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Fv-1 N- and B-Tropism-Specific Sequences in Murine Leukemia Virus and Related Endogenous Proviral Genomes

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Oligonucleotide probes specific for the *Fv-1* N- and B-tropic host range determinants of the *gag* p30-coding sequence were used to analyze DNA clones of various murine leukemia virus (MuLV) and endogenous MuLV-related proviral genomes and chromosomal DNA from four mouse strains. The group of DNA clones consisted of ecotropic MuLVs of known *Fv-1* host range, somatically acquired ecotropic MuLV proviruses, xenotropic MuLV isolates, and endogenous nonecotropic MuLV-related proviral sequences from mouse chromosomal DNA. As expected, the prototype N-tropism determinant is carried by N-tropic viruses of several different origins. All seven endogenous nonecotropic MuLV-related proviral sequence clones derived from RFM/Un mouse chromosomal DNA, although not recognized by the N probe, showed positive hybridization with the prototype B-tropism-specific probe. The two xenotropic MuLV clones derived from infectious virus (one of BALB:virus-2 and one of AKR xenotropic virus) failed to hybridize with the N- and B-tropic oligonucleotide probes tested and with one probe specific for NB-tropic Moloney MuLV. One of two endogenous xenotropic class proviruses derived from HRS/J mouse chromosomal DNA (J. P. Stoye and J. M. Coffin, J. Virol. 61:2659–2669, 1987) also failed to hybridize to the N- and B-tropic probes, whereas the other hybridized to the B-tropic probe. In addition, analysis of mouse chromosomal DNA from four strains indicates that hybridization with the N-tropic probe correlates with the presence or absence of endogenous ecotropic MuLV provirus, whereas the B-tropic probe detects abundant copies of endogenous nonecotropic MuLV-related proviral sequences. These results suggest that the B-tropism determinant in B-tropic ecotropic MuLV may arise from recombination between N-tropic ecotropic MuLV and members of the abundant endogenous nonecotropic MuLV-related classes including a subset of endogenous xenotropic proviruses.

It has been known for many years that some strains of mice carry one or more loci for inducible ecotropic retrovirus, and in almost every case these viruses have an *Fv-1* tropism as defined by their ability to replicate in the cells of mice carrying the $Fv\text{-}1^{n/n}$ alleles and be restricted by cells of mice carrying the $Fv\text{-}1^{b/b}$ alleles (2, 13, 17, 22, 26, 36, 39, 40). Viruses with an *Fv-1* B tropism emerge later in life in animals with the $Fv\text{-}1^{b/b}$ alleles, where they have a selective advantage over the endogenous N-tropic virus (12, 32). The origin of the B-tropic virus has not been precisely defined, but early observations suggested that it might be a variant of the endogenous N-tropic virus (34). The first experimental evidence to suggest that a recombination event was involved comes from the serological characterization by Benade et al. (3) and tryptic peptide mapping by Gautsch et al. (10, 11). Their experiments and observations suggested that B tropism was acquired by recombination of an N-tropic ecotropic virus with an endogenous xenotropic virus. Experiments of Aaronson and Barbacid (1) suggested that the inducible endogenous xenotropic virus (class II) of BALB/c mice, BALB:virus-2, carried an N-tropism determinant, whereas the work of Gautsch et al. (10) demonstrated that a xenotropic virus of the uninducible class III was cryptically B tropic. Subsequently, Sakai et al. (37) investigated the *Fv-1* host range of xenotropic virus by using an assay based on pseudotypes of xenotropic and ecotropic virus. With the exception of a single isolate from SL mice which appeared to be N tropic, they were unable to demonstrate either N or B tropism associated with xenotropic murine leukemia viruses (MuLVs). The one apparently N-tropic xenotropic virus may have been a recombinant, acquiring that host range from another virus (37). This may be similar to B-MuX, a xenotropic virus with an ecotropic *gag* region, most likely acquired by recombination (16). The cryptic *Fv-1* tropism of B-MuX was not reported. More recently Rassart et al. (33) have described B-tropic MuLV isolates from C57BL/Ka mice that are recombinants with some endogenous nonecotropic (xenotropic) sequence in the *gag-pol* region. This work also suggests that the B-tropism determinant was derived from an endogenous component.

Based on the known nucleotide sequence coding for the N- and B-tropic determinants of p30, the *Fv-1* target (9, 30), we have approached the question of the origin of B-tropic MuLV by specific oligonucleotide probe hybridization analysis of various recombinant DNA clones of MuLV isolates and endogenous proviral sequences and chromosomal DNA from four mouse strains. In this report we demonstrate homology of the N probe to clones of known *Fv-1* N-tropic ecotropic viruses and somatically acquired ecotropic proviruses and to chromosomal DNA of mouse strains known to carry an endogenous ecotropic provirus but not to a strain known to lack an endogenous ecotropic provirus. The B probe hybridizes to a clone of the prototype *Fv-1* B-tropic ecotropic virus and to each of seven endogenous MuLV-related proviral sequence clones distinguished by a characteristic long terminal repeat (LTR) and primer-binding site (27, 28) and to multiple copies of MuLV-related sequences in mouse chromosomal DNA. The BALB:virus-2 and AKR

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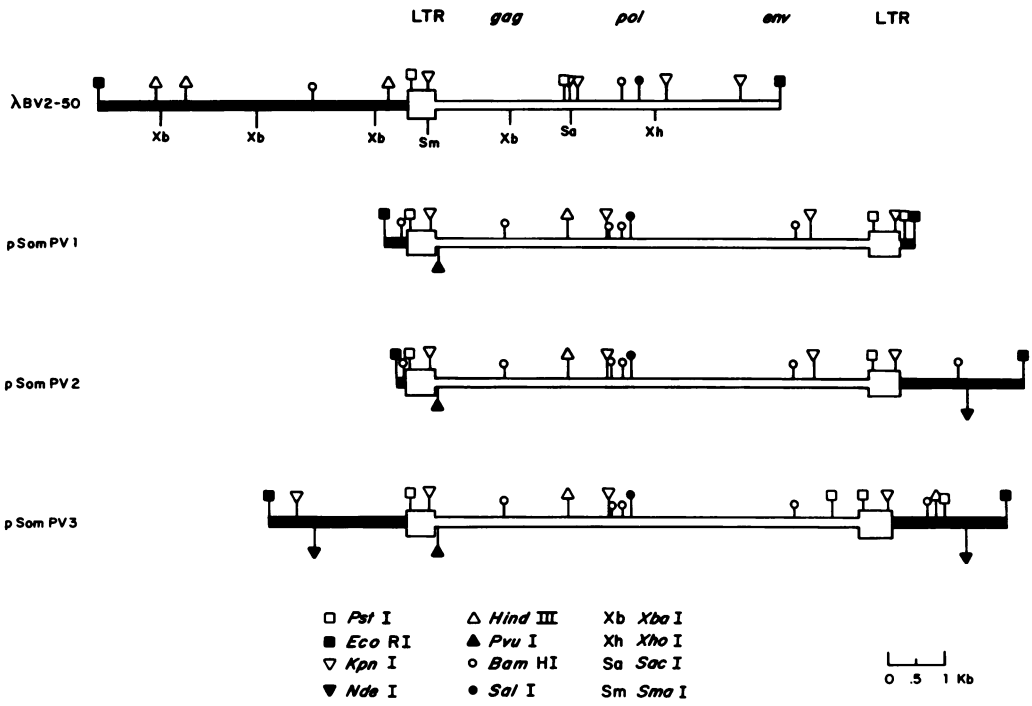

FIG. 1. Restriction endonuclease cleavage site maps of xenotropic MuLV and somatically acquired ecotropic MuLV. Sites indicated below the line for λBV2-50 have not been mapped for the ecotropic proviruses. Sites indicated below the line for the ecotropic proviruses have not been mapped in λBV2-50.

xenotropic isolates cloned from somatically infected cells hybridized to neither probe. Of two endogenous proviruses classified as xenotropic by Stoye and Coffin (41), one hybridized to neither probe, whereas another hybridized to the B probe but not to the N probe.

MATERIALS AND METHODS

The prototype N- and B-tropic virus clones pWN41 and pWB5 and the Gross leukemia virus (passage A) clone pGN104 were previously described (5), as was the endogenous N-tropic virus from RFM/Un mice, pRFV105 (25). The clone of N-tropic FBJ MuLV, pFBJMLV1 (8), was obtained from the American Type Culture Collection. The xenotropic BALB:virus-2 clone λBV2-50 is a provirus isolated from the DNA of human melanoma cell line A673 infected with BALB:virus-2. Approximately 20% of the genome (*env* gene region and right LTR) is missing to the right of the *Eco*RI site in the *env* gene due to cloning at that site into Charon 4A. The virus-specific portion of the 12.1-kilobase-pair (kbp) insert of λBV2-50 is 6.6 kbp (Fig. 1). The AKR xenotropic clone λC1.2-4.3 was provided by Dave Joseph of The University of North Carolina at Chapel Hill. The unintegrated form I DNA was cloned into λ Charon 28 at the *Bam*HI site (24). Two endogenous xenotropic proviral clones derived from *Hin*dIII-digested HRS/J mouse chromosomal DNA, pMX22 and pMX30, were provided by Jonathan Stoye, Tufts University School of Medicine (41). pSomPV1, pSomPV2, and pSomPV3 are recombinant DNA clones of ecotropic MuLV proviruses isolated from *Eco*RI-digested spleen DNA of an RFM/Un mouse. They are all somatically acquired reintegrations of the endogenous ecotropic provirus. Screening was based on hybridization to an ecotropic specific *env* probe (4). Within the proviral specific portion of the insert, pSomPV1 and pSomPV2 are indistinguishable from the other ecotropic MuLV maps (5), whereas pSomPV3 has an apparent alteration in the *env* region resulting in loss and gain of restriction enzyme sites and a loss of approximately 150 bp (Fig. 1). Of the three, only pSomPV1 resulted in release of infectious virus after DNA transfection into NIH 3T3 cells (unpublished data).

MuLV-related endogenous proviral sequences were cloned from *Hin*dIII-digested spleen DNA of an RFM/Un mouse (27, 28). Selection was based on positive hybridization to an LTR probe and failure to hybridize to the ecotropic specific *env* probe. For the present analysis a further selection was based on positive hybridization to a *gag-pol* probe consisting of the 2.4-kbp fragment from the *Pvu*I site in the 5′ leader sequence to the *Hin*dIII site in *pol*, located 413 bp beyond the *gag* termination codon. All seven selected clones also contain an insertion sequence within the LTR characteristic of this class of endogenous provirus (20, 27, 28, 31). pRFM3 and pRFM11 have not previously been described, nor has the restriction enzyme site been mapped in detail. Restriction maps of the other five clones were published previously (27, 28).

Restriction enzyme-digested DNAs were subjected to electrophoresis in submerged horizontal agarose gels and blot transferred to a Nytran (Schleicher & Schuell Co.) nylon membrane or dried down in the gel (for hybridization of oligonucleotide probes to chromosomal DNA). The *Ava*I fragment *gag* probe consists of the 3′-terminal 10 bp of p12, all of p30 (789 bp), and the 5′-terminal 137 bp of p10 from a pWN41 subclone and was ^{32}P labeled by nick translation to a specific activity of approximately 10^8 cpm/μg. Oligonucleotides 18 bases in length were synthesized by OCS Laboratories Inc., Denton, Tex., and end labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP to a specific activity of 2 ×

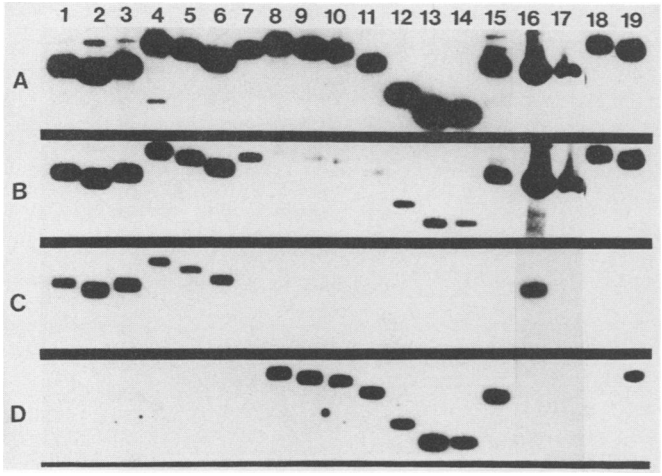

FIG. 2. Hybridization analysis of MuLV recombinant DNA clones. DNA blots were hybridized as indicated in Materials and Methods. Lanes: 1, pWN41; 2, pGN104; 3, pRFV105; 4, pSomPV1; 5, pSomPV2; 6, pSomPV3; 7, λBV2-50; 8, pRFM16; 9, pRFM6; 10, pRFM1; 11, pRFM3; 12, pRFM9; 13, pRFM17; 14, pRFM11; 15, pWB5; 16, pFBJMLV1; 17, λC1.2-4.3; 18, pMX22; 19, pMX30. Hybridization was with 0.9-kbp *Ava*I p30 *gag* probe; preparations were washed under moderate-stringency conditions (A) and then under high-stringency conditions (B). Oligonucleotide probes hybridized were N (C) and B (D). See Fig. 3 for oligonucleotide probes. The NB (Moloney) probe did not anneal to any clone, and the blank autoradiograph is not shown.

10^6 to 5×10^6 cpm/ng. Nick-translated *gag* probe (3×10^6 cpm) or oligonucleotide probe (3×10^7 to 4×10^7 cpm) was hybridized to the membrane or dried gel in 6× SSC (1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 47°C for approximately 18 h. The posthybridization wash (for oligonucleotide probes) was in 2× SSC–0.1% sodium dodecyl sulfate at 47°C for 1 h, and autoradiography exposure was usually 1 to 6 h for clones and 1 to 3 days for chromosomal DNA. Annealed oligonucleotide probes were melted off by washing in 0.2× SSC at 65°C for 1 h to use the same membrane or gel for different probes. After hybridization with the 0.9-kbp *Ava*I fragment *gag* probe the membrane was washed for 1 h at a moderate stringency (2× SSC–0.1% sodium dodecyl sulfate at 65°C) and exposed for autoradiography. Subsequently, the same membrane was washed for 1 h at a higher stringency (0.2× SSC–0.1% sodium dodecyl sulfate at 65°C) and exposed for autoradiography.

RESULTS

Hybridization with *gag* p30 probe. Many of the recombinant DNA clones used in this study have been previously described and are cited in Materials and Methods. Restriction endonuclease maps for those described for the first time in this report are shown in Fig. 1. DNA from each MuLV recombinant clone was digested with the restriction enzyme which cleaves between insert and vector, subjected to electrophoresis in agarose, and transferred to a nylon membrane for hybridization. A 0.9-kbp *Ava*I-cleaved DNA fragment from the ecotropic MuLV clone pWN41 was used as a broadly reacting *gag* p30 gene probe (see Materials and Methods). Posthybridization washing was done at a moderate stringency to detect the *gag* gene of all clones and then at

TABLE 1. *Fv-1* tropism-specific probes

Designation	Host range[a]	Form[b]	Insert (kbp)	Hybridization with probe[c]			
				*Ava*I	N	B	NB
pWN41	N-ECO	CCC	8.8	+	+	−	−
pGN104	N-ECO	CCC	8.3	+	+	−	−
pWNB5	B-ECO	CCC	8.9	+	−	+	−
pFBJMLV1	N-ECO	CCC	8.2	+	+	−	−
pRFV105	N-ECO	CCC	8.8	+	+	−	−
pSomPV1	N-ECO	Som PV	9.5	+	+	−	−
pSomPV2	N-ECO	Som PV	11.0	+	+	−	−
pSomPV3	N-ECO	Som PV	13.0	+	+	−	−
λBV2-50	XENO	Som PV (incomp)	12.1	+	−	−	−
λC1.2-4.3	XENO	CCC	9.0	+	−	−	−
pMX22	XENO	Endo PV	15.4	+	−	−	ND
pMX30	XENO	Endo PV	14.2	+	−	+	ND
pRFM1	POLY	Endo PV (incomp)	12.4	+/*	−	+	−
pRFM3	POLY	Endo PV (not mapped)	10.9	+/*	−	+	−
pRFM6	POLY	Endo PV	9.2	+/*	−	+	−
pRFM9	POLY	Endo PV (incomp)	6.1	+/*	−	+	−
pRFM11	POLY	Endo PV (not mapped)	5.1	+/*	−	+	−
pRFM16	POLY	Endo PV	13.6	+/*	−	+	−
pRFM17	POLY	Endo PV (incomp)	5.1	+/*	−	+	−

[a] N-ECO, Ecotropic MuLV (replicates in mouse only) with an *Fv-1*^{n} tropism; B-ECO, ecotropic MuLV with an *Fv-1*^{b} tropism; XENO, inability to replicate in mouse and ability to replicate in foreign species; POLY, ability to replicate in mouse and foreign species. The endogenous MuLV-related proviruses are not infectious and are designated POLY only to indicate that this class of provirus is thought to donate *env* sequences confering polytropism to recombinant viruses. Only RFM6 and RFM16 have *env* sequences in the clone.

[b] CCC, Cloned as an unintegrated form I DNA molecule (covalently closed circle); Som PV, somatically acquired proviruses cloned with flanking cellular sequences; Endo PV, endogenous germ line proviruses also cloned with flanking cellular sequences; incomp, clones which are incomplete due to cloning with a restriction enzyme which has a site within the proviral genome.

[c] The *Ava*I fragment p30 *gag* probe is described in Materials and Methods: +, hybridization detected; −, no hybridization detected; +/*, high-stringency posthybridization wash resulted in more melting of probe than the homologous paring with pWN41; ND, not done. N, B, and NB oligonucleotide probes are shown in Fig. 3.

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-Tyr-Thr-Thr-Thr-Glu-Gly-Arg-          B TARGET
                 *   *
-Tyr-Thr-Thr-Gln-Arg-Gly-Arg-          N TARGET
                     *
-Tyr-Thr-Thr-Gln-Ala-Gly-Arg-          NB

3'  G TGG TGA TGT CTT CCA TC  5'       B OLIGONUCLEOTIDE
          * **  **
3'  G TGG TGG GTT TCT CCA TC  5'       N OLIGONUCLEOTIDE
              * **
3'  G TGG TGG GTC CGT CCA TC  5'       NB OLIGONUCLEOTIDE
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FIG. 3. Sequence of p30 *gag* amino acid residues 106 through 112 in B-, N-, and Moloney NB-tropic MuLV is shown in the upper portion of the figure. Differences between N-tropic MuLV and the other two are indicated by asterisks (*). The corresponding nucleotide sequence of the minus strands chosen as tropism-specific oligonucleotide hybridization probes are shown in the lower portion of the figure. Differences are indicated by asterisks (*).

high stringency to determine whether divergence could be detected with this probe (Fig. 2A and B). At the moderate stringency, all clones hybridized. Although there were limitations in quantitating sequence homology on blot hybridizations and some discrepancies in the amounts of DNA loaded in each lane, it was quite clear from the high-stringency wash (Fig. 2B) that the probe had less homology to the seven endogenous MuLV-related proviruses than the ecotropic viruses, which hybridized equally as expected. The xenotropic isolates λBV2-50 and λC1.2-4.3 and the endogenous xenotropic proviruses pMX22 and pMX30 also showed a high degree of homology.

Fv-1 tropism-specific oligonucleotide probes. N- and B-tropism-specific oligonucleotide probes are shown in Fig. 3 and are derived from the published sequence of MuLV clones (9, 30). The N-tropic probe demonstrated homology to the four known N-tropic MuLVs (Fig. 2C, lanes 1 through 3 and 16) and the three somatically acquired ecotropic proviruses (Fig. 2C, lanes 4 through 6). No other clone hybridized with the N-tropic oligonucleotide probe. The B-tropic probe showed homology to the known B-tropic virus (Fig. 2D, lane 15), to all seven of the endogenous provirus clones from RFM/Un mouse DNA (Fig. 2D, lanes 8 through 14), and to pMX30 (Fig. 2D, lane 19), one of two endogenous xenotropic proviruses from HRS/J mouse DNA. No other clone hybridized with the B-tropic oligonucleotide probe. The results are summarized in Table 1. The BALB: virus-2 xenotropic provirus clone, λBV2-50 (Fig. 2, lane 7), the AKR xenotropic MuLV clone, λC1.2-4.3 (Fig. 2, lane 17), and pMX22, an endogenous xenotropic provirus of HRS/J (Fig. 2, lane 18), did not hybridize to N or B oligonucleotide probes, although each contained sequences hybridizing to the broadly reacting *gag* p30 probe.

The analogous sequence from NB-tropic Moloney MuLV (38) (Fig. 3) was also used as a probe and failed to hybridize to any clone tested. These data are also summarized in Table 1, although the negative autoradiograph is not shown in Fig. 2.

If the N- and B-tropism-specific oligonucleotide sequences exist only within the context of proviral genomes in mouse chromosomal DNA, we would expect the N probe to detect the ecotropic MuLV and the B probe to detect many or all of the members of the proviral family represented by the seven RFM/Un MuLV-related proviruses as well as a subset of endogenous xenotropic proviruses. Restriction enzyme digestion and hybridization analysis of chromosomal DNA from mouse strains differing in their content of MuLV proviruses would confirm this prediction. We chose four strains to examine. BALB/c is known to contain a single endogenous ecotropic provirus, *Emv-1* (18), and is the strain of origin of WN1802N and WN1802B, the prototype N- and B-tropic MuLV isolates. RFM/Un also contains a single ecotropic provirus, *Emv-1*, and is the strain from which our MuLV-related proviral sequence clones were derived. SJL contains two endogenous ecotropic proviral loci, *Emv-9* (apparently defective) and *Emv-10*, and NFS/N has none (18). Four different restriction enzymes were used. *Pst*I has a conserved site in the LTR of ecotropic MuLVs and MuLV-related proviruses, and conserved internal *Pst*I sites also exist in MuLV-related sequences. Therefore fragment size does not depend on flanking sequences, and relative hybridization intensity reflects copy number. Endogenous ecotropic MuLV proviruses *Emv-1* and *Emv-10* are expected to give an 8.2-kbp *Pst*I fragment, MuLV-related proviruses represented by RFM16 will give a 7.8-kbp fragment, and MuLV-related proviruses represented by RFM1 or RFM9 will give a 4.6-kbp band. *Emv-9* has a 5.1-kbp *Pst*I fragment detected by an ecotropic *env* probe (18), but it is not known what size fragment, if any, is detected by the *gag* p30 N or B oligonucleotide probe. *Xba*I recognizes a single site in ecotropic MuLV, and in a double digest with *Pst*I will result in a 7.1-kbp fragment. *Xba*I sites in the MuLV-related proviruses have not been mapped. Likewise, with all four enzymes used, it is not known what size fragments to expect from the subset of endogenous xenotropic proviruses detected by the B probe. The ecotropic MuLV provirus has a single *Hin*dIII site and no *Eco*RI site, whereas MuLV-related proviruses have a conserved *Eco*RI site and some have a *Hin*dIII site (see discussion of polytropic and modified polytropic proviruses [41] below). Digestion with *Hin*dIII or *Eco*RI generally results in proviral fragments which include flanking sequences and thus unique sizes for each integration site. The nonecotropic proviruses will thus

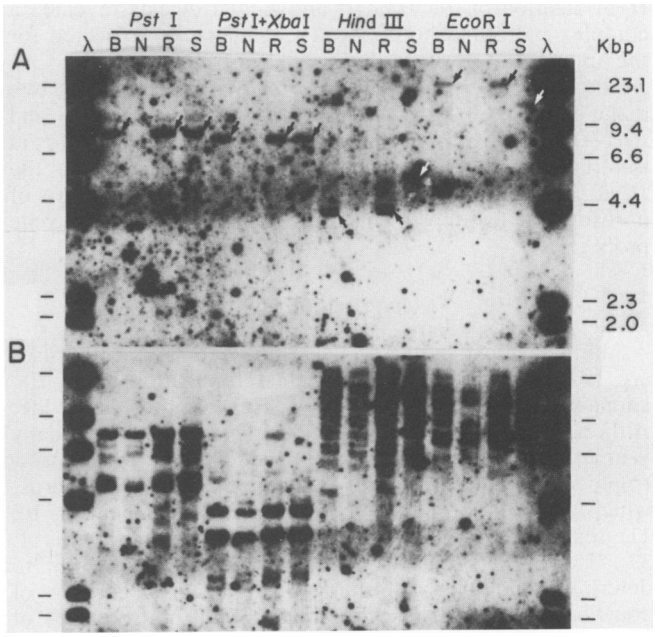

FIG. 4. Hybridization analysis of mouse chromosomal DNA. Mouse spleen DNA digested with the indicated restriction enzyme and subjected to electrophoresis in agarose gel was hybridized to N oligonucleotide (A) or B oligonucleotide (B). Abbreviations: B, BALB/c; N, NFS/N; R, RFM/Un; S, SJL. Arrows in panel A point to the single band in each lane.

generate a complex pattern of approximately 30 bands, whereas the ecotropic MuLV proviruses will yield zero, one, or possibly two bands. The size of the 5′ *Hin*dIII fragment of *Emv-1* of BALB/c (and predicted to be in RFM/Un) is approximately 4 kbp based on the cloned endogenous provirus map of Horowitz and Risser (15). The *Eco*RI fragment of *Emv-1* in RFM/Un is known to be 19 kbp (4) and is predicted to be the same in BALB/c. The 5′ *Hin*dIII or *Eco*RI fragments of *Emv-9* and *Emv-10* are not known.

The N-tropic oligonucleotide (Fig. 4A) detected an 8.2-kbp *Pst*I band and a 7.1-kbp *Pst*I-*Xba*I band in BALB/c, RFM/Un, and SJL DNA, as predicted for *Emv-1* and *Emv-10*, and no band in NSF/N, also as predicted since this strain lacks an endogenous ecotropic MuLV provirus. The failure to detect a fragment corresponding to *Emv-9* suggests that this locus has a deletion, consistent with its apparent defectiveness (18), or does not have the prototype N-tropic sequence. BALB/c and RFM/Un had a single 4.2-kbp *Hin*dIII band and a single 19-kbp *Eco*RI band as predicted; SJL had a single 5.4-kbp *Hin*dIII band and a single 13.0-kbp *Eco*RI band. It is assumed that this single fragment detected with each restriction enzyme is *Emv-10*, since the single fragment in the *Pst*I digest corresponded to the predicted *Emv-10* fragment (18). NFS/N had no detectable hybridization with the N-tropic oligonucleotide probe.

Hybridization of the *Pst*I and *Pst*I-*Xba*I digests with the B-tropic probe revealed two bands of relatively high intensity in all four strains of mice (Fig. 4B). These are the result of multiple copies of MuLV-related proviruses and represent the two major classes predicted to yield a 7.8-kbp or a 4.2-kbp fragment. Several other bands were common among two or more strains, and some unique bands were observed, due to xenotropic class proviruses or polymorphism of the *Pst*I (and *Xba*I) sites and deletions within certain MuLV-related proviruses. Double digestion with *Pst*I-*Xba*I revealed a similar result, simply shifted to smaller fragments. Hybridization of the *Hin*dIII and *Eco*RI digests revealed a complex pattern of unresolved fragments, as expected for the approximately 30 MuLV-related proviruses.

The *Ava*I *gag* probe (data not shown) detected the two major families of MuLV-related proviruses in the *Pst*I and *Pst*I-*Xba*I digests of all strains plus the ecotropic provirus in the three strains indicated above. Hybridization to the *Hin*dIII and *Eco*RI digests revealed a complex pattern of unresolvable bands, similar to those found with the B-tropic probe.

DISCUSSION

Although most inducible germ line ecotropic proviruses are N tropic, the selection against replication in $Fv\text{-}1^{b/b}$ animals is strong and B-tropic viruses emerge late in life. Although the concept has been in the literature for many years that B-tropic virus was a recombinant between N-tropic ecotropic virus and endogenous xenotropic virus, which was the donor for the B-tropism determinant (3, 10, 11), results from some studies appeared to contradict this (1, 37). The present study was undertaken in an attempt to determine the class of endogenous provirus capable of contributing the B-tropic determinant and the frequency of its occurrence in the mouse genome.

In recent years the structure and relationships of various endogenous proviral elements with specific ecotropic and mink cell cytopathic *env* sequences have become better understood (18, 29). It is apparent that many of the endogenous viral sequences of laboratory mice are nonecotropic yet distinct from xenotropic virus and are the nonecotropic parent for dual-tropic, mink cell-cytopathic viruses (6, 7, 19, 21, 27, 29, 35). Recently Stoye and Coffin (41) characterized a group of clones of endogenous proviruses of HRS/J mice. They distinguished three classes of nonecotropic proviruses and designated them as xenotropic, polytropic, and modified polytropic. The strain distribution of these three classes has been examined with class-specific oligonucleotide probes (42). There is considerable polymorphism among strains of mice for both the number and location of each of the proviral classes; however, the polytropic and modified polytropic provirus loci appear to be more conserved than the xenotropic loci.

We previously made a distinction among endogenous MuLV-related proviral clones based on the size of the *Pst*I-to-*Kpn*I fragment in the LTR (31). The large 600-bp fragment class is designated a, whereas the medium 550-bp fragment class is designated b. A small 370-bp fragment designated c is only observed in ecotropic and xenotropic MuLVs (27, 28, 31). It is interesting to note that pRFM6, pRFM16, and AL10 (31), which are all type b, resemble the polytropic proviruses described by Stoye and Coffin (41) by the absence of a characteristic *Hin*dIII site at 5.6 kbp. Clones pRFM1, pRFM9, and pRFM17, which are all type a, resemble the modified polytropic class by the presence of the characteristic *Hin*dIII site at 5.6 kbp. The *Hin*dIII site in pRFM17 is shifted to the left due to an undefined deletion. pRFM3 and pRFM11 have not been adequately mapped to assign them with respect to this characterization. From the work presented here it is suggested that in addition to providing *env* gene sequences for recombinant mink cell focus-forming viruses both the polytropic and modified polytropic proviral genomes could contribute the B-tropism-specific determinant to ecotropic virus. These MuLV-related (polytropic and modified polytropic) genomes have several other characteristics in common, including a glutamine tRNA primer binding site (rather than proline tRNA of ecotropic MuLV) and a 170 to 200-bp insertion sequence within the LTR (20, 27, 28, 31).

Our data indicate that clones derived from two replication competent xenotropic viruses do not hybridize to either the N- or the B-tropic oligonucleotide probe. BALB/c and AKR, the strains from which these two viruses were isolated, carry the same locus for inducible xenotropic virus, *Bxv-1* (23). One of two endogenous xenotropic proviruses from the HRS/J mouse does hybridize to the B-tropic probe. Thus, a subset of xenotropic proviruses is also capable of donating the B-tropism-specific determinant.

The analysis of mouse chromosomal DNA demonstrated that the N-tropic oligonucleotide is specific for the endogenous ecotropic provirus in the strains examined. This is the first endogenous ecotropic specific *gag* probe described and allows the detection of the 5′ *Hin*dIII fragment of *Emv-1* (4.2 kbp) predicted by Horowitz and Risser (15) and *Emv-10* (5.4 kbp). All four mouse strains had a similar copy number of sequences detected by the B-tropic oligonucleotide probe, apparently including some members of all three classes of nonecotropic proviruses described by Stoye and Coffin (41, 42).

Some apparently contradictory observations concerning the cryptic *Fv-1* tropism of xenotropic viruses and the origin of B tropism cannot be completely resolved. However, it is clear that the B-tropic determinant of WN1802B exists in many endogenous MuLV-related proviruses and is absent in some xenotropic MuLV genomes. The N-tropic determinant is also absent from the xenotropic proviruses examined;

therefore it is likely that some xenotropic MuLVs have neither *Fv-1* phenotype (i.e., are NB tropic), whereas others do, either inherently or acquired by recombination. Whether it is a polytropic or modified polytropic MuLV-related provirus or a xenotropic provirus that contributes the B-tropic determinant to B-tropic ecotropic MuLV probably depends on the specific tissues in which these sequences are expressed.

The Moloney NB oligonucleotide probe did not hybridize to any clone tested. The determinant for NB tropism is not precisely known. Previous *gag* p30 sequence comparisons have revealed many differences between N- or B-tropic and NB-tropic MuLVs, and regions other than amino acid residues 109 and 110 may be responsible for the NB-tropic phenotype (9, 30).

The results from hybridization with the 0.9-kbp *Ava*I probe indicate that ecotropic MuLV p30 is more closely related to xenotropic MuLV p30 than to endogenous MuLV-related provirus p30.

In summary, the N-tropism-specific oligonucleotide probe hybridizes to three other infectious N-tropic ecotropic MuLVs, is homologous to the published sequence of MuLV strain AKV (14), and hybridizes to three somatically acquired proviruses in $Fv\text{-}1^{n/n}$ RFM/Un mice (one of these three is infectious and N tropic [Table 1]). In mouse chromosomal DNA this probe detects the endogenous ecotropic provirus of *Emv-1* and *Emv-10*. The B-tropic probe hybridizes to the more abundant nonecotropic proviral sequences in the four mouse strains tested, to each of seven endogenous MuLV-related (polytropic and modified polytropic) provirus clones from the RFM/Un mouse, and to one of two endogenous xenotropic clones of the HRS/J mouse. Neither probe hybridizes to BALB:virus-2 or AKR xenotropic MuLV clones or the other endogenous xenotropic provirus of the HRS/J mouse. A probe for the homologous region of NB-tropic Moloney MuLV fails to hybridize to any clone tested. We suggest that there are many endogenous MuLV-related proviruses of the polytropic and modified polytropic classes as well as some, but not all, xenotropic proviruses from which the B-tropism determinant of WN10802B could be derived.

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