unique oligonucleotides and schematic diagrams are presented in Fig. 3 and 5, respectively. The interpretation of genome structure is based on comparison of relative electrophoretic mobilities of the unique oligonucleotides and our previous studies of the map order of the unique oligonucleotides of the Akv, Gross A/NIH, and SL3-2 viruses.

The SL5 and SL11 viruses share many se-

FIG. 4. RNase T₁ fingerprints of viral RNA from the SL2 cell line (A) and a schematic interpretation (B). Open circles represent oligonucleotides shared with Akv; solid circles represent oligonucleotides unique to SL2 which are present at unimolarity; cross-hatched circles represent oligonucleotides unique to SL2 which are present at less than unimolarity.

FIG. 5. RNase T₁ fingerprints and schematic interpretations of viral RNA from SL5 (A and B) and SL 11 (C and D) cell lines. Open circles represent oligonucleotides unique to the SL virus; small circles represent weakly labeled oligonucleotides.

FIG. 5—continued

quences in common with the Akv genome. All the oligonucleotides characteristic of the *gag-pol* region of the Akv genome are present in the fingerprints of both viruses. Most of the oligonucleotides characteristic of the p15(E) region are also present. However, both isolates differ markedly from the Akv virus genome in the region that codes for the gp70 envelope glycoprotein and in the region closest to the 3' terminus of the viral RNA. A large block of oligonucleotides characteristic of the gp70 region of the Akv virus genome is missing from the fingerprints of these viruses. More Akv gp70-specific oligonucleotides are missing from the fingerprint of SL5 RNA than are missing from that of SL11 RNA. The lower intensity of several oligonucleotides derived from the 3' portion of the gp70 region of SL11 may reflect localized sequence heterogeneity of the SL11 virus RNA sample. Oligonucleotides located in the extreme 3' terminal region of the Akv virus genome are also missing from the fingerprints of the SL5 and SL11 viral RNAs.

The fingerprints of SL5 and SL11 RNAs also contain a number of non-Akv oligonucleotides, many of which are identical to oligonucleotides which map in the gp70 region of the SL3-2 isolate. More such oligonucleotides are present in SL5 than in SL11. Neither SL5 nor SL11 RNAs express the extreme 3'-terminal oligonucleotide 36B. However, 208B is present in fingerprints of SL11 but not SL5 viral RNA.

We conclude that these two cell lines produce a single major viral RNA species. We note that the structure of the virion RNA closely resembles that of the MCF viruses isolated from the tissues of preleukemic or leukemic AKR mice (9, 13, 19). For comparison, we also present the oligonucleotide map of the MCF 247 virus in Fig. 3. The SL5 and SL11 viral RNAs contain a substitution similar to that of the MCF viruses in the gp70 region. The SL5 and SL11 viruses differ in structure from the Akv virus and the SL3-like viruses in the region proximal to the 3' terminus of the genome. In this region, the SL5 and SL11 viruses resemble the MCF viruses more closely than they do either the Akv or SL3-like viruses. Neither the SL5 nor the SL11 cell line produced virus capable of accelerating AKR leukemia (11; unpublished data).

SL9 virus. The fingerprint of the virus produced by the SL9 cell line differs from that of the viruses produced by the other SL cell lines (Fig. 6). The RNA appears to be a mixture of at least two species, a major and a minor component. The minor component contains many oligonucleotides characteristic of the Akv virus, but we cannot deduce the probable structure, as it is present in small amounts.

The fingerprint of the major component of the

SL9 viral RNA differs from that of the Akv virus and other SL viruses in the *gag-pol* region. At least eight oligonucleotides characteristic of this region of the Akv and SL3 virus genomes are absent in the major RNA component of the SL9 virus. A number of oligonucleotides characteristic of the gp70 gene of the Akv virus are also missing. The oligonucleotides characteristic of the p15(E) region of the Akv virus genome are present in the fingerprint of the major component of the SL9 virus. However, most of the oligonucleotides characteristic of the extreme 3' regions of the Akv genome are absent. Several oligonucleotides characteristic of the gp70 region of the SL3-2 and MCF viruses are present in the fingerprint of the major component of the SL9 virus. Oligonucleotides 36B and 208B are not expressed in SL9 viral RNA.

We conclude that the viral RNA of the SL9 virus has a major and a minor component. The major component is substantially different from that of the other SL viruses in the *gag-pol* region. It probably contains a substitution in the gp70 region similar to that of the MCF and SL3-2 viruses, is similar to the Akv virus in the p15(E) region, and differs from the Akv virus in the region near the 3' terminus of the viral genome. However, the RNA of the major component resembles that of the SL5, 11, and MCF viruses in the region immediately adjacent to the 3' terminus of the viral genome. The SL9 cell does not produce virus capable of accelerating AKR leukemia (unpublished data).

DISCUSSION

In our previous studies of the genome structure of the leukemogenic AKR viruses, we observed that several cloned isolates of leukemogenic AKR viruses differed from the non-leukemogenic Akv virus by the same changes in sequence in the 3' region of the genome (1, 14). This variant 3' region is characterized by oligonucleotides 208B and 36B. The observation that oligonucleotides 208B and 36B are present in the viral RNAs produced by five of the eight lymphoid lines suggests that the variant 3' terminus reflects the expression of an endogenous locus of proviral DNA that encodes this sequence. We consider the alternative possibility that the same changes in sequence arose by independent mutations to be unlikely. This possibility is rendered more remote by the observation that those cell lines that express the variant 3' region as indicated by oligonucleotides 36B and 208B also express a variant *gag-pol* RNA sequence. These 5' sequences differ from that of the Akv virus by the absence of three Akv-specific oligonucleotides and the presence of one non-Akv oligonucleotide. These changes in the *gag-pol* region do

FIG. 6. RNase T₁ fingerprint of viral RNA from the SL9 cell line (A) and a schematic interpretation (B). Symbols same as those for Fig. 5.

not represent strain differences in the *Akv* loci as they are not present in the RNAs of *Akv* virus isolated from normal tissues of mice from the same colony that gave rise to the SL viruses (16; unpublished data). The coordinate expression of

3' and 5' variant region of the genome suggests that these two sequences may be part of the same endogenous provirus locus. We refer to this hypothetical locus as the spontaneous lymphoma virus locus.

The spontaneous lymphoma virus locus may encode the information for an entire replication-competent virus. The SL3-1 virus may represent expression of this virus. Alternatively, this locus may not contain the information for an entire provirus. In such case, recombination with other endogenous viruses such as the Akv virus or the AKR xenotropic virus may be required to produce replication-competent viruses. Recombination of the spontaneous lymphoma virus locus product with the Akv virus or an endogenous xenotropic virus to produce leukemogenic viruses such as SL3-1 and SL3-2 viruses could account for the delayed onset of the AKR disease. In this view the production of the leukemogenic virus would require simultaneous expression of the RNA of the Akv or xenotropic loci in the same cell.

Our previous studies have linked the expression of the variant 3' region characterized by the presence of oligonucleotides 36B and 208B with the leukemogenic activity of some AKR viruses. We observe that five of the eight cell lines investigated here express both of these marker oligonucleotides and all of these lines produce virus capable of accelerating leukemia in AKR mice. One cell line, SL11, which expresses 208B but not 36B, does not produce leukemia-accelerating virus, nor do the two cell lines which express neither marker oligonucleotide. This is not to say that all leukemogenic viruses of AKR origin must possess this variant 3' region. However, the association between its expression and the oncogenic potential of the viruses produced by these cell lines is striking.

How could the different genomic structure of viruses coded by the Akv and spontaneous lymphoma virus loci influence the leukemogenic activity of the viruses? Our previous study of the Gross A/NIH and cloned viruses showed that the 3' variant sequences would result in a change in amino acid sequence in a protein that might be coded for by the open reading frame located at the 3' terminus of the *env* gene region. An analogous region of the Moloney murine leukemia virus is translated (23, 24). The change in the amino acid sequence of the protein coded for by this region could affect its function. On the other hand, oligonucleotide 36B is related by a single base change to a similar oligonucleotide, f, in the 5' part of the U₃ region of the Akv virus. This change could affect the replication functions of the viruses.

Although the SL3-like viruses are produced by most of the lymphoid cell lines, two of the cell lines produce virus that resembles the AKR MCF viruses in structure. Portions of the gp70 gene of these viruses are substituted with sequences related to endogenous AKR xenotropic viruses. The 3' region of these viruses also

differs from that of the Akv virus as well as from that of the SL3-like viruses. Many of the same changes in the gp70 and 3' regions are also characteristic of the AKR MCF viruses (see the oligonucleotide map of the MCF 247 virus, Fig. 3). Our preliminary studies of the fingerprint of several independent isolates of the AKR xenotropic viruses indicate that these viruses have some of the same sequences as do the MCF-like viruses in this region of the genome. However, the region between the gp70 gene and the 3' portion of the p15(E) gene is identical to that of the Akv virus and different from that of the xenotropic viruses. Therefore, we believe it is likely that the AKR MCF-like viruses arose by a double recombinatorial event between the Akv virus and a xenotropic virus, an event that results in substitution of the Akv genome with parts of xenotropic virus in the gp70 and 3' terminal regions of the genome. The structure of the SL9 virus resembles that of the MCF viruses in the 3' half of the genome and may have a similar origin.

For some time it has been clear that the pattern of virus expression in preleukemic and leukemic AKR mice is complex. Consistent with these studies we find that the SL lines produce a number of different viruses. We suggest that the apparent complexity can be understood by a simpler process, that is, the recombination between RNA products of three types of proviral loci: the locus that codes for the Akv virus, proviral loci related to inducible xenotropic viruses, and a locus that codes for at least the *gag-pol* and 3' region of the SL virus that is here designated the spontaneous leukemia virus locus. Further studies of the structure and expression of such endogenous viral loci may contribute to our understanding of the etiology of spontaneous AKR lymphoma.

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