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Tissue-Specific Expression of the Newly Acquired Ecotropic *Emv*-18 Provirus in *Fv-2* Congenic Mice

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Expression of endogenous retroviral sequences in *Fv-2* congenic mouse strains was examined by Northern blot analysis. Endogenous ecotropic virus transcripts were observed in total spleen RNA of B6.S (*Fv-2*^{ss}) mice. Endogenous ecotropic transcripts were not detected in spleen RNA of C57BL/6, the *Fv-2*^{rr} congenic partner of B6.S, nor in spleens of the C57BL/10 (*Fv-2*^{rr}) and B10.C (*Fv-2*^{ss}) congenic strains. Mendelian segregation analysis revealed that only backcross mice segregating the newly acquired *Fv-2*-linked endogenous ecotropic provirus had endogenous ecotropic transcripts in spleen RNA. Examination of different tissues of B6.S mice showed that *Emv*-18 transcription was highest in spleen and bone marrow, tissues in which *Fv-2* has been shown to function. These results support the conclusion that chromosomal location is an important factor controlling *Emv*-18 expression in B6.S mice. We also report the presence in the spleen of a novel xenotropic virus transcript detectable only in B6.S mice.

Chromosomal location appears to be a major factor in the control of expression of endogenous retroviruses. For example, Jaenisch and his colleagues have generated mouse strains in which a Moloney murine leukemia virus (M-MuLV) provirus is transmitted in the germline as an endogenous locus, each strain carrying an M-MuLV genome inserted into a unique chromosomal location (14, 15). The proviruses of different strains have been shown to be expressed at different times during embryogenesis or the postnatal life of these mice (15). This stage-specific virus expression occurs in all mice of a given strain, suggesting that virus activation occurs as a consequence of developmentally regulated activation of adjacent cellular genes (15).

The C57BL/6 strain (B6) of genotype *Fv-2*^{rr} has one endogenous ecotropic provirus, designated *Emv*-2 (17), located on chromosome 8. Recently, we have reported the identification of an endogenous ecotropic provirus which is closely linked to the *Fv-2* locus (24), on chromosome 9 in the B6 *Fv-2*^{ss} congenic partner strain, B6.S (1). Thus, B6.S harbors both *Emv*-2 and the new *Fv-2*-linked ecotropic provirus, designated *Emv*-18. Two observations suggest that the *Emv*-18 provirus is the result of a reinsertion of the *Emv*-2 provirus during the construction of the B6.S strain. One is that the donor of the *Fv-2*^{s} allele in B6.S mice, the SIM strain, has no endogenous ecotropic virus loci detectable by Southern blot analysis, using an ecotropic endogenous virus envelope probe (24). The other is that, at the level of resolution of restriction enzyme mapping, no differences are observed in the structure of the *Emv*-2 and *Emv*-18 proviruses (24). Thus, these congenic strains represent a system in which the same endogenous viral genome is integrated at two distinct chromosomal locations.

The close proximity of the *Emv*-18 and the *Fv-2* loci provided an opportunity to examine possible effects of known tissue-specific loci on provirus expression. *Fv-2* is the major host locus governing susceptibility to erythroleukemia induction by Friend spleen focus-forming virus (SFFV) (20). In addition, the *Fv-2* locus has been shown to regulate specifically DNA synthesis in an erythroid progenitor cell, BFU-E (burst-forming unit, erythroid), in the spleen and bone marrow of normal uninfected mice (30).

In this paper, we have examined the expression of the *Emv*-18 provirus in B6.S mice. Experiments are presented which show high levels of endogenous ecotropic virus sequences in the spleen of B6.S mice which cannot be detected in the spleen of B6 or other *Fv-2* congenic mice. Genetic crosses were performed which establish that these endogenous ecotropic sequences in B6.S mice are transcribed from *Emv*-18. We also show that the *Emv*-18 provirus is expressed to highest levels in hemopoietic tissues.

It has previously been reported that B6.S mouse spleen contains RNA sequences which hybridize with a cDNA probe derived from Friend SFFV (21). In this paper, transcripts in B6.S spleen are identified which hybridize to DNA probes derived from molecular clones of the SFFV *gag* and SFFV envelope regions. The sequences detected with the SFFV-envelope probe are novel xenotropic virus-related transcripts not seen in spleen RNA of B6 mice or other *Fv-2* congenic mouse strains.

MATERIALS AND METHODS

Mice. Two pairs of mouse strains congenic at the *Fv-2* locus were used. C57BL/6J (B6) mice of genotype *Fv-2*^{rr} are congenic with the B6.S strain of genotype *Fv-2*^{ss} (1). The donor of the *Fv-2*^{s} allele was the SIM strain. These mice are maintained at the Ontario Cancer Institute. C57BL/10Sn (B10) mice of genotype *Fv-2*^{rr} are congenic with B10.C of genotype *Fv-2*^{ss} (27). The donor of the *Fv-2*^{s} allele in B10.C was the BALB/c strain. These mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Genetic crosses were done at the Ontario Cancer Institute. RNA and DNA of backcross generation mice were extracted when the mice were between 5 and 7 weeks of age.

RNA extraction and Northern blotting. RNA was isolated from whole spleens by a modification of the method of

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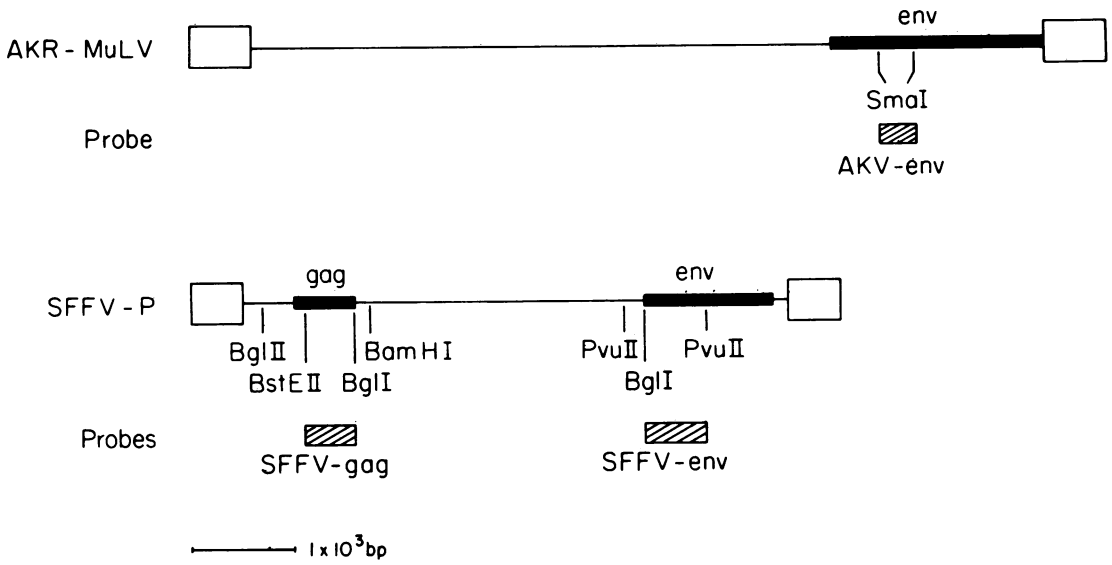

FIG. 1. Restriction endonuclease maps of integrated AKR-MuLV (4) and $SFFV_p$ (34) DNA. Open boxes represent long terminal repeats; shaded boxes represent viral protein-coding regions. Probe fragments are indicated by hatched boxes below the restriction map.

Chirgwin et al. (5). Whole spleens were placed in 20 ml of guanidine thiocyanate stock solution (4 M guanidine thiocyanate, 0.1 M Tris-hydrochloride, pH 7.5, 0.1 M 2-mercaptoethanol, 0.5% Sarkosyl) and immediately homogenized for 20 s, using a Polytron tissue homogenizer at full speed. This homogenate was centrifuged at approximately 2,500 rpm in a Beckman TJ-6 bench-top centrifuge for 15 min to remove air bubbles and then layered on a 15-ml cushion of cesium chloride solution (5.7 M $CsCl_2$, 0.1 M EDTA, pH 7.0) in a Beckman SW27 polyallomer centrifuge tube. Tubes were centrifuged in a Beckman SW27 rotor at 25,000 rpm and 20°C for 24 h to pellet the RNA. The supernatant was removed, and the RNA pellets were resuspended in 2 ml of 10 mM Tris-hydrochloride (pH 7.5)–5 mM EDTA (pH 7.0)–1% sodium dodecyl sulfate. This was extracted twice with water-saturated phenol, once with a 1:1 mixture of phenol and chloroform and once with chloroform. RNA was precipitated from the final aqueous phase by the addition of 0.1 volume of 3 M sodium acetate, pH 5.5, and 2.5 volumes of absolute ethanol and incubation overnight at −20°C. RNA was pelleted by centrifugation at 12,000 × *g* and 4°C for 20 min, washed once with 70% ethanol, dried in vacuum, and resuspended in 0.2 ml of water. RNA was stored frozen at −20°C. Total RNA (10 μg) was denatured with glyoxal, electrophoresed in agarose gels, and blotted onto nitrocellulose (Schleicher & Schuell) or Zetabind (AMF Corp.) filters according to the method of Thomas (31). Filters were hybridized with 2 × 10^6 cpm of nick-translated probe (25) per ml of hybridization solution (50% formamide, 0% dextran sulfate for nitrocellulose, 10% dextran sulfate for Zetabind) for 20 to 24 h at 42°C. Filters were then washed, with the highest stringency being 50°C, 0.1× SSC (1× SSC = 0.15 M NaCl plus 0.015 M sodium citrate). Filters were air dried and exposed to Kodak XAR-5 film with one DuPont Cronex Lightning-Plus intensifying screen at −70°C.

DNA extraction and Southern blotting. DNA was extracted by a modification of the technique of Gross-Bellard et al. (12). Whole fresh or frozen kidney was homogenized in 2 ml of buffer A (10 mM Tris-hydrochloride, pH 7.5 to 8.0, 10 mM EDTA, 10 mM NaCl) in a 5-ml Dounce homogenizer to yield a single cell suspension, which was then added to 2 ml of DNA buffer B (same as buffer A with 2% sodium dodecyl sulfate) to lyse the cells. Proteinase K was added to a final concentration of 100 μg/ml and incubated at 37°C for 2 h. This was then extracted twice with phenol, once with a 1:1 mixture of phenol and chloroform, and once with chloroform. The DNA was then precipitated by addition of 0.1 volume of 5 M NaCl and 2 volumes of absolute ethanol and spooled with a sealed Pasteur pipette. The DNA was redissolved in 2 ml of TE buffer (10 mM Tris-hydrochloride, 1 mM EDTA) and stored at 4°C.

DNA (20 μg) was digested with restriction enzymes for 4 h in conditions specified by the suppliers (Bethesda Research Laboratories; Boehringer Mannheim Corp.) and electrophoresed on 0.8-cm-thick 0.8% agarose gels in TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA) at 20 V overnight. DNA in the gels was acid depurinated and cleaved by the method of Wahl et al. (33) before blotting onto nitrocellulose or Zetabind membranes as described by Southern (28). Filters were hybridized and washed as described above for Northern blots.

Probes. The AKV-env probe was purified from plasmid p400eco containing a 400-base-pair (bp) ecotropic virus-specific fragment of AKR-MuLV DNA (4; kindly provided by D. Lowy, National Institutes of Health, Bethesda, Md.). This probe is derived from a *Sma*I fragment from within the envelope protein-coding region of AKV (Fig. 1).

The SFFV-gag probe was purified from plasmid pSFFVgag containing a 1,045-bp *Bgl*II-*Bam*HI fragment from the molecularly cloned Friend SFFV (34). The probe used was a 473-bp *Bst*EII-*Bgl*II fragment contained within the *gag* protein-coding sequences (Fig. 1).

The SFFV-env probe was purified from plasmid pYY320 containing an 810-bp *Pvu*II fragment from the molecularly cloned SFFV provirus. The probe used was a 598-bp *Bgl*I-*Pvu*II fragment contained within the envelope protein-coding sequence (Fig. 1).

The tubulin probe was purified from plasmid pT2 (32) containing a 1.7-kilobase-pair (kb) cDNA copy of the chicken β-tubulin message. The probe used was a 1.2-kb *Pst*I fragment containing only tubulin cDNA sequences.

All probes used were DNA fragments isolated from the

vector and other viral sequences by preparative agarose gel electrophoresis, followed by electroelution from excised gel segments and phenol extraction.

RESULTS

Endogenous ecotropic virus expression in *Fv-2* congenic mouse spleens. To determine whether the *Fv-2*-linked provirus *Emv*-18 is expressed in B6.S mice, transcription of endogenous ecotropic virus loci in spleens of B6 and B6.S mice was examined by Northern blot analysis, using a cloned DNA probe specific for endogenous ecotropic viruses. This probe, designated AKV-env, is derived from a 400-bp *Sma*I fragment which is contained within the viral envelope protein-coding region of the AKR ecotropic provirus (Fig. 1) and has been shown to hybridize specifically with endogenous ecotropic viral sequences (4). Figure 2 shows that high levels of endogenous ecotropic virus sequences were present in cellular RNA from B6.S mouse spleen, but that these sequences could not be detected in the RNA from B6 mouse spleen.

To rule out the possibility that the *Fv-2*^{s} allele is activating in *trans* transcription from the *Emv*-2 provirus present in all C57BL mice (17), total spleen RNA from another pair of *Fv-2* congenic mouse strains was examined with the endogenous ecotropic virus-specific probe. B10 and B10.C are congenic strains made by substitution of the BALB/c *H-7* histocompatibility locus onto the C57BL/10Sn (B10) genetic background (27). As *H-7* and *Fv-2* are closely linked, B10 and B10.C are congenic at both loci, B10.C being homozygous for the *Fv-2*^{s} allele from BALB/c. As shown previously, B10 and B10.C have only the C57BL provirus *Emv*-2 (24). Ecotropic viral RNA sequences were not observed in B10 or B10.C mouse spleens (Fig. 2). These results demonstrate the association of endogenous ecotropic virus transcripts with the new *Emv*-18 provirus and not with the *Fv-2*^{s} allele. Hybridization of the Northern blot shown in Fig. 2 with a tubulin probe indicates that approximately equal amounts of RNA were present in each lane (data not shown).

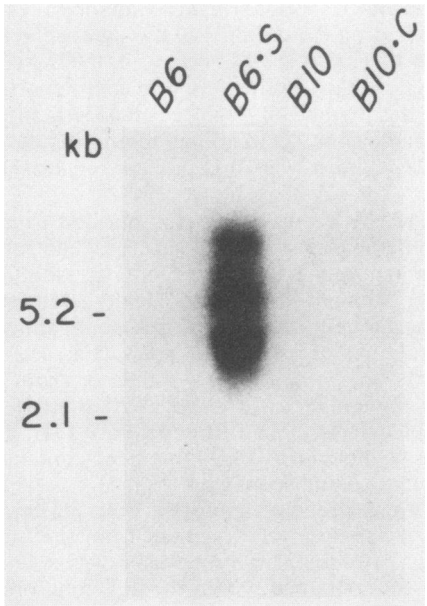

FIG. 2. Endogenous ecotropic virus expression in *Fv-2* congenic mouse spleens. Total RNA was denatured with glyoxal, electrophoresed in an agarose gel, blotted onto nitrocellulose paper, and hybridized with AKV-env probe as described in the text. Ribosomal 28S and 18S RNAs, corresponding to approximately 5.2 and 2.1 kb, respectively, were used as molecular weight standards.

TABLE 1. Linkage[a] of *Emv*-18 with presence of ecotropic retroviral transcripts in (B6 × B6.S)F1 × B6 backcross mice

Possible phenotype	Presence of:		No. of animals observed
	Emv-18[b]	Ecotropic retroviral RNA[c]	
Nonrecombinant	+	+	17
	−	−	7
Recombinant	+	−	0
	−	+	0

[a] $\chi^2 = 22.04$; $P < 0.001$.
[b] Mice were tested for *Emv*-18 by Southern blot analysis as shown in Fig. 3A.
[c] Mice were tested for ecotropic retroviral transcripts by Northern blot analysis as shown in Fig. 3B.

Endogenous ecotropic RNA in B6.S mice is transcribed from the *Emv*-18 provirus. To determine more directly whether the endogenous ecotropic RNA sequences in B6.S mice are transcribed from the *Emv*-18 provirus, genetic linkage of *Emv*-18 with the presence of ecotropic viral RNA in the spleen was tested. B6 and B6.S mice were crossed to give F1 mice heterozygous for *Emv*-18. These F1 generation mice were then backcrossed to B6 mice, which are *Emv*-18 negative. The resulting backcross generation, N1, was expected to include homozygous *Emv*-18-negative mice and heterozygous mice with one copy of the *Emv*-18 provirus. If the viral RNA expression observed in B6.S mice is derived from transcription from the *Emv*-18 provirus, complete cosegregation of the new provirus with ecotropic viral RNA sequences would be expected. Figure 3A shows a Southern blot of kidney DNA digested with *Hin*dIII and Fig. 3B shows a Northern blot of the corresponding total spleen RNA from the same sample of N1 mice, both hybridized with the ecotropic virus-specific probe. It is clear from Fig. 3 that ecotropic virus-specific RNA was observed only in those mice which also have the *Emv*-18 provirus. Table 1 summarizes results of segregation analysis of 24 N1 mice. Of 24 mice, 17 carried the *Emv*-18 provirus, whereas 7 did not. In every mouse containing the *Emv*-18 locus, ecotropic virus-related RNA sequences were detected in the spleen, whereas in those mice that did not harbor *Emv*-18 these sequences could not be detected. These observations suggest that this *Fv-2*-linked provirus is expressed in B6.S mice.

Tissue-specific expression of *Emv*-18. The *Fv-2* locus is known to play a role in regulating the cell cycle status of erythroid progenitor cells in marrow and spleen (30). It was therefore of interest to determine whether *Emv*-18 was preferentially expressed in hemopoietic tissues. Expression of *Emv*-18 in various tissues was examined by hybridization of Northern blots of equal amounts of total RNA from spleen, bone marrow, thymus, liver, kidney (Fig. 4), and brain (data not shown) with the ecotropic specific probe. Highest levels of expression were detected in the spleen, lower levels were detected in bone marrow and kidney, trace amounts were detected in thymus and liver, and none was detected in brain. No ecotropic virus sequences could be detected in the same tissues from a B6 mouse.

SFFV-derived probes detect endogenous virus sequences in B6 and B6.S mouse spleen RNA. Sequences homologous to

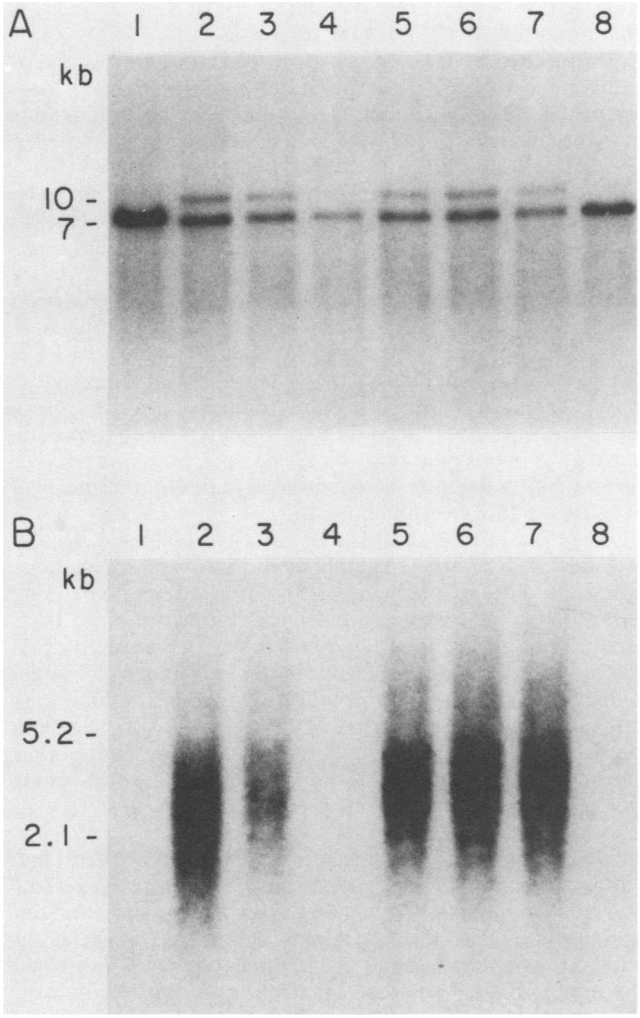

FIG. 3. Cosegregation of *Emv*-18 provirus with endogenous ecotropic RNA sequences in the spleen in (B6 × B6.S)F1 × B6 backcross mice. High-molecular-weight DNA from eight individual backcross generation mice was digested with *Hin*dIII, electrophoresed, and blot hybridized as described in the text. Sizes of DNA fragments were calculated from *Hin*dIII-digested λ DNA size standards. Total RNA from the same eight individual generation backcross mice was analyzed as in the legend to Fig. 2. Both DNA and RNA filters were hybridized with the AKV-env probe as described in the text. (A) DNA from eight individual backcross mice. Upon digestion with *Hin*dIII, the *Emv*-18 provirus generates the 10-kb junction fragment and the *Emv*-2 provirus generates the 7-kb junction fragment. (B) RNA from the same eight individual backcross mice.

Friend SFFV have previously been detected with a cDNA probe derived from SFFV in the spleen and bone marrow of B6.S mice, but not B6 mice (21). Using molecular clones derived from different parts of the SFFV genome, we prepared defined probes with which to examine expression of endogenous ecotropic *gag* and xenotropic-related envelope sequences in total spleen RNA of B6 and B6.S mice. Three cloned probes were utilized in these experiments (see above) (Fig. 5). The AKV-env probe is a 400-bp *Sma*I fragment derived from the envelope region of the cloned AKR endogenous ecotropic provirus (4). The SFFV-gag probe is derived from a 473-bp *Bst*EII-*Bgl*I fragment contained within the *gag* protein-coding region and has been shown to be highly homologous to the *gag* region of the endogenous ecotropic AKR provirus (6, 7). The SFFV-env probe is a 598-bp *Bgl*I-*Pvu*II fragment contained within the envelope protein-coding sequence. This region of SFFV has been shown to be derived from an endogenous mink cell focus-forming (MCF) virus (18) and to hybridize specifically with MCF and xenotropic virus sequences (3).

Hybridization with the AKV-env probe (Fig. 5A) shows the position of the *Emv*-18 transcripts for comparison with transcripts detected with the other probes.

Because the SFFV-gag probe is highly homologous to the corresponding region of AKR virus, transcripts from *Emv*-18 should also be detected with this probe. In Fig. 5B it can be seen that indeed the SFFV-gag probe detects high levels of transcripts in B6.S spleen that are identical in size to the *Emv*-18 transcripts shown in Fig. 5A. The SFFV-gag probe also detects a transcript in B6 spleen. As SFFV *gag* and xenotropic *gag* regions are sufficiently related to form a heteroduplex (2), this transcript in B6 may be a xenotropic transcript which is cross-hybridizing with the SFFV-gag probe.

Hybridization with the SFFV-envelope probe shown in Fig. 5C revealed a common RNA transcript in spleen RNA from both B6 and B6.S mice. In addition, a novel transcript in B6.S spleen RNA was observed with this probe. As this probe is specific for MCF and xenotropic virus envelope sequences (3), this observation suggests that xenotropic or MCF virus expression is specifically elevated in B6.S mice.

DISCUSSION

Northern analysis of total spleen RNA from *Fv-2* congenic strains B6, B6.S, B10, and B10.C showed that endogenous ecotropic RNA is detectable only in B6.S mice. The absence of endogenous ecotropic RNA sequences in the spleen of B10.C mice, also of genotype $Fv\text{-}2^{ss}$, indicated that the $Fv\text{-}2^{s}$ allele is not activating in *trans* transcription from the *Emv*-2 provirus. The most direct demonstration that the *Emv*-18

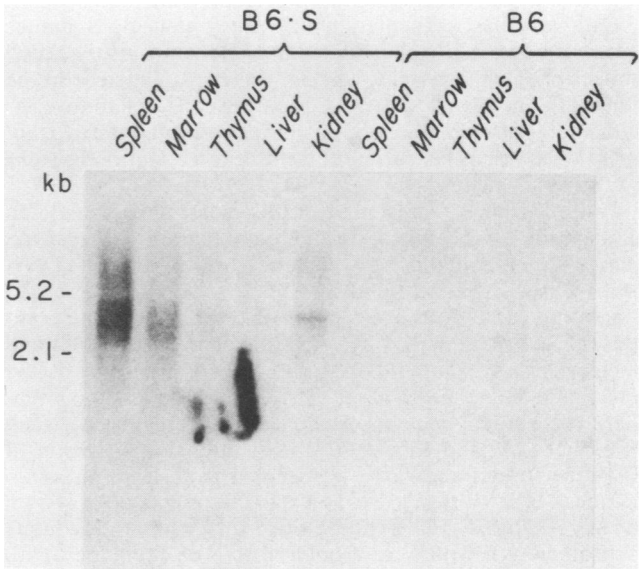

FIG. 4. Tissue-specific expression of *Emv*-18. Total RNA from various tissues of B6 and B6.S mice was analyzed as in the legend to Fig. 2. Blot hybridized with the AKV-env probe and exposed for 1 week.

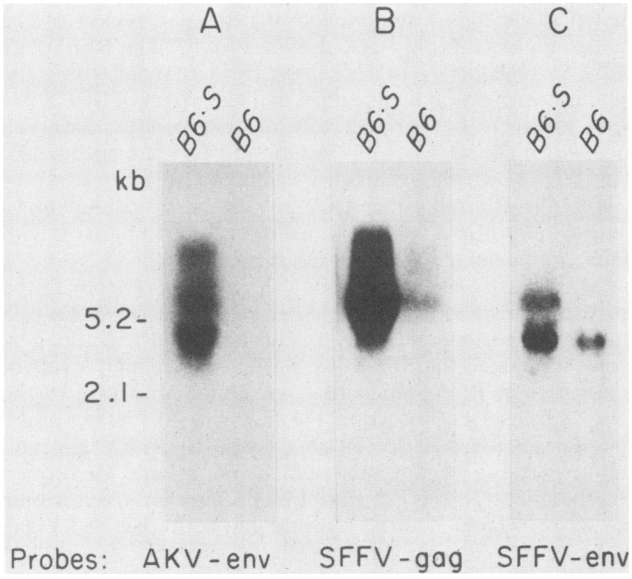

FIG. 5. SFFV-derived probe detection of endogenous virus sequences in B6 and B6.S mouse spleen RNA. Total RNA from B6 and B6.S mouse spleen was blotted as described in the text. Triplicate blots, electrophoresed in the same gel, are shown. (A) Blot hybridized with the AKV-env probe and exposed for 1 week. (B) Blot hybridized with the SFFV-gag probe and exposed for 1 week. (C) Blot hybridized with the SFFV-env probe and exposed overnight.

provirus is transcribed in B6.S mice derives from experiments with (B6 × B6.S)F1 × B6 backcross mice. These experiments demonstrated that ecotropic transcripts are only observed in the spleens of mice which have *Emv*-18 in their genome and not in mice lacking *Emv*-18. An examination of various tissues of B6.S mice revealed that *Emv*-18 transcripts are present at highest levels in spleen and bone marrow, in trace amounts in thymus, kidney, and liver, and not at all in brain. Interestingly, the *Fv-2* gene has also been shown to be functioning in the spleen and bone marrow (30).

Data are also presented which show that B6.S mouse spleen contains a novel endogenous xenotropic virus-related transcript. This transcript was not seen in B6 mice or in the other *Fv-2* congenic strains, B10 and B10.C. Therefore, as with the ecotropic transcripts, the xenotropic-related transcript does not appear to be the result of activation in *trans* by the $Fv\text{-}2^s$ allele.

The experiments presented in this paper also provide an explanation of previous results demonstrating that B6.S mouse spleen contains RNA which hybridizes with a cDNA probe derived from Friend SFFV (21). This cDNA probe was homologous to those regions of the SFFV genome not present in Friend MuLV RNA. Molecular cloning (34) and nucleotide sequencing (6) of SFFV, and comparison with the nucleotide sequence of other retroviruses, have shown that there are several regions of sequence divergence between Friend MuLV and SFFV (7, 18). The envelope region of SFFV has been shown to be derived from a (xenotropic-related) MCF virus (7, 18). The *gag* region has been shown to have almost 100% homology with the endogenous ecotropic AKR-MuLV and only 70 to 80% homology with M-MuLV, which is thought to be closely related to Friend MuLV (7). Thus, the SFFV-specific cDNA probe used in the previous experiments likely contained both SFFV *env* and SFFV *gag* sequences. The results presented in this study show that B6.S mice have elevated levels of RNA sequences which hybridize to both of those probes.

Several factors have been shown to control expression of endogenous viruses. These include the chromosomal location of the proviral genome, the structure of the endogenous viral genome itself, host genes, and recombination between viral genomes (8, 26).

The results presented in this paper are consistent with the hypothesis that the chromosomal location of the *Emv*-18 provirus is a major factor controlling its expression. As described previously, there is evidence that the *Emv*-18 provirus in B6.S mice is the result of a germ line reinsertion from the *Emv*-2 provirus (24). At its location on chromosome 8, the *Emv*-2 provirus is expressed, but at a very low level (22). We have shown in this paper that the *Emv*-18 provirus in B6.S mice, at its new location on chromosome 9 closely linked with the *Fv-2* locus, is expressed in spleen and bone marrow. It is possible that the tissue-specific expression of *Emv*-18 may reflect the transcriptional activation of the *Fv-2* locus to which *Emv*-18 is linked.

Our results also establish that the *Fv-2* congenic B6 and B6.S mice differentially regulate transcription of endogenous xenotropic virus-related sequences. The factors controlling endogenous xenotropic or MCF virus transcription in B6.S remain to be determined. One possibility is that high levels of *Emv*-18 transcription result in the formation of novel viruses which are recombinants between endogenous ecotropic and xenotropic or MCF genomes. It has been suggested that such recombination events can occur in other mouse strains, such as AKR and HRS, in which endogenous ecotropic virus expression is high and high levels of xenotropic and MCF virus are observed (11).

Endogenous virus expression can result in germ line reintegration and the formation of new endogenous virus loci. Such reintegration events can be mutagenic. For example, the dilute, *d*, and agouti yellow, A^y, coat color mutations are both associated with an endogenous ecotropic provirus (9, 16). By contrast, the allelic difference at the *Fv-2* locus in B6.S mice does not appear to be the result of the insertion of the *Emv*-18 provirus, as *Fv-2* and *Emv*-18 loci have been shown to segregate in B6 × B6.S crosses (24). Other evidence indicating that ecotropic endogenous virus integration does not play a role in the *Fv-2* phenotype is that $Fv\text{-}2^s$ alleles of other inbred strains, e.g., SIM and BALB/c, are not linked with endogenous ecotropic proviruses (24). In this paper, we have shown that neither the endogenous ecotropic virus nor the xenotropic virus-related transcripts are present in the spleen of the $Fv\text{-}2^{ss}$ B10.C strain mouse. Thus, it is unlikely that the *Emv*-18 transcripts or the novel xenotropic virus-related RNA sequences have a role in the Friend virus susceptibility phenotype.

Sublines of the high-virus AKR strain have been shown to be highly polymorphic in endogenous ecotropic virus content (13, 29). Although endogenous ecotropic virus polymorphism is not common in low-virus strains such as C57BL, there are now several reports of ecotropic virus germ line reintegrations in congenic strains of C57BL (10, 19, 23, 24). The reintegration and high-level expression of *Emv*-2 at its new chromosomal location at *Emv*-18 provides a further example of the generation of genetic polymorphism between inbred and closely related strains of mice due to the presence of endogenous retrovirus proviruses in the mouse germ line. Thus, acquisition of novel retrovirus proviruses in the mouse germ line may have important phenotypic consequences for the host, depending on the site of integration and regulation of viral gene expression in different tissues.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Axelrad, A. A., M. Ware, and H. van der Gaag.** 1972. Host cell susceptibility and resistance to murine leukemia viruses and their genetic control, p. 239–254. *In* P. Emmelot and P. Bentvelzen (ed.), RNA viruses and host genome in oncogenesis. North-Holland Publishing Co., Amsterdam.
2. **Bosselman, R. A., L. J. L. D. van Griensven, M. Vogt, and I. M. Verma.** 1980. Genome organization of retroviruses. IX. Analysis of the genome of Friend spleen focus-forming (F-SFFV) and helper murine leukemia viruses by heteroduplex-formation. Virology **102**:234–239.
3. **Chattopadhyay, S. K., M. W. Cloyd, D. L. Linemeyer, M. R. Lander, E. Rands, and D. R. Lowy.** 1982. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. Nature (London) **295**:25–31.
4. **Chattopadhyay, S. K., M. R. Lander, E. Rands, and D. R. Lowy.** 1980. Structure of endogenous murine leukemia virus DNA in mouse genomes. Proc. Natl. Acad. Sci. U.S.A. **77**:5774–5778.
5. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**: 5294–5299.
6. **Clark, S. P., and T. W. Mak.** 1983. Complete nucleotide sequence of an infectious clone of Friend spleen focus-forming provirus: gp55 is an envelope fusion glycoprotein. Proc. Natl. Acad. Sci. U.S.A. **80**:5037–5041.
7. **Clark, S. P., and T. W. Mak.** 1984. Fluidity of a retrovirus genome. J. Virol. **50**:759–765.
8. **Coffin, J.** 1982. Endogenous viruses, p. 1109–1203. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Molecular biology of tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Copeland, N. G., N. A. Jenkins, and B. K. Lee.** 1983. Association of the lethal yellow (A^y) coat color mutation with an ecotropic murine leukemia virus genome. Proc. Natl. Acad. Sci. U.S.A. **80**:247–249.
10. **Datta, S. K., P. Tsichlis, R. S. Schwartz, S. K. Chattopadhyay, and C. J. M. Melief.** 1978. Genetic differences unrelated to *H-2* in *H-2*-congenic mice. Immunogenetics **7**:359–365.
11. **Famulari, N. G.** 1983. Murine leukemia viruses with recombinant *env* genes: a discussion of their role in leukemogenesis. Curr. Top. Microbiol. Immunol. **103**:76–108.
12. **Gross-Bellard, M., P. Oudet, and P. Chambon.** 1973. Isolation of high-molecular-weight DNA from mammalian cells. Eur. J. Biochem. **36**:32–38.
13. **Herr, W., and W. Gilbert.** 1982. Germ-line MuLV reintegrations in AKR/J mice. Nature(London) **296**:865–868.
14. **Jaenisch, R.** 1976. Germ line integration and Mendelian transmission of the exogenous Moloney leukemia virus. Proc. Natl. Acad. Sci. U.S.A. **73**:1260–1264.
15. **Jaenisch, R., D. Jahner, P. Nobis, I. Simon, J. Lohler, K. Harbers, and D. Grotkopp.** 1981. Chromosomal position and activation of retroviral genomes inserted into the germ line of mice. Cell **24**:519–529.
16. **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.
17. **Jenkins, N. A., N. G. Copeland, B. A. Taylor, and B. K. Lee.** 1982. Organization, distribution, and stability of endogenous ecotropic murine leukemia virus DNA sequences in chromosomes of *Mus musculus*. J. Virol. **43**:26–36.
18. **Khan, A. S.** 1984. Nucleotide sequence analysis establishes the role of endogenous murine leukemia virus DNA segments in formation of recombinant mink cell focus-forming murine leukemia viruses. J. Virol. **50**:864–871.
19. **Langdon, W. Y., T. S. Theodore, C. E. Buckler, J. H. Stimpfling, M. A. Martin, and H. C. Morse III.** 1984. Relationship between a retroviral germ line reintegration and a new mutation at the ashen locus in B10.F mice. Virology **133**: 183–190.
20. **Lilly, F.** 1970. *Fv-2*: identification and location of a second gene governing the spleen focus response to Friend leukemia virus in mice. J. Natl. Cancer Inst. **45**:163–169.
21. **Mak, T. W., A. A. Axelrad, and A. Bernstein.** 1979. *Fv-2* locus controls expression of Friend spleen focus-forming virus-specific sequences in normal and infected mice. Proc. Natl. Acad. Sci. U.S.A. **76**:5809–5812.
22. **McCubrey, J., and R. Risser.** 1982. Genetic interactions in the spontaneous production of endogenous murine leukemia virus in low leukemic mouse strains. J. Exp. Med. **156**:337–349.
23. **Moll, B., J. W. Hartley, and W. P. Rowe.** 1979. Induction of B-tropic and N-tropic murine leukemia virus from B10.Br/SgLi mouse embryo cell lines by 5-iodo-2′-deoxyuridine. J. Natl. Cancer Inst. **63**:213–217.
24. **Mowat, M., and A. Bernstein.** 1983. Linkage of the *Fv-2* gene to a newly reinserted ecotropic retrovirus in *Fv-2* congenic mice. J. Virol. **47**:471–477.
25. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
26. **Risser, R., and J. M. Horowitz.** 1983. Endogenous mouse leukemia viruses. Annu. Rev. Genet. **17**:85–121.
27. **Snell, G. D., and H. P. Bunker.** 1965. Histocompatibility genes of mice. V. Five new histocompatibility loci identified by congenic resistant lines on a C57BL/10 background. Transplantation (Baltimore) **3**:235–252.
28. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
29. **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.
30. **Suzuki, S., and A. A. Axelrad.** 1980. *Fv-2* locus controls the proportion of erythropoietic progenitor cells (BFU-E) synthesizing DNA in normal mice. Cell **19**:225–236.
31. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
32. **Valenzuela, P., M. Quiroga, J. Zaldivar, W. J. Rutter, M. W. Kirschner, and D. W. Cleveland.** 1981. Nucleotide and corresponding amino acid sequences encoded by α and β tubulin mRNAs. Nature (London) **289**:650–655.
33. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.
34. **Yamamoto, Y., C. L. Gamble, S. P. Clark, A. Joyner, T. Shibuya, M. E. MacDonald, D. Mager, A. Bernstein, and T. W. Mak.** 1981. Clonal analysis of early and late stages of erythroleukemia induced by molecular clones of integrated spleen focus-forming virus. Proc. Natl. Acad. Sci. U.S.A. **78**:6893–6897.