Organization, Distribution, and Stability of Endogenous Ecotropic Murine Leukemia Virus DNA Sequences in Chromosomes of *Mus musculus*

NANCY A. JENKINS,* NEAL G. COPELAND, BENJAMIN A. TAYLOR, AND BARBARA K. LEE

The Jackson Laboratory, Bar Harbor, Maine 04609

Received 18 January 1982/Accepted 4 March 1982

The endogenous ecotropic murine leukemia virus DNA content and integration sites were characterized for 54 inbred strains and substrains of mice by restriction enzyme digestion, Southern blotting, and hybridization with an ecotropic murine leukemia virus DNA-specific probe. More than 75% of these strains carried endogenous ecotropic proviruses which were located in at least 29 distinct integration sites in chromosomes of *Mus musculus*. Fourteen of these proviruses have been assigned specific locus designations. Most, but not all, of the endogenous ecotropic proviruses were structurally indistinguishable by this analysis from the prototype AKR ecotropic virus, and the distribution of these proviruses followed known relationships among the inbred strains and substrains of mice. These results suggest that, in general, viral DNA integration preceded the establishment of inbred mouse strains and that these integrations are relatively stable.

Mice of many inbred strains have been shown to carry multiple copies of murine leukemia virus (MuLV) DNA sequences as part of their normal genetic complement (23, 39). These endogenous viral genes are replicated and transcribed as cellular genes and are inherited in a Mendelian manner. MuLVs can be grouped into three classes depending on their host range: ecotropic MuLVs, which infect only murine cells; xenotropic MuLVs, which infect cells of heterologous species; and amphotropic MuLVs, which infect cells of murine and several heterologous species (13, 28). Endogenous MuLVs are structurally related, with xenotropic and ecotropic MuLVs sharing greater than 60% sequence homology (4).

Although cells of all inbred strains carry multiple copies of xenotropic virus-related DNA sequences, the number of ecotropic MuLV sequences varies among different inbred strains (23). In AKR/N mice, two loci for ecotropic virus inducibility have been mapped to chromosomes 7 and 16 (21, 31). The single ecotropic virus inducibility locus of BALB/c and C3H/He mice has been assigned to chromosome 5 (18, 20), and that of C57BL/10 mice has been assigned to chromosome 8 (22). The single DBA/2J ecotropic provirus is closely associated with the dilute (*d*) coat color mutant allele on chromosome 9 (19).

Recently, the detailed structure of several integrated retrovirus genomes has been determined (9, 16, 24, 33, 41). In all cases, the

proviral DNA showed the structural features characteristic of transposable elements: long direct repeats terminated by short inverted repeats at the ends of viral DNA; short sequences of cellular DNA that are duplicated during integration and flank each provirus; and, upon integration, a precise site in viral DNA that is joined to nonhomologous sites in host cellular DNA. Retroviruses, like transposable elements, can also act as insertional mutagens and interrupt genetic units by the integration of viral DNA (14, 42). Furthermore, closed circular DNA copies of the *Drosophila* transposable element *copia* have been identified which closely resemble the circular forms of retrovirus proviruses (11). However, to date there is no information available to suggest that proviruses can transpose to new sites in host chromosomes by means other than transcription into RNA, subsequent reverse transcription, and reintegration. Analysis of ecotropic MuLV DNA sequences in a DBA/2J dilute coat color revertant (*d*^{+2J}), however, has suggested that ecotropic proviruses can be excised from mouse chromosomes (19). The deletion of viral DNA sequences in DBA/2J mice may be mediated by homologous recombination involving the viral long terminal repeat sequences in a manner analogous to the excision of Moloney MuLV DNA sequences from Rous sarcoma virus-transformed rat cells (42) or of the transposable element *Ty1* from the yeast *his4* gene (10).

In view of the relationship between transpos-

able elements and retroviruses, we were interested in determining the stability of endogenous ecotropic MuLVs at several defined locations within mouse chromosomes. In the present study, we used a hybridization probe that is specific for ecotropic MuLV DNA sequences (6) to characterize the ecotropic proviral DNA content and integration sites of many related inbred strains and substrains of mice and to determine the frequency at which ecotropic proviral DNA sequences are deleted, rearranged, or transposed in mouse chromosomes.

MATERIALS AND METHODS

Isolation of high-molecular-weight DNA from mouse spleens. Single frozen spleens from 8- to 10-week-old mice (stored at -70°C) were placed, without thawing, in a 7-ml Dounce tissue homogenizer containing 5 ml of disruption buffer (0.25 mg of pronase per ml, 0.5% sodium dodecyl sulfate, 0.15 M NaCl, 0.015 M sodium citrate) and homogenized with a "B" pestle. The homogenate was transferred to a 50-ml disposable polypropylene conical centrifuge tube, the homogenizer was rinsed with 5 ml of disruption buffer, and the combined homogenates were incubated at 37°C for 30 min. A 10-ml amount of phenol saturated with $1\times$ SSC (0.15 M NaCl plus 0.015 M sodium citrate) was added and mixed well, and the solution was allowed to stand at room temperature for 5 min. Chloroform-isoamyl alcohol (10 ml; 24:1) was then added and mixed well, and the phases were separated by centrifugation at room temperature for 3 min in an IEC centrifuge. The aqueous phase was removed, transferred to a clean 50-ml centrifuge tube, and reextracted with 20 ml of chloroform-isoamyl alcohol, and the DNA was wound onto a glass rod after the addition of 20 ml of 95% ethanol to the resulting aqueous phase.

The DNA was redissolved in 10 ml of $0.1\times$ SSC. The solution was brought to $1\times$ SSC with $10\times$ SSC and incubated at 37°C for 30 min after the addition of 0.5 ml of 2-mg/ml RNase A. A 0.5-ml amount of 5-mg/ml pronase was then added, and the solution was incubated for an additional 30 min at 37°C . The DNA was then extracted once with phenol and chloroform-isoamyl alcohol and twice with 20 ml of chloroform-isoamyl alcohol. The DNA was finally collected by winding and dissolved in 1 ml of $0.1\times$ SSC. The average yield from one mouse spleen is 1.0 to 1.5 mg of DNA, which can be stored indefinitely at -70°C .

Restriction enzyme analysis, electrophoresis, DNA transfers, and hybridization. DNAs (10 μg per lane) were digested to completion (10 U of enzyme, 2 h, 37°C) under reaction conditions described by the manufacturer (New England Biolabs, Inc., Beverly, Mass.), electrophoresed through 0.6% agarose gels, and transferred to nitrocellulose filters as previously described (8). The filters were baked under vacuum at 80°C for 2 h and then placed in heat-sealable plastic bags (up to four filters per bag). To each bag was added 50 ml of prehybridization buffer ($4\times$ SSCP [$20\times$ SSCP contains 43.7 g of Na_2HPO_4 , 12.7 g of $\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}$, 88.2 g of sodium citrate, and 140.3 g of NaCl per liter], $1\times$ Denhardt solution [$20\times$ Denhardt solution contains 0.4 g of Ficoll, 0.4 g of polyvinylpyrrolidone, and 0.4 g of bovine serum albumin per 100 ml

of distilled H_2O], 100 μg of sonicated denatured salmon sperm DNA per ml). The bags were sealed and incubated for 2 h in a shaking 65°C water bath.

After the 2-h incubation, the buffer was removed, 12 ml of hybridization buffer was added to each bag, and the filters were hybridized overnight in a shaking 65°C water bath. Hybridization buffer contained 100 μg of sonicated denatured salmon sperm DNA per ml, 0.2% sodium dodecyl sulfate, $4\times$ SSCP, $1\times$ Denhardt solution, and 5×10^6 cpm of denatured ^{32}P -labeled hybridization probe per ml. The probe ($>2\times 10^8$ cpm/ μg of DNA) was prepared by nick translation (29) of the entire plasmid carrying the 400-base-pair ecotropic type-specific fragment of AKR MuLV DNA (6).

The filters were washed in a 65°C shaking water bath with 500-ml changes of $4\times$ SSCP- $1\times$ Denhardt solution-0.1% sodium dodecyl sulfate, first for 15 min, and then three times for 30 min each and three times for 15 min each. After the 65°C washes, the filters were rinsed once at room temperature with 1 liter of $0.3\times$ SSC and then twice for 5 min each with 500 ml of 3 mM Tris-base. The filters were air dried and autoradiographed at -70°C with Kodak XAR-5 X-ray film and Du Pont Lightning Plus intensifying screens. Bands were detectable by overnight exposure, with optimal exposures normally requiring 2 to 3 days.

RESULTS

Endogenous ecotropic MuLV DNA content and integration sites in DNA of 54 inbred strains and substrains of mice. High-molecular-weight DNA was prepared from the spleens of mice of each inbred strain, digested to completion with *Pvu*II, *Hind*III, or *Xba*I, electrophoresed through agarose gels, blotted onto nitrocellulose paper, and hybridized with a ^{32}P -labeled ecotropic MuLV DNA-specific probe. For each distinct AKR-like provirus (as determined by restriction enzyme analysis of internal viral DNA fragments), digestion with *Hind*III or *Pvu*II will produce a single detectable 3' cell-virus DNA junction fragment containing 5.8 or 3.0 kilobases (kb), respectively, of viral DNA (27). Likewise, *Xba*I digestion will produce a 5' cell-virus junction fragment containing 7.8 kb of viral DNA.

Of 54 inbred strains analyzed, 12 did not produce detectable viral DNA-containing fragments. These strains were AU/SsJ, BUB/BnJ, CBA/CaJ, CBA/H-T6J, CBA/N, CE/J, C57L/J, NZB/B1NJ, RIIS/J, SWR/J, 129/J, and 129/SvJ. Some of these strains, including C57L, NZB, and 129, have previously been shown to lack ecotropic MuLV DNA sequences (6, 23). That mice of the SWR/J strain do not carry endogenous ecotropic MuLV DNA sequences is surprising since Stephenson and Aaronson (38) reported chemical induction of a B-tropic retrovirus from SWR/J mouse embryo cells in tissue culture.

Forty-two inbred strains carried endogenous ecotropic proviruses. In most cases, *Pvu*II digestion generated the most diagnostic viral DNA-containing junction fragments, and representative

PvuII digestions are shown in Fig. 1 and 2. A summary of the size of the viral DNA fragments produced by digestion with all three enzymes is shown in Table 1. Of the 42 strains found to carry endogenous ecotropic proviruses, 30 carried only one ecotropic provirus and, with the exception of strains LG/J, LP/J, ST/bJ, and DA/HuSn (Fig. 2), could be grouped into three distinct classes (Fig. 1). The number of ecotropic MuLVs carried by the other 12 inbred strains varied between two and six copies (Fig. 2).

For most strains, the size of the viral DNA-containing fragments produced by *PvuII*, *HindIII*, or *XbaI* digestion was larger than that of the corresponding fragment of unintegrated linear AKR MuLV DNA, suggesting that these viral DNA sequences are linked to cellular DNA (Table 1). However, DNA from mice of five inbred strains, ST/bJ, MA/MyJ, SJL/J, SJL/Wt, and PL/J, produced *XbaI* fragments that were less than 7.8 kb (Table 1, parts D and E). This result suggested that these strains carry proviruses that either are defective or are structurally unrelated to the AKR ecotropic provirus.

Analysis of the endogenous ecotropic MuLV genomes with restriction enzymes that generate detectable internal viral DNA fragments. Since some strains appeared to carry aberrant ecotropic MuLV genomes, we analyzed the internal organization of the endogenous proviruses in more detail by using enzymes that generate detectable internal viral DNA fragments. Digestion of AKR/J or AKR/N DNA with *KpnI*, *BamHI*, or *PstI* produced single detectable internal viral DNA fragments of 4.4, 3.3, or 8.2 kb, respectively (Table 1). The sizes of these fragments were similar to that of the corresponding fragment of unintegrated linear AKR MuLV DNA (27). Since *PstI* cleaves unintegrated AKR MuLV DNA only within the long terminal repeat sequences, the presence of an 8.2-kb *PstI* fragment suggests the presence of an AKR-like endogenous provirus containing long terminal repeat sequences.

Digestion with *KpnI*, *BamHI*, or *PstI* of DNA from the 12 strains that were shown to be ecotropic virus negative again demonstrated that these strains do not carry detectable ecotropic MuLV genomes. Digestion of DNAs from the 42 strains that do carry endogenous ecotropic proviruses produced detectable viral DNA fragments. Most of the strains that appeared to carry normal AKR-like proviruses after *PvuII*, *HindIII*, or *XbaI* digestion produced normal *KpnI*, *BamHI*, or *PstI* fragments (Table 1). Therefore, digestion of DNA with enzymes that produce detectable internal viral DNA fragments confirmed that most of these endogenous ecotropic proviruses are structurally similar, including the presence of long terminal repeat sequences, to the prototype endogenous AKR provirus.

Digestion of DNA from the five strains that carried aberrant MuLV genomes (as defined by

the size of *XbaI* viral DNA-containing fragments) also produced aberrant internal viral DNA fragments (Fig. 3). In addition, DNA from mice of the LT/Sv and C58/J strains produced an abnormal 23-kb *BamHI* fragment (Fig. 3), suggesting that these strains may also carry an aberrant provirus. The aberrant proviruses of the C58/J and LT/Sv strains are probably identical since the LT/Sv strain is a recombinant inbred strain derived from C58 and BALB/c mice (34). The aberrant provirus of the C58/J and LT/Sv strains is structurally dissimilar to that of PL/J mice (Table 1, parts F and G). However, the aberrant proviruses of the SJL/J, SJL/Wt, MA/MyJ, and ST/bJ strains appear to be identical, with the same flanking cellular DNA sequences detected in SJL/J, SJL/Wt, and MA/MyJ mice, but not in ST/bJ mice (Table 1, parts D and E).

Classification of endogenous ecotropic MuLV genomes. Previously, endogenous ecotropic MuLV loci have been named according to the strain in which they were identified. However, this nomenclature becomes difficult when the same virus locus is carried by many different inbred strains (Table 1). In addition, previous nomenclature has not clearly distinguished between endogenous ecotropic virus loci and xenotropic virus loci. Therefore, we have designated the different endogenous ecotropic loci that we have characterized *Emv* (for endogenous ecotropic murine leukemia virus locus), followed by a numerical designation. Loci detectable with xenotropic virus-specific probes might then be designated *Xmv* (xenotropic murine leukemia virus locus). This nomenclature is consistent with that previously used for the endogenous avian leukosis virus loci (2, 3) and endogenous mouse mammary tumor virus loci (40).

(i) *Emv-1*. The endogenous ecotropic provirus present in the DNA of BALB/c mice, previously referred to as *Cv*, has been designated *Emv-1* and appears to be carried as a single endogenous provirus in 15 of the 54 inbred strains analyzed (Table 1). Although we have not shown that the endogenous proviruses carried by these 15 strains map to the same chromosomal location, we believe that this is the case because: (a) all of the restriction enzyme fragments detected in these strains are identical; (b) historical records indicate that the A, BALB/c, and C3H strains are related (34); (c) the ecotropic proviruses of the A/HeJ and BALB/cJ strains do not recombine in genetic crosses (our unpublished data); (d) SEC/1ReJ was derived from a cross of NB and BALB/c mice (34); and (e) the HTG/GoSfSn strain was derived from BALB/c mice (12, 34).

Of the four CBA strains analyzed, only CBA/J carried an ecotropic provirus that appeared to be identical to *Emv-1*. As the C3H (which carries *Emv-1*) and CBA strains had common origins

FIG. 1. Characterization of the endogenous ecotropic MuLV sequences in DNA of 26 inbred mouse strains. High-molecular-weight DNAs (10 μ g per lane) prepared from spleens of 26 different inbred strains and substrains of mice were digested to completion with *Pvu*II. The sizes (in kb) of viral DNA-containing fragments detected after electrophoresis through 0.6% agarose gels and Southern analysis were calculated using 32 P-labeled *Hind*III-digested λ DNA electrophoresed in parallel lanes of the same gels. The bands indicated by ● do not represent hybridization of the probe to cellular DNA sequences, as the size of the fragment (3.9 kb) is unaltered by digestion with various restriction enzymes and is also detected in lanes lacking DNA but containing tracking dye.

(34) and the CBA/J and CBA/Ca related substrains diverged at about the F30 generation of inbreeding, it is possible that the progenitors of the CBA/J and CBA/Ca strains carried *Emv-1* sequences that were still segregating at the time of substrain divergence. Alternatively, CBA/J mice may have been unintentionally crossed to C3H mice since both strains are identical in appearance and CBA/J mice have some genetic markers characteristic of C3H but not of CBA/Ca mice (30).

(ii) *Emv-2*. The endogenous ecotropic provirus present in the DNA of C57BL mice, previously referred to as *Blv*, has been designated *Emv-2*. This virus is carried by all five substrains of C57BL mice analyzed, as well as by C57BR/cdJ mice. The C57BL and C57BR sublines were separated in the first generation from a mating of

female 57 $\times$ male 52 from the Lathrop stock (34), and both strains have inherited the same ecotropic provirus. Male 52 was also mated with female 58 to give rise to the C58 strain (34) which does not carry *Emv-2*. Interestingly, the leaden coat color mutation of the C57L/J strain occurred at the F22 generation of inbreeding in a C57BR subline which is now extinct (34). C57L/J mice are ecotropic virus negative, suggesting either that this C57BR subline did not carry *Emv-2* or that *Emv-2* sequences were still segregating in C57BR mice at the F22 generation.

(iii) *Emv-3*. The endogenous ecotropic provirus present in the DNA of DBA mice, previously referred to as *Dbv*, has been designated *Emv-3*. This virus was found in all three substrains of DBA mice analyzed, as well as in two other related inbred strains, BDP/J and P/J (34). Re-

FIG. 2. Characterization of the endogenous ecotropic MuLV sequences of 16 inbred mouse strains. DNAs from 16 strains and substrains of mice were digested to completion with *Pvu*II and analyzed as described in the legend to Fig. 1. The bands indicated by • do not represent hybridization of the probe to cellular DNA sequences (see legend to Fig. 1).

cently, *Emv-3* was shown to be closely linked to the dilute (*d*) coat color mutation of DBA/2J mice (19). The BDP/J and P/J strains carry the *d* allele (34) and have therefore inherited both *Emv-3* and the dilute mutation from DBA mice.

The HRS/J and SEA/GnJ strains also carried *Emv-1* as well as *Emv-3*. The HRS/J strain, which carries the hairless (*hr*) mutation, was derived from the cross of a noninbred hairless male (*hr/hr*) with a BALB/cGn female (E. L. Green, personal communication). Dilute (*d/d*) mice were observed among the descendants of this cross, and *Emv-1*, *Emv-3*, and *d* became fixed in HRS/J mice. SEA/GnJ is a recombinant inbred strain derived from a cross of the BALB/c and P strains (34) and has inherited both *Emv-1* (BALB/c) and *Emv-3* as well as *d* (P).

(iv) *Emv-4* through *Emv-7*. Four strains, LG/J, LP/J, ST/bJ, and DA/HuSn, carried single endogenous ecotropic proviruses that were unrelated to each other or to *Emv-1*, *Emv-2*, and *Emv-3*. These loci have been designated *Emv-4* through *Emv-7*, respectively (Table 1).

(v) *Emv-8* through *Emv-10*. In addition to the HRS/J and SEA/GnJ strains, three other strains were identified (MA/MyJ, SJL/J, and SJL/Wt) that carried two ecotropic proviruses (Table 1, part E). All three of these strains carried an identical ecotropic provirus that was non-AKR-like, and this provirus has been designated *Emv-9*. The limited distribution of *Emv-9* suggests that the MA/MyJ and SJL strains are related. The additional proviruses carried by MA/MyJ, SJL/J, and SJL/Wt mice have been designated

TABLE 1. Endogenous ecotropic type-specific MuLV DNA sequences of 42 inbred mouse strains^a

Mouse strains and associated proviruses	Viral DNA-containing fragment size (kb) ^b					
	<i>Pvu</i> II	<i>Hind</i> III	<i>Xba</i> I	<i>Kpn</i> I	<i>Bam</i> HI	<i>Pst</i> I
A. Strains carrying a single endogenous ecotropic provirus, designated <i>Emv-1</i> (A/HeJ, A/J, A/WySnJ, BALB/cByJ, BALB/cGnEi, BALB/cJ, BALB/cWtEi, CBA/J, C3HeB/FeJ, C3H/HeJ, C3H/HeSnJ, FS/Ei, SEC/1ReJ, SM/J, HTG/GoStSn)	4.3	7.0	10.5	4.4	3.3	8.2
B. Strains carrying a single endogenous ecotropic provirus, designated <i>Emv-2</i> (C57BL/KsJ, C57BL/6ByJ, C57BL/6J, C57BL/10J, C57BL/10SnJ, C57BR/cdJ)	5.2	7.0	11.5	4.4	3.3	8.2
C. Strains carrying a single endogenous ecotropic provirus, designated <i>Emv-3</i> (DBA/1J, DBA/2J, DBA/2DeJ, BDP/J, P/J)	5.4	6.4	8.4	4.4	3.3	8.2
D. Additional strains carrying single endogenous ecotropic proviruses						
LG/J, <i>Emv-4</i>	4.8	8.4	8.4	4.4	3.3	8.2
LP/J, <i>Emv-5</i>	6.4	15.0	9.0	4.4	3.3	8.2
ST/bJ, <i>Emv-6</i>	13.0	19.0	5.3	3.8	2.1	5.1
DA/HuSn, <i>Emv-7</i>	5.6	6.0	9.8	4.4	3.3	8.2
E. Strains carrying two endogenous ecotropic proviruses ^c						
HRS/J ^d or SEA/GnJ						
<i>Emv-1</i>	4.3	7.0	10.5	4.4	3.3	8.2
<i>Emv-3</i>	5.4	6.4	8.4			
MA/MyJ						
<i>Emv-8</i>	5.8	8.4	8.0	4.4	3.3	8.2
<i>Emv-9</i>	4.8	6.6	6.3	3.8	2.1	5.1
SJL/J or SJL/Wt						
<i>Emv-9</i>	4.8	6.6	6.3	3.8	2.1	5.1
<i>Emv-10</i>	4.1	11.0	11.5	4.4	3.3	8.2
F. Strains carrying three endogenous ecotropic proviruses ^e						
AKR/N ^f						
<i>Emv-11</i> (Akv-1)	4.8	5.9	13.5	4.4	3.3	8.2
<i>Emv-12</i> (Akv-2)	5.0	7.0	11.5			
<i>Emv-13</i> (Akv-3)	3.9	21.0	13.5			
AKR/J ^g						
<i>Emv-11</i> (Akv-1)	4.8	5.9	13.5	4.4	3.3	8.2
<i>Emv-13</i> (Akv-3)	3.9	21.0	13.5			
<i>Emv-14</i> (Akv-4)	8.3	6.0	13.5			
RF/J, <i>Emv-1</i>	4.3	7.0	10.5	4.4	3.3	8.2
	6.1	23.0	14.0			
	4.8	8.7	9.6			
LT/Sv, <i>Emv-1</i>	4.3 ^h	7.0 ^h	10.5	4.4	3.3	8.2

TABLE 1—Continued

Mouse strains and associated proviruses	Viral DNA-containing fragment size (kb) ^b					
	<i>PvuII</i>	<i>HindIII</i>	<i>XbaI</i>	<i>KpnI</i>	<i>BamHI</i>	<i>PstI</i>
	6.4	8.6	23.0		23.0	
	4.1 ^h	6.7 ^h	8.8			
G. Strains carrying more than three endogenous ecotropic proviruses ^c						
C58/J	9.3	11.0	23.0	ND ⁱ	23.0	8.2
	8.0	10.0	10.5		3.3	
	6.4	8.6	8.8			
	4.1	7.6				
	3.9	6.7				
	3.3					
I/LnJ						
<i>Emv-1</i>	4.3	7.0	10.5	4.4	3.3	8.2
<i>Emv-3</i>	5.4	6.4	8.4			
	6.4	17.5	13.5			
	4.8	14.0	9.0			
	3.9	7.8				
PL/J	17.0	20.0	20.0	ND ⁱ	9.5	8.2
	5.8	13.5	13.5		3.3	3.2
	4.5	10.5	8.0			
		8.4	5.3			

^a The endogenous ecotropic type-specific MuLV DNA sequences of 42 inbred mouse strains (1 to 5 mice per inbred strain) that carried endogenous ecotropic proviruses were analyzed by digestion with six different restriction enzymes (*PvuII*, *HindIII*, *XbaI*, *KpnI*, *BamHI*, and *PstI*), electrophoresed through agarose gels, blotted onto nitrocellulose paper, and hybridized with an ecotropic type-specific probe as described in the text. The proviral loci have been designated *Emv-1* through *Emv-14* for the endogenous ecotropic MuLV locus.

^b The sizes in kb of the viral DNA-containing fragments detected were calculated from ³²P-labeled *HindIII*-digested λ DNA electrophoresed in parallel lanes of the same gels.

^c Restriction enzyme fragments representing *Emv-9* were assigned from fragments that were common to the MA/MyJ, SJL/J, and SJL/Wt strains. Since *Emv-9* was non-AKR-like (by *XbaI* digestion), *KpnI*, *BamHI*, and *PstI* fragments that were also non-AKR-like were assigned to *Emv-9*. Fragments specific to *Emv-8* and *Emv-10* were specified after the assignment of fragments to *Emv-9*. Although there was only one detectable *KpnI*, *BamHI*, and *PstI* fragment in HRS/J and SEA/GnJ DNAs, we assumed that these fragments were doublets.

^d HRS/J mice that were homozygous for the *hr* allele were identical, by restriction enzyme analysis, to HRS/J +/- mice.

^e In some cases, fragments have been assigned to specific *Emv* loci. Where no assignments have been made, the fragments were listed in order of decreasing size.

^f Restriction enzyme fragments specific to *Emv-12* were assigned by subtracting the fragments that were known to be specific to *Emv-11* and *Emv-13*.

^g The assignment of restriction enzyme fragments to *Emv-11*, *Emv-13*, and *Emv-14* was done by restriction analysis of DNAs from three AKXL recombinant inbred mouse strains (derived from a cross of the AKR/J and C57L/J strains), which were previously shown to carry only *Emv-11*, *Emv-13*, or *Emv-14* (37). In addition, all three AKR/J loci were shown to produce normal *KpnI*, *BamHI*, and *PstI* fragments by analysis of DNAs of the AKXL recombinant inbred strains, but this has not been determined for *Emv-12*.

^h These closely migrating fragments are difficult to resolve but are presumed to be present based on the origin of the LT/Sv strain and the presence of three *XbaI* fragments, including a 10.5-kb *XbaI* fragment characteristic of *Emv-1*.

ⁱ ND, Not determined.

Emv-8 (MA/MyJ) and *Emv-10* (SJL/J and SJL/Wt).

(vi) *Emv-11* through *Emv-14*. DNA from all AKR/N and AKR/J mice examined carried at least three ecotropic proviruses. Two of these proviruses were present in both substrains and have been termed *Akv-1* and *Akv-3* (5, 17, 35).

The additional proviruses carried by AKR/N and AKR/J mice have been termed *Akv-2* and *Akv-4*, respectively (*Akv-4* was previously referred to as *Akv-2J*, but has recently been renamed by Steffen et al. [37]). Digestion of AKR/N DNA with *PvuII* generated three viral DNA-containing junction fragments that corre-

FIG. 3. Restriction enzyme analysis of DNA of inbred mouse strains that carry non-AKR-like ecotropic MuLV proviruses. DNAs prepared from spleens of MA/MyJ, SJL/J, ST/bJ, LT/Sv, C58/J, or PL/J mice were digested to completion with *Kpn*I, *Bam*HI, *Xba*I, or *Pst*I (as indicated) and analyzed as described in the legend to Fig. 1. The bands indicated by • do not represent hybridization of the probe to cellular DNA sequences (see legend to Fig. 1).

sponded to *Akv-1* (4.8 kb), *Akv-2* (5.0 kb), and *Akv-3* (3.9 kb) (Fig. 2). Digestion of AKR/J DNA with *Pvu*II produced the 4.8- and 3.9-kb fragments characteristic of *Akv-1* and *Akv-3* and an 8.3-kb fragment that represented *Akv-4*. The assignment and complete restriction enzyme analysis of *Akv-1*, *Akv-3*, and *Akv-4* was done using DNA from three AKXL recombinant inbred strains (derived from a cross of AKR/J and C57L/J mice). These three strains were previously shown to carry only *Akv-1*, *Akv-3*, or *Akv-4* after segregation of these loci during inbreeding of the AKXL recombinant inbred strains (37). The assignment of specific fragments to *Akv-2* was done by restriction enzyme analysis of AKR/N DNA and subtraction of fragments representing *Akv-1* and *Akv-3*.

In addition to the three viral DNA fragments normally found in AKR/J DNA, DNA from the two AKR/J mice used in these experiments also produced a 3.6-kb fragment (Fig. 2). This recently acquired provirus is probably the result of germ line infection of an AKR ecotropic MuLV

during inbreeding of the AKR/J strain, a form of gene duplication that has been shown to occur in congenic NIH *Akv-1* sublines of mice (32) and in two series of AKR/J-derived recombinant inbred strains (19, 37). This additional ecotropic provirus has not been analyzed in detail and is not listed in Table 1. In keeping with our previously used nomenclature, we have renamed these loci *Emv-11* (*Akv-1*), *Emv-12* (*Akv-2*), *Emv-13* (*Akv-3*), and *Emv-14* (*Akv-4*).

The five other strains that we analyzed, RF/J, LT/Sv, C58/J, I/LnJ, and PL/J, also carried multiple ecotropic proviruses. In most cases, assignment of these proviruses to specific *Emv* loci will require that they be separated by appropriate genetic crosses and analyzed individually. However, certain *Emv* loci have been tentatively assigned to some of these strains based on the data summarized in Table 1.

DISCUSSION

Of the 54 inbred mouse strains that we studied, 42 carried endogenous ecotropic MuLV

DNA sequences which, in most cases, were structurally related to the AKR ecotropic provirus. These results are consistent with those reported by Steffen et al. (36) and Chattopadhyay et al. (6) and further demonstrate that most inbred strains carry endogenous proviruses. Although restriction enzyme analysis of cell-virus DNA junction fragments indicated that these viral DNA sequences are located in at least 29 distinct integration sites, many strains carried ecotropic proviruses that were indistinguishable, including flanking cellular DNA sequences, from three prototype proviruses found in BALB/c, C57BL, and DBA mice. These viral DNA loci have been designated *Emv-1*, *Emv-2*, and *Emv-3*, respectively. As retroviruses are thought to integrate at random sites, the presence of common proviruses shared by different strains suggests that these strains are related. These strain relationships have been confirmed by historical records in most instances. However, strain-specific differences in virus expression have been observed in some strains carrying the same *Emv* locus. For example, BALB/c and C3H mice carry the same endogenous ecotropic provirus, yet this provirus is expressed at different ages in mice of these two strains (18). These differences in virus expression may be due to host factors or may reflect small structural differences in the *Emv* locus itself. The availability of several strains carrying the same endogenous provirus should be useful in identifying genes that regulate virus expression.

It has been suggested that the AKR ecotropic provirus is of Asian origin (7, 36) and is closely related to all of the endogenous ecotropic proviruses found in American laboratory mice. This conclusion is supported by the similarity in restriction enzyme cleavage patterns of proviral DNAs carried by the mice that we have characterized. However, we have identified six strains that carry non-AKR-like MuLV DNAs. The origin of these proviruses is unclear but may result from a deletion or insertion within the viral genome that occurred at the time of virus integration or during inbreeding, from the presence of a related but different class of MuLV, or from a mutation within the viral genome that leads to alterations in restriction enzyme cleavage sites.

The distribution of ecotropic MuLVs followed known relationships among the inbred strains and substrains of mice. In many cases, the substrains have been maintained independently for more than 30 to 50 years (34), suggesting that most viral integrations preceded inbreeding and were relatively stable. By combining the number of years that the strains and substrains carrying only *Emv-1*, *Emv-2*, or *Emv-3* have been maintained separately, one can estimate that no new

proviruses have become fixed in the germ line of these mice in 1,000 years, or 2,500 generations of inbreeding. Therefore, it is difficult to account for the diversity of ecotropic proviral DNA integration sites by transposition or other forms of gene duplication alone. These results are in contrast to those obtained in highly viremic strains, such as AKR, which have been shown to acquire new endogenous ecotropic MuLV genomes at low frequencies. Amplification of viral DNA sequences in these strains probably results from germ line infection of the virus that they spontaneously produce (32) and was found to have occurred in the AKR/J mice used in these experiments, as well as in several other AKR substrains and two AKR/J-derived sets of recombinant inbred strains (19, 26, 37). An estimate of the rate at which new endogenous ecotropic proviruses are becoming fixed in these viremic strains is one new provirus every 15 to 50 years. Therefore, it seems likely that many of the ecotropic proviruses carried by the lowly viremic strains were acquired by germ line infection of progenitor strains rather than by transposition or other forms of gene duplication and rearrangement.

Although the distribution of endogenous ecotropic proviruses suggests that virus integration is relatively stable, it does not preclude occasional transposition or deletion of viral DNA sequences. In particular, it was not possible to determine if some of the ecotropic proviruses identified in strains carrying multiple proviruses were acquired by transposition, from ancestor strains, or by germ line infection. For example, *Emv-6* found in the ST/bJ strain and *Emv-9* found in the MA/MyJ and SJL strains are non-AKR-like and share what are thought to represent internal viral DNA fragments, but have distinctive 5' and 3' cell-virus junction fragments. These two proviruses may represent separate germ line integrations of a non-AKR-like MuLV. Alternatively, one of these proviruses may have originated by transposition. We are currently analyzing these possibilities in more detail.

Among the highly leukemic strains characterized, all were found to carry at least two endogenous ecotropic proviruses. However, the significance of this result is unclear since some strains having a low incidence of leukemia that carry multiple ecotropic proviruses were also identified. For example, the I/LnJ strain carries five ecotropic proviruses and has a low incidence of leukemia (1). In addition, HRS/J mice homozygous for the autosomal recessive mutant allele *hr* (hairless) show a 45% incidence of leukemia by 8 to 10 months of age, whereas the incidence in heterozygotes (*hr/+*) is low (1% at 8 to 10 months) (25). Yet, HRS/J *hr/hr* and HRS/J *+/+*

mice carry the same two endogenous ecotropic proviruses, and both *hr/hr* and *hr/+* mice spontaneously produce high titers of ecotropic virus (15). Furthermore, SEA/GnJ mice carry the same *Emv* loci as HRS/J mice, but in SEA/GnJ mice the proviruses are only occasionally expressed early in life (our preliminary data), and SEA/GnJ is a strain having a low incidence of leukemia (1). Recently, we have identified a third strain, *cw* (curly whiskers, maintained by the Mutant Stock Center, Jackson Laboratory, Bar Harbor, Maine), that also carries the same *Emv* loci as HRS/J and SEA/GnJ mice (19), spontaneously produces ecotropic MuLV early in life (H. G. Bedigian, unpublished data), and has a high incidence of reticulum cell sarcoma (J. B. Roths and E. D. Murphy, unpublished data). The availability of several inbred strains carrying the same *Emv* loci and differing in virus expression and leukemia incidence should be useful in identifying other genes that are important in leukemogenesis.

ACKNOWLEDGMENTS

We thank Eva Eicher, Marianna Cherry, Leroy Stevens, Leonard Shultz, and Wesley Beamer for providing various inbred mouse strains and Margaret Green, Eva Eicher, and Donald Bailey for helpful discussions and critical reading of this manuscript. We are especially grateful to the late Hans Meier for his encouragement.

This work was supported by grants MV-124 (N.A.J.) and MV-119 (N.G.C.) from the American Cancer Society and Public Health Service research contract NO1 CP33255 from the Division of Cancer Cause and Prevention, National Cancer Institute (B.A.T.).

LITERATURE CITED

- Altman, P. L., and D. D. Katz. 1979. Inbred and genetically defined strains of laboratory animals. Part 1. Mouse and rat, p. 418. Federation of American Societies for Experimental Biology, Bethesda, Md.
- Astrin, S. M. 1978. Endogenous viral genes of the white leghorn chicken: common site of residence and sites associated with specific phenotypes of viral gene expression. *Proc. Natl. Acad. Sci. U.S.A.* 75:5941-5945.
- Astrin, S. M., H. L. Robinson, L. B. Crittenden, E. G. Buss, J. Wyban, and W. S. Hayward. 1979. Ten genetic loci in the chicken that contain structural genes for endogenous avian leukosis viruses. *Cold Spring Harbor Symp. Quant. Biol.* 44:1105-1109.
- Callahan, R., R. E. Benveniste, M. M. Lieber, and G. J. Todaro. 1974. Nucleic acid homology of murine type-C viral genes. *J. Virol.* 14:1394-1403.
- Canaani, E., and S. A. Aaronson. 1979. Restriction enzyme analysis of mouse cellular type C viral DNA: emergence of new viral sequences in spontaneous AKR/J lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* 76:1677-1681.
- Chattopadhyay, S. K., M. R. Lander, E. Rands, and D. R. Lowy. 1980. The structure of endogenous murine leukemia virus DNA in mouse genomes. *Proc. Natl. Acad. Sci. U.S.A.* 77:5774-5778.
- Chattopadhyay, S. K., M. R. Lander, and W. P. Rowe. 1980. Close similarity between endogenous ecotropic virus of *Mus musculus molossinus* and AKR virus. *J. Virol.* 36:499-505.
- Copeland, N. G., A. D. Zelenetz, and G. M. Cooper. 1979. Transformation of NIH/3T3 mouse cells by DNA of Rous sarcoma virus. *Cell* 17:993-1002.
- Dhar, R., W. L. McClements, L. W. Enquist, and G. F. Vande Woude. 1980. Nucleotide sequences of integrated Moloney sarcoma provirus long terminal repeats and their host viral junctions. *Proc. Natl. Acad. Sci. U.S.A.* 77:3937-3941.
- Farabaugh, P. J., and G. R. Fink. 1980. Insertion of the eukaryotic transposable element *Ty1* creates a 5-base pair duplication. *Nature (London)* 286:352-356.
- Flavell, A. J., and D. Ish-Horowitz. 1981. Extrachromosomal circular copies of the eukaryotic transposable element *copia* in cultured *Drosophila* cells. *Nature (London)* 292:591-595.
- Gorer, P. A., and Z. B. Mikulska. 1959. Some further data on the *H-2* system of antigens. *Proc. R. Soc. London Ser. B* 151:57-69.
- Hartley, J. W., and W. P. Rowe. 1976. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. *J. Virol.* 19:19-25.
- Hayward, W. S., B. G. Neel, and S. M. Astrin. 1981. Activation of a cellular *onc* gene by promoter insertion in ALV-induced lymphoid leukemia. *Nature (London)* 290:475-480.
- Hiai, H., P. Morrissey, R. Khirya, and R. S. Schwartz. 1977. Selective expression of xenotropic virus in congenic HRS/J (hairless) mice. *Nature (London)* 270:247-249.
- Hishinuma, F., P. J. DeBona, S. Astrin, A. M. Skalka. 1981. Nucleotide sequence of acceptor site and termini of integrated avian endogenous provirus *ev-1*: integration creates a 6 bp repeat of host DNA. *Cell* 23:155-164.
- Ihle, J. N., and D. R. Joseph. 1978. Serological and virological analysis of NIH (NIH $\times$ AKR) mice: evidence for three AKR murine leukemia virus loci. *Virology* 87:287-297.
- Ihle, J. N., D. R. Joseph, and J. J. Domotor. 1979. Genetic linkage of C3H/HeJ and BALB/c endogenous ecotropic C-type viruses to phosphoglucomutase-1 on chromosome 5. *Science* 204:71-73.
- Jenkins, N. A., N. G. Copeland, B. A. Taylor, and B. K. Lee. 1981. Dilute (*d*) coat colour mutation of DBA/2J mice is associated with the site of integration of an ecotropic MuLV genome. *Nature (London)* 293:370-374.
- Kozak, C. A., and W. P. Rowe. 1979. Genetic mapping of the ecotropic murine leukemia virus-inducing locus of the BALB/c mouse to chromosome 5. *Science* 204:69-71.
- Kozak, C. A., and W. P. Rowe. 1980. Genetic mapping of the ecotropic virus-inducing locus *Akv-2* of the AKR mouse. *J. Exp. Med.* 152:1419-1423.
- Kozak, C. A., and W. P. Rowe. 1980. Chromosomal mapping of ecotropic and xenotropic leukemia virus-inducing loci in the mouse, p. 171-176. In B. Fields and R. Jaenisch (ed.), *Animal virus genetics*. Academic Press Inc., New York.
- Lowy, D. R., S. K. Chattopadhyay, N. M. Teich, W. P. Rowe, and A. S. Levine. 1974. AKR murine leukemia virus genome: frequency of sequences in DNA in high, low, and non-virus-yielding mouse strains. *Proc. Natl. Acad. Sci. U.S.A.* 71:3555-3559.
- Majors, J. E., and H. E. Varmus. 1981. Nucleotide sequence at host-proviral junctions for mouse mammary tumor virus. *Nature (London)* 289:253-258.
- Meier, H., D. D. Myers, and R. J. Huebner. 1969. Genetic control by the *hr*-locus of susceptibility and resistance to leukemia. *Proc. Natl. Acad. Sci. U.S.A.* 3:759-766.
- Quint, W., W. Quax, H. van der Putten, and A. Berns. 1981. Characterization of AKR murine leukemia virus sequences in AKR mouse substrains and structure of integrated recombinant genomes in tumor tissues. *J. Virol.* 39:1-10.
- Rands, E., D. R. Lowy, M. R. Lander, and S. K. Chattopadhyay. 1981. Restriction endonuclease mapping of ecotropic murine leukemia viral DNAs: size and sequence heterogeneity of the long terminal repeat. *Virology* 108:445-452.
- Rasheed, S., M. B. Gardner, and E. Chan. 1976. Ampho-

- tropic host range of naturally occurring wild mouse leukemia viruses. *J. Virol* 19:13-18.
29. Rigby, P. W., M. Dieckmann, C. Rhodes, and P. J. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237-251.
 30. Roderick, T. 1978. Further information on subline differences, p. 485-494. *In* H. C. Morse (ed.), *Origins of inbred mice*. Academic Press Inc., New York.
 31. Rowe, W. P., J. W. Hartley, and T. Bremner. 1972. Genetic mapping of a murine leukemia virus-inducing locus of AKR mice. *Science* 178:860-862.
 32. Rowe, W. P., and C. A. Kozak. 1980. Germline reinfections of AKR murine leukemia virus genomes in *Akv-1* congenic mice. *Proc. Natl. Acad. Sci. U.S.A.* 77:4871-4874.
 33. Shimotohno, K., S. Mizutani, and H. M. Temin. 1980. Sequence of retrovirus provirus resembles that of bacterial transposable elements. *Nature (London)* 285:550-554.
 34. Staats, J. 1980. Standardized nomenclature for inbred strains of mice: seventh listing. *Cancer Res.* 40:2083-2128.
 35. Steffen, D., S. Bird, W. P. Rowe, and R. A. Weinberg. 1979. Identification of DNA fragments carrying ecotropic proviruses of AKR mice. *Proc. Natl. Acad. Sci. U.S.A.* 76:4554-4558.
 36. Steffen, D. L., S. Bird, and R. A. Weinberg. 1980. Evidence for the Asiatic origin of endogenous AKR-type murine leukemia proviruses. *J. Virol.* 35:824-835.
 37. Steffen, D. L., B. A. Taylor, and R. A. Weinberg. 1982. Continuing germ line integration of AKV proviruses during the breeding of AKR mice and derivative recombinant inbred strains. *J. Virol.* 42:165-175.
 38. Stephenson, J. R., and S. A. Aaronson. 1976. Induction of an endogenous B-tropic type C RNA virus from SWR/J mouse embryo cells in tissue culture. *Virology* 70:352-359.
 39. Stephenson, J. R., R. K. Reynold, R. S. Tronick, and S. A. Aaronson. 1975. Distribution of three classes of endogenous type-C RNA viruses among inbred strains of mice. *Virology* 67:404-414.
 40. Traina, V. L., B. A. Taylor, and J. C. Cohen. 1981. Genetic mapping of endogenous mouse mammary tumor viruses: locus characterization, segregation, and chromosomal distribution. *J. Virol.* 40:735-744.
 41. Van Beveren, C., J. G. Goddard, A. Berns, and I. M. Verma. 1980. Structure of Moloney murine leukemia viral DNA: nucleotide sequence of the 5' long terminal repeat and adjacent cellular sequences. *Proc. Natl. Acad. Sci. U.S.A.* 77:3307-3311.
 42. Varmus, H. E., N. Quintrell, and S. Ortiz. 1981. Retroviruses as mutagens: insertion and excision of a nontransforming provirus alter expression of a resident transforming provirus. *Cell* 25:23-36.