A Locus That Enhances the Induction of Endogenous Ecotropic Murine Leukemia Viruses Is Distinct from Genome-Length Ecotropic Proviruses

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The segregation of genes that enhance the induction of ecotropic murine leukemia viruses (*In* loci) has been compared with the segregation of ecotropic-specific nucleotide sequences in 12 low-leukemic mouse strains and 18 recombinant inbred strains. Endogenous ecotropic viruses of these strains are of genome length and structurally similar to AKR ecotropic proviruses. Low-leukemic strains of related pedigree contain ecotropic proviruses at common integration sites. Loci previously identified which enhance induction of ecotropic viruses (*In* genes) were correlated with the inheritance of ecotropic viral sequences in inbred low-leukemic mouse strains and in CXB recombinant inbred mouse strains. However, four BXH recombinant inbred strains were observed to possess an *In* gene(s) yet lack the probed envelope gene region for the corresponding endogenous ecotropic virus. These observations indicate that at least one gene that enhances ecotropic virus expression in vitro is encoded by DNA sequences outside ecotropic proviruses or by subgenomic viral sequences.

High-leukemic mouse strains differ from low-leukemic mouse strains in the pattern of spontaneous in vivo expression and in vitro induction of endogenous ecotropic murine leukemia virus (MuLV) (16, 16a, 21, 22). High-leukemic strains express high titers of virus and yield high titers of MuLV after treatment of cultures of virus-nonproducing cells with halogenated pyrimidines (14, 30), whereas low-leukemic strains rarely express high titers of MuLV until late in life (12, 16a, 18) and yield very little ecotropic MuLV after in vitro induction (1, 16, 28, 29). Congenic mouse strains have been produced through the selected breeding of virus-inducing genes of high-leukemic mouse strains onto the genetic background of ecotropic-virus-negative strains. Study of the DNA of these strains has provided correlational evidence for an equivalence between the chromosomal loci conferring in vitro inducibility of ecotropic viruses and ecotropic virus structural genes (2-4, 22, 23, 26). Though limited by the constraints of fine structure mapping in the mouse, this evidence implies that the ecotropic proviruses of high-leukemic mouse strains are necessary and sufficient for the virus phenotype of high-leukemic strains.

The analysis of in vitro induction of ecotropic viruses carried by low-leukemic mice has demonstrated a more complex series of interactions leading to virus expression. Studies in this labo-

ratory have identified loci that in combination enhance ecotropic virus induction in cells of (BALB/c × C57BL/6) F_1 hybrid mice (15, 16, 16a). The number of virus-producing cells observed after treatment of cells with 5-iododeoxyuridine (IUdR) is 10- to 50-fold higher in cultures derived from hybrid mice compared with either parental strain. Genetic studies demonstrated that a dominant gene of BALB/c, termed *Inc-1*, and a dominant gene of C57BL/6 (B6), termed *Inb-1*, are responsible for enhanced virus induction. Genetic mapping experiments localized *Inc-1* to chromosome 5, closely linked to the chromosomal location for the BALB/c virus-inducing gene, *Cv-1*. *Inb-1* was mapped to chromosome 8, probably near the chromosomal position of the C57BL/6 virus-inducing gene, *Bv-1* (6, 7, 10, 16). Loci allelic to *Inc-1* of BALB/c mice were found in C3H/He, A/J, and SEC/J mice, whereas a locus allelic to the *Inb-1* gene of C57BL/6 mice was found in C57BR/cdJ mice (15). Ecotropic-virus-negative strains lack *Inc-1* or *Inb-1*, and crosses involving these strains have never led to progeny exhibiting enhanced ecotropic virus expression following in vitro induction (15, 16).

Using a molecular probe specific for endogenous ecotropic viruses, we have studied the relationship between induction loci and ecotropic virus structural genes in low-leukemic mouse

strains. Our studies indicate that at least one *In* locus is distinct from the ecotropic envelope gene.

MATERIALS AND METHODS

Mice. A/J, BALB/cByJ, CBA/J, C57BL/6ByJ, C57BL/10J, C57BR/cdJ, C3H/HeJ, DBA/2J, HRS/J, NZB/BinJ, SEA/GnJ, SEC/1ReJ, and CXB recombinant inbred mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. The BXH recombinant inbred strains were a generous gift of Benjamin Taylor of the Jackson Laboratory. Mice were housed and bred at the McArdle Laboratory under standard conditions.

Cells and induction assay. Cells were prepared from mouse embryos and were analyzed for inducibility as has been previously described (16). Briefly, cultures were prepared from 14- to 18-day embryos, and when confluent, cells were transferred to a series of 60-mm petri dishes at 2.5×10^5 cells per plate. The following day, cells were treated with IUdR (Sigma Chemical Co., St. Louis, Mo.) in medium (20 μ g/ml), and they were overlaid with 10^5 SC-1 cells per 60-mm plate 2 days later. Cells were cocultivated for 11 days and then screened for ecotropic virus expression by the UV-XC plaque assay (9, 24).

DNA extraction, restriction enzyme digestion, and agarose gel electrophoresis. DNA used in these studies was prepared from tissue culture cells and spleens from splenectomized animals. Spleens were surgically removed from young mice and immediately frozen in liquid nitrogen. The frozen spleens were ground to a fine powder, and the powdered tissues were transferred to tubes containing 4.7 ml of STE (100 mM NaCl, 20 mM Tris [pH 7.2], 10 mM EDTA). After solubilization of the ground spleens, the solution was raised to 200 μ g of predigested Proteinase K (Sigma) per ml and 0.4% sodium dodecyl sulfate (Sigma). The resulting viscous solution was gently mixed and incubated at 37°C for 60 min. Each sample was then extracted with an equal volume of buffered phenol (Mallinkrodt, Inc., Paris, Ky.) and then was subjected to two further rounds of extraction with equal volumes of phenol and chloroform-isoamyl alcohol (24:1). DNAs were spooled after the addition of ice-cold 100% ethanol and then were resuspended in TE buffer (10 mM Tris [pH 7.2], 0.1 mM EDTA) and stored at -4°C. This protocol reliably resulted in DNAs of molecular weight greater than 3.0×10^7 and in ratios of optical density at 260 nm to optical density at 280 nm of greater than 1.85. DNAs derived from mouse embryo tissue culture cells were prepared as described above after trypsinization of cells and two rounds of washing with phosphate-buffered saline.

Restriction enzymes were purchased from New England Biolabs, Inc. (Beverly, Mass.) and were used under buffer conditions recommended by the manufacturer. Reactions typically contained two- to threefold-greater amounts of enzyme per microgram of input DNA than suggested. Digestions were performed for 3 to 4 h at 37°C. After 60 min of incubation, an appropriate portion of each reaction was added to 0.5 μ g of simian virus 40, pBR322, or λ DNA and incubated along with cell DNA reactions to monitor completeness of digestion. Test reactions were electrophoresed on 0.4% agarose gels (Seakem), stained with ethidium

bromide, and photographed under shortwave UV light. Those reactions judged complete were raised to 10 mM EDTA, 10 mM Tris (pH 8.1), 10% sucrose, and 0.01% bromophenol blue and then were electrophoresed through 7-mm-thick 0.7% agarose gels with a Tris-acetate buffer. *Hind*III-digested λ DNA was electrophoresed in parallel with cell DNA digests as molecular size standards. Those reactions judged incomplete were treated with additional enzyme and incubated as previously described. Gels were electrophoresed until the bromophenol blue dye front had migrated 17 cm.

Southern blotting and filter hybridization. Agarose gels were denatured in 400 ml of 1.5 M NaCl-0.5 M NaOH for 60 min at room temperature on an orbital shaker. They were then neutralized for 120 min in 400 ml of 0.5 M Tris (pH 7.5)-1.5 M NaCl, shaking slowly at room temperature. DNAs were then allowed to transfer to nitrocellulose (Schleicher & Schuell Co., Keene, N.H.) in a stream of $6 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) essentially by the method of Southern (25) for 48 h. After transfer, nitrocellulose sheets were baked in vacuo at 80°C for 3 h and preannealed for 18 h in an incubator at 41°C using 5 ml of a buffer containing 50% formamide (Sigma), 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.4), $2 \times$ Denhardt solution (5), 25 μ g of yeast RNA per ml, 100 μ g of denatured salmon sperm DNA per ml, 0.4% sodium dodecyl sulfate, and $3 \times$ SSC. Filters were then hybridized with 5×10^6 cpm of nick-translated ecotropic-specific probe in 5 ml of preannealing buffer for 48 h at 41°C. Filters were washed as follows. Each filter was rinsed for 15 min at room temperature on an orbital shaker with 400 ml of a solution of $2 \times$ SSC and $1 \times$ Denhardt, then again for 45 min at room temperature with 400 ml of $2 \times$ SSC, and finally at 53°C with three changes, 400 ml each, of a solution of 0.1% sodium dodecyl sulfate and $0.1 \times$ SSC, with gentle agitation for a total of 90 min. Filters were air dried and exposed to Kodak XAR-5 X-ray film supplemented with a Dupont Cronex Lightning-Plus intensifying screen. Films were placed at -70°C for up to 5 days before development.

Nick translation of the ecotropic-specific probe. The ecotropic-specific clone pEC-B4 was a generous gift of Malcolm Martin; its origin and construction have been described elsewhere (2, 13). Nick translation reactions were performed essentially as described by Rigby et al. (20). [α - 32 P]dCTP (600 to 800 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was used exclusively in all nick translation reactions. After phenol-chloroform extraction and ethanol precipitation of polymerase reactions, specific activities were found to be between 1.5×10^8 and 2.5×10^8 cpm per μ g of input clone DNA.

RESULTS

Ecotropic proviral sequences are found in genome-length fragments in DNA of low-leukemic mice. To analyze the relationship between the ecotropic virus induction loci of low-leukemic mouse strains and endogenous ecotropic viral genes we have analyzed the DNA of these strains for the presence of ecotropic proviral genomes by the method of Southern (25) using a molecularly cloned viral probe. This probe,

FIG. 1. Restriction endonuclease map of endogenous ecotropic MuLV. The genes for viral core antigens (GAG), DNA polymerase (POL), and envelope glycoproteins (ENV) are given as well as the proviral long terminal repeats found at the termini of viral DNA. The ecotropic-virus-specific fragment derived from the envelope gene and carried by clone pEC-B is represented by the shaded box.

which detects only ecotropic-specific sequences, was derived from a 500-base pair fragment in the envelope gene of AKR ecotropic virus. Cleavage sites for nine restriction endonucleases as well as the 500-base pair DNA fragment (shaded box) contained by the ecotropic-specific clone are illustrated (Fig. 1).

To determine if low-leukemic mouse strains carry ecotropic-specific sequences in retroviral genome-length fragments, cellular DNA was digested with the enzyme *Pst*I, which recognizes a single cleavage site within each ecotropic virus long terminal repeat and, therefore, would be expected to generate an 8.9-kilobase (kb) fragment. DNA was electrophoresed through agarose gels and transferred to nitrocellulose filters, and the filters were hybridized with the ecotropic-specific probe. The results of this analysis with a representative sample of DNAs of low-leukemic mouse strains are presented in Fig. 2. A single 8.9-kb fragment exhibiting homology with the 500-base pair ecotropic-specific probe was found in each of those strains which have been previously observed to be ecotropic virus positive in biological experiments. No ecotropic-specific sequences were observed in DNA isolated from NZB/BinJ mice, a strain that lacks ecotropic virus expression (12). The other ecotropic-virus-negative mouse strains 129/J, NFS/N, and SWR/J also showed no hybridization to the ecotropic-specific probe (data not shown).

Digestion of low-leukemic mouse DNAs with restriction endonucleases that cleave at multiple locations within ecotropic proviruses generates internal fragments with homology to the ecotropic-specific probe. *Bam*HI and *Kpn*I were used to compare the internal organization of ecotropic proviruses harbored by the low-leukemic strains BALB/c, B6, and DBA/2 with those of AKR/J mice. With these enzymes, a 3.3-kb fragment was observed after *Bam*HI digestion and a 4.4-kb fragment after *Kpn*I digestion (data not shown). Fragments of similar sizes were generated upon digestion of DNA from AKR/J

FIG. 2. *Pst*I digestion of DNAs from low-leukemic mouse strains. DNAs were extracted, digested, and electrophoresed as described in the text. A 12- μ g amount of cleaved DNA was applied to each gel lane. After transfer to nitrocellulose, DNA was hybridized with the 32 P-labeled clone pEC-B4. *Hind*III-digested λ DNA was electrophoresed in parallel as fragment size standards. kbp, Kilobase pair.

mice with these enzymes (Fig. 1) (2; N. Jenkins and N. Copeland, personal communication). Thus, the results of experiments with these three restriction endonucleases support the contention that the ecotropic proviruses of these low-leukemic mice are similar to those of the high-leukemic mouse strain AKR/J.

Distribution of ecotropic proviruses in low-leukemic mouse strains correlates with identification of induction loci. Restriction endonuclease digestion of mouse DNAs with *Eco*RI followed

FIG. 3. *Eco*RI digestion of DNAs from low-leukemic mouse strains. DNAs were treated as in Fig. 2. Strains that have been characterized for their inheritance of induction genes are given: *Inc-1*⁺ denotes the BALB/c-like gene; *Inb-1*⁺ denotes the C57BL/6-like gene. kbp, Kilobase pair.

TABLE 1. Low-leukemic strain distribution pattern of endogenous ecotropic viruses using *EcoRI*

Mouse strains sharing <i>EcoRI</i> fragments of:		
26 kb	22 kb	20 kb
C57BL/6J	BALB/cByJ	DBA/2J
C57BL/10J	A/J	SEA/GnJ
C57BR/cdJ	CBA/J	HRS/J
CXBD	C3H/He	
CXBE	SEC/1ReJ	
CXBG	SEA/GnJ	
CXBI	HRS/J	
CXBJ	CXBE	
CXBK	CXBH	
BXH 3	CXBI	
BXH 9	CXBJ	
BXH 10	CXBK	
BXH 11	BXH 3	
BXH 12	BXH 6	
BXH 14	BXH 8	
BXH 19	BXH 9	
	BXH 12	
	BXH 19	

by filter hybridization with the ecotropic-specific probe allows for the estimation of the minimum number of endogenous ecotropic proviruses harbored by each strain. A cleavage site for *EcoRI* is not contained within ecotropic viruses; hence, digestion of cellular DNA with *EcoRI* will resolve each endogenous ecotropic virus to a fragment characteristic of its chromosomal location (26). Figure 3 shows the results of such an analysis performed on a variety of low-leukemic mouse strain DNAs. It is apparent that three distinct families of proviruses are resolved by digestion with *EcoRI*: (i) A/J, C3H/HeJ, CBA/J, SEC/J, SEA/J, HRS/J, and BALB/cJ mice share a common 22-kb fragment with homology to the ecotropic-specific probe; (ii) DBA/2J, SEA/J, and HRS/J mice share a common fragment of 20 kb; (iii) C57BL/6J, C57BL/10J, and C57BR/cdJ mice share a 26-kb fragment with homology to the ecotropic-specific probe (Table 1). Digestion of DNA isolated from C3H/He, CBA/J, BALB/c, B6, and C57BL/10 mice with the restriction endonucleases *XbaI* and *PvuII* followed by hybridization with the ecotropic-specific probe has confirmed the patterns of comigrating fragments observed with *EcoRI* (data not shown).

It is interesting to note that the strains found to share an ecotropic provirus on a similar *EcoRI* fragment have been derived from common progenitor strains. Unlike the instability characteristic of endogenous ecotropic viruses of high-leukemic strains of mice, this analysis points out the stability of ecotropic viruses within the mouse genome since these strains were separated 60 to 70 years ago. Furthermore, the

inheritance of ecotropic proviruses by the BALB-related and B6-related strains correlates completely with the *Inc-1* or *Inb-1* inheritance genes, respectively (15, 16). Characterization of the induction loci inherited by SEA, HRS, and DBA mice has not as yet been completed.

Analysis of induction loci and inheritance of endogenous ecotropic viruses in the CXB recombinant inbred strains. To analyze further the relationship between the ecotropic proviruses of BALB/c and C57BL/6 mice and the corresponding *Inc-1* and *Inb-1* loci responsible for enhanced virus induction, DNAs from the CXB recombinant inbred strains were digested with *EcoRI* and, after transfer to nitrocellulose, were hybridized with the ecotropic-specific probe (Fig. 4). Below each lane of Fig. 4 are mean values of in situ plaque assays for ecotropic virus induction accumulated in the phenotypic analysis of these strains (16). Clearly, those strains which show enhanced virus induction, i.e., CXBE, I, J, and K, contain both BALB/c and C57BL/6 ecotropic proviruses. Strains which carry only the BALB/c or B6 proviruses show much lower virus induction. Genetic experiments have indicated that CXBH carries the *Inc-1* gene inherited from BALB/c and that CXBD and CXBG carry the *Inb-1* gene inherited from B6. The

FIG. 4. *EcoRI* digestion of DNAs from the CXB recombinant inbred strains. DNAs were treated as in Fig. 2. Below each lane are mean UV-XC plaque values observed in in situ induction analysis of these strains (16) as well as the strain distribution patterns of inheritance of *Inc-1*, the BALB/c induction gene; *Inb-1*, the C57BL/6 induction gene; *Cv-1*, the BALB/c ecotropic provirus; and *Bv-1*, the C57BL/6 ecotropic provirus. kbp, Kilobase pair.

TABLE 2. *Inc* and *Inb* classification of BXH recombinant inbred strains

Strain designation	Crosses to BALB/c or C3H mice		Crosses to C57BL/6 mice	
	Mean syncytia per culture (no. of cultures examined)	<i>Inb-1</i> genotype	Mean syncytia per culture (no. of cultures examined)	<i>Inc</i> genotype
BXH				
3	43 (4)	+	50 (8)	+
4	1 (6)	-	0 (8)	-
6	0 (8)	-	58 (4)	+
7	0 (17)	-	14 (27)	+
8	0 (9)	-	17 (6)	+
9	95 (6)	+	121 (16)	+
10	32 (13)	+	7 (19)	+
11	110 (3)	+	11 (13)	+
12	ND ^a		260 (4)	+
14	159 (15)	+	44 (16)	+
19	161 (14)	+	132 (15)	+
Parental				
C57BL/6	15 (38)	+	0.5 (44)	-
C3H/He	0 (10)	-	15 (38)	+

^a ND, Not determined.

molecular hybridization experiments indicate that CXBH has inherited the BALB/c provirus and that the CXBD and G strains have inherited the B6 provirus.

The data presented thus far indicate that the BALB/c ecotropic provirus cosegregates with *Inc-1* and that the B6 ecotropic provirus cosegregates with *Inb-1* (Fig. 3) (16). Because *Inc-1* and *Inb-1*, detected by assays for enhanced virus induction, have been shown to cosegregate with the *Cv-1* and *Bv-1* genes, respectively, which determine virus presence or absence in crosses with virus-free strains (6, 7, 10, 11, 17), the molecular results presented here support the contention that, as expected, the virus-inducing genes *Cv-1* and *Bv-1* are ecotropic virus structural genes.

Relationship between ecotropic proviral inheritance and induction phenotype in the BXH recombinant inbred strains. McCubrey and Risser, using classical genetic methods, demonstrated allelism between the ecotropic induction loci (*In*) of BALB/c and C3H/He mice (15). *EcoRI* digestion of DNAs from these strains, as illustrated in Fig. 3, produces identically migrating fragments with homology to the ecotropic-specific probe. To determine if the linkage of the BALB/c ecotropic induction locus and proviral structural genes would also be observed for C3H/He mice, the BXH recombinant inbred strains were analyzed for their individual complement of ecotropic proviruses as well as their induction phenotype in crosses with BALB/c or

C3H/He mice (*Inc-1*⁺) and C57BL/6 mice (*Inb-1*⁻).

To test for the presence of genes that enhance ecotropic MuLV induction, each BXH strain was mated to BALB/c or C3H/He (*Inc-1*^{+/+}, *Inb-1*^{-/-}) and to B6 (*Inc-1*^{-/-}, *Inb-1*^{+/+}). Cultures were prepared from individual F₁ embryos and analyzed for the number of virus-producing cells in situ after IUDR induction. In such tests, the parental strains BALB/c or C3H/He produced an average of 0.2 syncytia per 2.5 × 10⁵ induced cells, and the parental strain B6 produced an average of 0.5 syncytia per 2.5 × 10⁵ induced cells. (C3H/He × B6)F₁ hybrid cells produced an average of 15 syncytia per 2.5 × 10⁵ induced cells. The data in Table 2 indicate that 6 of the 10 BXH strains in hybrids with C3H/He or BALB/c showed enhanced virus induction. Therefore, these six BXH strains have inherited a gene from their B6 progenitor, which in combination with a gene of C3H/He or BALB/c enhances ecotropic virus induction. In accord with our previous study of BALB/c and B6 mice, we designate this gene *Inb-1* (16).

When the same BXH strains were mated to B6 (*Inc-1*^{-/-}, *Inb-1*^{+/+}), cells from 10 of the 11 hybrids showed enhanced virus induction (Table 2 and Fig. 5). Three of these strains, BXH 7, 10, 11, were analyzed in more detail by determining

FIG. 5. Frequency of virus-producing cells in induced cultures of BXH and F₁ mice. Cultures were prepared and analyzed as described in the text.

FIG. 6. *EcoRI* digestion of DNAs from BXH recombinant inbred strains. DNAs were treated as in Fig. 2. Below each lane are the strain distribution patterns for the four loci studied as in Fig. 4. Discordant inheritance patterns are designated by boxes. kbp, Kilobase pair.

the number of virus-producing cells observed in induction tests with cells of the parental mice. The results of that experiment indicate that cells of BXH 7 showed no virus induction in situ, and cells of BXH 10 and BXH 11 show enhanced virus induction (Fig. 5), as would be predicted from their *Inb-1*⁺ and *Inc*⁺ genotypes. (We have not numbered the *Inc* gene[s] inherited by BXH strains because there is no genetic evidence to indicate that the C3H/He progenitor strain carries only *Inc-1* and no other *Inc*-like genes.)

To determine the inheritance of proviral genomes by these BXH strains, DNAs extracted from each BXH strain were cleaved with *EcoRI*, electrophoresed in agarose gels, transferred to nitrocellulose, and hybridized with the ecotropic-specific probe. Figure 6 illustrates the resulting autoradiogram as well as the strain distribution patterns for the inheritance of the C3H/He and C57BL/6 induction loci (from Table 2). Cosegregation of the B6-derived ecotropic provirus and *Inb-1* was observed in the 10 strains tested for both traits. BXH strains 4 and 7 show no sequence homology with the ecotropic-specific probe and, therefore, have not inherited the ecotropic envelope gene fragment detected with this probe. BXH strains 3, 6, 8, 9, 12, and 19 have inherited the C3H/He ecotropic provirus, and each scored as *Inc*⁺ in induction tests.

Unexpectedly, four of the BXH strains that lack the 22-kb C3H/He ecotropic-containing fragment were scored as *Inc*⁺ in induction tests. Three of these four do possess the ecotropic provirus contributed by the C57BL/6 progenitor, whereas DNA of one strain, BXH 7, shows no homology to the ecotropic-specific probe. Thus, BXH strain 7 provides evidence that at least one induction locus (*In*) of low-leukemic mouse

strains is not equivalent to the envelope gene of endogenous ecotropic viruses. To ensure that the mice used in our breeding and hybridization experiments were the appropriate BXH strains generated by Taylor, our mice were examined for inheritance of the alloantigenic markers *Es-3*, *Hbb*, *Mup-1*, *Pep-3*, and *Pgm-1*. The results of that analysis corresponded to the independent typing of Taylor (personal communication).

Further study of one such discordant strain, BXH 11, is shown in Fig. 7. Mouse embryo cells derived from the cross of (C57BL/6 × [BXH 11]) were assayed for their inducibility by IUDR, and DNA was extracted and characterized for inherited proviruses. The DNAs were cleaved in this case with *PvuII*, a restriction enzyme which generates a single fragment for each provirus homologous to the ecotropic-specific probe. DNA derived from the CXBI recombinant inbred strain and DNA from BXH 11 were digested and electrophoresed in parallel as genotypic controls. As one would predict, the four resulting embryo cultures possess the single 5-kb *PvuII*-generated fragment characteristic of the B6 ecotropic provirus. Yet, each embryo culture expresses ecotropic virus at levels comparable to the CXBI strain, a known *Inc-1*⁺, *Inb-1*⁺ mouse strain. Induction of cells from the parental BXH 11 strain results in significant frequencies of virus induction (Fig. 5 and 7). Despite the fact that cells of (B6 × [BXH 11]) or BXH 11 mice lack an intact C3H/He-donated ecotropic provirus, these cells show enhanced virus induction characteristic of (B6 × C3H/He)_{F1} mice. This result indicates that not all examples of enhanced virus induction require two different intact ecotropic proviral genomes.

FIG. 7. *PvuII* digestion of (C57BL/6 × [BXH 11]) embryo cell DNAs. DNAs were treated as in Fig. 2. Mean plaque values for each induced embryo culture as well as for cells from induced CXBI and BXH 11 cultures are given below each lane. kbp, Kilobase pair.

DISCUSSION

We have characterized 12 low-leukemic mouse strains and 18 recombinant inbred strains for their complements of endogenous ecotropic viruses using a molecular probe specific for ecotropic viral sequences. Moreover, we have characterized these strains for the inheritance of genes that enhance ecotropic virus expression after IUdR induction. This analysis established that the inheritance of an ecotropic provirus identified on a characteristic 26-kb *EcoRI* fragment occurs concurrently with the inheritance of the *Inb-1* gene. The 26-kb *EcoRI* fragment is indicative of the ecotropic proviruses carried by the C57BL family of mouse strains and is correlated with low expression of ecotropic virus throughout life and negligible expression of ecotropic virus in IUdR induction studies (16a, 17). Those low-leukemic and recombinant inbred strains that have inherited a single ecotropic provirus identified on a 22-kb *EcoRI* fragment exhibit the phenotype indicative of the *Inc-1* induction gene. The 22-kb *EcoRI* fragment is characteristic of BALB/c-related mouse strains as is the infrequent expression of ecotropic virus early in life (16a). A similar strain distribution pattern for ecotropic proviral genomes has also been found by numerous other investigators (2, 8, 19, 27).

McCubrey and Risser have observed an enhanced frequency of IUdR-induced ecotropic virus expression in cells that contain the *Inc-1* and *Inb-1* induction genes (16). Our analysis of the CXB recombinant inbred strains using the ecotropic-virus-specific probe has correlated the enhanced expression of ecotropic virus with the inheritance of an intact ecotropic provirus from both progenitor strains, BALB/c and C57BL/6.

However, our analysis of the BXH recombinant inbred strains has identified four strains (BXH 7, 10, 11, and 14) that show discordant inheritance of *Inc* function and ecotropic proviral sequences. These strains do not exhibit inheritance of the C3H/He ecotropic provirus, as judged by hybridization with the ecotropic-specific envelope gene fragment, yet they possess the *Inc*-like trait as assayed by the induction of F₁ cells from crosses with *Inb-1*-containing strains. Induction of cells from BXH strains 10 and 11 results in significant expression of ecotropic virus, a result consistent with their *Inc*⁺, *Inb-1*⁺ genotype. Induction of cells from BXH 7 mice results in no ecotropic virus expression, consistent with their *Inc*⁺, *Inb-1* designation. The observation of enhanced virus induction from induced F₁ cells of the (B6 × [BXH 7]) cross establishes that heterozygosity of the *In* gene(s) and the B6 ecotropic provirus are sufficient preconditions for enhanced virus induc-

tion. These results also indicate that at least one locus that enhances ecotropic virus induction is not an intact ecotropic proviral genome.

Several explanations exist for the apparent discrepancy between inheritance of the *Inc* function and the C3H/He ecotropic proviral marker fragment in BXH strains. (i) The C3H/He progenitor strain may have multiple *Inc*-like genes, only one of which cosegregates with the ecotropic provirus. (ii) During the generation of BXH recombinant strains, genetic recombination has taken place within C3H/He ecotropic proviral sequences, leaving a fragmented provirus that contains the *Inc* function, e.g., *gag* or *pol* genes, but does not contain the ecotropic-specific marker fragment. (iii) The *Inc* function is encoded by sequences adjacent to but not within ecotropic proviral sequences, and genetic recombination has dissociated these genes in BXH strains. Further molecular and genetic experiments, currently in progress, will resolve these possibilities and provide insight into the expression of endogenous viral genomes in low-leukemic mouse strains.

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