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Molecular Genetic Characterization of the RD-114 Gene Family of Endogenous Feline Retroviral Sequences

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RD-114 is a replication-competent, xenotropic retrovirus which is homologous to a family of moderately repetitive DNA sequences present at ca. 20 copies in the normal cellular genome of domestic cats. To examine the extent and character of genomic divergence of the RD-114 gene family as well as to assess their positional association within the cat genome, we have prepared a series of molecular clones of endogenous RD-114 DNA segments from a genomic library of cat cellular DNA. Their restriction endonuclease maps were compared with each other as well as to that of the prototype-inducible RD-114 which was molecularly cloned from a chronically infected human cell line. The endogenous sequences analyzed were similar to each other in that they were colinear with RD-114 proviral DNA, were bounded by long terminal redundancies, and conserved many restriction sites in the *gag* and *pol* regions. However, the *env* regions of many of the sequences examined were substantially deleted. Several of the endogenous RD-114 genomes contained a novel envelope sequence which was unrelated to the *env* gene of the prototype RD-114 *env* gene but which, like RD-114 and endogenous feline leukemia virus provirus, was found only in species of the genus *Felis*, and not in other closely related *Felidae* genera. The endogenous RD-114 sequences each had a distinct cellular flank which indicates that these sequences are not tandem but dispersed nonspecifically throughout the genome. Southern analysis of cat cellular DNA confirmed the conclusions about conserved restriction sites in endogenous sequences and indicated that a single locus may be responsible for the production of the major inducible form of RD-114.

The chromosomal DNAs of a variety of vertebrate species have been shown to harbor moderately repetitive gene families that are related to the genomes of retroviruses (40). Three distinct retroviral gene families have been detected in genomic DNA from the domestic cat, *Felis catus*. The first consists of a group related to the exogenous feline leukemia virus (FeLV) (5). The endogenous FeLV sequences are generally thought to be defective since many of the sequences are substantially deleted (35) and also because FeLV is yet to be isolated from normal feline tissues. The second feline retrovirus family is distantly related to the MAC-1 primate retrovirus genome and also has not been isolated as a replicating virus from cat tissues (8). The third family includes a sequence (or sequences) capable of coding for a replication-competent, C-type retrovirus, RD-114. RD-114 was originally isolated from human rhabdomyosarcoma (RD) cells which had been passaged in fetal kittens (22). Subsequent in vitro isolations of RD-114 from cultured feline cells by cocultivation with heterologous cells (13, 20, 38) demonstrated the inducibility of this xenotropic feline virus. Liquid DNA hybridization experiments in several laboratories (1, 16, 24, 30, 34) showed that the genomic DNA of normal cat tissues contained multiple copies of RD-114-related sequences. Cat cells and tissues seldom produce RD-114 virions; however, many tumors and several apparently normal embryonic tissues have been shown to transcribe RD-114 RNA and to produce *gag*-specific antigens (15, 25, 33). Cellular sequences homologous to RD-114 were detected in some but not all species of the cat family *Felidae*, indicating a precipitous introduction of the virus into the felid germ line (6). A striking degree of DNA sequence homology to baboon endogenous virus (BaEV) further suggested that the feline sequences resulted from a cross species infection by an ancestor of the primate retrovirus (6, 7, 12).

We report here a molecular genetic analysis of the RD-114 family of domestic cats. Molecular clones of infectious RD-114 were derived from a gene library of infected human cells. A group of endogenous feline RD-114 genomes was likewise cloned, and the restriction maps were compared with each other and with that of the inducible virus. Endogenous proviruses were each bounded by long terminal redundancies (LTRs) and by distinct flanking cellular sequences, suggesting nontandem and nonspecific integration. The endogenous sequences exhibited substantial sequence conservation within the *gag* and *pol* genes but were characterized by deletions and substitutions within the *env* region. The endogenous sequences analyzed contained *env* information unrelated to that of replication-competent retrovirus and may have been derived from a recombinant virus. The combined results of analyses of molecular clones of exogenous and endogenous RD-114 sequences indicated that the virogene family consists of ca. 20 members, most of which have undergone substantive divergence away from virulence with only 1 or 2 replication-competent loci remaining.

MATERIALS AND METHODS

Unintegrated viral DNA. RD-114 retrovirus was obtained from tissue culture fluid of MXC, a chronically infected human cell line (22). Unintegrated viral DNA was isolated from acutely infected human RD cells 24 to 48 h after infection by the method of Hirt (17).

Viral RNA. RD-114 virions were collected by centrifugation of filtered (0.45 μm), 12-h harvests of culture medium from MXC cells and double banding on sucrose gradients as previously described (3). After lysis and phenol extraction of virion preparations, viral RNA banding in the 70S region on 15 to 30% sucrose gradients was collected and concentrated by ethanol precipitation.

Radiolabeled probes and hybridization. cDNA probes were

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synthesized from an RD-114 viral RNA template in an exogenous reaction (37). Nick-translated probes were prepared from double-stranded DNAs by the method of Rigby et al. (32). Restriction endonucleases were obtained from Bethesda Research Laboratory or New England Biolabs. DNA was incubated for 2 to 4 h with >2 U of enzyme per μg of DNA in the buffer suggested by the manufacturer. In the case of cellular DNAs, the extent of digestion was monitored by the addition of 1 μg of adenovirus-2 DNA. Gel electrophoresis and transfer to nitrocellulose filters were accomplished by the method of Southern (36). Hybridizations were carried out at 42°C in a preheated mixture containing 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× Denhardt solution, 20 mM sodium phosphate, 50% deionized formamide, 100 μg of sheared calf thymus DNA per ml, 10% dextran sulfate, and 0.5×10^6 cpm of denatured radiolabeled probe per ml. After 24 to 48 h of incubation, filters were washed for 1 h in 1× Denhardt solution plus 2× SSC at room temperature, followed by three washes of 20 min each in 0.2× SSC–0.1% sodium dodecyl sulfate at 50°C. The filters were rinsed in 0.2× SSC, blotted dry, and sealed in cassettes with Kodak X-Omat XR-5 film at −70°C.

Construction of DNA clone libraries. High-molecular-weight, cellular DNA isolated from kidneys of domestic cats or from RD-114-infected human cells was partially digested with *Eco*RI, and the resulting fragments were fractionated on sucrose density gradients. DNA fragments of 10 kilobases (kb) or greater were pooled and concentrated under ethanol, suspended and ligated with purified *Eco*RI "arms" of bacteriophage λ WES:λBgt (19) under standard conditions, and packaged by the method of Hohn and Murray (18). Packaged phage were infected into the *Escherichia coli* strain LE 392 for titration. These procedures typically produced phage titers of ca. 10^6 PFU per μg of DNA in the ligation reaction. Approximately 85% of all plaques hybridized to ^{32}P-labeled cat cellular DNA. Recombinant libraries were screened with specific radiolabeled probes by the filter hybridization procedure of Benton and Davis (4).

RESULTS

Derivation of RD-114 restriction map and comparison with map of BaEV. Human RD cells were infected with RD-114, and extrachromosomal DNA was extracted 24 to 48 h later by the procedure of Hirt (17). The uncleaved viral DNA consisted of a predominant linear species of 8.8 kb and a minor species which represented a covalently closed, circular form. A map of recognition sites for 10 restriction enzymes was derived by Southern analysis of the products of restriction digests of unintegrated RD-114 DNA and confirmed with molecular clones of integrated RD-114 which were isolated from a library of infected human cell DNA (Fig. 1). The 5′ to 3′ orientation was determined by reciprocal Southern hybridization experiments with subclones of BaEV and of RD-114, as well as by homology of their restriction maps (Fig. 1; see below).

To determine the extent and character of divergence between BaEV and RD-114, we have aligned the RD-114 restriction map with that of an inducible form of BaEV (Fig. 1). Recognition sites for eight of the restriction endonucleases used in this study have been mapped in BaEV as well (2, 11). With the formula for similarity of Nei and Li (23), S = 2 × (number of common sites)/(number of sites in sequence a + number of sites in sequence b), in which "a" and "b" denote the two sequences being examined, the similarity between the replication-competent forms of RD-114 and BaEV is 18/32 = 56%. The conserved sites were distributed over the full length of the viral genomes; however, as reported by Cohen et al. (12), the homology detected by hybridization was reduced near the 3′-end of the viral genome.

Molecular cloning of exogenously integrated and endogenous RD-114 proviruses. A clone library was constructed from the DNA of human RD cells which were chronically infected with RD-114 and producing virus (22). Restriction maps of two RD-114 clones (Rex.2a and Rex.9) and derivative probe fragments used in this study are shown in Fig. 2. Clone Rex.2a contained 3.9 kilobase pairs of sequence from the 5′ half of the virus plus 2.6 kb of flanking human DNA. An overlapping clone, Rex.9, contained the entire virus except 700 base pairs from the 5′ end of RD-114 plus 5.0 kb of flanking human DNA. Derivative probe fragments used in this study are indicated in Fig. 2.

To isolate endogenous RD-114 sequences for detailed molecular analysis, we constructed a clone library by ligating the products of a partial *Eco*RI digest of kidney DNA from a domestic cat into the bacteriophage lambda cloning

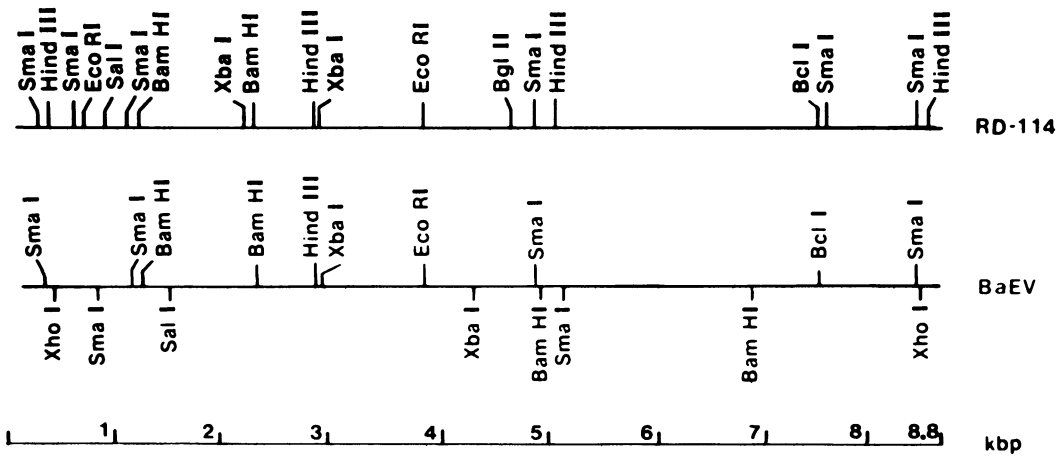

FIG. 1. Comparison of the physical maps of restriction endonuclease recognition sites in the RD-114 and BaEV genomes. BaEV map is from Battula and Todaro (2) and Cohen et al. (10). Restriction sites shared between the two viruses are shown above the BaEV map. An unexpected 7.9-kb fragment was visualized after EcoRI digestion. This fragment is a modified RD-114 genome which is produced de novo during viral replication due to the loss of the centrally located *Eco*RI restriction site (J. Casey and J. Mullins, personal communication).

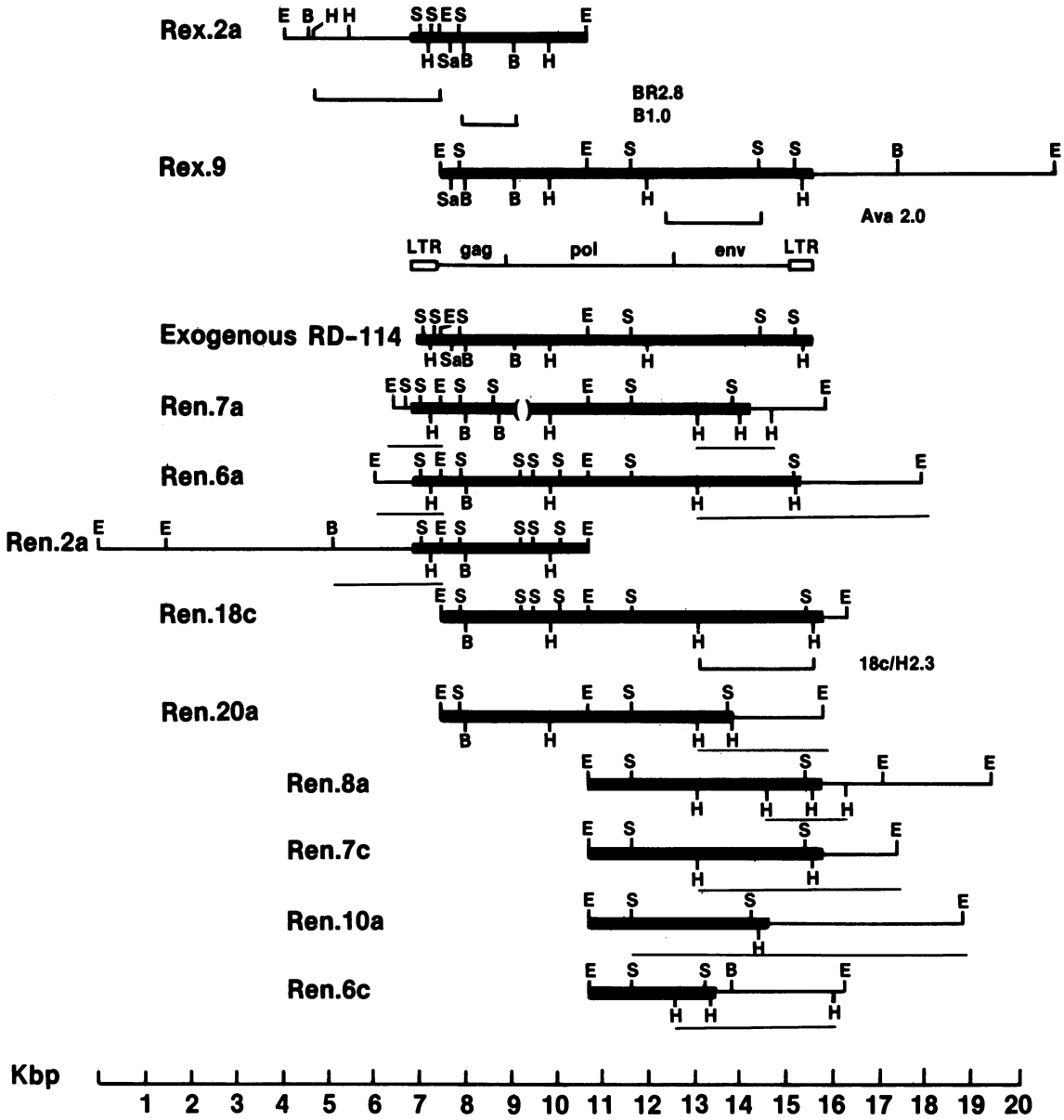

FIG. 2. Restriction maps of RD-114 containing clones derived from a genomic library of RD-114-infected human RD cells (Rex) and maps of molecular clones of RD-114 endogenous sequences derived from a cat genomic library (Ren). Viral information in each clone is indicated by a heavy line and was determined by hybridization with RD-114-specific probes and by localization of LTRs. Light lines indicate flanking cat (or human) cellular DNA. Underscores indicate restriction fragments which hybridize to the LTR probe BR2.8. Abbreviations: S, *Sma*I; H, *Hin*dIII; E, *Eco*RI; Sa, *Sal*I; B, *Bam*HI. For orientation, the genetic map of BaEV is aligned above the RD-114 maps. Brackets with names to the right are restriction fragments used as probes in these experiments. Scale is in kilobase pairs.

vector, λ WES: λ Bgt (19). Nine molecular clones containing RD-114 information were isolated and subjected to restriction enzyme analysis. The derived restriction maps (Fig. 3) are aligned at the central *Eco*RI and *Sma*I sites conserved between the exogenous virus and each endogenous sequence (located at 10.7 and 11.8 kb, respectively, on the scale in Fig. 3). The limits of the RD-114 sequences were determined by Southern analysis of each endogenous RD-114 clone with RD-114 specific probes (Rex.2a and Rex.9; Fig. 2).

Several important points become evident upon examination of the maps presented in Fig. 2. First, the nine endogenous RD-114 clones represent at least eight distinct integrations as determined by the different restriction sites in nonviral (flanking) DNA at the 3'-ends of the sequences. Ren.2a is the only clone which does not include the 3' flank and as such could represent the 5'-end of any one of five other cloned sequences (18c, 8a, 7c, 10a, or 6c). The viral portion of clone Ren.7c has the same map as the 3'-end of Ren.18c, but these sequences appear to be integrated into different cellular DNA sequences because their 3' flanking DNA maps are different. Second, although none of the cloned sequences is identical to the inducible provirus, several restriction sites were common to all. These conserved sites were found in the *gag* and *pol* regions but not in *env*. Finally, comparison of the different restriction maps suggests that multiple deficiencies and rearrangements have

occurred in the evolutionary history of these sequences. For example, the 5′ half of the RD-114 sequence in clone Ren.7a has retained several restriction sites in the same order as those in other endogenous sequences. However, the fragments generated from the 5′-end of Ren.7a after digestion with *Hin*dIII or *Eco*RI are 200 base pairs shorter than the corresponding fragments from other sequences, suggesting that a deletion (see parenthesis, Fig. 2) has taken place within this region. In addition, the positions of the 3′ viral termini relative to the conserved *Eco*RI and *Sma*I sites in the *pol* gene are different for each clone, suggesting several independent rearrangements of the *env* regions of these proviral segments.

Endogenous RD-114 sequences are bounded by LTRs. Two of the enzymes used to construct the RD-114 restriction map, *Hin*dIII and *Sma*I, had recognition sites close to both ends of the linear provirus and generated fragments whose total length was slightly less than the length of the uncut provirus. These observations suggested that the enzymes cut within the LTR sequences which are common to all retroviruses. Recognition sites for *Hin*dIII and *Sma*I were also found near the limit of material hybridizing to RD-114 at both the 5′- and 3′-ends of all endogenous viruses analyzed. To confirm the existence of LTRs around each endogenous sequence, we hybridized the LTR-specific BR2.8 probe (shown in Fig. 2) to restriction digests of endogenous RD-114 clones. In every case, hybridization occurred with restriction fragments containing the junction of viral and flanking DNA (see underscore, Fig. 2).

To precisely localize the LTR within the hybridizing

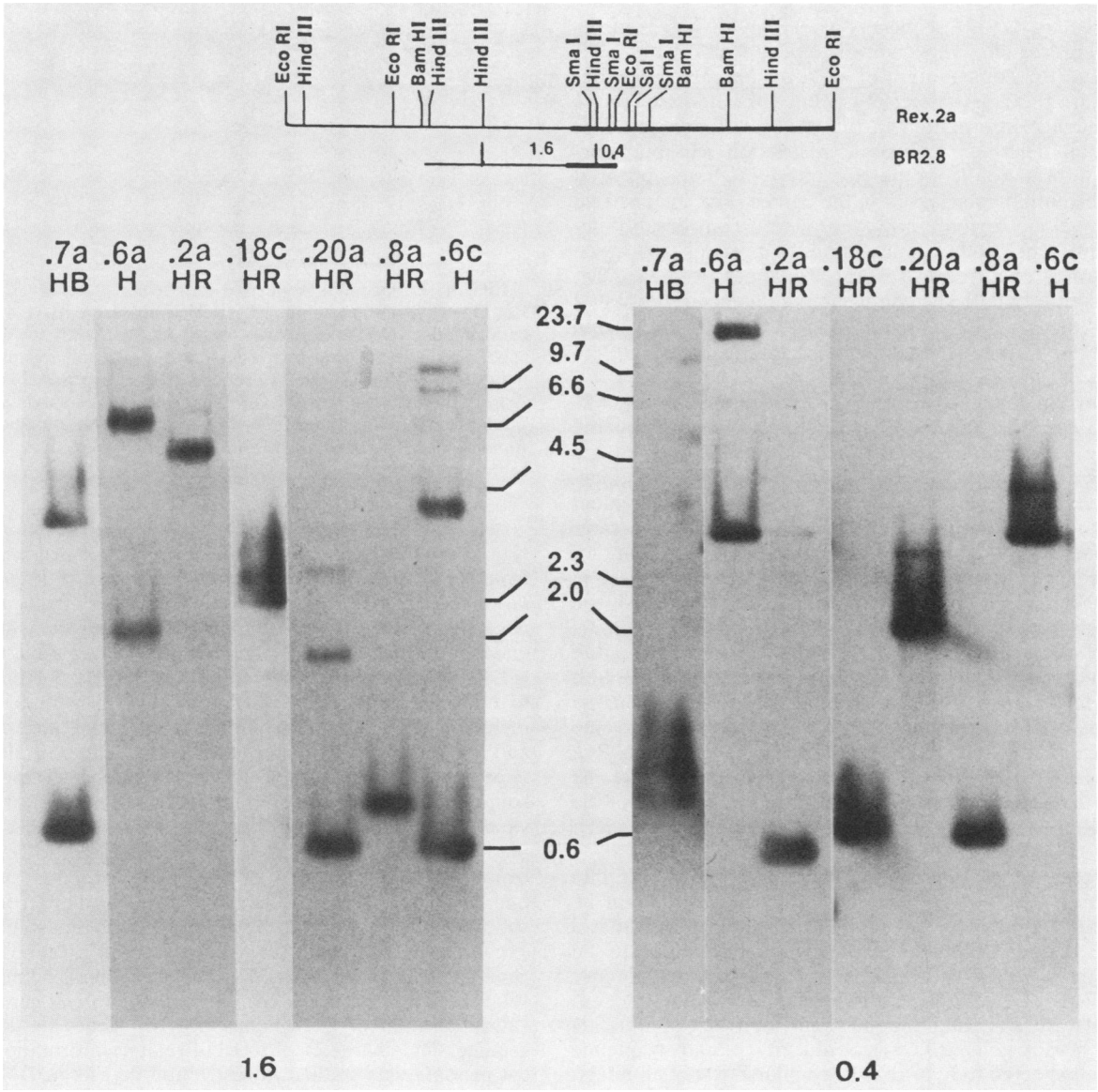

FIG. 3. Localization of LTRs about endogenous RD-114 sequences. Subclone BR2.8 was digested with *Hin*dIII-*Eco*RI, and the resulting 1.6- and 0.4-kb fragments were used as probes to restriction endonuclease digestions of RD-114 endogenous sequence clones. The 1.6-kb probe hybridized only to fragments to the left of the LTR *Hin*dIII site in each clone: the 0.4-kb fragment recognized only sequences to the right of this site. Compare with the restriction maps in Fig. 2. Faintly hybridizing bands detected by the 1.6-kb probe in clones .20a and .6c are due to incomplete restriction digests of these DNAs. Abbreviations for restriction enzymes are as follows: H, *Hin*dIII; R, *Eco*RI; B, *Bam*HI. Double letters (e.g., HB) indicate double digests (e.g., *Hin*dIII-*Bam*HI).

regions, the 1.6- and 0.4-kb *Hin*dIII-*Eco*RI digestion products of BR2.8 were isolated for use as probes (Fig. 3). These probes recognized fragments to the left and right, respectively, of the *Hin*dIII sites in the LTRs of endogenous sequences (Fig. 3). For example, if the *Hin*dIII site in the LTR of Rex.2a is the same site as the *Hin*dIII site at the 5'-end of Ren.2a viral sequences, then one would predict recognition of a 5.1-kb fragment with the leftward probe (1.6 kb) and a 0.5-kb fragment with the rightward probe (0.4 kb) after digestion with *Eco*RI and *Hin*dIII. This is precisely what is observed (Fig. 3, lane 3). The other clones also revealed the predicted right and left fragments when analyzed with the two probes (Fig. 3). These experiments proved that the *Hin*dIII sites in the LTRs of endogenous sequences correspond to those in the exogenous retrovirus and provide accurate markers for the limits of the endogenous viral sequences.

Endogenous RD-114 sequences are highly divergent in the *env* coding region. The hybridization results with LTR probes and the conservation of adjacent *Sma*I and *Hin*dIII sites within the LTRs precisely defined the limits of retroviral sequence information in the endogenous clones. Despite small deletions and some restriction site polymorphisms, information from the *gag* and *pol* regions appeared to be substantially conserved in the clones (Fig. 2). The size of the envelope regions, however, varied considerably between different clones, suggesting that major *env* gene deletions had occurred in several sequences (Ren.7a, .20a, Ren.8a, and Ren.6c). The endogenous sequence represented in clone Ren.7a, for example, contained only 7 kb of viral information, whereas the exogenous viral genome is 8.8 kb long.

The envelope gene of each of the endogenous clones was found to be even further diverged than expected by the following experiment. Restriction digests of clones of the exogenous virus, Rex.9, of BaEV and of each of the endogenous Ren clones were hybridized to a *pol-env* fragment (Ava2.0; Fig. 2) derived from the replication-competent, viral clone, Rex.9. Although this probe recognized homologous *env* segments in Rex.9 and weakly detected them in BaEV, there was no hybridization detected with any of the envelope segments from the various Ren clones (Fig. 4). As expected, the *pol* fragments of the same clones were readily detected (Fig. 4). Thus, the Ava2.0 probe detects the 3.4-kb *Hin*dIII and 2.3-kb *Sma*I *env* fragments of Rex.9 and the 1.65-kb *env* LTR fragment of BaEV but fails to detect the *Hin*dIII-generated *env* LTR fragments of any of the Ren clones (see arrows, Fig. 4). These results demonstrate the extreme divergence, or substitution of the *env* regions, of the studied Ren sequences from the *env* gene of exogenous RD-114.

The presence of sequences bounded by RD-114 LTRs which are not homologous to the inducible virus might be explained in a number of ways. One possibility was that a nonviral, cellular DNA sequence had been transduced to the *env* position of these endogenous viral genomes in a manner reminiscent of retroviral recombination with cellular oncogenes (14). Because total unique sequence DNA of all felids is very homologous (6), a transduced cellular sequence would be expected to hybridize to cellular DNA of all felids. Conversely, if the novel *env* sequences was introduced to the cat germ line horizontally at a point subsequent to the divergence of the Felidae (as occurred with RD-114), then it would be found in a subset of *Felidae* species. To test these possibilities, a probe was prepared from a 2.3-kb *Hin*dIII fragment of the *env* region of Ren.18c (.18c/H2.3)

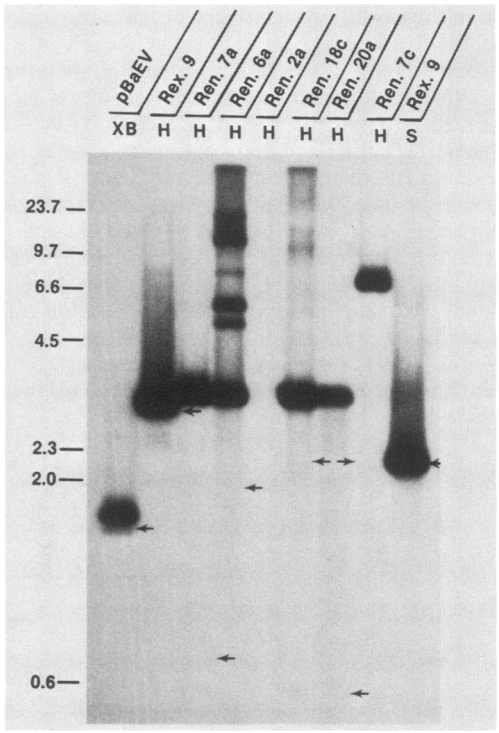

FIG. 4. Endogenous sequence *env* regions are diverged from RD-114. DNAs from clones of BaEV, exogenous RD-114, and six endogenous RD-114 sequences were digested with the indicated restriction endonucleases, transferred to a nitrocellulose filter, and hybridized with the Ava2.0 probe derived from Rex.2a (Fig. 2). Arrows indicate the positions of *env* region fragments visualized by ethidium bromide staining of the same gel. Abbreviations: XB, *Bam*HI-*Xho*I; H, *Hin*dIII; S, *Sma*I.

(Fig. 2) and hybridized to *Hin*dIII digests of genomic DNA from six RD-114-positive species (*F. margarita*, *F. chaus*, *F. nigripes*, *F. lybica*, and *F. sylvestris*) and from RD-114-negative species (Fig. 5). Only those species which had endogenous RD-114 contained sequences related to .18c/H2.3. In similar experiments, the Ren.18c/H2.3 probe failed to hybridize with FeLV and with several primate DNAs, including yellow baboon, stump-tailed macaque, colobus, and colobus virus-infected dog cells. Thus, it does not appear that these variant RD-114 sequences represent a subpopulation of primate viruses horizontally transmitted to small felids in conjunction with the ancestor of RD-114 nor recombination with a known endogenous feline retrovirus from the MAC-1-related or FeLV endogenous sequence families. The absence of the unusual *env* segment from feline species which lack endogenous RD-114 virogenes effectively excludes the possibility of viral rescue of a feline cellular gene and affirms the horizontal introduction of the novel *env* sequences into an ancestor of the modern *Felis* genus.

Organization of endogenous proviral genes in the feline genome. The character of RD-114-related information in the cat genome was studied in restriction digests of DNA from kidney, spleen, and liver. No tissue-specific differences were detected in nucleic acid hybridizations with LTR-specific (Fig. 6a), *gag*-specific (Fig. 6b), and representative (*rep*) (Fig. 6c) RD-114 probes. Furthermore, the restriction patterns were nearly identical in DNA tested from several individuals, suggesting very limited genetic polymorphism of

endogenous sequences. Although the patterns of RD-114 fragments were quite complex, they emphasize several important points. The LTR probe, clone pBR2.8, revealed a minimum of 30 distinct bands with several restriction endonucleases, suggesting that RD-114 is present in at least 15 integrations in cat cellular DNA. This estimate is in approximate agreement with previous measurements of the RD-114 copy number based on solution hybridization (6).

The *rep* and *gag*-specific probes were useful in determining whether the molecular clones described in Fig. 2 were representative of the entire family of RD-114 sequences. Conserved *Eco*RI and *Hin*dIII fragments (3.2 and 2.7 kb, respectively) were seen as heavy regions of hybridization in genomic blots analyzed with the *gag*-specific probe (Fig. 6b), suggesting that these fragments are conserved in multiple endogenous copies as predicted by the clone analysis in Fig. 2. An additional, highly reiterated *Hin*dIII fragment of 3.7 kb was detected with the *rep* probe and corresponds to the conserved *pol-env* region fragment seen in several endogenous sequences (Fig. 6c).

Three restriction endonucleases (*Bam*HI, *Sal*I, and *Sma*I) produced primarily high-molecular-weight, RD-114-containing fragments. This result was expected for *Bam*HI, which cleaved most of the proviruses analyzed at a single site, and for *Sal*I, which failed to cleave the endogenous sequences. The predominance of high-molecular-weight fragments from apparently complete digestions with *Sma*I (based on ethidium bromide staining and the cleavage of marker DNAs) may be due to the occurrence of a methylation site within the *Sma*I recognition sequence. Since methylation prevents cleavage by this enzyme, a reiterated *Sma*I fragment could be masked if endogenous sequences are differentially methylated.

No highly repeated *Sma*I or *Bam*HI fragments were observed. A *Bam*HI fragment of 1.0 kb, the size of the internal fragment from exogenous RD-114, hybridized with the approximate intensity of a single copy band in cat cellular DNA. Since such a *gag* fragment was absent from all of the Ren clones, it seemed likely that this fragment was diagnostic for the replication-competent RD-114 genome and that the inducible sequence was present in few (or one) copies per genome. The genetic mapping of this *Bam*HI fragment in a panel of somatic cell hybrids (29; R. Reeves, W. Nash, and S. O'Brien, manuscript in preparation) to a specific feline chromosome (as opposed to several) lends support to this conclusion.

DISCUSSION

This report presents a molecular and genetic analysis of the RD-114 gene family of domestic cats, including the exogenously replicating, inducible retrovirus. Several (or all) of these viral segments have undergone some genomic divergence as evidenced by the comparison of restriction maps of individual clones. In addition, the associated cellular DNAs flanking each clone were found to be unique, suggesting that the viruses are integrated in different chromosomal positions. Analysis of the maps of each of nine molecular clones examined showed that the endogenous proviruses are genetically distinct from the infectious virus and provided a means of identifying specific virogenes on the basis of unique sequence flanks or of diagnostic, internal, restriction fragments. A 1.0-kb *Bam*HI restriction fragment characteristic of the inducible virus hybridized at an intensity characteristic of single-copy fragments in genomic blots of cat DNA and has been genetically mapped to a single cat chromosome. Thus, the inducible, replication-competent genome of RD-114 appears to be present as a single copy in the feline genome.

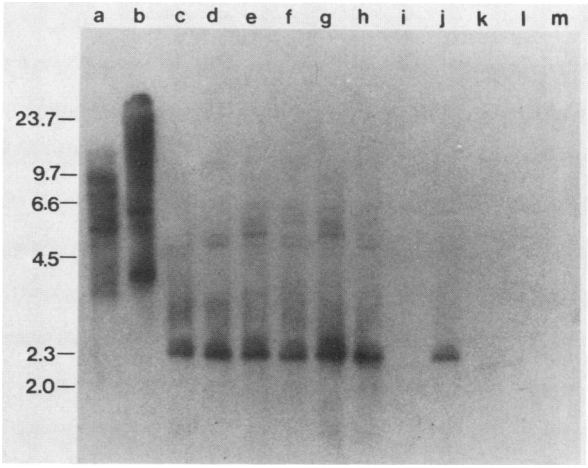

FIG. 5. Information related to a variant *env* region from an endogenous sequence is found only in felids which contain endogeous RD-114 sequences. Equivalent quantities of cellular DNA from domestic and exotic cats were hybridized with probe .18c/H2.3 (Fig. 2). Lanes a and b, Domestic cat DNA restricted with *Eco*RI and *Sma*I, respectively. All other DNAs were digested with *Hin*dIII. Lanes c to h, Domestic cats; lane i, Pallas cat; lane j, black-footed cat; lane k, leopard cat; lane l, tiger; lane m, lion.

Comparison of the physical maps of endogenous RD-114 sequences revealed a number of features of this gene family. Within the *gag* and *pol* regions, there is considerable homology between the replicating virus and other endogenous viruses. It may be important to note, however, that the endogenous clones examined all lacked a *Sal*I site which was present in the infectious virus ca. 300 base pairs downstream from the 5′ LTR. This genomic region has been shown to be essential for encapsidation of spleen necrosis virus and murine leukemia virus (21, 39). If the analogous region were functionally deleted in the endogenous RD-114 viral segments, then genomic viral RNA transcripts from these sequences (if any) would not be packaged or transmitted to neighboring cells. We have not studied the transcriptional activity of these sequences; however, the presence of LTRs in each endogenous provirus raises the possibility that they may be transcribed. Transcriptional and translational products of RD-114 have been observed in embryonic tissues of the domestic cat (15, 25) and in tissues derived from feline lymphomas, sarcomas, and carcinomas (26, 27, 33).

Although the *gag* and *pol* regions of the endogenous RD-114 clones were rather conserved, the *env* regions displayed considerable divergence from the exogenous RD-114 *env* gene, both in size (as demonstrated by the limits defined by LTRs; Fig. 3) and in sequence homology (Fig. 4). A similar pattern of *env* divergence has been reported for a family of endogenous retroviruses in the human genome (31). However, this pattern is different from that observed among endogenous FeLV sequences (35). Analysis of molecular clones of four members of the FeLV gene family detected substantial deletions from the *gag* and *pol* regions, whereas relatively small segments were deleted from *env*. These contrasting patterns of evolutionary divergence suggest that different selective pressures have resulted in the apparent inactivation of members of these two gene families. The fact that the pattern of deletions is conserved within each gene family suggests that, in both cases, inactivation occurred before the

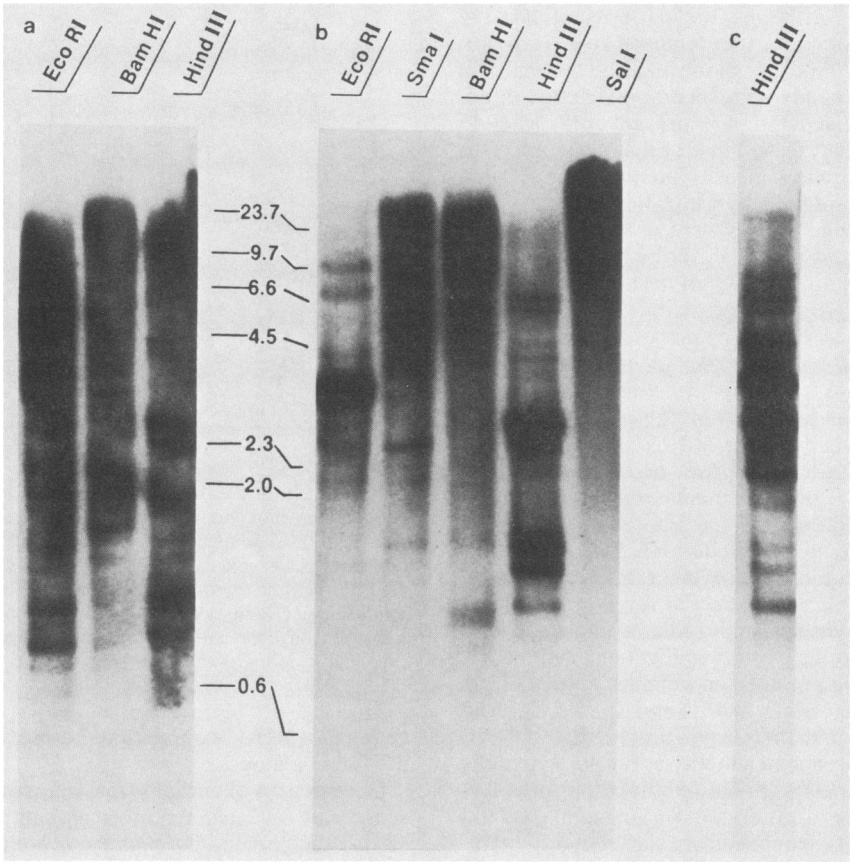

FIG. 6. Southern analysis of cat cellular DNA with subgenomic and representative probes. Hybridization of cat cellular DNA to: (a) an LTR-specific probe (BR2.8, Fig. 2); (b) a *gag*-specific probe (probe B1.0, Fig. 2); or (c) a *rep* RD-114 cDNA probe.

amplification and dispersion of these sequences in the cat genome.

The amplification mechanism for the two families may be different as well. The FeLV sequences examined by Soe et al. (35) had similar restriction maps in DNA flanking the endogenous sequences, suggesting that amplification (and transposition?) involved the replication of a relatively large segment of cellular DNA. Restriction sites in DNA flanking RD-114 sequences in this analysis were distinct in every clone. Furthermore, in the case of clones Ren.2a and .6c, the flanks were shown to represent unique sequence cat DNA segments from different chromosomes. Thus, the endogenous RD-114 sequences appear to have been integrated as discrete retrovirus units. In this regard, it may be important that the LTRs of endogenous RD-114 sequences are similar to those of the replication-competent virus, whereas the U3 portion of the LTR in replicating FeLV is distinct from that of endogenous FeLV sequences (19). Considered together, these data suggest that the original feline RD-114 transposition(s) involved discrete viral units, whereas the FeLV amplification involved blocks of DNA segments, including virus and flanks.

Several RD-114 sequences contained *env* regions which are substantially different from those of RD-114 or of its nearest relative, BaEV. The origin of this diverged *env* region is not clear. The simplest explanation is that the *env* gene is simply more subject to genetic drift than *gag-pol* and therefore has accumulated substantial mutations, rearrangements, and other genetic abberations. An observation which is not consistent with this interpretation is the conservation of a 2.3-kb *Hin*dIII *env* fragment within the genomes of the domestic cat and of several other RD-114-positive members of the genus *Felis* (Fig. 5). Such conservation is not consistent with a rapid stochastic mutational history of this region. An alternative hypothesis (which we favor) would suggest that the novel *env* region was introduced into the RD-114 genome as a result of recombination between RD-114 and an as-yet-unidentified retrovirus. Since the novel envelope and the endogenous RD-114 *gag-pol* sequences show the same species distribution, they both seemed to have been introduced precipitously into the felid germ line at a point before radiation of the genus *Felis* but subsequent to the split of that group from the other species of the family *Felidae* (see reference 28). The recombination event probably occurred at this time as well, since the novel *env* sequence shows no homology to endogenous envelope sequences of baboons and other Old World monkeys from which RD-114 was originally derived.

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