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Identification and cloning of endogenous retroviral sequences present in human DNA

(endogenous primate proviruses/retrovirus evolution/cloned human DNA/recombinant DNA)

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ABSTRACT **Using nonstringent annealing conditions and a 2.75-kilobase segment of cloned African green monkey DNA that specifically hybridizes to the proviruses of AKR ecotropic murine leukemia virus (MuLV) and baboon endogenous virus (BaEV) as a probe, we detected related sequences in three different preparations of human brain DNA fragments. The blot-hybridization pattern obtained with cleaved human DNA was similar to that previously reported for the interaction of MuLV cDNA and cleaved mouse DNA and suggested the presence of numerous copies of retrovirus-related sequences in the human genome. The labeled 2.75-kilobase fragment derived from cloned monkey DNA was used to screen a human DNA library in Charon 4A. One clone obtained hybridized to three contiguous MuLV-and BaEV-reactive fragments of the cloned monkey DNA and to multiple fragments of human DNA including a prominent 1.0-kilobase *Eco*RI fragment also present in the clone.**

In the mouse, certain endogenous type C proviruses have been shown to be vertically transmitted (1–3), have been mapped to specific chromosomal loci (4–6), and have the potential of being expressed as infectious ecotropic and xenotropic (7) murine leukemia viruses (MuLV). Comparison of results from nucleic acid hybridization experiments, which indicate the existence of numerous copies of endogenous mouse proviruses (8–10), with those derived from genetic and virus isolation studies, which point to a few defined inducible loci in the genomes of certain inbred mouse strains (4–6, 11), strongly suggests that the majority of the MuLV-reactive sequences in mouse DNA consist of incomplete viral DNA segments whose function is unknown.

Type C endogenous retroviruses have also been recovered from several different Old World monkeys including the baboon (12), stump-tail macaque (13), rhesus (14), and colobus (15). Solution hybridization experiments with baboon endogenous virus (BaEV) cDNA probes have demonstrated the existence of multiple copies of reactive type C proviruses in the chromosomal DNAs of the baboon and related simian species (16, 17). Although sequences hybridizing to labeled BaEV RNA have been reported in some human leukemic cellular DNAs (18), no endogenous type C retroviral DNA has been detected in preparations of normal human DNA (13, 15, 17) despite the use of nonstringent reaction conditions (19) or the sensitive blot-hybridization technique (20).

We recently reported the molecular cloning of a 17-kilobase (kb) segment from African green monkey (AGM) DNA which had nearly 5 kb of homology with AKR ecotropic MuLV DNA (21). In the present manuscript we show that the internal organization of the endogenous type C AGM and baboon proviruses, including 8 of 10 restriction enzyme cleavage sites, has been highly conserved. Furthermore, by using a radiolabeled 2.75-kb *Bam*HI fragment of the cloned AGM DNA, which specifically hybridizes to a similarly sized *Bam*HI fragment (22) of the BaEV provirus, hybridization to several bands in three different restricted preparations of human DNA was observed. Based on our ability to detect type C virus-related sequences in human DNA, we cloned an 11-kb segment from human DNA which hybridizes to AGM and mouse proviral DNAs.

MATERIALS AND METHODS

Preparation and Cleavage of Cellular DNA. High molecular weight AGM and rhesus monkey liver DNAs were purified from fresh tissue as described (23). Baboon cellular DNA was prepared from a cell line (CP 21) established from primary skin fibroblasts. DNA was isolated from three human brain specimens as outlined (24). Cellular DNAs were digested with restriction enzymes, electrophoresed in 0.6% horizontal agarose slab gels (23, 25), and transferred to nitrocellulose membranes as outlined by Southern (26).

Preparation of DNA Probes. *Bam*HI fragments of a recombinant Charon 4A phage (λ AGM-1) containing a 17.4-kb insert of AGM DNA with nearly 5 kb of homology with the AKR ecotropic provirus (21) were cloned in pBR322 as described (27). Recombinant plasmids (pAGM-B10, pAGM-B6, and pAGM-B9) containing the 0.9-, 2.75-, and 1.2-kb λ AGM-1 *Bam*HI fragments (designated 10, 6, and 9, respectively, in Fig. 1) were labeled by the nick-translation procedure (28) and had specific activities of $1.0–1.5 \times 10^8$ cpm/μg.

The BAB8-K isolate of BaEV (12) was prepared from chronically infected HT 1080 cells (29) by harvesting supernatant fluids at 3- to 4-hr intervals and purifying virus particles by centrifugation through 25–45% (wt/vol) sucrose gradients. Pelleted virus was deproteinized with phenol in the presence of 2% $NaDodSO_4$ and the resulting viral RNA was sedimented through a 5–20% (wt/vol) sucrose gradient containing 100 mM NaCl, 50 mM Tris·HCl (pH 7.4), 1.0 mM EDTA, and 0.2% $NaDodSO_4$ in an SW 41 rotor for 140 min at 35,000 rpm and 4°C. Single-stranded cDNA was synthesized by adding 1 μg of purified BaEV RNA to a reaction mixture (100 μl) containing 50 mM·Tris·HCl (pH 8.0), 50 mM KCl, 8 mM $MgCl_2$, 4 mM dithiothreitol, dCTP, dGTP, and dTTP at 1 mM each, 0.2 mCi of [α-^{32}P]dATP (400 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), 34.5 units of avian myeloblastosis virus polymerase, and 1 mg of fragmented calf thymus DNA. After a 2-hr incubation at 37°C, the reaction was stopped by the addition of $NaDodSO_4$ to 0.5%, EDTA to 20 mM, and self-digested Pronase to 0.5 mg/ml, and the mixture was incubated at 37°C for an additional 45 min. The reaction mixture was then extracted with an equal volume of phenol/chloroform, 2:1 (vol/vol), and the aqueous phase was treated with alkali (0.5 M NaOH, 3 hr, 37°C), neutralized, and passed through a Sephadex G-50 (coarse) column to remove unincorporated [α-^{32}P]dATP.

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Abbreviations: AGM, African green monkey; BaEV, baboon endogenous virus; kb, kilobase(s); LTR, long terminal repeat; MuLV, murine leukemia virus; NaCl/Cit, standard saline citrate.

Cloned Proviral DNA. pBR322 containing the AKR ecotropic provirus, previously cloned in Charon 4A (30) and inserted at the *Eco*RI and *Hin*dIII sites (pAKR 623), was a generous gift of Douglas Lowy (National Cancer Institute). A λ Charon 4A clone of an integrated BaEV provirus was kindly provided by Maurice Cohen (Frederick Cancer Research Center).

Nucleic Acid Hybridization. Conditions for "standard" blot-hybridization experiments have been described (25) and include a 30-min wash of nitrocellulose membranes with 0.1 × standard saline citrate (NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) (31). When nonstringent hybridization conditions were used, the reaction mixtures containing ^{32}P-labeled DNA, 6 × NaCl/Cit, 0.1% NaDodSO$_4$, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and fragmented denatured salmon sperm DNA (50 μg/ml) were incubated at 55°C for 16–36 hr as described (21). Subsequent to hybridization, the nitrocellulose membranes were extensively washed in 6 × NaCl/Cit containing 0.1% NaDodSO$_4$ at 55°C.

Molecular Cloning of Type C Viral DNA Sequences from Human DNA. A partial *Alu* I and *Hae* III human DNA library (32) in λ Charon 4A (33), kindly provided by Tom Maniatis (California Institute of Technology, Pasadena, CA), was screened for the presence of type C retrovirus sequences, by using ^{32}P-labeled recombinant plasmid (pAGM-B6) DNA consisting of pBR322 joined to the 2.75-kb *Bam*HI fragment 6 of λ AGM-1 DNA (see Fig. 1). The *in situ* plaque hybridization assay was carried out under the nonstringent hybridization conditions described above.

A 1.8-kb *Hin*dIII fragment of cloned human DNA (λ H51) (see Fig. 5) was inserted into the *Hin*dIII site of pBR322 as described (27).

RESULTS

Relationship of Cloned AGM DNA to a Known Type C Primate Virus. The molecular cloning of a 17.4-kb segment of AGM liver DNA which contains sequences that react with AKR ecotropic MuLV DNA has been accomplished (21). The MuLV-related portion (4.8 kb) of the AGM DNA clone (λ AGM-1) was localized to three *Bam*HI fragments [*Bam*HI λ AGM-1 DNA fragments 10, 6, and 9 (Fig. 1)] which hybridized in a reciprocal fashion to the 1.9- and 2.95-kb internal *Bam*HI fragments of the AKR ecotropic provirus. To characterize further the MuLV-reactive segment present in the cloned AGM DNA and to determine its relationship, if any, to a known type C primate retrovirus, λ AGM-1 DNA was digested with *Bam*HI and the fragments were electrophoresed through a 0.6% horizontal agarose slab gel, transferred to a nitrocellulose membrane, and hybridized to ^{32}P-labeled BaEV cDNA. The labeled BaEV cDNA reacted not only with MuLV-related 0.9-, 2.7- and 1.2-kb *Bam*HI cleavage products (*Bam*HI fragments 10, 6, and 9) of the cloned AGM DNA but also with the 8.8-kb fragment located immediately to the 5′ side of *Bam*HI fragment 10 and the 1.9-kb fragment to the 3′ side of *Bam*HI fragment 9 (Fig. 2).

To localize more precisely the polynucleotide sequence homology observed between the cloned AGM DNA and BaEV cDNA, a clone of integrated BaEV proviral DNA in λ Charon 4A was digested with *Xho* I and *Bam*HI. *Xho* I cleaves the BaEV provirus within the long terminal repeat (LTR), whereas *Bam*HI digestion of unintegrated BaEV DNA generates five fragments (22). After electrophoresis, the fragments were transferred to a nitrocellulose membrane, and individual 0.7 × 1.0 cm strips were exposed to various labeled DNA probes. Lane 2 of Fig. 2 shows that a BaEV cDNA probe hybridized to the expected 2.75-, 1.85-, 1.75-, 1.0-, and 0.92-kb *Bam*HI fragments of the BaEV provirus (22). Under these electrophoresis conditions, the 1.0- and 0.92-kb fragments cannot be resolved. The faintly

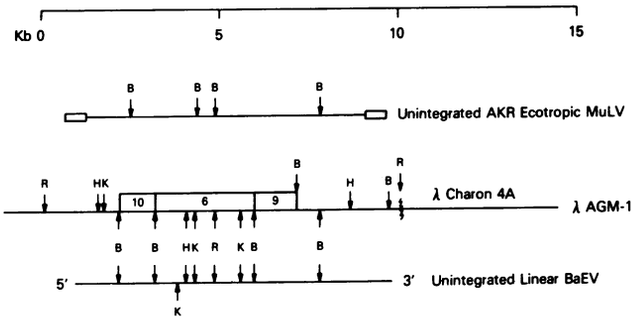

FIG. 1. The relationship of MuLV-reactive cloned AGM DNA to AKR ecotropic and BaEV proviruses. See ref. 21 for the cloning, restriction enzyme mapping, and the hybridization properties of λ AGM-1 DNA. The region of polynucleotide sequence homology shared by λ AGM DNA (middle line) and AKR ecotropic MuLV (top line) DNA was determined in a series of reciprocal hybridization experiments and shown to encompass *Bam*HI fragments 10, 6, and 9 of λ AGM-1 DNA (21). The cloned monkey DNA segment was aligned with the cleavage map of the BaEV provirus (22) (bottom line) on the basis of the results presented in Fig. 2. B, *Bam*HI; R, *Eco*RI; K, Kpn I; H, *Hin*dIII.

reactive 5.5-kb band represents a partial cleavage product. When the nitrocellulose strips were separately hybridized to ^{32}P-labeled cloned *Bam*HI fragments of λ AGM-1 DNA, hybridization to single *Xho* I/*Bam*HI-fragments of BaEV-cloned DNA was observed (Fig. 2, lanes 3–5). The hybridization of contiguous λ AGM-1 *Bam*HI fragments 10, 6, and 9 to the contiguous 1.0-, 2.75-, and 1.85-kb *Bam*HI fragments, respectively, of the BaEV provirus indicates the conservation of *Bam*HI restriction sites in the cloned AGM DNA and the endogenous baboon provirus. Comparison of *Bam*HI, *Kpn* I, *Eco*RI, and *Hin*dIII restriction sites present in a 5.7-kb segment of λ AGM-1 (21) and BaEV DNAs (22) indicates that 8 of 10 sites are conserved (Fig. 1).

Identification of Endogenous Type C Virus Sequences in Primate DNA. Sequences related to type C endogenous primate viruses have been detected in cellular DNAs prepared from primate and nonprimate tissues by solution-hybridization techniques (13–19). When labeled BaEV cDNA was used to monitor polynucleotide sequence homology in such experi-

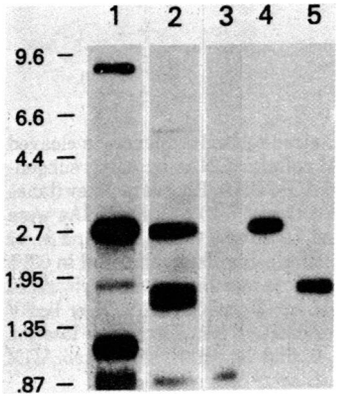

FIG. 2. Reciprocal hybridization reactions of BaEV and cloned AGM DNAs. λ AGM-1 DNA (0.3 μg per 1-cm lane) was digested with *Bam*HI; the fragments were electrophoresed in a 0.6% horizontal agarose slab gel and transferred to a nitrocellulose membrane. A 1-cm strip from the nitrocellulose sheet was exposed to ^{32}P-labeled BaEV cDNA under nonstringent hybridization conditions (lane 1). Nitrocellulose strips containing *Bam*HI/*Xho* I-digested λ Charon 4A–BaEV DNA (0.08 μg per 1-cm strip) were treated with ^{32}P-labeled (4 × 10^6 cpm) BaEV cDNA (lane 2), pAGM-B10 (lane 3), pAGM-B6 (lane 4), or pAGM-B9 (lane 5) DNAs under nonstringent hybridization conditions. The numbers indicate the size (kb) of a marker mixture consisting of λ DNA cleaved with *Hin*dIII or *Sma* I and φX174 DNA cleaved with *Hpa* II or *Hae* III.

ments, thermally stable hybrids were detected with rhesus and, to a lesser extent, with AGM DNAs (34, 17). To analyze nucleotide sequences related to type C endogenous viruses in Old World monkeys, cloned, subgenomic, and BaEV-reactive segments of λ AGM-1 DNA were nick-translated and hybridized to restricted preparations of rhesus, baboon, and AGM DNAs (Fig. 3 *A–C*). Sequences present in λ AGM-1 *Bam*HI fragments 6 and 9 (Fig. 1), which hybridize to segments of the unintegrated BaEV provirus that map 2.2–4.9 and 4.9–6.1 kb, respectively, from the 5′ terminus, are highly conserved in the three monkey DNAs as judged by the presence of multiple, discrete, and comigrating bands (Fig. 3 *B* and *A*). For example, four comigrating bands (1.2, 1.85, 2.8, and 3.8 kb) were detected in *Bam*HI-cleaved rhesus, baboon and AGM DNAs, which hybridized to ^{32}P-labeled pAGM-B9 DNA.

The labeled pAGM-B9 DNA, which contains the 1.2-kb *Bam*HI fragment 9 of the cloned AGM DNA, hybridized to a 1.85-kb *Bam*HI fragment in baboon cellular DNA [presumably corresponding to the 1.85-kb *Bam*HI fragment of unintegrated BaEV (Fig. 1)] as well as to a 1.2-kb *Bam*HI product of baboon DNA (Fig. 3*A*, lane 5), which comigrated with a fragment in *Bam*HI-restricted AGM DNA (Fig. 3*A*, lane 8). These 1.2-kb cellular DNA fragments comigrate with the *Bam*HI fragment 9 present in cloned monkey DNA. Fewer comigrating bands (e.g., a 3.7-kb *Bam*HI fragment) were observed when pAGM-B10 DNA was used as a hybridization probe and reactivity to baboon cellular DNA was markedly decreased (Fig. 3*C*). This probe, which contains the 0.9-kb *Bam*HI fragment 10 of λ AGM-1 DNA, hybridized to a single, similarly sized *Bam*HI fragment of the BaEV provirus that maps 1.25–2.2 kb from the 5′ terminus (22). ^{32}P-Labeled BaEV cDNA hybridized to cleavage products of the three monkey cellular DNA preparations (Fig. 3*D*), which comigrated with some of the restriction fragments hybridizing to pAGM-10, pAGM-6, and pAGM-9 probes (Fig. 3 *A–C*).

In light of the sensitivity of the blot-hybridization technique and the observed conservation of specific segments of type C retroviruses, DNAs prepared from three human brains were digested with *Eco*RI, *Bam*HI, and *Kpn* I; the fragments were electrophoresed in an agarose slab gel, transferred to a nitrocellulose membrane and hybridized to ^{32}P-labeled pAGM-B6 which contains the MuLV- and BaEV-reactive *Bam*HI fragment 6 (Fig. 1) of cloned monkey DNA. The autoradiogram presented in Fig. 4 shows the presence of multiple, discrete bands in each of the three human DNA samples. A prominent 1.0-kb *Eco*RI fragment and four or five comigrating *Bam*HI fragments were present in all of the human DNAs analyzed. No obvious conservation of restriction enzyme sites present in the endogenous proviruses of man or Old World monkeys was apparent (compare with Fig. 3*B*). *Bam*HI fragment 9 of λ AGM-1 DNA hybridized to the 1.0-kb *Eco*RI fragment present in the human brain DNA preparations (data not shown). No hybridization of *Bam*HI fragment 10 of cloned AGM DNA to the three human DNAs was observed (data not shown). The human DNAs used in this experiment were prepared several years ago prior to the time that work with retroviruses was initiated in our laboratory. These brain DNA preparations were isolated from patients with renal neoplasms and served as "normal" DNA controls for comparison with tumor DNA from the same individual (24).

Molecular Cloning of Type C Virus-Related Sequences from Normal Human DNA. The ability to detect discrete fragments of human DNA that were related to type C viral sequences present in λ AGM-1 DNA suggested that reactive sequences could be identified and cloned from a human DNA library (32) in λ Charon 4A (33). Of the several λ clones containing type C virus-related sequences initially identified by using ^{32}P-labeled pAGM-B6 DNA under nonstringent hybridization conditions, one (λ H51) contained an 11-kb DNA insert that hybridized to the three MuLV-related *Bam*HI fragments present in the cloned AGM DNA. A partial restriction map of λ H51 DNA and a "best-fit" positioning of reactive λ AGM-1 DNA sequences are presented in Fig. 5.

Although subgenomic segments of the AKR ecotropic provirus hybridized to restricted AGM cellular DNA under nonstringent hybridization conditions (21), no reaction was detected with preparations of cleaved human cellular DNA (data not shown). These experiments included use of labeled MuLV probes that map to the highly conserved central 5.0-kb region of the AKR provirus (see Fig. 1). To detect polynucleotide sequence homology between the type C virus-reactive sequences present in the cloned human DNA and cloned MuLV proviral

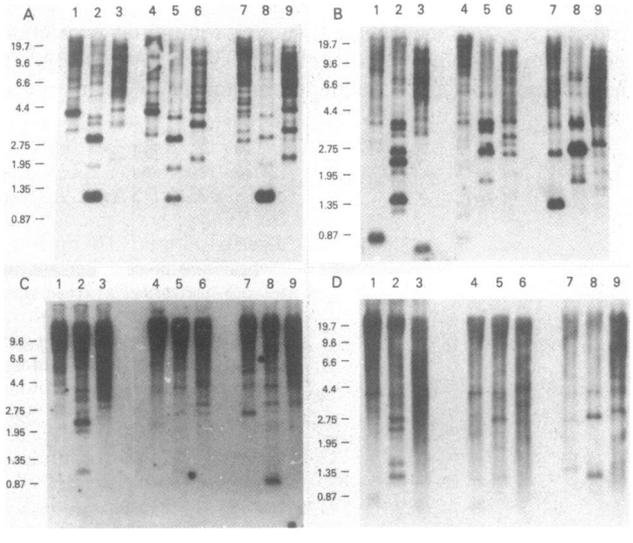

FIG. 3. Identification of type C virus-related sequences in cleaved rhesus monkey, baboon, and AGM cellular DNAs by using subgenomic probes derived from the cloned AGM DNA. Rhesus monkey (lanes 1–3), baboon (lanes 4–6), and AGM (lanes 7–9) cellular DNAs were digested with *Eco*RI (lanes 1, 4, and 7), *Bam*HI (lanes 2, 5, and 8), or *Kpn* I (lanes 3, 6, and 9). The fragments were electrophoresed in 0.6% agarose gels, transferred to nitrocellulose membranes, and hybridized to ^{32}P-labeled pAGM-B9 (*A*), pAGM-B6 (*B*), pAGM-B10 (*C*), or BaEV cDNA (*D*) under nonstringent hybridization conditions. The location of λ AGM-1 *Bam*HI fragments 9, 6, and 10 within the cloned AGM DNA is shown in Fig. 1.

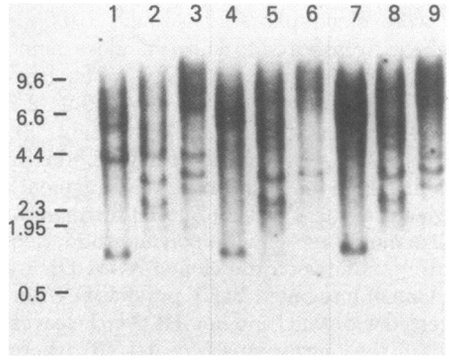

FIG. 4. Detection of type C virus-related sequences in human DNA by using a subgenomic probe derived from cloned AGM DNA. Cellular DNAs, prepared from three human brain samples (lanes 1–3, 4–6, and 7–9), were digested with *Eco*RI (lanes 1, 4, and 7), *Bam*HI (lanes 2, 5, and 8), and *Kpn* I (lanes 3, 6, and 9); the fragments were electrophoresed through 0.6% horizontal agarose slab gels, transferred to nitrocellulose membranes, and hybridized to ^{32}P-labeled pAGM-B6 DNA under nonstringent conditions.

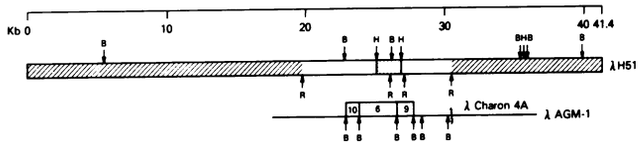

FIG. 5. Cleavage map of λ H51 DNA. Hatched segments within λ H51 DNA, position of λ Charon 4A DNA sequences; stippled region, position of the 1.8-kb internal *Hin*dIII fragment. λ AGM-1 DNA is positioned to show "best-fit" regions of polynucleotide sequence homology. R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII.

DNA, ^{32}P-labeled λ H51 DNA was hybridized to *Bam*HI-cleaved pAKR 623 DNA. Hybridization was observed to the internal 3.0- and 1.9-kb *Bam*HI fragments of the cloned AKR provirus as well as to a 6.6-kb fragment containing viral, cellular, and plasmid DNA sequences under nonstringent hybridization conditions (Fig. 6, lane 2). When the nitrocellulose strips were treated under the stringent conditions described by Jeffreys and Flavell (31), which included a 30-min wash in 0.1 × NaCl/Cit, reactivity was observed with the 3.0-kb internal *Bam*HI fragment of AKR MuLV DNA as well as with the 6.6-kb fragment (Fig. 6, lane 3). The hybridization of labeled λ H51 DNA to the 6.6-kb *Bam*HI fragment most likely reflects homology between pBR322 and λ phage DNAs because no hybridization of the ^{32}P-labeled *Bam*HI/*Xba* I (at 7.1–7.7 kb) or *Bam*HI/*Kpn* I (at 7.1–8.7 kb) fragment of the AKR ecotropic provirus to λ H51 DNA was observed (data not shown).

To ascertain the relative abundance and restriction patterns of human chromosomal DNA segments related to λ H51 DNA, a 1.8-kb *Hin*dIII fragment of the cloned human DNA, which reacted strongly with the highly conserved internal *Bam*HI fragment 6 of λ AGM-1 DNA (Fig. 5), was inserted into the *Hin*dIII site of pBR322 DNA, nick-translated, and used in a series of blot-hybridization experiments. This labeled recombinant plasmid (pH51-H1) DNA hybridized to many of the same fragments of restricted human DNA (Fig. 7) which had hybridized to ^{32}P-labeled pAGM-B6 DNA including the prominent 1.0-kb *Eco*RI cleavage product (Fig. 4). This strongly reacting *Eco*RI fragment is identical in size to the 1.0-kb *Eco*RI segment present in λ H51 DNA which overlaps the 1.8-kb *Hin*dIII segment included in the pH51-H1 probe (Fig. 5). At least six comigrating *Bam*HI fragments, which ranged in size from 1.9 to 5.7 kb and contained sequences homologous to pH51-H1 DNA, were present in the three human brain DNA preparations (Fig. 7, lanes 2, 5, and 8). One of these bands was identical in size (3.3 kb) to a *Bam*HI fragment present in λ H51 DNA that hybridized to the pH51-H1 probe (Fig. 5). *Hin*dIII digestion of human DNA generated 18 discrete bands that hybridized to pH51-H1 DNA, including a prominent 1.8-kb fragment that presumably was identical to the 1.8-kb *Hin*dIII insert in pH51-H1 (data not shown).

DISCUSSION

A 17-kb AGM DNA segment that has nearly 5 kb of homology with AKR ecotropic MuLV DNA has been studied (21). This region of homology was mapped to contiguous fragments between 1.9 and 7.0 kb (from the 5′ terminus) on the MuLV provirus; it encodes the M_r 30,000 and 10,000 viral structural proteins and most of the reverse transcriptase. The study of the cloned AGM DNA described in this paper reveals extensive homology to cloned type C BaEV DNA. Annealing of labeled BaEV cDNA was observed to MuLV-reactive segments of the cloned AGM DNA as well as to restriction fragments thought to contain putative LTR sequences. An unexpected finding was the hybridization of individual labeled MuLV-related *Bam*HI fragments from the cloned AGM DNA to separate (and corresponding) *Bam*HI fragments of the BaEV provirus. Closer examination of the location of *Bam*HI, *Eco*RI, *Kpn* I, and *Hin*dIII sites in the MuLV-reactive portion of the cloned AGM DNA (21) and in the unintegrated BaEV provirus (22) indicated that 8 of 10, spanning 5.5 kb of DNA, were conserved. This conservation of endogenous baboon and AGM proviral DNA organization is even more remarkable in view of the divergence of the baboon and AGM lineage from a common simian ancestor 7–8 and 12 million years ago, respectively (17).

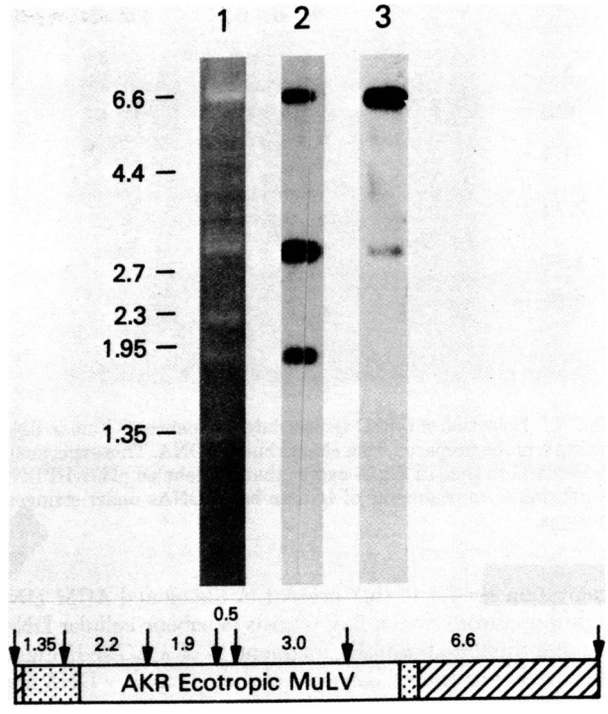

FIG. 6. Hybridization of cloned human DNA to cloned AKR ecotropic proviral DNA. *Bam*HI fragments of pAKR 623 DNA (0.35 μg/per lane) were electrophoresed and transferred to a nitrocellulose membrane as in Fig. 2. Strips (1 cm) from the membrane were exposed to ^{32}P-labeled λ H51 DNA under nonstringent (lane 2) and stringent (lane 3) conditions. The strips shown in lanes 2 and 3 were exposed at −70°C for 1 and 5 days, respectively. Lane 1 is a photograph of an ethidium bromide-stained gel of *Bam*HI-digested pAKR 623 DNA. The location of *Bam*HI sites in pAKR 623 is shown diagrammatically at the bottom of the figure. Hatched areas, pBR322 DNA; stippled regions, mouse DNA sequences that flank the cloned AKR ecotropic provirus.

When specific segments of AGM-cloned DNA were used as hybridization probes to assess polynucleotide homology among the endogenous baboon, rhesus monkey, and AGM proviruses, fragments mapping 2.25–6.1 kb from the putative 5′ terminus exhibited the greatest reactivity. The conservation of this 4-kb proviral DNA segment which encodes the M_r 30,000 and 10,000 proteins and reverse transcriptase is consistent with the reported crossreactivity of these proteins with analogues specified by different type C retroviruses in competition radioimmunoassays (35, 36). It is interesting to note the preservation of common *Bam*HI sites within endogenous baboon, rhesus, and AGM proviruses and the relative paucity of conserved *Kpn* I sites. In this regard, integrated and unintegrated BaEV proviral DNA contains a single 1.85-kb *Bam*HI fragment (at 5.0–6.8 kb from the 5′ terminus) (37), which corresponds to the 1.2- and 0.65-kb *Bam*HI fragments in the cloned AGM DNA (Fig. 1). When the labeled 1.2-kb *Bam*HI fragment of λ AGM-1 DNA was hybridized to *Bam*HI-digested baboon genomic DNA, reactivity with several bands including 1.85- and 1.20-kb fragments was observed. This finding suggests that a provirus with an internal

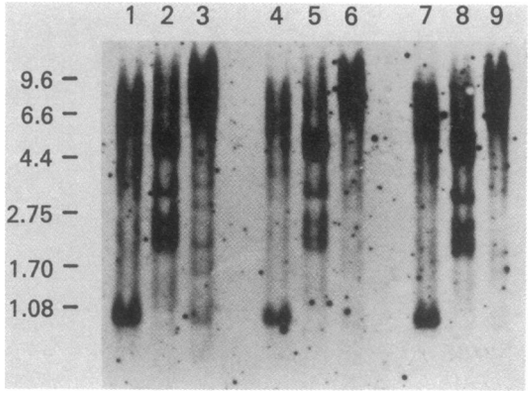

FIG. 7. Detection of type C virus-related sequences in human DNA by using a probe prepared from cloned human DNA. This experiment was identical to that in Fig. 4 except that ^{32}P-labeled pH51-HI DNA was hybridized to fragments of human brain DNAs under stringent conditions.

organization similar to that present in the cloned AGM DNA but different from cloned BaEV exists in baboon cellular DNA. Although the 0.9-kb *Bam*HI fragment 10 of λ AGM-1 (Fig. 1) hybridized to restricted baboon and rhesus monkey DNA fragments, the presence of fewer, less-discrete, and noncomigrating reactive cleavage products suggests significantly less conservation in the corresponding portions (1.25–2.25 kb from the 5′ terminus) of endogenous simian proviruses.

The hybridization of the MuLV-reactive *Bam*HI fragment 6 of λ AGM-1 DNA to three different human brain DNAs demonstrates the presence of endogenous type C virus-related sequences in normal human tissue. Cloned subgenomic segments of MuLV proviral DNA failed to hybridize to human DNA fragments under various conditions. The detection of multiple discrete bands in the three human DNA preparations was facilitated by using a cloned, highly conserved, radiolabeled segment of AGM "proviral" DNA in blot-hybridization reactions carried out under nonstringent conditions. Although the DNA probe used to identify type C virus-related sequences in human cellular DNA was derived from a clone of AGM DNA, our results should not be interpreted as indicating that the endogenous viral genomes present in the AGM are more closely related to those in man than the virogenes present in other Old World monkeys. In fact, the conservation of several restriction endonuclease cleavage sites in the BaEV provirus and the cloned AGM DNA, including the two generating the 2.75-kb *Bam*HI fragment which specifically hybridized to human DNA, suggests that this segment of the baboon virus would react with human DNA. *Bam*HI fragments 10 and 9 of cloned AGM DNA, which map to the 5′ and 3′ sides of the reactive *Bam*HI fragment 6, respectively, hybridized only weakly to restricted human DNA. The molecular cloning of a human DNA segment which hybridized to the internal 1.9- and 3.0-kb *Bam*HI fragments of cloned AKR ecotropic proviral DNA provides further evidence for the existence of a highly conserved region in mammalian type C proviruses.

The presence of several bands in human DNA that hybridize to an MuLV-reactive fragment of cloned AGM DNA is similar to the patterns observed after hybridization of retroviral cDNA probes to cleaved cellular DNAs. Because the radiolabeled DNA probes used to identify type C viral DNA sequences in human DNA were derived from internal proviral DNA fragments, the detection of multiple, comigrating *Bam*HI fragments in each of the human brain preparations indicates the presence of related proviruses that differ from one another by the location *Bam*HI cleavage sites. A similar pattern has been observed when specific segments of MuLV proviruses are hybridized to cleaved mouse DNA (unpublished data).

To date, we have observed no differences in the blot-hybridization patterns of five different human DNAs, including two preparations of leukemic spleen DNA digested with *Eco*RI, *Bam*HI, *Kpn* I, *Hin*dIII, *Hpa* I, *Xho* I, *Pst* I, or *Bgl* II. This may reflect the use of two labeled DNA probes which map to the highly conserved region of MuLV and BaEV proviruses encoding reverse transcriptase.

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