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Nucleotide Sequences of a Feline Leukemia Virus Subgroup A Envelope Gene and Long Terminal Repeat and Evidence for the Recombinational Origin of Subgroup B Viruses

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Molecular clones of the subgroup A feline leukemia virus FeLV-A/Glasgow-1 have been obtained. Nucleotide sequence analysis of the 3′ end of the proviral genome and comparison with the published sequence of FeLV-B/Gardner-Arnstein showed that the most extensive differences are located within the 5′ domain of the *env* gene. Within this domain, several divergent regions of *env* are separated by more conserved segments. The 3′ end of *env* is highly conserved, with only a single amino acid coding difference in p15*env*. The proviral long terminal repeats are also highly conserved, differing by only eight base substitutions and one base insertion. Specific probes constructed from the FeLV-A or FeLV-B *env* genes were used to compare the *env* genes of various exogenous FeLV isolates and the endogenous FeLV-related proviruses of normal cat DNA. An FeLV-A-derived *env* probe showed no hybridization to normal cat DNA but detected all FeLV-A and FeLV-C isolates tested. In contrast, an FeLV-B *env* probe detected independent FeLV-B isolates and a family of endogenous FeLV-related proviruses. Our observations provide strong evidence to support the hypothesis that FeLV-B viruses have arisen by recombination between FeLV-A and endogenous proviral elements in cat DNA.

Three subgroups of feline leukemia virus (FeLV), A, B, and C, have been identified by viral interference tests, suggesting that feline retroviruses can interact with at least three distinct host cell receptors (21, 48). Although viruses of each subgroup can be separated readily and grown independently to high titer in feline embryonic fibroblast cells, the subgroup composition of FeLV field isolates (A, AB, AC, ABC) suggests that they are interdependent in vivo. Two explanations have been considered for the apparent dependence of FeLV-B and FeLV-C on FeLV-A in vivo. The first explanation is that the other subgroups must be in a phenotypic mixture with FeLV-A for horizontal transmission to occur. Experimental studies have shown that FeLV-A is efficiently transmitted from cat to cat and that FeLV-B can be passed on in phenotypic mixture with FeLV-A (22). There is no evidence that under natural conditions FeLV-B or FeLV-C can be horizontally transmitted without FeLV-A. The second explanation, which was first suggested by Russell and Jarrett (44), is that the other subgroups may arise by recombination between FeLV-A and endogenous proviral elements in normal cat DNA. Sequence homology between the FeLV-B *env* gene and that of murine mink cell focus-forming (MCF) viruses (9, 51), which appear to be derived by recombination between exogenous and endogenous proviral elements (6, 10, 23), prompted us to ask whether FeLV-B viruses have a similar origin.

Further interest in the subgroups of FeLV is provided by their association with specific diseases in cats. The clearest example is the association of FeLV-C isolates with erythroid hypoplasia, a disease that can be reproduced rapidly after inoculation of FeLV-C isolates into newborn cats (12, 39). There is less conclusive evidence that FeLV-B isolates may be associated with higher rates of viremia and leukemia in susceptible cat populations (20). In addition, some of the most highly leukemogenic isolates of FeLV, such as the Rickard strain (41), are FeLV-AB mixtures.

In view of these interesting biological properties, we undertook the molecular cloning and detailed analysis of the FeLV-A strain that has been used as the prototype subgroup A strain in many experimental studies, FeLV-A/Glasgow-1. Comparison of our sequence data with the published sequences of two FeLV-B isolates (9, 37, 51) showed that the 3′ end of *env*, including the entire p15*env* domain, and the long terminal repeat (LTR) are highly conserved. The most divergent region, predicted to encode the receptor recognition function, is found at the 5′ end of the *env* gene. Subcloned DNA probes from the divergent region of FeLV-A showed specificity for FeLV-A and C isolates, while probes from the equivalent region of FeLV-B hybridized to FeLV-B isolates and to endogenous FeLV-related sequences, providing support for the hypothesis that FeLV-B isolates have arisen by recombination between FeLV-A and proviral elements of the normal feline genome.

MATERIALS AND METHODS

FeLV strains and molecular clones. The various field strains of FeLV used in this study were supplied by O. Jarrett of the Department of Veterinary Pathology, University of Glasgow, Glasgow, U.K. These were provided as infected cultures of feline embryo cells, and all cultures had been typed by viral interference. Molecular clones were available for FeLV-B/Gardner-Arnstein (pFGB) (32), FeLV-B/Rickard (pFRB) FeLV-C/Sarma (pFSC), and two endogenous FeLV proviruses, pBCM-3 and pLCM-1 (31; (J. I. Mullins, R. C. Binari, Jr., J. H. Elder, G. Beltz, E. A. Hoover, and V. A. Hirsch, submitted for publication).

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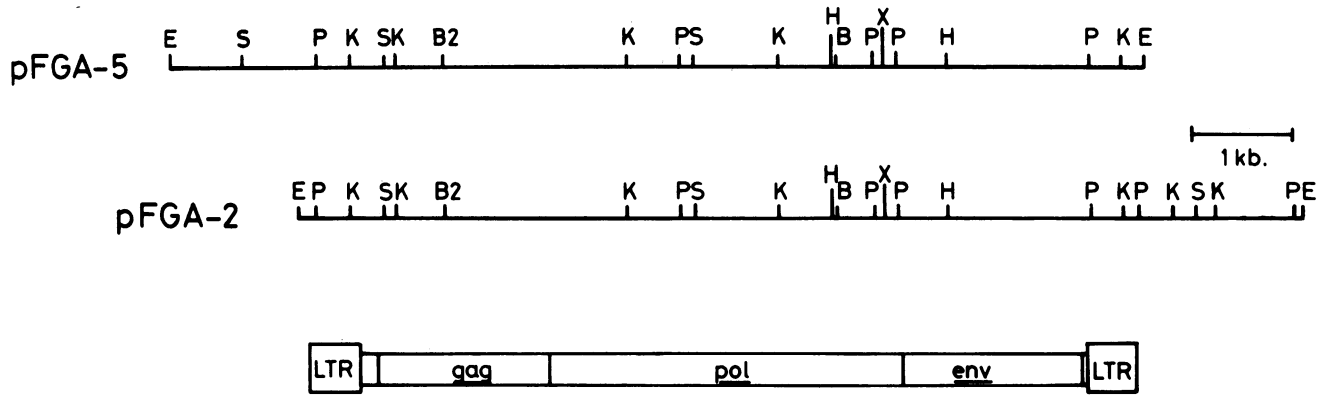

FIG. 1. Restriction maps of *Eco*RI inserts of two biologically active clones of FeLV-A/Glasgow-1, pFGA-2 and pFGA-5. Bacteriophage clones in λgtWES were subcloned into plasmid pUC8, and restriction maps were determined by double-digest and partial-digest analyses. The genetic map of a typical FeLV provirus is shown underneath, aligned with consensus restriction enzyme sites which predict the location of proviral sequences (31, 32). The two clones have indistinguishable restriction maps within proviral sequences but are clearly identified as independent clones by differences in flanking sequence restriction sites. In addition, restriction mapping and DNA sequence analysis shows an unusual feature at the 3′ end of the pFGA-2 provirus, where the 3′ LTR is interrupted 10 base pairs from its 3′ end by another LTR sequence (see text). This unusual structure appears not to affect the recovery of infectious FeLV after transfection of pFGA-2 into feline embryo cells. Restriction enzyme abbreviations: B, *Bam*HI; B2, *Bgl*II, E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; S, *Sst*I.

Cloning of FeLV-A/Glasgow-1 proviruses. Cultures of feline embryonic fibroblast cells (FEA; 21) infected with a biologically cloned stock of FeLV-A/Glasgow-1 were used as the source of DNA for cloning. Cellular DNA was digested with *Eco*RI, and DNA fragments of 8 to 15 kilobases (kb) were purified from low-melting-point agarose gels. This fraction of the DNA (1 μg) was ligated to *Eco*RI arms (300 ng) of the bacteriophage vector λgtWES (obtained from Amersham International plc). After in vitro packaging, a library of 1.5×10^6 recombinants was obtained and screened with a hybridization probe derived from the U3 region of the FeLV LTR (3). Five positive recombinants were isolated from 5×10^5 phage clones. After plaque purification and isolation of phage DNA, we screened the recombinants for their ability to yield infectious FeLV-A after transfection into FEA cells by the calcium phosphate technique (13). On the basis of these results, we selected two recombinants (λFGA-2 and λFGA-5) for subcloning into plasmid vectors and further analysis.

Biological testing of FeLV-A clones. Testing for infectious FeLV was performed according to published procedures. Two tests were used; transfected cultures were tested for focus induction on cocultivation with S+L− cells (C81; 11) or for interference to superinfection with murine sarcoma virus (FeLV) pseudotypes (43). Transfected cells were grown for at least 3 weeks before interference testing to allow virus spread to all cells.

DNA sequence analysis. Sequence analysis was carried out using the methods of Maxam and Gilbert (30) and Sanger et al. (46). A 2.0 kb *Pst*I fragment of pFGA-2, encompassing the *env* gene of FeLV, was purified and digested to completion with *Sau*3A or *Hae*III. The products were cloned into *Bam*HI-cut (*Sau*3A products) or *Sma*I-cut (*Hae*III products) vector m13mp10. Single-stranded DNA was prepared, and dideoxynucleotide sequencing was performed. [α-^{35}S]dATP was used in the sequencing protocol.

To sequence regions not covered by dideoxy sequencing or which proved difficult to sequence because of secondary structure effects, the chemical method of Maxam and Gilbert was utilized. For this method, fragments were labeled either at the 5′ ends (using [γ-^{32}P]ATP and polynucleotide kinase) or at the 3′ ends (using [α-^{32}P]ddATP and deoxynucleotide terminal transferase).

Sequencing of the LTRs was carried out using the dideoxy method in conjunction with the recently developed m13mp18 and mp19 phages (35).

Sequencing products were separated on 0.3-mm denaturing 6 or 8% polyacrylamide gels. After fixing and drying, gels were exposed to Kodak RP film and stored at −70°C (^{32}P) or at room temperature (^{35}S) overnight.

Construction of *env* gene-specific probes. From the results of sequence analyses we expected that the 5′ end of the *env* gene would yield probes capable of distinguishing between FeLV-A/Glasgow-1 and FeLV-B/Gardner-Arnstein (GA). This was confirmed by blot hybridization analyses using subclones containing the entire *env* region of each virus (not shown). To prepare convenient reagents for further analyses, two subclones were made, as follows. (i) For subclone A/HH, a *Hin*cII-*Hin*dIII fragment of the FeLV-A/Glasgow-1 *env* gene was cloned into plasmid pUC8 between the *Sma*I and *Hin*dIII sites. The inserted fragment can be isolated by digestion with *Eco*RI and *Hin*dIII. For subclone B/S, a *Sau*3A fragment from the *env* gene of FeLV-B/GA was cloned into the *Bam*HI site of pUC8. The B/S clone contains two copies of the FeLV fragment, which can be isolated as a single fragment after digestion with *Eco*RI and *Hin*dIII. The restriction sites delineating these fragments are indicated on the sequence in Fig. 3 and in a separate diagram in Fig. 5.

RESULTS AND DISCUSSION

Molecular cloning and preliminary analysis of FeLV-A/Glasgow-1 proviruses. As described above, we isolated two biologically active clones from a bacteriophage library of DNA from embryonic fibroblast cells infected with FeLV-A/Glasgow-1. These were subcloned into bacterial plasmids and analyzed further. The restriction maps of the two plasmid subclones (pFGA-2 and pFGA-5) are shown in Fig. 1 with a genetic map of FeLV shown underneath for reference. The *Eco*RI inserts were 10 kb (pFGA-2) and 9.6 kb (pFGA-5) in length. As expected from the infectious nature

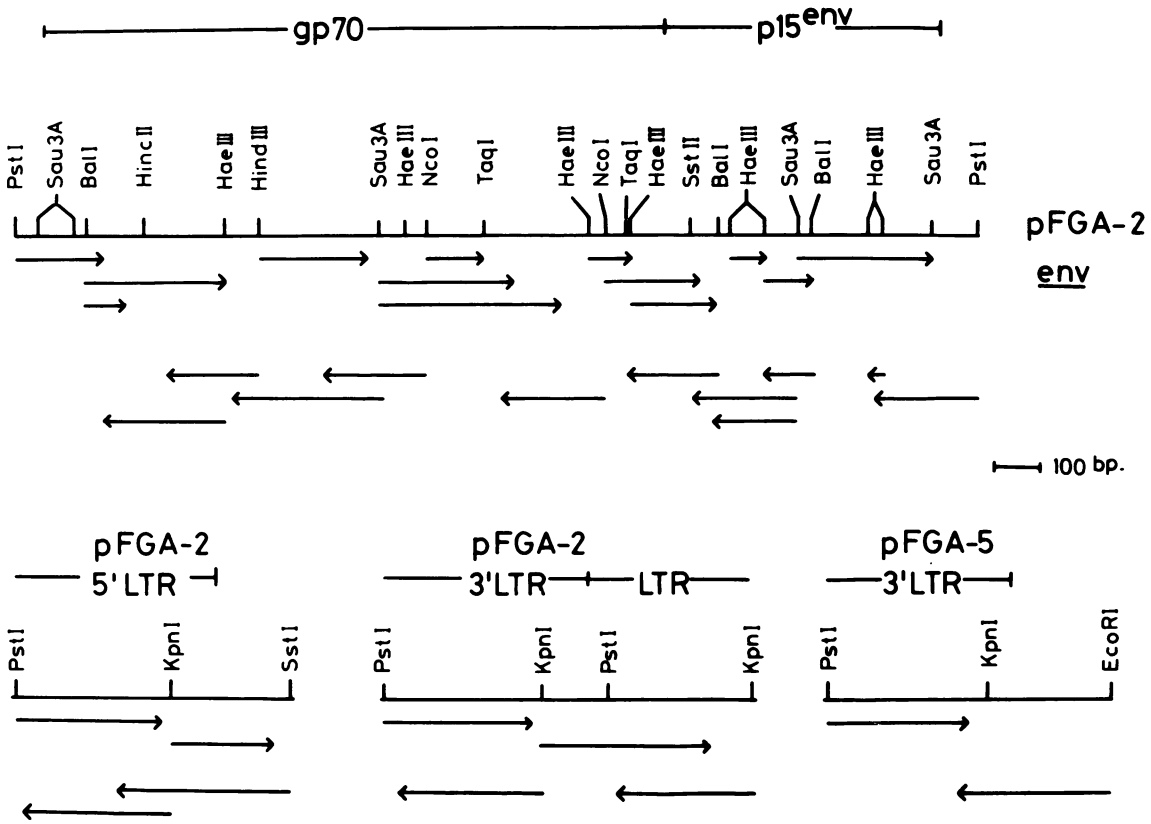

FIG. 2. In the upper part of the figure, the strategy employed in determining the sequences of the *env* gene and 3′ LTR of FeLV-A/Glasgow-1 are shown. Both Maxam-Gilbert and dideoxynucleotide sequencing methods were used (see Materials and Methods). Restriction sites used for sequencing are indicated. Arrows indicate the direction in which sequencing was carried out. For each of the arrows shown, at least two complete sequencing experiments were performed. The lower part of the figure shows sequencing strategies for the LTRs of the pFGA-2 and pFGA-5 clones. These additional determinations were performed to understand the duplicated LTR structure seen at the 3′ end of pFGA-2.

of these clones, the *Eco*RI inserts contained apparently full-length copies of FeLV proviruses, with characteristic LTR restriction enzyme sites for *Kpn*I (31, 32) at either end. Heteroduplex analysis using pFGA-5 and the cloned FeLV-B/GA was performed and confirmed that our clones were colinear with the FeLV-B genome with only a short contiguous stretch (0.5 to 1 kb) of low homology which could be assigned to the 5′ end of the *env* gene (33). To analyze the nature and extent of the subgroup differences within the *env* gene, we undertook direct DNA sequence analysis of portions of each of these clones (Fig. 2).

Sequencing strategy. The strategy used for sequence analysis of the FeLV-A/Glasgow-1 genome is illustrated in Fig. 2. By a combination of dideoxy and Maxam-Gilbert methods, we determined the sequence of 2,522 base pairs corresponding to the 3′ end of the FeLV-A/Glasgow-1 genome. As indicated in the legend to Fig. 1, the pFGA-2 clone showed a highly unusual structure such that the 3′ end of the FeLV LTR was interrupted by another FeLV provirus in the same orientation. It is possible that this represents a rare case where a double integration event occurred or that there had been a recombination between two unintegrated FeLVs before proviral integration. However, since the clone came from cells infected in mass culture, it was not possible to test for the existence of this structure in cellular DNA prior to molecular cloning. The additional FeLV sequences did not represent a complete provirus, and the 3′ terminal *Eco*RI site of the clone came from cat cell DNA. Despite the unusual structure, the pFGA-2 provirus encodes an uninterrupted copy of the FeLV RNA genome, and the recovery of infectious FeLV after transfection was unimpaired by the 10-base-pair truncation of U5. We obtained further confirmation of the sequence of the FeLV-A/Glasgow-1 LTR by sequencing the 3′ LTR of the pFGA-5 provirus and the 5′ LTR of the pFGA-2 provirus. The sequence shown in Fig. 3 takes this information into account.

Sequence of the *env* gene: basic features. The sequence analyzed showed a long open reading frame for a protein product of 71,067 molecular weight. Comparison with published sequences of murine leukemia virus (MuLV) and FeLV *env* genes confirmed this as the precursor for the mature *env* gene products, gp70*env* and p15*env* (9, 18, 24, 25, 28, 37, 51). Several other short open reading frames were noted, but these showed no significant conservation in other feline and murine retroviruses. The predicted *env* precursor showed the expected hydrophobic leader sequence characteristic of molecules which are glycosylated and inserted into cellular membranes (2). In addition, other regions of the molecule showed significant hydrophobicity (Fig. 4), including the N and C termini of the gp70*env* domain and portions of the p15*env* molecule, particularly at the N terminus. There were 13 potential N-linked glycosylation sites in the sequence, and these were clustered mainly in the C-terminal half of gp70. A single potential glycosylation site in FeLV

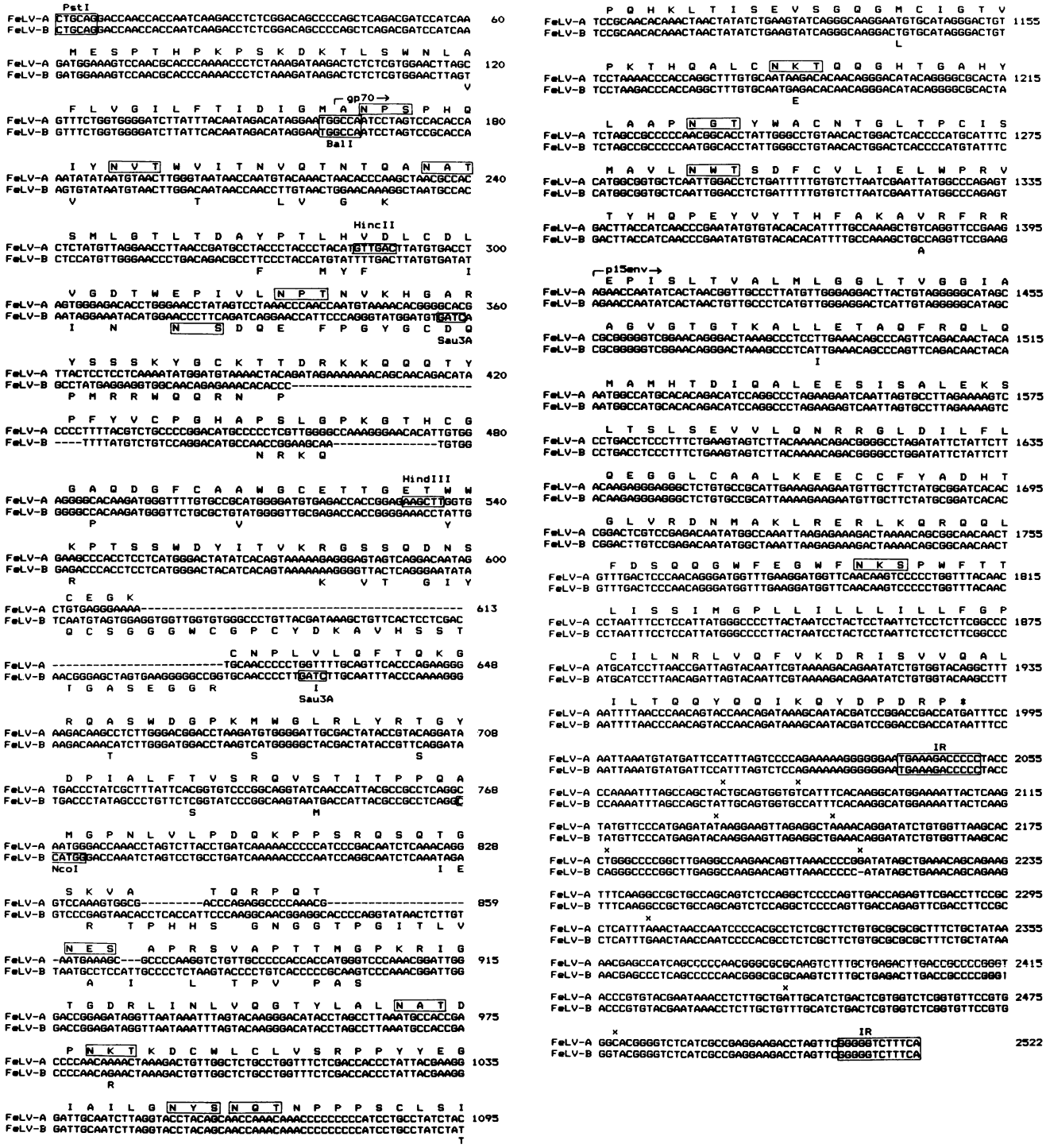

FIG. 3. Comparison of the *env* genes and 3′ LTRs of FeLV-A/Glasgow-1 and FeLV-B/GA. The nucleotide sequences are shown with the predicted amino acid sequence for the FeLV-A *env* gene precursor protein Pr80*env* and its mature gene products gp70 and p15*env*. The nucleotide sequence of FeLV-B/GA (9) is shown underneath for comparison, and amino acid coding changes are highlighted underneath the nucleotide sequence. Other features illustrated in boxes are restriction sites used in generating the subgroup-specific probes, inverted repeats delineating the LTRs, and potential N-linked glycosylation sites; base differences within the LTR are indicated by an asterisk above the sequence.

p15*env* is presumably not used since this molecule is not labeled by radioactive carbohydrate precursors (34). It is not known which of the potential gp70 glycosylation sites are used.

The FeLV *env* products are presumably translated from a spliced, subgenomic mRNA, by analogy with related retroviruses. A splice acceptor site used for the MuLV *env* mRNA (18) is conserved in FeLV, but is not seen in the

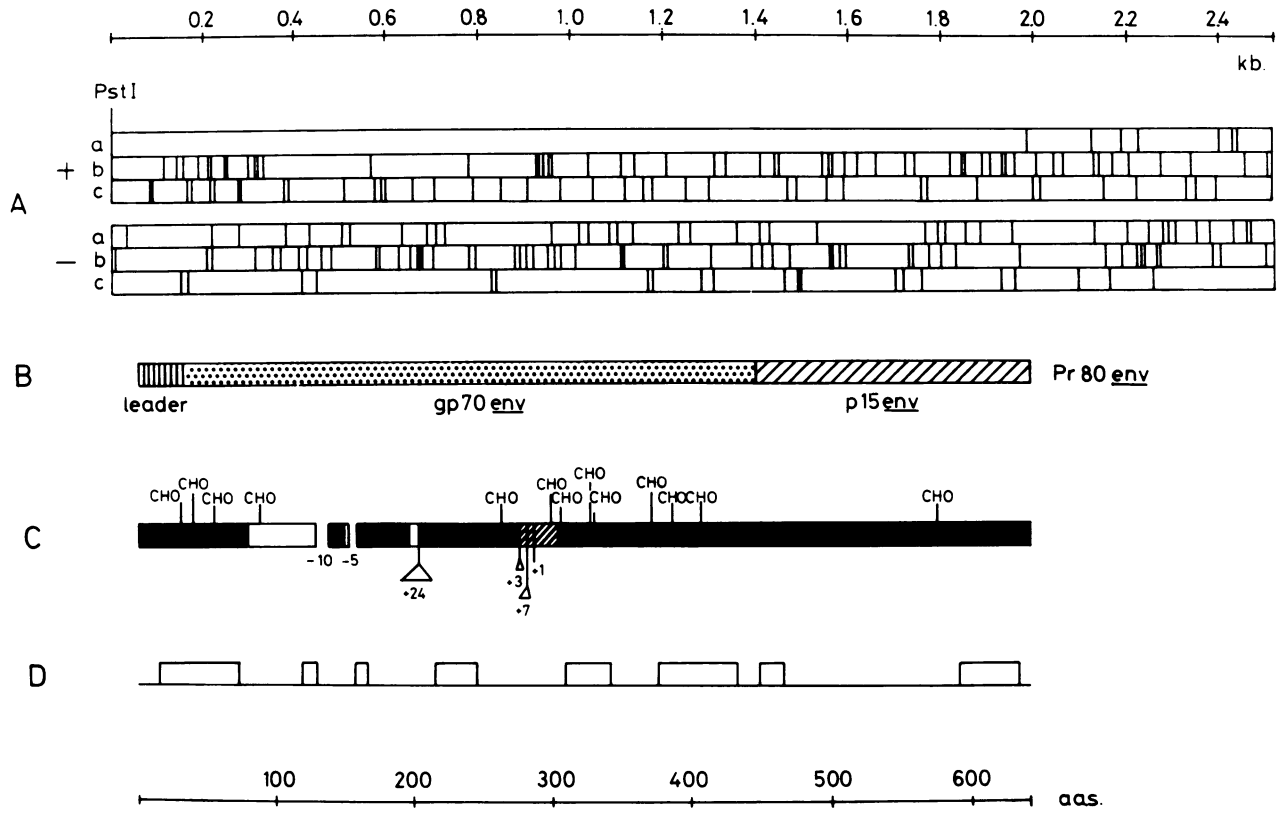

FIG. 4. Characteristics of the *env* gene of FeLV-A/Glasgow-1 and its products. (A) Distribution of termination codons in the coding (+) and noncoding (−) strands in all reading frames (a, b, c). (B) Representation of the *env* polypeptide and individual protein products. (C) Comparison of the FeLV-A/Glasgow-1 and FeLV-B/GA *env* precursor polypeptides. Block shading indicates >70% homology between the A and B proteins, hatched shading indicates 30 to 70% homology, and open boxes indicate <30% homology. Numbers refer to amino acid deletions (−) and insertions (+) in the B protein with respect to the FeLV-A protein. Potential N-linked glycosylation sites (CHO) in the A protein are indicated. These are completely conserved in the FeLV-B sequence with the exception of one site which occurs in an area of low protein sequence homology. (D) Open boxes indicate hydrophobic stretches of more than 11 amino acids. These regions were identified by analysis of the sequence using the Hopp and Woods algorithm (19).

sequence shown in Fig. 3 since it is located 220 base pairs 5′ to the *Pst*I site upstream of *env* (M. Stewart and J. Neil, unpublished data).

Sequence of the FeLV-A/Glasgow-1 LTR. The FeLV-A/Glasgow-1 LTR showed strong homology to previously published FeLV LTR sequences (9, 15), particularly that of FeLV-B/GA, with conservation of the consensus sequences for transcriptional initiation (CAAT, 2311–2314; TATA, 2351–2355) and termination (AATAAA, 2427–2432). Only 9 base pair differences were noted between the LTRs of FeLV-A/Glasgow-1 and FeLV-B/GA. These were scattered throughout the LTR, but not in a completely random fashion. Most of the changes were in the 5′ half of the LTR, and three base changes were noted within a region of homology to the repeated sequences of the MuLV LTR (50) which have been shown to contain transcriptional enhancer elements (26). Although no detailed functional analyses of enhancer elements of the FeLV LTR have yet been published, their homology to well-characterized enhancers in MuLV LTRs predicts a similar function. Furthermore, it has been established that the FeLV LTR can direct the expression of the bacterial neomycin resistance gene in mammalian cells and can also provide enhancer function in this assay (N. L. Fregien and N. Davidson, personal communication). The importance of enhancer elements for the tissue specificity and leukemogenicity of MuLV isolates (4, 5, 7, 8, 27) suggests that even the minor differences we have noted could be of biological significance. The FeLV-A/Glasgow-1 isolate is not highly leukemogenic, although it can infect and replicate efficiently in vivo (22). It will be of interest to compare the sequence and functions of the FeLV-A LTR with those of more highly leukemogenic FeLV isolates.

Comparison of FeLV-A/Glasgow-1 and FeLV-B *env* sequences. The predicted *env* gene products of FeLV-A/Glasgow-1 have been aligned with those of FeLV-B/GA in Fig. 3 and 4, showing the extent of amino acid homology along the length of the *env* gene precursor. The *env* sequence of the FeLV-A virus is 20 amino acids shorter than that of FeLV-B, due entirely to differences within the gp70 coding region. With the exception of the N-terminal half of gp70*env*, we noted a very good match between the sequences. The conservation among the feline and murine retroviruses of various features of the *env* gene product, such as the clustering of potential glycosylation sites and the position of blocks of hydrophobic amino acids in gp70 and p15*env*, allows us to apply similar structural models for the spatial relationships of the mature gene products on the virion surface (28, 51). According to the model proposed by Lenz and co-workers (28), the divergent N-terminal region projects away from the surface of the virion outer mem-

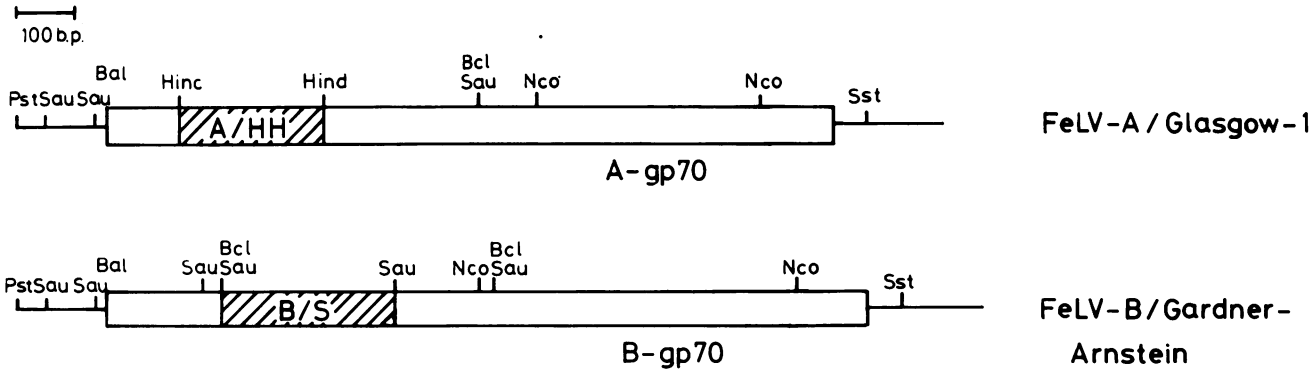

FIG. 5. Origin of specific probes derived from the *env* genes of FeLV-A/Glasgow-1 and FeLV-B/GA. The A/HH and B/S probes were obtained by subcloning, respectively, a *Hinc*II-*Hin*dIII fragment and a *Sau*3A fragment into the plasmid pUC8. Plasmid inserts were prepared by extracting the DNA from low-melting-point agarose gels by means of melting the gel at 70°C, removing the agarose by repeated phenol extractions, and precipitating the DNA with ethanol in the presence of 0.8 M ammonium acetate. Cleavage sites are indicated for the restriction enzymes *Bal*I, *Bcl*I, *Hinc*II, *Hin*dIII, *Nco*I, *Pst*I, *Sau*3A, and *Sst*II, and the coding regions of the FeLV-A and B gp70 molecules are enclosed within open boxes.

brane. Furthermore, the region of greatest divergence between FeLV-A and FeLV-B gp70*env* includes one of the most hydrophilic stretches of the molecule. Since FeLV-A and B are expected to recognize different cell surface receptors (21, 48), our data lend support to a model in which the divergent N-terminal region of FeLV gp70 is involved directly in receptor binding. The divergence of this region was further illustrated by the requirement for deletions and insertions to align the two amino acid sequences (Fig. 4). Apart from the divergent N-terminal domain, the predicted FeLV-A/Glasgow-1 *env* gene products showed strong similarity to those of FeLV-B/GA with fewer mismatches than noted in a published comparison of two different FeLV-B isolates (37). For example, we did not observe the cluster of noncoding or conservative changes in the *env* leader peptide domain of FeLV-B/Snyder-Theilen (37), and fewer differences were noted in the C terminus of gp70. The predicted N-linked glycosylation sites were all conserved, with one exception (Fig. 4). However, at the variant sites in both FeLV-A and FeLV-B, glycosylation may be suppressed by an adjacent proline residue (Asn-Pro-Ser/Thr).

The p15*env* protein of FeLV has been implicated as a possible effector of the immunosuppressive disease associated with FeLV, since partially purified FeLV p15*env* can

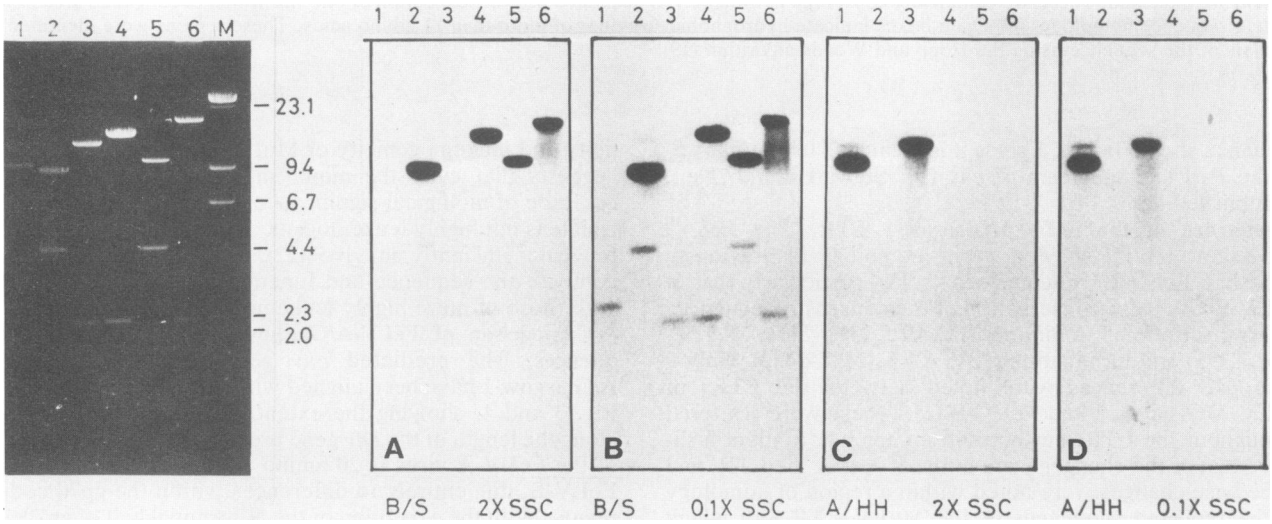

FIG. 6. Hybridization of *env* probes to cloned FeLV proviruses. (Left) Agarose gel stained with ethidium bromide showing one of the four identical gels used in the blot hybridization analyses. For lanes 1 through 6, plasmid DNAs containing full-length clones of exogenous or endogenous FeLV-related proviruses and cellular flanking sequences were cut with *Eco*RI to separate insert from vector sequences. Lanes: 1, FeLV-A/Glasgow-1 in pUC8; 2, FeLV-B/GA in pBR322; 3, FeLV-C/Sarma in pK125; 4, FeLV endogenous provirus (pLCM-1) in pK125; 5, FeLV endogenous provirus (pBCM-3) in pBR322; 6, FeLV-B/Rickard in pK125. Lane M contains *Hin*dIII-digested DNA molecular weight markers. Panels A through D show hybridization of multiple blots of lanes 1 through 6 with the three probes described in the legend of Fig. 5. Probes were labeled by nick-translation to at least 10^8 cpm/μg and used at 1 ng/ml in blot hybridization. Hybridization was carried out in 50% formamide–5× SSC–0.1% sodium dodecyl sulfate–5× Denhardt solution at 42°C, and filters were washed at 60°C in either 2× SSC–0.5% sodium dodecyl sulfate or 0.1× SSC–0.5% sodium dodecyl sulfate for 1 h. After drying, the filters were exposed to Kodak XRP X-ray film with intensifying screens at −70°C for (A) 4, (B) 24, (C) 16, or (D) 72 h. The weak hybridization to vector sequences seen in blots probed with the B/S insert is due to a small amount of contaminating pUC8 in the probe.

suppress lymphocyte functions in vitro (17, 29). We found only a single amino acid difference between FeLV-A/Glasgow-1 and FeLV-B/GA p15*env*. It will be of interest to compare this highly conserved gene product in FeLV strains with more pronounced immunosuppressive activity.

Derivation of *env*-specific probes. Comparison of the nucleotide sequences of the FeLV-A and B *env* genes encouraged us to search for probes that would distinguish between FeLV viruses in a subgroup-specific manner. A diagram showing the origin of the probes we have used is given in Fig. 5, and the restriction enzyme sites used are highlighted in the sequence in Fig. 3. The subgroup B probe (B/S) was a *Sau*3A fragment of the FeLV-B *env* gene encompassing the domain of greatest mismatch between the A and B *env* genes. The subgroup A probe (A/HH) was derived from a domain of the *env* gene which overlaps with the corresponding B/S probe. These probes have proved useful, allowing us to screen many more FeLV strains than would have been practical by molecular cloning and DNA sequence analyses.

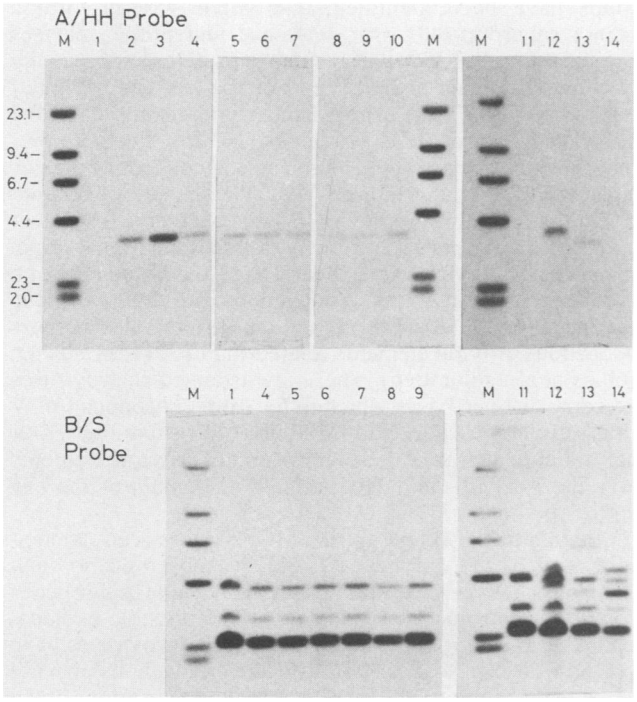

FIG. 7. Use of subgroup-specific *env* gene probes in hybridization analysis of virus-infected cell DNA. FEA cells (21) were infected in mass culture with various FeLV isolates and passaged for 3 weeks to allow virus spread to all cells. DNA was purified from these cell cultures for use in Southern blot hybridization analyses. DNAs were digested with *Kpn*I, and the fragments were separated on 0.8% agarose gels. Southern blots of these gels were analyzed with the A/HH or B/S probes as indicated. Hybridization and washing conditions were as outlined in the legend of Fig. 6, except that 10% dextran sulfate was included during hybridization, the probes were used at 5 ng/ml, and only high-stringency washing (0.1× SSC) was used. Molecular weight markers (M) were end-labeled, *Hin*dIII-digested lambda DNA fragments, and the cellular DNA digests were from uninfected FEA cells (lanes 1 and 11) or FEA cells infected with (lane 2) FeLV-A/Glasgow-H38, (lane 3) FeLV-A/Glasgow-H40, (lane 4) FeLV-A/Glasgow H41, (lane 5) FeLV-A/Glasgow-1, (lane 6) FeLV-C/Glasgow-FA27, (lane 7) FeLV-C/Glasgow-FS246, (lane 8) FeLV-C/Glasgow-FY981, (lane 9) FeLV-C/Glasgow-FZ215, (lanes 10 and 13) FeLV-AB/OF142, (lane 12) FeLV-B/Snyder-Theilen, or (lane 14) FeLV-B/GA (pFGB).

Properties of a subgroup A FeLV *env* gene probe (A/HH). The subgroup A-derived probe was tested for hybridization to cloned FeLV proviruses (Fig. 6). Strong hybridization was detected to the parental clone pFGA-2 (and to pFGA-5, not shown) as well as to an FeLV subgroup C clone, pFSC. However, no hybridization was detected to either of two FeLV subgroup B clones (pFGB, pFRB) or to two cloned endogenous FeLV-related proviral elements (pLCM-1 and pBCM-3). This lack of hybridization to endogenous proviruses was confirmed by hybridization of the A/HH probe to Southern blots of normal feline DNA (Fig. 7). The A/HH probe showed no reactivity with DNA from FEA feline embryonic fibroblasts unless these were infected with an FeLV of subgroup A or C. Only one of four FeLV-B strains showed hybridization to the A/HH probe, as discussed below. Digestion of FeLV-infected cell DNA with *Kpn*I yielded, in most cases, a 3.7-kb restriction fragment corresponding to the 3′ end of the exogenous FeLV genome (see Fig. 1 and references 31 and 32). Since this is an internal proviral fragment, its size is unaffected by site of viral integration and it can readily be detected in mass-infected cells. All six FeLV-A and five FeLV-C strains that were tested hybridized to the A/HH probe. Some examples are shown in Fig. 7. The intensity of hybridization correlated closely with that seen with a U3 probe (not shown), suggesting that any variations were due to differences in proviral copy number rather than extent of relatedness to the probe. All FeLV-B strains tested failed to show hybridization to the A/HH probe at appropriate stringencies, with the exception of an uncloned stock of FeLV-B/Snyder-Theilen, in which one of the two viral components in this mixture hybridized to A/HH (Fig. 7). However, the same viral component also hybridized with the FeLV-B *env* probe, B/S, indicating that this virus complex includes a provirus with portions homologous to each of the probes. Interference testing revealed only subgroup B, suggesting that both components carry the same receptor-binding determinant or that one of the genomes is defective in *env* expression and thereby fails to block superinfection.

Properties of a subgroup B *env* gene probe (B/S). A *Sau*3A subfragment of the FeLV-GA *env* gene (B/S) hybridized with contrasting specificity to the A/HH probe and would detect only the FeLV-B viruses and the endogenous FeLV proviruses, even if the blots were washed under conditions of reduced stringency (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.5% sodium dodecyl sulfate, 60°C). In addition, this probe hybridized to a larger family of endogenous proviral elements in normal cat DNA (see Fig. 7). Digestion of FEA DNA with *Kpn*I yielded three bands which hybridized with the B/S probe at 4.2, 3.0, and 2.4 kb. The intensity of the 2.4-kb band suggests it corresponds to the most abundant class of endogenous proviral elements as represented in clones pBCM-3 and pLCM-1 (31; Mullins et al., submitted). The B/S probe could also detect infection of feline cells with exogenous FeLV-B viruses, which appeared as a new *Kpn*I fragment of 3.7 kb or greater. Each of the FeLV-B-infected cell DNAs yielded more than one new viral band (Fig. 7), and in the case of FeLV-B/ST and FeLV-AB/OF142 this was due to the fact that these were uncloned, mixed viral stocks. Cells infected with FeLV-B/GA were obtained after transfection of FEA cells with the pFGB plasmid clone, which was expected to yield a 3′ *Kpn*I fragment of 3.7 kb. We detected the expected fragment and, in addition, a band at 4.7 kb which we suggest may have arisen from some deleted form of the provirus generated during the transfection process.

The B/S probe will be useful for selective cloning of FeLV-B proviruses, particularly if it is used in sequential screening with a U3 probe. We intend to apply this strategy to clone proviruses from the FeLV-GM1 complex, as there is circumstantial evidence to implicate a replication-defective FeLV-B virus in the genesis of the acute myeloid leukemias associated with this virus strain (D. Onions et al., submitted for publication). The B/S probe detected a novel 5-kb *Kpn*I fragment in FEA cells infected with pooled tumor material from cases of FeLV-GM1 disease (FeLV-AB/OF142) (Fig. 7). Furthermore, a similar 5-kb *Kpn*I fragment was observed in independent tumors induced by FeLV-GM1 (not shown).

Recombinant origin of FeLV-B viruses. Our results show that the A/HH probe, like the U3 portion of exogenous FeLV LTR elements (3), represents a sequence which has no close counterpart in normal cat DNA. In contrast, an equivalent region of the FeLV-B*env* gene, represented by the B/S probe, is closely matched to all FeLV-B viruses and to a family of endogenous FeLV-related proviruses of normal cat DNA. This leads us to suggest that FeLV-B viruses arise by recombination between exogenous FeLV-A viruses and the endogenous proviruses, yielding progeny viruses with exogenous U3 elements and *env* genes derived at least in part from the endogenous proviruses. The distribution of FeLV subgroups in nature such that all isolates contain FeLV-A while only a subset contain FeLV-B (20) would also be compatible with this proposal. Furthermore, sequence analysis of an endogenous FeLV *env* gene (pBCM-3) now provides direct evidence for our proposal. The analysis presented here provides no evidence that the FeLV-C viruses arise by recombination with endogenous proviral elements, but this also is inferred from direct sequence analysis of the FeLV-C/Sarma *env* gene (Mullins et al., submitted; N. Riedel, R. C. Binari, Jr., J. H. Elder, G. Beltz, E. A. Hoover, and V. A. Hirsch, submitted for publication). If the rare FeLV-C viruses arise by recombination between FeLV-A and endogenous genetic elements, then the common AC sequences detected by the A/HH probe would be expected to be derived consistently from the exogenous parental FeLV-A virus. Alternatively, a minority of cats may harbor endogenous proviruses which carry a subgroup C *env* determinant which would match to the A/HH probe.

Comparison of *env*-specific probes with viral interference and antigenic analyses of FeLV *env* genes. There is a good correlation between the grouping of viruses by hybridization with the A/HH and B/S probes and subgroup as defined by viral interference testing. The B/S probe, for instance, was found to hybridize only to FeLV-B or endogenous FeLV proviruses. The A/HH probe hybridized to all FeLV-A and C strains tested but not to FeLV-B strains, with only one anomalous exception. The lack of complete correlation could be explained if the regions determining receptor specificity are significantly smaller than the regions covered by the DNA probes (>80 codons). Our sequence analysis should provide a basis for experiments involving mutagenesis or exchange of small regions of *env* which would provide a more direct approach to identifying crucial domains determining interference subgroup.

Virus groupings according to antigenic analyses are less easily compared with our results, although some clear parallels can be drawn. Nunberg and colleagues (36), using a bacteriophage expression vector, cloned a small coding domain of gp70 from FeLV-B/GA. The fusion product was detected with a monoclonal antibody which neutralizes FeLV-A, B, and C (14). We found that the peptide encoded by this domain is completely conserved in FeLV-A/Glasgow-1 and that it is located well away from the region of greatest mismatch between FeLV-A and B. Also, the results of Russell and Jarrett (44, 45), based on the neutralizing activity of cat sera for various FeLV strains, provide a parallel with our results since they reported strong shared antigenic determinants between FeLV-A and C strains.

Parallels with other retroviruses. Our study extends the parallel which has been observed between the MuLV and FeLV families. Common patterns can be discerned in viral properties such as host range and host cell receptor use, in the sequence and structure of the *env* genes, and in the relationship between the exogenous retroviruses and their host genomes. For example, FeLV-A isolates are generally strictly ecotropic (21) like various well-characterized MuLV strains, e.g., Friend, Moloney, and AKR MuLV (47). FeLV-B and C strains have a broader host range, as do the MCF viruses (16). The existence of distinct interference subgroups of FeLV has been known for some time (48), but the value of this method of viral classification has been seen relatively recently for MuLV isolates, where several interference groups have been identified. The MCF viruses form a distinct subgroup (40). Our sequence and probe analyses now show that FeLV-A and B subgroups diverge principally in a limited domain at the 5′ end of the *env* gene. Similar conclusions have been drawn from comparisons of Friend MuLV and Friend MCF *env* genes (24, 25). Furthermore, comparison of the predicted amino acid sequences of the *env* genes of FeLV-B and murine MCF viruses shows relatedness in the region where the MCF viruses diverge from their ecotropic counterparts (9). Finally, a recombinational origin for murine MCF viruses has been suggested by comparison of exogenous MuLV and endogenous proviral *env* gene sequences (6, 10, 23). The normal cat genome also harbors endogenous proviral elements related to FeLV (1, 31, 38, 49; Mullins et al., submitted). These are arranged as proviruses and carry distinct LTRs which differ from exogenous FeLV principally in the U3 region (Mullins et al., submitted). Our analysis indicates that these endogenous proviral elements carry the FeLV-B- and MCF-related 5′ domain of the *env* gene.

Although FeLV-B and murine MCF viruses are related at the 5′ end of the *env* gene (9, 51), we observed no such relatedness between the FeLV-A *env* gene and the *env* genes of various ecotropic MuLV isolates. One possible explanation is that the exogenous FeLV and MuLV proviruses have evolved from the MCF or FeLV-B-like endogenous proviral elements in the relatively recent past. Their alterations in the U3 region of the LTR and the *env* gene receptor-binding region could reflect the requirements for mutations in these regions for the viruses to escape host restrictions on replication. A similar proposal has been made for the evolution of the exogenous, horizontally transmitted strains of avian leukosis virus, which also differ from their endogenous counterparts both in U3 and at the 5′ end of *env* (42). Further studies will be aimed at establishing the significance of this parallel between the various retroviral families and examining the roles of the variant *env* genes of FeLV in pathogenesis.

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