JOURNAL OF VIROLOGY, July 1987, p. 2225–2231
0022-538X/87/072225-07$02.00/0

Vol. 61, No. 7

Amphotropic Proviral Envelope Sequences Are Absent from the *Mus* Germ Line

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Received 29 January 1987/Accepted 8 April 1987

We derived an amphotropic murine leukemia virus (MuLV) type-specific probe for use in Southern blot hybridizations with cloned and genomic DNAs. A 133-base-pair *RsaI-RsaI* fragment from the 5′ *env* region of the amphotropic viral isolate 4070A was subcloned into M13mp18 and radiolabeled in vitro. The probe detected the proviral DNAs in mink cells infected with seven different amphotropic MuLV isolates. The probe did not cross hybridize with the DNAs of molecular clones of ecotropic, mink cell focus-forming, or xenotropic MuLVs; nor did it anneal to the proviral DNAs of four xenotropic or six mink cell focus-forming viral isolates grown in mink cells. DNAs of 12 inbred laboratory mouse strains and more than 15 different wild mouse species and subspecies were examined for the presence of endogenous amphotropic *env*-related fragments. Amphotropic *env*-related sequences were found only in the DNAs of wild mice trapped in southern California in an area previously shown to harbor mice producing infectious amphotropic virus. Restriction enzyme analyses of DNAs from these mice showed that amphotropic sequences were not present as germ line copies but were the result of congenital or horizontal infection or both in this population. The DNAs of 11 various mammalian and avian species, including both natural predators of mice and squabs from the farms with virus-positive mice, lacked amphotropic envelope-related sequences.

There are three general methods by which retroviruses can be transmitted from one animal to another: (i) horizontal transmission through infection by exogenous virus, (ii) vertical transmission from parent to progeny by congenital infection with exogenous virus released by the mother, or (iii) genetic transmission of proviral DNAs from either parent. It is well documented that ecotropic and xenotropic murine leukemia viruses (MuLVs) are genetically transmitted in inbred mice (2, 3, 8, 30, 31). Through the use of foster-nursing and breeding experiments, it was previously demonstrated (10) that amphotropic MuLVs are congenitally transmitted in some populations of wild California mice. These experiments did not resolve whether germ line copies are also transmitted. Liquid hybridization experiments with cDNA probes initially indicated that amphotropic-related sequences are also present in the *Mus* germ line (4); however, subsequent experiments suggested that amphotropic proviral sequences were not present in the limited populations of normal mice that were examined (1, 27). The absence of specific hybridization probes for amphotropic proviral genes has prevented any definitive identification of germ line copies.

Although molecularly cloned hybridization probes specific for the envelope gene of ecotropic MuLVs have been available since 1980 (3, 6), probes specific for either xenotropic or mink cell focus-forming (MCF)-related DNAs have only recently been derived (24). Here we report an amphotropic-specific probe reactive with DNAs of all tested members of the amphotropic class of MuLVs. We used this probe to show that amphotropic-specific *env*-related segments were not present in the DNAs of inbred laboratory mice, most wild mice, or in 11 other species. In individual mice obtained from infected populations of California wild mice, we found that amphotropic proviral sequences were present as infectious agents but were not integrated in the germ line. Thus, amphotropic MuLVs do not appear to be genetically transmitted even in populations in which they are endemic.

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MATERIALS AND METHODS

Mice. Wild mice (Table 1) were provided by Michael Potter, National Cancer Institute. Additional wild mice were trapped at Lake Casitas, Calif., by John Estes and provided by S. Rasheed, University of Southern California. S. Rasheed also supplied frozen liver from squabs from these same farms. Maryland pigeons were trapped in a barn in Boonsboro by Charles Shifler and South Carolina squab were obtained from Sutton Place Gourmet, Bethesda, Md.

Cells, viruses, and DNAs. Mink lung cells (16) were propagated and infected with 4070A (14), 1504A (14), CasBr-A (14), C2S-A, 15029 spl-A (14), Cas L1-A, and Cas E no. 2-A as previously described (14). C2S-A and Cas L1-A are amphotropic MuLVs isolated from normal spleens and lymphomatous lymph nodes, respectively, of wild mice which were trapped in the Lake Casitas area of southern California and kindly supplied by Murray Gardner, University of California at Davis. Cas E no. 2-A was isolated from primary cell cultures exposed to 5-iododeoxyuridine (20 μg/ml for 48 h) from embryos from the donor of isolate C2S-A. Mink lung cells producing AKR-L1 ecotropic MuLV introduced by phenotypic mixing, as well as mink cells infected with four xenotropic and six MCF MuLVs, have been individually referenced (24). DNA was prepared by using standard techniques from virus-infected mink cells (24) or from mouse liver DNAs (3, 18).

Restriction enzymes, gel electrophoresis, and DNA hybridization. Cellular DNAs cleaved by restriction enzymes were run in 0.4% agarose gels, transferred bidirectionally to nitrocellulose membranes, and hybridized as previously

TABLE 1. Wild mouse populations (*Mus* genus) examined for amphotropic MuLV envelope sequences

Subgenus	Species	Origin
Coelomys	*pahari*	Tak Province, Thailand
Pyromys	*saxicola*	Mysore, India
Mus	*cookii*	Loei, Tak Province, Thailand
	caroli	Chanburi Province, Thailand
	cervicolor cervicolor	Thailand
	spicelegus (formerly *hortulanus*)	Pancevo, Yugoslavia
	spretus	Puerto Real, Spain
	musculus musculus	Vejrumbro, Denmark
	musculus musculus	Skive, Denmark
	musculus musculus (CzI)	Morovia, Czechoslavakia
	musculus musculus (CzII)	Slovakia, Czechoslavakia
	musculus molossinus	Kyushu, Japan
	musculus castaneus	Thailand
	musculus domesticus (formerly *brevirostris*)	Azrou, Morocco
	musculus domesticus (formerly *praetextus*)	Erfound, Morocco
	musculus domesticus	Bouquet Canyon, Calif.
	musculus domesticus (Centreville Light)	Centreville, Md.
	musculus domesticus (J. J. Downs)	Ridgley, Md.
	musculus domesticus (Sanner's Farm)	Davidsonville, Md.
	musculus domesticus (Lewes)	Lewes, Del.
	musculus domesticus (formerly *poschiavinus*)	Tirano, Italy
	musculus domesticus	Zalende, Switzerland
	musculus (NYD)	Abu Rawash, Egypt

described (18). The 455-base-pair (bp) *Bgl*II-*Eco*RI *env*-specific segment of NFS-Th-1 xenotropic proviral DNA (p*Xenv* probe [2]) was nick translated to a specific activity of 10^8 cpm/μg (22). Filters that had been hybridized with the p*Xenv* probe were washed at 62°C as previously described (24), and filters that were incubated with the amphotropic-specific probe were washed in the identical manner, except that solutions were maintained at 50°C. Molecular weight markers included 3.1-kilobase (kb) *Bam*HI and 12.6-kb *Xho*I cleavage products of amphotropic MuLV 4070A cloned into pBR322 at the *Eco*RI site (7). The 3.1-kb *Bam*HI fragment results from cleavage at 4.1 kb in the amphotropic proviral DNA and at base 375 of pBR322. These fragments were mixed with xenotropic and MCF-related marker fragments (24; C. A. Kozak and R. R. O'Neill, submitted for publication), thereby providing documentation for the absence of cross hybridization in each experiment.

Construction of the amphotropic-specific hybridization probe. We have previously derived both xenotropic- and MCF-specific envelope probes (24) from the 5′ end of the envelope gene of xenotropic and MCF MuLVs. An analogous 133-bp *Rsa*I-*Rsa*I fragment of amphotropic MuLV 4070A is only 70% homologous to xenotropic MuLV sequences and 56% homologous to MCF-related sequences (Fig. 1). A 60-μg sample of cloned 4070A DNA (7) was digested with *Rsa*I and separated on a 10% acrylamide gel. The 130- to 140-bp region was removed from the gel, electroeluted, ethanol precipitated, and resuspended. One-fifth of the yield was ligated to 0.4 μg of *Hin*cII-cleaved and alkaline phosphatase-treated M13mp18 DNA. After transformation of *Escherichia coli* JM103 cells, 24 clear bacteriophage plaques were screened for the presence of the desired 133-bp insert by sizing single-stranded bacteriophage DNAs in agarose gels, followed by dideoxy sequencing (33).

Radiolabeled probes were made by using modifications of the M13 dideoxy sequencing method (33) as previously described (24), except that endonuclease digestion was performed only with *Eco*RI and the sample was heat denatured before the acrylamide preparatory gel was loaded. In this way a single-stranded probe was produced which could not self-anneal and thus could be reused in subsequent experiments. Samples containing 2×10^6 cpm in 15 ml were used for hybridizations with cloned proviral DNAs; 12×10^6 to 23×10^6 cpm in 15 ml was incubated with membranes containing restricted cellular DNAs. Kodak X-AR film was exposed at −70°C with intensifying screens for 40 min for cloned DNAs or for 1 to 3 days and then reexposed for 3 weeks for cellular DNAs.

RESULTS

Hybridization specificity of the cloned amphotropic *env* DNA fragment. The host range of murine retroviruses is determined by the ability of the envelope proteins to interact with specific cellular receptors. Although the *env* proteins of each class of MuLV interact with different cellular receptors (28), the *env* gene sequences of nonecotropic MuLVs are very similar to one another relative to the ecotropic sequences (5, 7, 23). Previously reported hybridization probes, such as the 455-bp p*Xenv* probe (2), cannot differentiate among amphotropic, xenotropic, and MCF MuLV sequences. However, a smaller segment of the p*Xenv* probe is xenotropic specific, and an analogous region of MCF247 is specific for MCF-related sequences (24). A segment of *env* DNA bounded by *Rsa*I sites from the nearly equivalent region of the amphotropic MuLV genome shows only 56% homology to MCF-related and 70% homology to xenotropic MuLV sequences (Fig. 1). With this degree of homology, one would expect this segment to hybridize only with amphotropic MuLV sequences under standard conditions of hybridization and washing.

The reactivity of the amphotropic *env* fragment cloned in M13mp18 was initially evaluated by hybridizing it to molecular clones of ecotropic, amphotropic, MCF, and xenotropic MuLVs. When filters were washed in 15 mM NaCl–1.5 mM sodium citrate–0.1% sodium dodecyl sulfate at 50°C or above, the amphotropic *env*-specific probe annealed specifically to the cloned amphotropic proviral DNA and failed to react with the other three proviral DNAs (Fig. 2C). The p*Xenv* probe reacted strongly with both the cloned MCF and xenotropic DNAs and weakly with the amphotropic proviral DNA even when membranes were washed in identical solutions at 62°C (Fig. 2B). Hybridization of pBR322 (p*Xenv* probe) sequences to a partial endonuclease digestion product of the pBR322-ecotropic MuLV recombinant resulted in the 16.4-kb band faintly visible in Fig. 2B, lane a.

The reactivity of the amphotropic *env* probe with various MuLV proviruses was also evaluated by Southern blot analyses of DNA from virus-infected mink cells. Since mink cells infected in tissue culture are polyclonal with respect to MuLV proviral integrations, an enzyme which cleaves within the viral genome on either side of the *env* region corresponding to the probes recruits internal proviral fragments from different integrations into a discrete band. The amphotropic-specific probe reacted strongly with all seven amphotropic proviral segments recruited by cleavage with

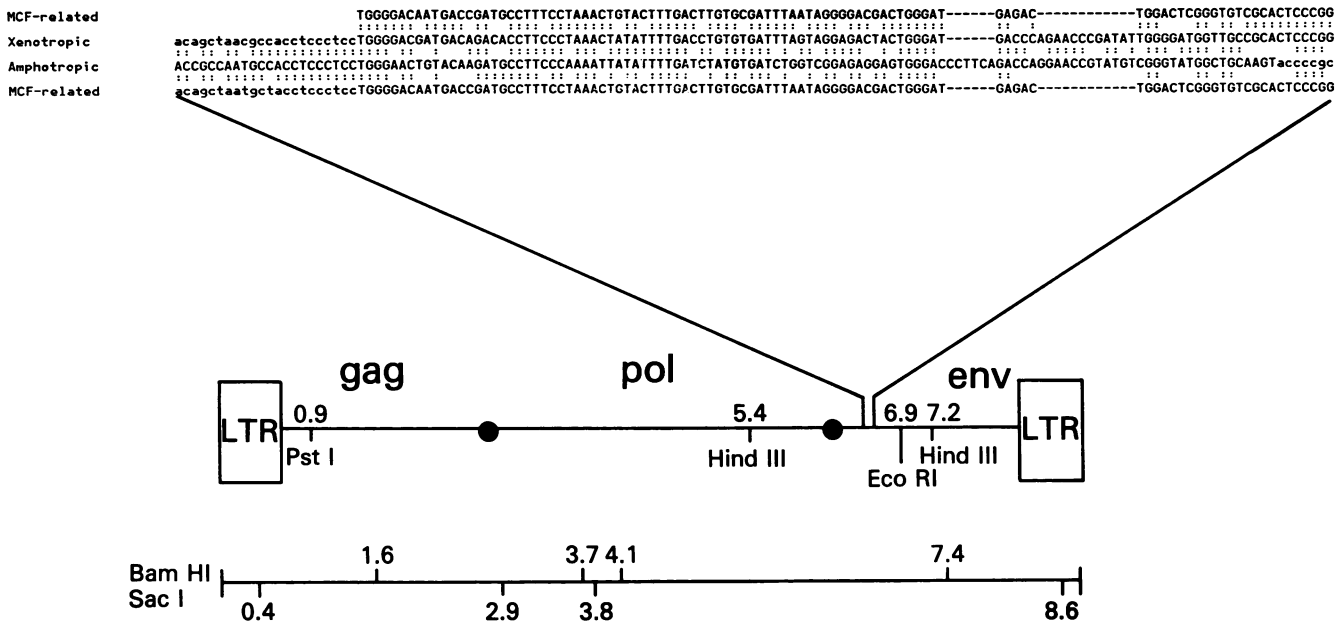

FIG. 1. Location and nucleotide sequence of the restriction fragment used as an amphotropic *env*-specific hybridization probe. The sequences of analogous probes specific for MCF-related MuLVs (100-bp *Bst*NI-*Xma*I [24]), for xenotropic MuLVs (112-bp *Bst*NI-*Xma*I [24]), and for amphotropic MuLVs (133-bp *Rsa*I-*Rsa*I) are aligned. Nucleotides represented by lowercase letters are present in proviral envelope sequences but were not subcloned during construction of type-specific hybridization probes. The restriction map of the molecular clone of amphotropic MuLV 4070A (7) indicates that *Pst*I and *Eco*RI cleaved only 5′ or 3′ to the envelope probe, wheras *Hin*dIII, *Bam*HI, and *Sac*I cleaved both 5′ and 3′ to the amphotropic-specific probe.

*Hin*dIII, *Bam*HI, or *Sac*I (Fig. 3A, B, and C). The sizes of the reactive 1.8-kb *Hin*dIII and 3.3-kb *Bam*HI fragments shown in Fig. 3 correspond to those predicted from previously reported restriction maps of 4070A, 1504A, 1740A, 1313A, and 292A (7). The use of *Sac*I revealed heterogeneity among amphotropic MuLVs (Fig. 3C and D). Different sizes of *Sac*I fragments (3.7 kb for CasBr-A, 15029 spl-A, and 1504A [lanes c, e, and b, respectively], 3.8 kb for Cas L1-A [lane g], and 4.8 kb for 4070A [lane a]) are consistent with a constant site at 3.8 kb and variable sites at 7.5, 7.6, and 8.6 kb as suggested for 1504A or 1313A, 1740A or 292A, and 4070A, respectively (7). C2S-A had a 6.7-kb *env* probe-reactive *Sac*I fragment (lane d), and Cas E no. 2-A had a 7.2-kb *Sac*I fragment (lane f) which could have resulted from cleavage at 0.4 and 7.6 kb in the proviral genome. Although *Pst*I cleaves most amphotropic MuLV DNAs only once, *Pst*I cleaves most nonamphotropic MuLV DNAs in each long terminal repeat as well as at additional sites (5). The amphotropic-specific probe did not anneal to *Pst*I-cleaved DNAs of mink cells infected by the ecotropic isolate AKR-L1, four xenotropic isolates (NZB-IU-6, NZB-6, AKR-6, and F/St Th-4), and six MCF MuLVs (MCF247, Friend MCF, AKR-13, Akv-1 C93, B10.F Thy-2, and 1504-M L2; data not shown).

Amphotropic *env*-related sequences in selected populations of California wild mice. Liver DNA from wild California mice trapped at squab farms near Lake Casitas was examined for the presence of viral *env* genes. According to previously reported restriction maps (7), restriction enzymes such as *Hin*dIII, *Bam*HI, and *Sac*I recruit internal amphotropic proviral fragments. Endonucleases such as *Eco*RI and *Pst*I produce provirus-cell junction fragments. Since ecotropic and xenotropic proviral DNAs are genetically transmitted from generation to generation, multiple discrete *env* probe-reactive bands are easily demonstrated with restric-

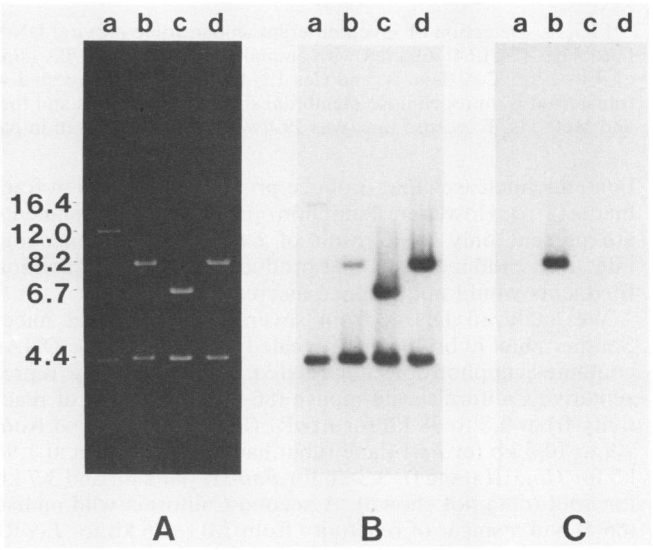

FIG. 2. Reactivity of the amphotropic-specific and pX*env* hybridization probes with ecotropic, amphotropic, MCF, and xenotropic MuLV cloned DNAs. The cloned proviral DNA (0.2 μg) of AKV623 (lane a; 21), amphotropic MuLV 4070A (lane b; 7), MCF247 (lane c; 19), and xenotropic MuLV NZB-IU-6 (lane d; 23) was released from pBR322 by digestion with the appropriate restriction endonucleases and run in 0.4% agarose gels. After the ethidium bromide-stained bands were visualized under UV transillumination (A), the DNA was blotted onto nitrocellulose filters and the membranes were hybridized to the pX*env* probe (B; 2) or to the amphotropic-specific probe (C) as described in Materials and Methods. The 4.4-kb bands in lanes a through d and the faint 16.4-kb band in lane a of panel B (pX*env* probe) reflect the hybridization of pBR322 sequences with full or partial cleavage products of cloned MuLV plasmid DNAs.

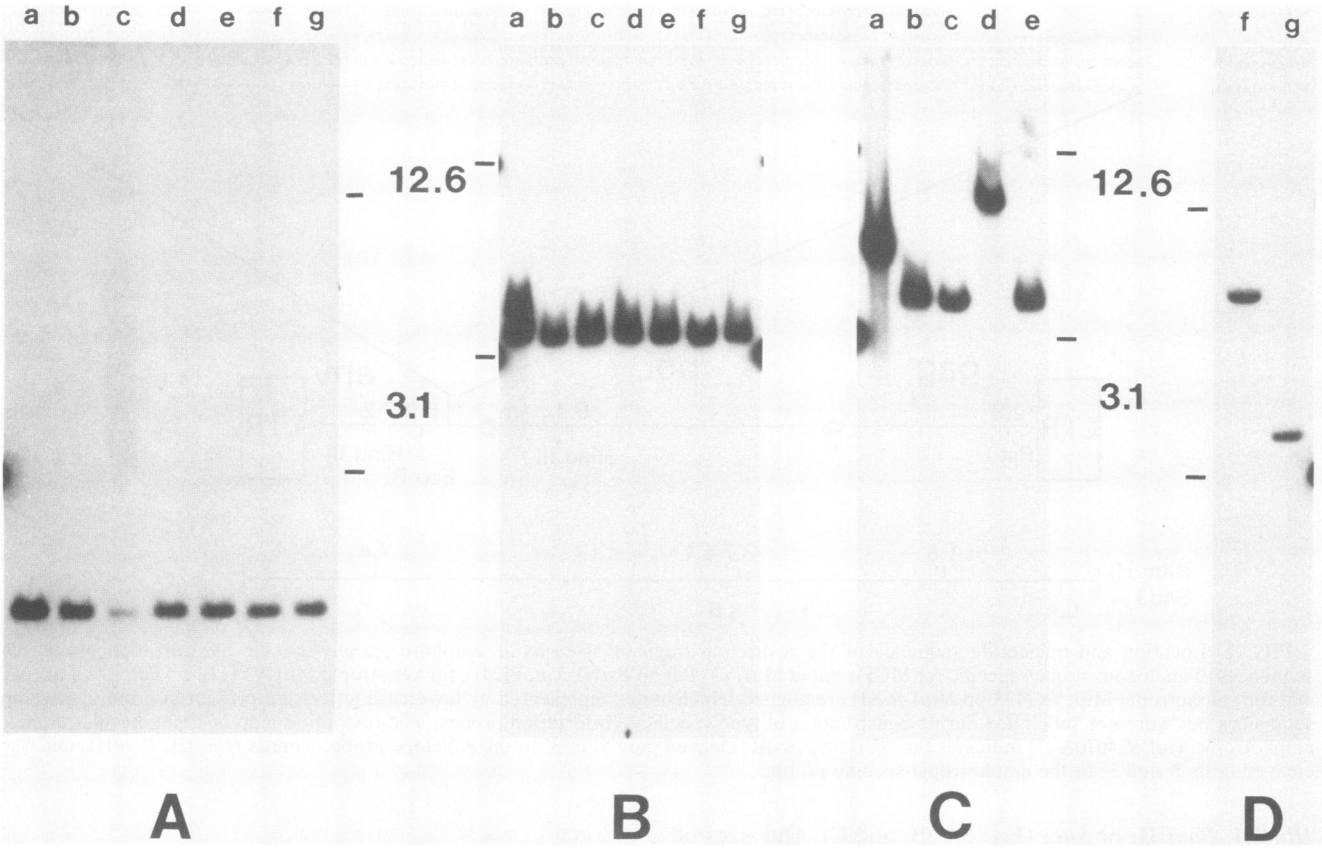

FIG. 3. Detection of seven different amphotropic proviral DNAs in mink cells infected in vitro. Samples (5 μg) of DNA from mink lung fibroblasts (CCL64) infected with amphotropic isolates 4070A (lane a), 1504A (lane b), CasBr-A (lane c), C2S-A (lane d), 15029 spl-A (lane e), Cas E no. 2-A (lane f), and Cas L1-A (lane g) were digested with *Hin*dIII (A), *Bam*HI (B), or *Sac*I (C and D). Restricted DNAs were transferred to nitrocellulose membranes from agarose gels and then hybridized to the amphotropic-specific probe as described in Materials and Methods. Exposure time was 19 h with flash and screen in panels A and D and 11 days without flash or screen in panels B and C.

tion endonucleases that produce provirus-cell junction fragments (2, 3). However, if amphotropic *env*-related fragments are present only as a result of exogenous or congenital infection, endonucleases that produce provirus-cell junction fragments would not produce discrete bands.

We analyzed DNAs from seven California wild mice. Southern blot hybridization revealed that five of these DNAs contained amphotropic *env*-related sequences. One representative California wild mouse (86-2) had a smear of reactivity from 9.8 to 38 kb for *Eco*RI (Fig. 4, lane h) and from 7.9 to 10.5 kb for *Pst*I (lane i) but had discrete bands at 1.95 kb for *Hin*dIII (lane f), 3.3 kb for *Bam*HI (lane g), and 3.7 kb for *Sac*I (data not shown). A second California wild mouse (86-4) had a smear of reactivity from 7.0 to 16 kb for *Eco*RI (lane l) and 7.9 to 12 kb for *Pst*I (lane m), and had 1.8-kb *Hin*dIII (lane j), 3.3-kb *Bam*HI (lane k), and 3.7-kb *Sac*I bands. DNAs from a third California wild mouse (86-3) required unusually long exposure times to demonstrate 1.8-kb *Hin*dIII, 3.3-kb *Bam*HI, and 4.8-kb *Sac*I bands. *Eco*RI or *Pst*I reactivity could not be observed after 3 weeks of exposure (data not shown). These data suggest that viral DNA is not present in these mice as germ line copies but that these mice harbor infectious amphotropic virus. The variation in hybridization intensity among these DNAs can most likely be attributed to variable numbers of infected cells present in each liver sample. Two of the five positive DNAs contained amphotropic sequences which produced the 1.95-kb *Hin*dIII fragment; the remaining three produced the smaller 1.8-kb fragment, indicating restriction enzyme polymorphisms among the amphotropic MuLVs and also indicating that each animal tested appeared to contain only a single type of amphotropic MuLV.

Lack of amphotropic *env*-specific segments in most mouse DNAs and 11 other species. The same methods used to demonstrate amphotropic proviral segments in infected mink cells and selected California wild mice were concurrently used to examine the DNAs of a wide variety of additional mice and other species. AKR/N and BALB/c mouse, California squab, rabbit, ground squirrel, human, and hamster DNAs digested with *Hin*dIII or *Bam*HI did not react with the amphotropic-specific envelope probe even after 3 weeks of autoradiographic exposure (Fig. 4). Eleven additional inbred laboratory strain or substrain DNAs (C3H/FgLw, 129/J, NZB/N, NFS, CBA/J, CBA/N, C57L/J, SWR/J, F/St, C57BL/6J, and AKR/J) were also negative when examined by using *Hin*dIII, *Bam*HI, *Sac*I, and *Eco*RI. The DNAs of various wild mouse populations (Table 1) and of the SC-1 cell line (derived from embryo cells from a pregnant female wild mouse trapped in Bouquet Canyon, Calif. [13]) were negative when examined by using *Hin*dIII, *Sac*I, *Bam*HI, *Kpn*I, and *Bgl*II (data not shown). In addition to the five nonmouse species in Fig. 4, we were unable to demonstrate amphotropic-related segments in the DNAs of mink, chicken, rat, cebus monkey, owl monkey, and cat after cleavage with *Hin*dIII, *Bam*HI, *Eco*RI, or *Pst*I.

Use of the amphotropic *env*-specific probe in the character-

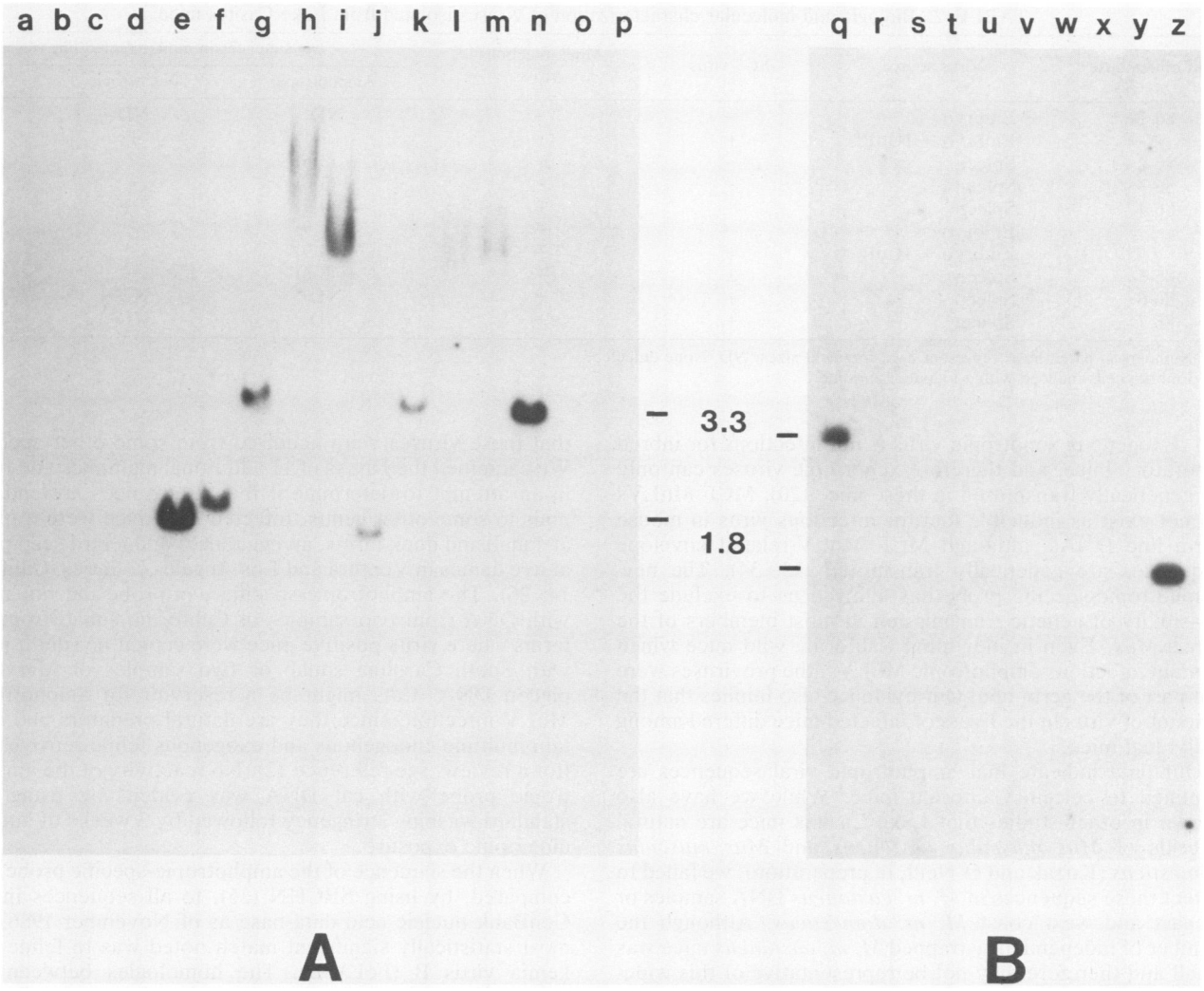

FIG. 4. Characterization of amphotropic *env*-specific proviral segments present in various DNAs. Lanes: a and b, 10 μg of DNA from an AKR/N inbred laboratory mouse digested with *Hin*dIII (a) or *Bam*HI (b); c and d, 10 μg of DNA from a BALB/c inbred laboratory mouse cleaved with *Hin*dIII (c) or *Bam*HI (d); f to i, 5 μg of DNA From California wild mouse 86-2 cut with *Hin*dIII (f), *Bam*HI (g), *Eco*RI (h), or *Pst*I (i); j to m, 5 μg of DNA from California wild mouse 86-4 cleaved with *Hin*dIII (j), *Bam*HI (k), *Eco*RI (l), or *Pst*I (m); o and p, 10 μg of DNA from California squab digested with *Hin*dIII (o) or *Bam*HI (p); r and s, 10 μg of rabbit DNA cleaved with *Bam*HI (r) or *Hin*dIII (s); t and u, 10 μg of ground squirrel DNA cut with *Bam*HI (t) or *Hin*dIII (u); v and w, 10 μg of human DNA digested with *Bam*HI (v) or *Hin*dIII (w); x and y, 10 μg of hamster DNA digested with *Bam*HI (x) or *Hin*dIII (y); e, z, n, and q, 5 μg of DNA from mink cells infected in vitro with 4070A and cleaved with *Hin*dIII (e and z) or *Bam*HI (n and q). These samples were run in 0.4% agarose gels, transferred to nitrocellulose membranes, and hybridized to the amphotropic *env*-specific probes as described in Materials and Methods. Exposure times were 3 days for panel A and 3 weeks for panel B with flash and screen.

ization of new MuLV isolates. Infectious MuLVs were isolated from all seven California wild mice from embryo cells, embryo cells induced with 5-iododeoxyuridine, or spleen cells cocultivated with mouse or mink cell lines (Table 2). Infectious ecotropic virus was isolated from five of the animals tested as detected by the XC test (32). Mink infectious virus was isolated from six of the seven mice as shown by focus formation on the mink $S^+ L^-$ cell line (25). DNAs were extracted from all infected mink lines and tested by Southern blot analyses for the presence of amphotropic, xenotropic, and MCF proviral sequences by using the appropriate *env*-specific probes (Table 2). One DNA preparation contained only xenotropic *env* sequences. All others tested contained amphotropic sequences alone or in conjunction with MCF MuLV *env* genes. Interestingly, the two mice with MCF-reactive fragments produced the same size *Sac*I fragment for both amphotropic and MCF *env* probes despite the fact that internal *Sac*I sites vary among endogenous MCF viruses (18). The infectious virus showed restriction enzyme polymorphisms compatible with that observed in the liver DNAs. Amphotropic *env*-reactive sequences were contained in 1.8- or 1.95-kb *Hin*dIII fragments and 3.7- or 4.8-kb *Sac*I fragments.

DISCUSSION

The derivation of specific hybridization probes for each of the four classes of MuLV has facilitated investigation of the different methods of mouse-to-mouse transmission used by these viruses. Ecotropic MuLVs are transmitted in inbred laboratory and wild mice through all three modes of transmission, i.e., horizontal, congenital, and genetic (8, 9, 11, 30,

TABLE 2. Biologic and molecular characterization of viruses isolated from Lake Casitas mice

California mouse	Virus source	XC+ virus	Mink infectious virus	*env* squences in mink infected lines[a]		
				Amphotropic	Xenotropic	MCF
86-1	Embryo	−	−	ND	ND	ND
	Embryo + IUdR[b]	+	+	−	+	−
86-2	Spleen	−	+	+ (3.6)	−	−
86-3	Spleen	+	+	+ (3.6)	−	+
86-4	Spleen	−	+	+ (4.9)	−	−
	Embryo	−	+	+ (4.9)	−	−
	Embryo + IUdR	+	+	+ (4.9)	−	−
86-5	Spleen	±	+	+ (3.6)	−	+
86-6	Spleen	+	+	ND	ND	ND
86-7	Spleen	+	+	ND	ND	ND

[a] Numbers in parenthesis represent *Sac*I fragment sizes. ND, None detected.
[b] Embryo cells induced with 5-iododeoxyuridine.

31). Exogenous xenotropic virus is not infectious for inbred laboratory mice, and therefore xenotropic viruses can only be genetically transmitted in these mice (20). MCF MuLVs do not exist as inducible loci for infectious virus in mouse germ line DNAs, although MCF MuLV-related envelope sequences are genetically transmitted (15, 34). The new amphotropic-specific probe has allowed us to exclude the possibility of genetic transmission in most members of the genus *Mus*. Even in individual California wild mice which contain infectious amphotropic MuLVs, the proviruses were not part of the germ line. Our evidence also implies that the amount of virus in the livers of infected mice differed among individual mice.

Our data indicate that amphotropic viral sequences are confined to certain California mice. While we have also shown in other studies that Lake Casitas mice are natural hybrids of *Mus musculus castaneus* and *Mus musculus domesticus* (Kozak and O'Neill, in preparation), we failed to detect these sequences in *M. m. castaneus* DNA samples or in east and west coast *M. m. domesticus*. Although the number of independently trapped *M. m. castaneus* mice was small and therefore may not be representative of this wide-ranging subspecies, these observations suggest that amphotropic MuLVs were introduced into the genus *Mus* in or around Lake Casitas.

While amphotropic MuLVs may be derived from other MuLV sequences in Lake Casitas mice, it is also possible that these viruses were acquired from some other species. We examined the DNAs of 11 additional mammals and birds in an attempt to determine if these sequences are endogenous to some other genus. Infected wild mice were trapped at squab and duck farms, an egg ranch, and a bird seed plant at five demes in Ventura and Los Angeles counties, Calif. (9, 14, 26). The amphotropic-specific *env* probe did not react with DNA from two samples of California squab from the farms where virus-positive mice were caught nor did it react with South Carolina squab or two samples of Maryland pigeon DNA. Cats might be a reservoir for amphotropic MuLV infection, since they are natural predators and contain multiple endogenous and exogenous feline retroviruses (for a review, see reference 12). No reactivity of the amphotropic probe with cat DNA was evident by using our standard washing stringency followed by 3 weeks of autoradiographic exposure.

When the sequence of the amphotropic-specific probe was compared, by using SRCHN (36), to all sequences in the GenBank nucleic acid data base as of November 1986, the most statistically significant match noted was to feline leukemia virus B (FeLV-B). The homologies between the nonecotropic MuLV family and strain A, B, and C FeLVs are shown in Table 3. Amphotropic MuLVs are approximately equidistant from xenotropic MuLVs and FeLV-B (Table 3). The FeLV A and C strains are 84% (with one insertion/deletion) related to one another in the region analogous to the amphotropic probe, and strains A and C are 58 and 59% (each with one insertion/deletion) homologous to strain B in this region. While this match is the most significant found, the disparity is still too great to detect cross-reactivity in Southern blots at the stringencies used; however, the similarities are close enough to suggest a possible evolutionary relationship.

The evolutionary origin of the amphotropic envelope gene is unknown. It was thought possible that a replicating virus could have recently transduced a normal cellular sequence for use as an envelope gene. If so, such a sequence must have diverged significantly after transduction, since the amphotropic *env*-specific probe did not detect any reactive sequences in most mouse DNAs or the DNAs of 11 other species. Use of the probe in low-stringency hybridizations to find ancestral sequences in mouse DNAs is precluded by the extensive number of endogenous xenotropic and MCF-related proviral segments.

The amphotropic-specific probe we have devised may be found useful in verifying that amphotropic virus-packaging systems for eucaryotic retroviral vectors are not releasing infectious amphotropic viruses. The specificity of the probe

TABLE 3. Nucleotide sequence homologies of specific envelope probes to various retroviral DNAs

Retroviral DNA[a]	% Homology (no. of gaps) with probe[b]:		
	Amphotropic	Xenotropic	MCF related
4070A amphotropic	100	66 (1)	48 (2)
NZB-IU-6 xenotropic	70 (1)	100	71 (1)
MCF247	56 (2)	71 (1)	100
GA-FeLV-B	69 (0)	59 (1)	56 (0)
FeLV-A	56 (1)	54 (0)	55 (0)
FeLV-C	59 (0)	53 (1)	53 (1)
AKV[c] ecotropic	23 (5)	28 (4)[d]	25 (5)

[a] The FeLV envelope sequences were obtained from Weiss et al. (35).
[b] The xenotropic and MCF-related specific probes were described by O'Neill et al. (24). Preliminary alignments were made by using NUCALN (36), with a K-tuple length of 3, window size of 20, and gap penalty of 7. The number of insertions or deletions (gaps) required in the alignment process is shown.
[c] The sequence of AKV was obtained from Herr (17).
[d] Alignment of nonecotropic to ecotropic *env* sequences followed the precedent of Fig. 3 in Repaske et al. (29).

could also be exploited for cloning putative oncogenes near the site of proviral integrations in amphotropic MuLV-induced tumors.

ACKNOWLEDGMENTS

We thank Roland Friedrich for distributing nucleotide sequencing data for the 4070A clone at the 1984 and 1985 Cold Spring Harbor RNA Tumor Virus Meetings, Stephen O'Brien for cat DNA, Debra Wetterskog for rabbit DNA, Roger Miller for ground squirrel DNA, Joan Austin for preparing mink cells infected by a wide variety of murine leukemia viruses, Susan Rosenfeld for typing the manuscript, and Sisir Chattopadhyay for providing the 4070A clone.

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