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Comparison of Two Host Cell Range Variants of Feline Immunodeficiency Virus

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Two molecular clones of feline immunodeficiency virus were compared. The first clone, 34TF10, was from a Petaluma, Calif., isolate; the second, PPR, was isolated from a cat in the San Diego, Calif., area. The cats from which the isolates were obtained suffered from chronic debilitating illnesses. The two molecular clones differed in their in vitro host cell range. The 34TF10 clone infected the Crandall feline kidney and G355-5 cell lines, but replicated less efficiently on feline peripheral blood leukocytes. In contrast, the PPR clone productively infected the primary feline peripheral blood leukocytes but not Crandall feline kidney or G355-5 cells. The 34TF10 and PPR clones had an overall sequence identity of 91%. The *env* gene was the least conserved (85% at the amino acid level). Additionally, the potential open reading frame for a Tat-like protein, ORF 2, contained a stop codon in the 34TF10 isolate which was not found in the PPR clone. This truncation did not prevent in vitro or in vivo replication of 34TF10. Two splice acceptor sites were identified in the 34TF10 clone. One was 5′ to the beginning of the putative *tat* open reading frame, and the other was 5′ to the putative *vif* product. Both of these acceptor sites were conserved in the PPR clone. The long terminal repeats of the viruses were 7% divergent between the two clones, with a lack of conservation in putative NF-κB, LBP-1, and CCAAT enhancer-promoter sites.

Feline immunodeficiency virus (FIV), a recently isolated virus of domestic cats (36), has a prevalence of 1.2% in low-risk animals and a 14% prevalence in high-risk groups (48). Although FIV is not known to infect people (36, 48), it is closely related to human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome. Both FIV and HIV are retroviruses, in the lentivirus subfamily, and cause a fatal immunodeficiency syndrome in their respective hosts. This syndrome is characterized by generalized lymphadenopathy and increased susceptibility to opportunistic infections (2, 20, 36, 37, 48, 49). FIV causes a significant disease in cats and also may serve as a model for the study of acquired immunodeficiency syndrome.

The nucleotide sequences of two closely related variants of the Petaluma isolate of FIV have recently been reported (32, 45). Here we report analysis of the complete nucleotide sequence of a new molecular clone of FIV (termed PPR), which originated from a cat from the San Diego, Calif., area. The PPR clone of the San Diego isolate differs substantially from two clones of the Petaluma isolate, not only in its nucleic acid sequence but also in its in vitro host cell range. The present studies contribute to our understanding of the genetic organization of FIV as well as providing a framework for the development of therapeutic and preventive approaches to lentivirus infections.

MATERIALS AND METHODS

Cells and virus. The Petaluma isolate of FIV was originally obtained from a Petaluma, Calif., cat (36). Prior to death, this cat demonstrated signs of severe immunodeficiency. This isolate was adapted to and propagated in an adherent cell line, Crandall feline kidney (CRFK) cells. The adherent feline cell line G355-5 used in the transfection studies was established from a cat fetal brain culture (13).

The PPR clone of the San Diego isolate was obtained from a cat suffering from a chronic debilitating illness. This cat originated from Cold Spring Harbor, N.Y. However, 6 years prior to viral isolation, the cat moved to the San Diego, Calif., area. Signs of FIV infection did not appear until approximately 3 years after relocation. Exactly when or where this cat contracted FIV was not known. The cat had a long and protracted illness characterized by lethargy, gingival ulceration, and tooth loss. The cat was FIV positive and feline leukemia virus negative. Symptomatic treatment was initiated and continued until the cat's condition deteriorated so much as to require humane euthanasia.

On necropsy examination of the cat, it was noted that the gastrointestinal tract was virtually devoid of ingesta, suggesting that the cat had not eaten in at least the last 24 h. Detailed histological and hematological evaluations were not performed. The San Diego isolate was obtained by culturing the cat's peripheral blood leukocytes (PBLs) in RPMI-1040 in the presence of 10% fetal bovine serum and recombinant human interleukin-2 (kindly provided by Hoffman-La Roche). PBLs from FIV-negative, specific-pathogen-free cats were added to the culture to maintain a 40% viability. The culture was monitored weekly for the development of Mg^{2+}-dependent reverse transcriptase activity (36).

Transfections. Full-length plasmid clones of 34TF10 and PPR were separately transfected into the G355-5 cells by the calcium phosphate precipitation method (34). The next day, noninfected feline PBLs were cocultivated with the transfected G355-5 cells for 24 h. After the cocultivation, the G355-5 cells and the cocultivated PBLs were maintained separately and monitored for the appearance of Mg^{2+}-dependent reverse transcriptase activity (36).

PCR amplification of cDNA. The polymerase chain reaction (PCR) was used to characterize the splice donor and splice acceptor sites of clone 34TF10. RNA was prepared

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from CRFK cells that were chronically infected with the Petaluma strain of FIV (40). The cDNA was made by conventional methods (43). Amplification of the cDNA was accomplished by selecting 5′ and 3′ PCR primers that had been altered to produce *Eco*RI sites to facilitate cloning into M13. The upstream primer corresponded to the 5′ end of the viral mRNA (bases 229 to 252 [45]). The downstream primer was from the 5′ region of the *env* gene (bases 6463 to 6489 [45]). The PCR products were cloned into M13 and sequenced.

Genomic libraries. DNA was prepared from FIV-infected CRFK cells (Petaluma strain) or PBLs (San Diego strain) as described previously (3, 46). The DNA was then partially digested with the restriction enzyme *Sau*3A to yield fragments with an average size of 20 kilobases (kb). Fragments were ligated into the *Bam*HI site of the bacteriophage lambda vector EMBL-4 (Stratagene), and six genomic equivalents of DNA were packaged. The library was then plated for subsequent screening with virus-specific ^{32}P-labeled probes derived from the *pol* gene of the 34TF10 clone of FIV (45). Positive clones were selected and taken through several cycles of purification prior to further analysis.

Nucleotide sequencing. Nucleotide sequencing was performed as described before (42). Specific proviral oligonucleotide primers were prepared to sequence the entire viral genome in both directions.

Computer analysis. The nucleotide and protein alignments as well as the percent identity and percent similarity were done with the University of Wisconsin Genetics Computer Group Sequence Analysis Software Package version 6.1.

Nucleotide sequence accession number. The complete PPR sequence has been deposited in the GenBank data base (accession no. M36968).

RESULTS

Transfection studies. Both of the FIV clones were infectious, as demonstrated by the rise in reverse transcriptase activity following transfection (Fig. 1). However, the clones differed in their in vitro host cell range. Substantial reverse transcriptase activity was only found in G355-5 cells after transfection with the 34TF10 clone. In contrast, reverse transcriptase activity was found only in PBLs transiently cocultivated with the PPR-transfected G355-5 cells. Long-term cultures of the G355-5 cells either transfected or infected with the PPR isolate remained negative for reverse transcriptase activity (not shown). However, we were able to establish infections of PBLs by the 34TF10 clone when higher multiplicities of infection were used (data not shown). The G355-5 cells transfected with the 34TF10 clone developed a directional growth pattern and became more elongated and spindle shaped relative to PPR-transfected and sham-transfected control cells (Fig. 2). Small syncytia were also apparent in the 34TF10-infected cultures (Fig. 2D). These cultures eventually died if not replenished with noninfected cells.

Nucleotide sequence analyses. The complete nucleotide sequence of the 34TF10 clone of the Petaluma isolate of FIV has been reported previously (45). The complete PPR sequence has been deposited in the GenBank data base, and regions of interest are presented here.

The PPR clone had a genome length of 9,468 base pairs (bp). Comparisons of the sequences of the 34TF10 and PPR clones revealed an overall nucleic acid sequence identity of 91%. The basic genomic organization of PPR and 34TF10 was similar. The only differences occurred in the small open reading frames (ORFs). Some of these small ORFs, previously noted in the 34TF10 strain (45), were not conserved in PPR. A comparison of the ORFs between 34TF10 and PPR clones and a summary diagram of the consensus genomic organization are shown in Fig. 3.

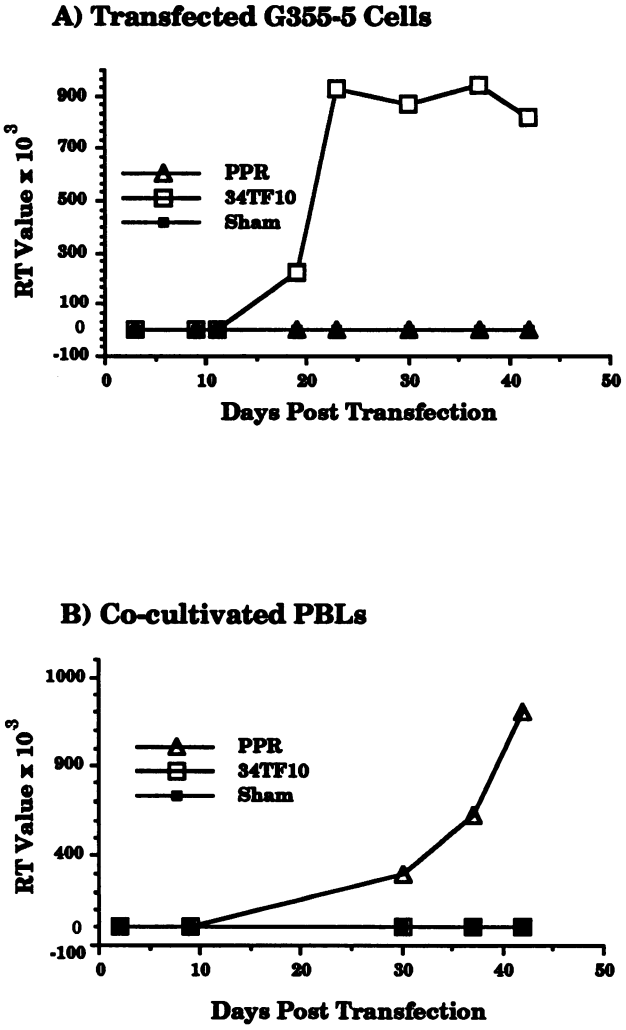

FIG. 1. Reverse transcriptase (RT) activity in culture supernatant after transfection with the molecular clones of FIV. The 34TF10 and PPR clones were separately transfected into noninfected G355-5 cells (13) by the calcium phosphate precipitation method (41). At 24 h posttransfection, noninfected PBLs were cocultivated with the transfected G355-5 cells for 24 h and then removed and cultured separately. The tissue culture supernatants from G355-5 cells (A) and PBLs (B) were monitored for Mg^{2+}-dependent reverse transcriptase activity (36). Expression of the PPR clone in G355-5 cells resulted in productive infection of PBLs during cocultivation. However, the PPR clone did not produce a detectable increase in RT activity in the G355-5 cells. In contrast, the 34TF10 clone established a productive infection in G355-5 cells after transfection. However, the amount of 34TF10 virus produced was insufficient to establish an infection in the cocultivated PBLs. Infection of PBLs by 34TF10 did occur but required a high multiplicity of infection (not shown).

LTRs. The long terminal repeats (LTRs) of the PPR and 34TF10 clones had 93% nucleic acid identity (Fig. 4). The TATA box of the promoter and the 2-base inverted repeats at the 5′ and 3′ ends of the LTR were perfectly conserved.

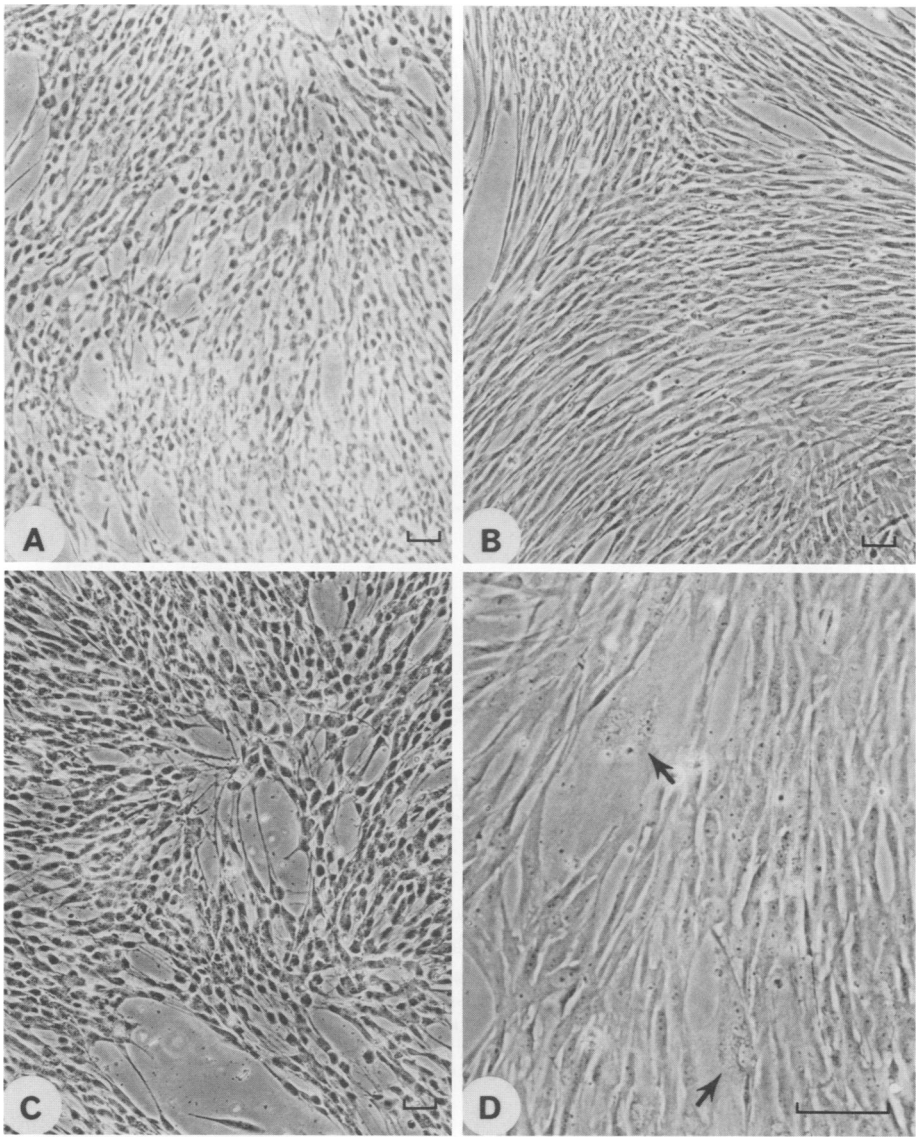

FIG. 2. Morphological changes of G355-5 cells associated with a productive FIV infection. PPR, 34TF10, and sham-transfected G355-5 cells are shown in panels A, B, and C, respectively, at low magnification (×100). Panel D is an ×200 magnification of the 34TF10-transfected cells. The arrows indicate the location of small syncytia observed in conjunction with productive infection by the 34TF10 clone. Note, in addition, that the productively infected cells (panels B and D) took on a more elongated, spindle-shaped appearance with loss of refractility, as well as a more organized growth pattern, relative to nonproductively infected cells (panels A and C). Bars, 20 μm.

The location of the polyadenylation site and, thus, the boundary between the R and the U5 regions of the LTR was confined to either base 287 or base 288 of the 34TF10 clone (Fig. 4). Since both of these bases were adenine residues, the exact location of the polyadenylation site was not determined. The 20-base region of the LTR from the start of the polyadenylation signal to the polyadenylation site was perfectly conserved between the two clones. In the U3 region of the LTR, several known upstream enhancer-promoter elements were common to both clones: AP-4 (28), AP-1 (28), and ATF (24) binding sites, as well as the TATA element (Fig. 4). However, the consensus sequence for a second AP-4 site and a CCAAT promoter element were present in the PPR clone but not conserved in the 34TF10 clone (Fig. 4). A putative LBP-1 (22) binding site and partial nucleotide match (8 of 10) for the consensus sequence of the NF-κB binding site (15) were found in the 34TF10 clone but not in the PPR clone (Fig. 4). Enhancers frequently take on the form of imperfect direct repeats. Two sets of imperfect direct repeats are shown in Fig. 4. Both sets of these imperfect direct repeats were conserved in the PPR and 34TF10 clones.

***gag* gene.** The *gag* gene of FIV is predicted to encode a polyprotein of 450 amino acids. Posttranslational cleavage of this polyprotein should yield the predicted matrix (MA), capsid (CA), and nucleocapsid (NC) proteins (45). This gene and its predicted protein products were highly conserved between the 34TF10 and PPR clones (Table 1).

***pol* gene.** The *pol* gene of FIV is most likely transcribed as a *gag-pol* polyprotein by ribosomal frameshifting (45). It has been suggested that through autodigestion, the following proteins result: protease (PR), reverse transcriptase (RT),

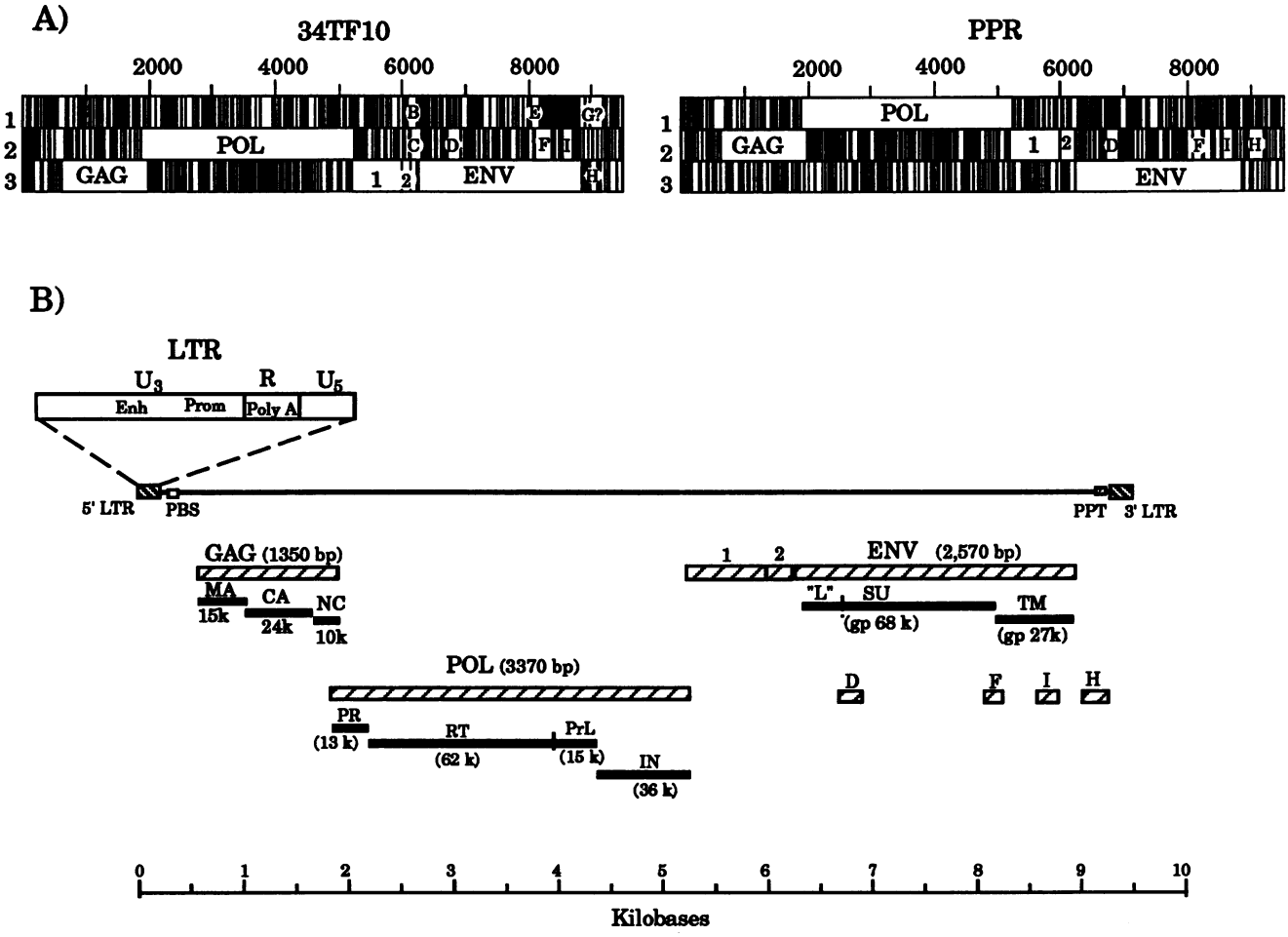

FIG. 3. (A) Comparison of the ORFs of the PPR and 34TF10 clones. (B) Consensus genomic organization of FIV. Features of both LTRs are shown in the 5′ LTR. *gag* region encodes the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins. *pol* region encodes protease (PR), reverse transcriptase (RT), proteaselike protein (PrL), and integrase (IN). *env* region encodes the putative L protein (9, 45) as well as major (SU) and minor (TM) glycoproteins of the viral envelope. PPT, Polypurine tract; PBS, primer-binding site. In addition to the above gene segments, six short ORFs, 1, 2, D, F, I, and H, were evident and conserved in both FIV clones. k, Kilodaltons.

proteaselike protein (PrL) (26, 27, 32, 45), and integrase (IN). The predicted protein products of the *pol* gene were highly conserved (Table 1).

***env* gene.** In addition to coding for the surface (SU) and the transmembrane (TM) proteins, the *env* gene of FIV has the potential to encode a third protein, of unknown function (45), similar to the L protein of visna virus (9). Of the large ORFs, the one with greatest nucleotide sequence variability

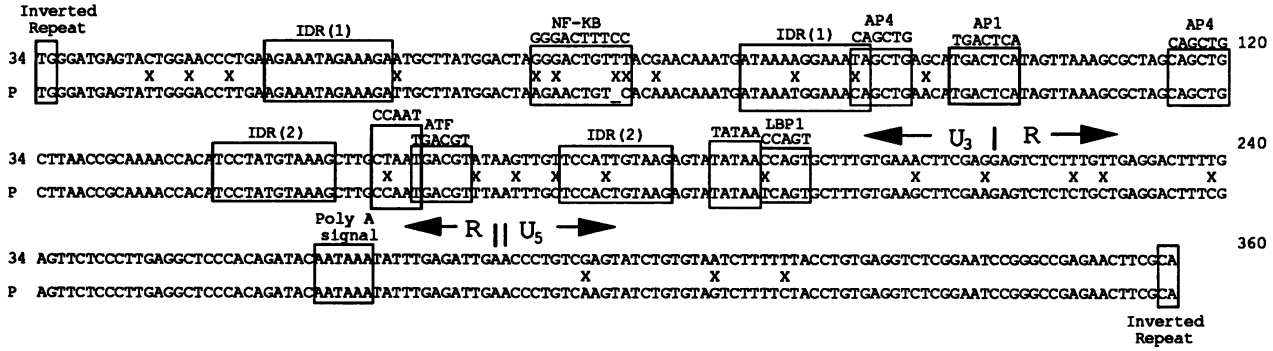

FIG. 4. Nucleotide sequence comparison of the LTR from the PPR and 34TF10 clones of FIV. Important structural features are boxed: the inverted repeats at the 5′ and 3′ ends of the LTR, two sets of imperfect direct repeats (IDR 1 and 2), CCAAT site, TATAA site, LBP-1 site, polyadenylation (Poly A) signal, and the recognition sequences of the enhancer proteins NF-κB, AP-1, AP-4, and ATF. The consensus sequences, for the enhancer-binding sites, are indicated above the appropriate box. Arrows indicate the boundaries of the U3, R, and U5 regions of the LTR. X's indicate nucleotide changes between the two clones. The underlined space indicates a relative deletion/insertion.

TABLE 1. FIV sequence comparison[a]

Gene and proteins	% Nucleic acid identity	% Amino acid identity	% Amino acid similarity
gag	95	96	98
Matrix	95	97	97
Capsid	95	96	99
Nucleocapsid	94	92	96
pol	95	95	97
Protease	95	98	98
Reverse transcriptase	94	95	97
Proteaselike protein	94	92	95
Integrase	95	95	98
env	86	85	92
L	87	82	91
Leader	67	55	69
Surface	88	87	92
Transmembrane	84	87	95
Small ORFs			
1	92	90	94
2	78	73	86
D	85	72	77
F	80	46	71
H	85	66	78
I	83	58	75

[a] Comparison of the nucleic acid identity, deduced amino acid identity, and predicted amino acid similarity from various protein coding regions of the PPR and 34TF10 clones of FIV.

was the *env* gene (Table 1). The predicted amino acid differences of the *env* gene products were not distributed randomly (Fig. 5). They clustered in six areas, which were called variable regions, 1 through 6 (V1 to V6). Nine areas of the predicted *env* gene product were well conserved, with few or no amino acid changes. These regions were designated conserved areas 1 through 9 (C1 to C9).

Although hydrophobic in both clones, the presumed leader sequence was contained within variable region 2. Two short variable regions, V5 and V6, were found within hydrophilic regions of the FIV TM protein. V5 was contained within a hydrophilic area that spanned the two hydrophobic regions of this protein, while V6 was located at the presumed cytoplasmic tail (hydrophobic and hydrophilic areas not shown). Although there was considerable variability in the predicted amino acid sequence of the *env* gene, the glycosylation sites and cysteine residues were highly conserved, with 31 of 33 cysteines and 21 of 22 glycosylation sites being maintained (Fig. 6). For all *env* regions, the percentage of amino acid similarity was substantially higher than the percentage of amino acid identity, particularly for the presumed L and TM proteins (Table 1).

Small ORFs. A number of small ORFs were present in both clones. Six ORFs (1, 2, D, F, I, and H) were greater than 120 bp in length and were conserved in both FIV clones (Fig. 3B, Table 1). ORF 1 was similar in size and location to the *vif* gene of the primate lentiviruses (6, 10, 12, 38, 41, 45, 47). However, no nucleotide sequence homology was evident. ORF 1 was conserved between the two clones to nearly the same degree as were the *gag* and *pol* genes (Table 1). ORF 2 resembled the first exon of *tat* by its size and location in the FIV genome (6, 10, 12, 38, 41, 47). Again, no nucleotide sequence homology was evident between ORF 2 and the primate Tat proteins. ORF 2 prematurely terminated in the 34TF10 clone due to a transition of a G to an A residue, resulting in the generation of a stop codon (45) (Fig. 7). Thus, this ORF of the PPR clone coded for a polypeptide that was approximately twice the size of that encoded by the 34TF10 clone. However, the premature stop codon of the 34TF10 clone does not appear to have a role in determining host cell range. This is because another clone of the Petaluma isolate (32) has been reported to have a host cell range similar to the 34TF10 clone but, like the PPR clone, codes for a full-length ORF 2.

Splice donor and acceptor sites. PCR amplification of cDNA was used to identify the splice donor and acceptor sites from two subgenomic mRNA species of clone 34TF10 (Fig. 8). Immediately upstream from the *gag* coding sequence, a 5′ splice donor site, common to both mRNA species, was found at base 604 of the 34TF10 sequence (45). The first mRNA species was spliced at least once, with a splice acceptor at base 5921, 70 bases 5′ to the start codon of the putative *tat* product. The second mRNA species was spliced at least twice, using the common 5′ splice donor site at base 604 and an acceptor site at base 5188. This acceptor site was located 68 nucleotides prior to the putative start of Vif. Seventy bases downstream, another classic splice donor site at base 5255 was used in this species. The message was then resumed at the previously described ORF 2 acceptor site, using base 5921. In neither of the mRNA species were

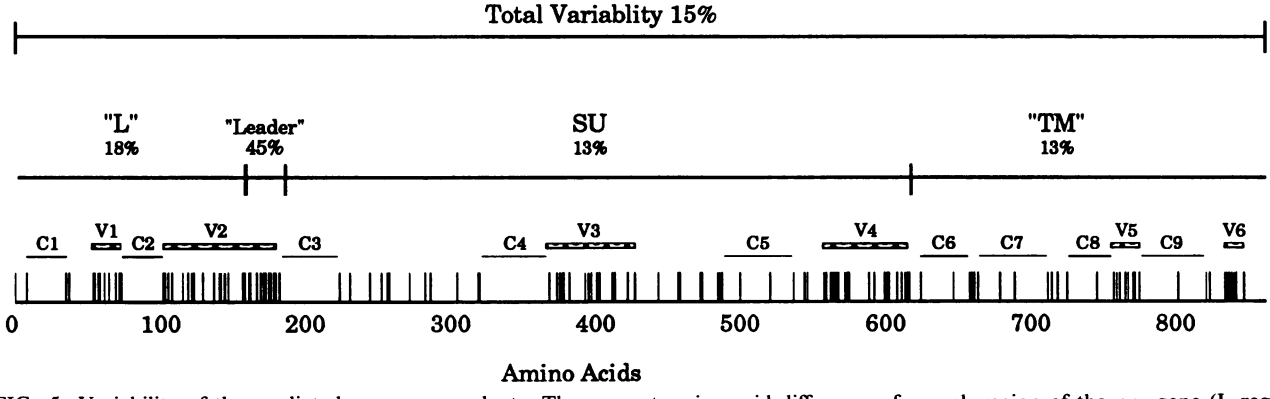

FIG. 5. Variability of the predicted *env* gene products. The percent amino acid differences for each region of the *env* gene (L region, leader, SU [surface protein], and TM [transmembrane protein]) are listed under the appropriate identifying symbols. The vertical lines represent the locations of the predicted amino acid differences. The six variable and nine conserved regions are indicated by V and C, respectively.

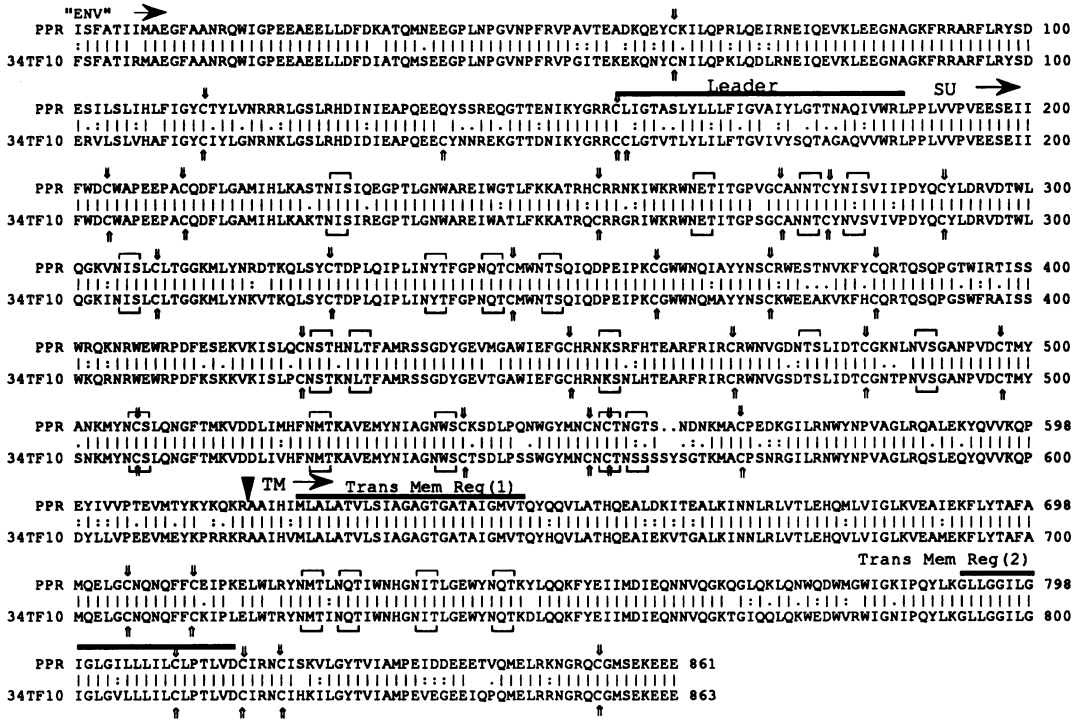

FIG. 6. Comparison of the deduced amino acid sequence of the *env* gene products from the 34TF10 and PPR clones of FIV. The vertical lines between amino acids indicate identity, two dots indicate a high degree of similarity, one dot shows a moderate degree of similarity, and a space represents dissimilar amino acids. Cysteines are indicated by small vertical arrows, while the brackets show potential glycosylation sites. The vertical arrowhead denotes the putative proteolytic cleavage site. SU, Major surface protein; TM, transmembrane protein. Horizontal lines are placed above the putative leader and the two presumed transmembrane regions (Trans Mem Reg 1 and 2).

ORFs maintained across the splice junctions. These 34TF10 splice acceptor sites were conserved in PPR (data not shown).

DISCUSSION

The purpose of this study was to compare two distinct FIV isolates. These two isolates originated from different cats at distinct geographic locations and varied in their in vitro host cell range. *env* variability has been used to determine the genetic similarity of HIV isolates (30), with the most distantly related isolates having an *env* variability of greater than 12% at the nucleic acid level. Thus, by the standards set for HIV, the two FIV clones, with an *env* diversity of 14%, would be considered distantly related isolates (30).

It is important to note that both FIV clones were infectious. Infectious clones have constraints on their sequence variability, as critical viral functions must be maintained for the virus to remain viable. Thus, essential functional domains of FIV should be conserved between these two clones. However, the FIV clones 34TF10 and PPR differed in an important biological property, their in vitro host cell range. In other retroviruses, host cell alterations have resulted from changes in the LTR and/or *env* regions of the virus (7, 8, 17, 18, 21, 25, 33, 39, 46). It is likely that one or both of these regions are also responsible for the host cell range of FIV. Further studies are needed before an exact determination of the factors influencing host cell range can be identified.

Similar to other lentiviruses, the *gag* and *pol* regions of FIV were highly conserved, up to 98% at the amino acid level. The *pol* gene of FIV, like that of visna virus and equine

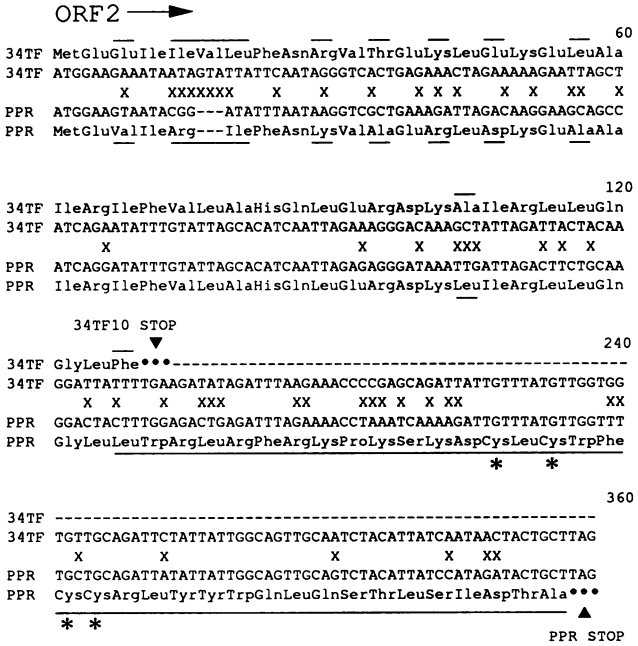

FIG. 7. Comparison of nucleotide sequence and deduced amino acid sequence of ORF 2 from the 34TF10 and PPR clones of FIV. X's indicate nucleotide changes between the two clones. The amino acid changes between the two clones are underlined. Cysteine residues are represented by *, and stop codons are shown as three black dots.

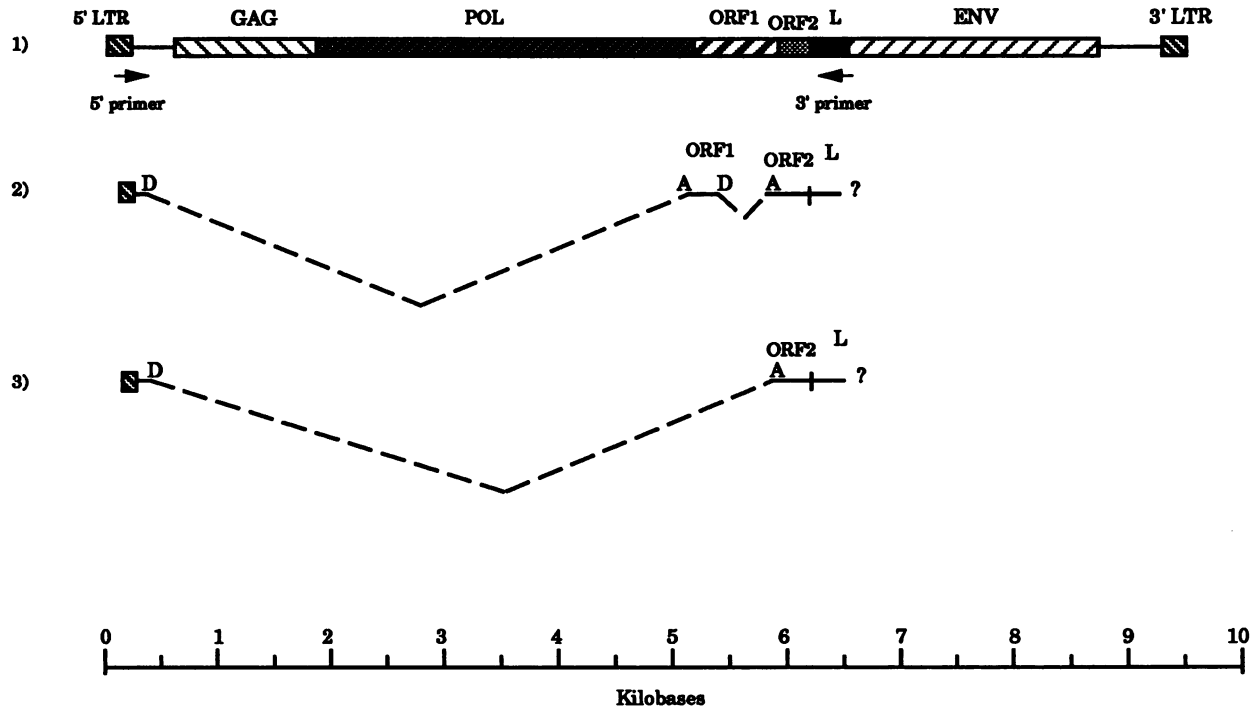

FIG. 8. Identification of the splice donor and splice acceptor sites of the 34TF10 isolate of FIV. The major ORFs of FIV are represented in line 1. Locations of plus- and minus-strand PCR primers are indicated by arrows. Line 2 represents a subgenomic mRNA of the 34TF10 clone, with splice donor sites (D) at bases 604 and 5255 and splice acceptor sites (A) at bases 5188 and 5921. Line 3 represents another subgenomic mRNA species of the 34TF10 clone of FIV with a single splice donor site at base 604 and splice acceptor site at base 5921.

infectious anemia virus, had the potential of coding for an extra proteaselike gene product, termed PrL (26, 27). Apparently, this gene product arose by tandem duplication and subsequent divergence of the simian retrovirus type I protease gene (26, 27). It is implied that the PrL gene was horizontally transferred to the precursor of the ungulate-feline lentiviruses after the divergence of HIV from this group. An alternative hypothesis is that PrL was transferred to the lentivirus precursor and was subsequently lost from the primate branch. Since this putative gene product is conserved to the same degree as the other *gag-pol* products of FIV and occurs in a group of viruses known for their efficient utilization of genetic material, it is likely that this putative gene product does have a function and is not being carried as a pseudogene. However, the exact function, if any, of PrL protein awaits further studies.

The LTRs of retroviruses determine, in part, the rate of viral transcription. Not surprisingly, with a 93% nucleic acid identity, many of the structural elements of the LTR were conserved between the two FIV clones. The identification of conserved enhancer-promoter binding sites does not ensure that they function in FIV transcription. However, these sites are located in the U3 region just upstream from the TATA site and are in an ideal location to exert enhancer function. Of particular interest is the presence of both AP-1 and AP-4 sites in the FIV clones, since they have been shown, in other systems, to synergistically enhance transcription (28). An ATF-binding site, which is also known as the cyclic AMP response element (14, 19), was conserved in both clones. The ATF protein has been shown to establish a preinitiation complex through interactions with its DNA-binding site and the mammalian TATA factor TFIID (14, 19). The virally encoded *trans*-activating protein $p38^{tax}$ of bovine leukemia virus may enhance transcription by binding to an ATF-binding site in its LTR (23). Thus, it is possible that an FIV-encoded protein may interact with the ATF-binding site in a manner similar to $p38^{tax}$.

A classic CCAAT promoter element was found in the PPR clone but not in the 34TF10 clone. Nucleotide substitutions anywhere within this element dramatically decrease transcription (31). Thus, this may be an important difference between these two clones.

The 34TF10 clone has an 8 of 10 match with the consensus sequence of the NF-κB binding site. However, the 34TF10 clone differs from the consensus NF-κB binding site sequence in two functionally critical bases, the two 3′ C residues (4, 15, 44). This change may be interpreted in three ways: (i) the putative NF-κB binding site is not really an enhancer binding site; (ii) the feline NF-κB protein recognizes a slightly different nucleic acid sequence; or (iii) an unknown enhancer protein, which is related to NF-κB, binds to this site. To complicate things further, the greatest LTR diversity between these two clones also occurred at the putative NF-κB binding site. The significance of PPR's changes at this site are currently under investigation in a collaborative study with P. Luciw and colleagues at the University of California, Davis. The LBP-1 site, also called UBP-1, was found in the 34TF10 clone but was not maintained in the PPR clone. Similar to the HIV LTR, the putative LBP-1 binding site of FIV was in close approximation to the TATA box. In HIV, the binding of the LBP-1 protein greatly increases transcription (22).

Although the LTR nucleic acid sequence of these two clones was highly conserved, some changes occurred in potentially critical areas. In particular, changes in the putative NF-κB binding site, one of the AP-4 binding sites, the LBP-1 site, and the CCAAT promoter element may be of functional importance. It is possible that one or several of

these changes are responsible for the different host cell ranges of these two FIV clones. Further experiments are needed to identify the role of the enhancer-promoter binding sites in FIV transcription and the resulting biological effect of these changes.

Of the large ORFs, the greatest predicted amino acid diversity was found in the *env* gene. There appeared to be certain constraints on this sequence variability, as both the cysteines and glycosylation sites were highly conserved. Additionally, many of the amino acid changes were of a conservative nature, as reflected in the high degree of amino acid similarity between the Env proteins.

The predicted amino acid changes of the *env* gene clustered in certain protein regions, allowing the identification of variable and conserved sites. This clustering of Env amino acid diversity appears to be a property of lentiviruses (1, 5, 11, 16, 29, 35). The variable regions of lentiviruses tend to occur at immunodominant sites. These regions may serve to divert immunologic recognition away from the conserved areas, which are more likely to encode important viral functions. The diversion of the immune system from the conserved regions may allow the virus to escape immune surveillance or to delay its neutralization. Through the use of recombinant DNA technology, it may be possible to produce an efficacious and broadly reactive lentivirus vaccine. The identification of the conserved regions of *env* is the first step toward the development of such a vaccine for FIV.

The second small ORF, ORF 2, is in the same location and is similar in size to the first exon of *tat* in the primate lentivirus. In the 34TF10 clone of the Petaluma isolate, this ORF terminated prematurely. This shortened ORF 2 appears to be unique to the 34TF10 clone, since this ORF was nearly twice as long in two other FIV clones (the PPR clone of this study and a second infectious clone of the Petaluma isolate [32]). Obviously, the full-length product of the putative *tat* gene is not needed for viral infectivity, since both the PPR and the 34TF10 clones were infectious.

As further evidence that these products are actually produced, we have identified two mRNA species that utilize splice acceptor sites just 5′ to the initiation codons of ORF 1 and ORF 2 of the 34TF10 clone. These sites are also conserved in the PPR clone. Further analyses to define additional spliced mRNA species and their protein products are in progress. Before the significance of the small ORFs can be discerned, the predicted gene products and their function must be identified.

Four other small ORFs were conserved between the two FIV clones (D, F, I, and H). Since they were found in both of these diverse FIV clones, it is possible that they have an important FIV function. However, the significance of these small ORFs awaits further investigations.

From this study, we have gained a better understanding of the genomic organization of FIV. Conserved areas of the virus were identified, as were potential regulatory elements. These regions may eventually be targeted for preventive or therapeutic intervention. This information is important, for FIV is not only an important pathogen of cats, but also should prove to be an excellent model for the study of HIV.

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