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Abstract: The complete nucleotide sequence of an isolate of puma lentivirus (PLV-14) was obtained by an inverse polymerase chain reaction (I-PCR) technique and confirmed by conventional PCR. Both methods were used to amplify overlapping regions of proviral DNA, for cloning and sequencing, from genomic DNA isolated from PLV-14 infected Florida puma (Felis concolor coryi) peripheral blood mononuclear cells (PBMC). The provirus has a total length of 9100 nucleotides and the genomic organization of presumed protein coding regions are similar to those seen in other members of the lentivirus family, i.e., three large open reading frames gag, pol, and env as well as smaller intergenic regions that apparently encode regulatory proteins vif and 3' rev by positional and sequence similarity to those seen in other lentiviruses. Two additional open reading frames were identified in the env region and their function (if any) is unknown. The length of the PLV-14 long terminal repeat (LTR) was found to be shorter than the LTRs of feline immunodeficiency virus (FIV). The sequence homology between PLV-14 and other lentiviruses demonstrates that PLV-14 is most closely related to FIV from domestic cats. However, the extent of sequence divergence of each retroviral gene segment is large (e.g., percentage sequence similarity between FIV and PLV-14 env is 8% amino acid and 37% nucleotide similarity), indicating relatively ancient divergence of these feline lentiviral genomes.The complete nucleotide sequence of an isolate of puma lentivirus (PLV-14) was obtained by an inverse polymerase chain reaction (I-PCR) technique and confirmed by conventional PCR. Both methods were used to amplify overlapping regions of proviral DNA, for cloning and sequencing, from genomic DNA isolated from PLV-14 infected Florida puma (Felis concolor coryi) peripheral blood mononuclear cells (PBMC). The provirus has a total length of 9100 nucleotides and the genomic organization of presumed protein coding regions are similar to those seen in other members of the lentivirus family, i.e., three large open reading frames gag, pol, and env as well as smaller intergenic regions that apparently encode regulatory proteins vif and 3' rev by positional and sequence similarity to those seen in other lentiviruses. Two additional open reading frames were identified in the env region and their function (if any) is unknown. The length of the PLV-14 long terminal repeat (LTR) was found to be shorter than the LTRs of feline immunodeficiency virus (FIV). The sequence homology between PLV-14 and other lentiviruses demonstrates that PLV-14 is most closely related to FIV from domestic cats. However, the extent of sequence divergence of each retroviral gene segment is large (e.g., percentage sequence similarity between FIV and PLV-14 env is 8% amino acid and 37% nucleotide similarity), indicating relatively ancient divergence of these feline lentiviral genomes.

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# Nucleotide Sequence Analysis of Puma Lentivirus (PLV-14): Genomic Organization and Relationship to Other Lentiviruses

RAYMOND J. LANGLEY,[1] VANESSA M. HIRSCH, STEPHEN J. O'BRIEN,* DIANE ADGER-JOHNSON, ROBERT M. GOEKEN, AND ROBERT A. OLMSTED†

*Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Rockville, Maryland 20852; *Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland 21702; and †Cancer and Infectious Diseases, Upjohn Laboratories, Kalamazoo, Michigan 49001*

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**The complete nucleotide sequence of an isolate of puma lentivirus (PLV-14) was obtained by an inverse polymerase chain reaction (I-PCR) technique and confirmed by conventional PCR. Both methods were used to amplify overlapping regions of proviral DNA, for cloning and sequencing, from genomic DNA isolated from PLV-14 infected Florida puma (*Felis concolor coryi*) peripheral blood mononuclear cells (PBMC). The provirus has a total length of 9100 nucleotides and the genomic organization of presumed protein coding regions are similar to those seen in other members of the lentivirus family, i.e., three large open reading frames *gag*, *pol*, and *env* as well as smaller intergenic regions that apparently encode regulatory proteins *vif* and 3′ *rev* by positional and sequence similarity to those seen in other lentiviruses. Two additional open reading frames were identified in the *env* region and their function (if any) is unknown. The length of the PLV-14 long terminal repeat (LTR) was found to be shorter than the LTRs of feline immunodeficiency virus (FIV). The sequence homology between PLV-14 and other lentiviruses demonstrates that PLV-14 is most closely related to FIV from domestic cats. However, the extent of sequence divergence of each retroviral gene segment is large (e.g., percentage sequence similarity between FIV and PLV-14 *env* is 8% amino acid and 37% nucleotide similarity), indicating relatively ancient divergence of these feline lentiviral genomes.** © 1994 Academic Press, Inc.

## INTRODUCTION

Lentiviruses are known to infect many mammalian species and cause a condition of persistent infection that can lead to varying degrees of morbidity and mortality depending on the virus and the host species involved (Narayan and Clements, 1989). The majority of these lentiviruses share similarities in their genome organization to the human immunodeficiency viruses, HIV-1 and HIV-2, the etiologic agents of acquired immunodeficiency syndrome (AIDS). One such virus, feline immunodeficiency virus (FIV) and the infection of its natural host, the domestic cat, has been described as a potential model for HIV infection of humans (Yamamoto *et al.*, 1988; Olmsted *et al.*, 1989a; Tompkins *et al.*, 1991). Cats naturally infected with this virus can progress to a lethal immunodeficiency syndrome (Pederson *et al.*, 1987; Yamamoto *et al.*, 1988) characterized by a progressive loss of CD4+ cells and numerous opportunistic infections (Ackley *et al.*, 1990; Barlough *et al.*, 1990). Thus, the disease manifests much in the same manner observed in HIV-1 infected AIDS patients (McCune, 1991) and nonhuman primates infected with the closest relative of the HIVs, simian immunodeficiency virus (SIV) (Hirsch *et al.*, 1989; Murphey-Corb *et al.*, 1986). SIV infection of nonhuman primates has been extensively studied as a model for HIV infection in humans and the genetic similarities of SIV and HIV-1 and HIV-2 suggest that HIVs had their origins in African primates (Doolittle *et al.*, 1989). Surprisingly, naturally infected African monkeys are free from many of the AIDS disease symptoms (Lowenstine *et al.*, 1986; Ohta *et al.*, 1988); however, when SIVs are inoculated into Asian macaque monkeys, immunodeficiency disease frequently occurs (Hirsch *et al.*, 1989). The genomic similarity of SIV strains isolated from several *Cercocebus* species to HIV-2, plus the phylogenetic overlap of HIV-2 isolates, implicates these primates as host reservoirs for the ancestors of HIV-2. The origin of HIV-1, the primary cause of AIDS outside of Africa, is less clear although genomic similarities of lentivirus isolates from captive chimpanzees in Gabon (Peeters *et al.*, 1989; Huet *et al.*, 1990) would support this species particularly if this chimp virus were to be discovered in free-ranging primate species.

Considerable interest has been focussed on FIV infection of the domestic cat as a potential small and inexpensive animal model for AIDS-like infections (Yamamoto *et al.*, 1988; Olmsted *et al.*, 1989a; Tompkins *et al.*, 1991). Natural FIV infections of domestic cats are prevalent worldwide and the viral isolates studied so far also exhibit significant genetic variability at the molecular level

[1] To whom correspondence and reprint requests should be addressed.



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similar to their human and nonhuman counterparts (Phillips *et al.*, 1990; Miyazawa *et al.*, 1991; Rigby *et al.*, 1993). Serological studies in wild and captive nondomestic felids have provided ample evidence that strains of FIV-like lentiviruses infect these larger cats (e.g., pumas, lions, and cheetahs; Lutz *et al.*, 1992; Olmsted *et al.*, 1992; Brown *et al.*, 1993). To date, a limited number of FIV isolates have been described at the molecular level. Limited sequence analysis of puma lentivirus (PLV) *pol* gene sequences indicated that the PLVs form a species-specific phylogenetic clade that is closely related to, but clearly divergent from, FIV isolates from domestic cats (Olmsted *et al.*, 1992). We have extended these studies and report here a complete nucleotide sequence and inferred genomic organization of one of these large felid lentiviruses, PLV-14, isolated from a free-ranging Florida puma (*Felis concolor coryi*; Roelke *et al.*, 1993). This virus shares antigenic determinants with FIV and has so far not been associated with any disease condition in *Felis concolor* (Olmsted *et al.*, 1992). Thus, a relationship that parallels infection of African nonhuman primates with SIVs and humans with HIVs may exist in domestic cats infected with FIV and pumas (and perhaps other nondomestic felids; Olmsted *et al.*, 1992) infected with PLV. We compared the genome organization of PLV to FIV and other related viruses to better understand the natural history, biology, and origins of the phylogenetically distinct group of lentiviruses that infect felids.

## MATERIALS AND METHODS

### Virus and cells

Peripheral blood mononuclear cells (PBMCs) from an FIV seronegative puma were used to propagate puma lentivirus-14 (PLV-14) isolated from Florida puma-14 as described (Olmsted *et al.*, 1992). At the peak of reverse transcriptase activity in infected cultures, genomic DNA containing PLV-14 proviruses was prepared and used as template for polymerase chain reaction (PCR) amplifications.

### Amplification of PLV-14 proviral DNA

Due to the low provirus copy number in the genomic DNA preparations, as determined by Southern blot analy-

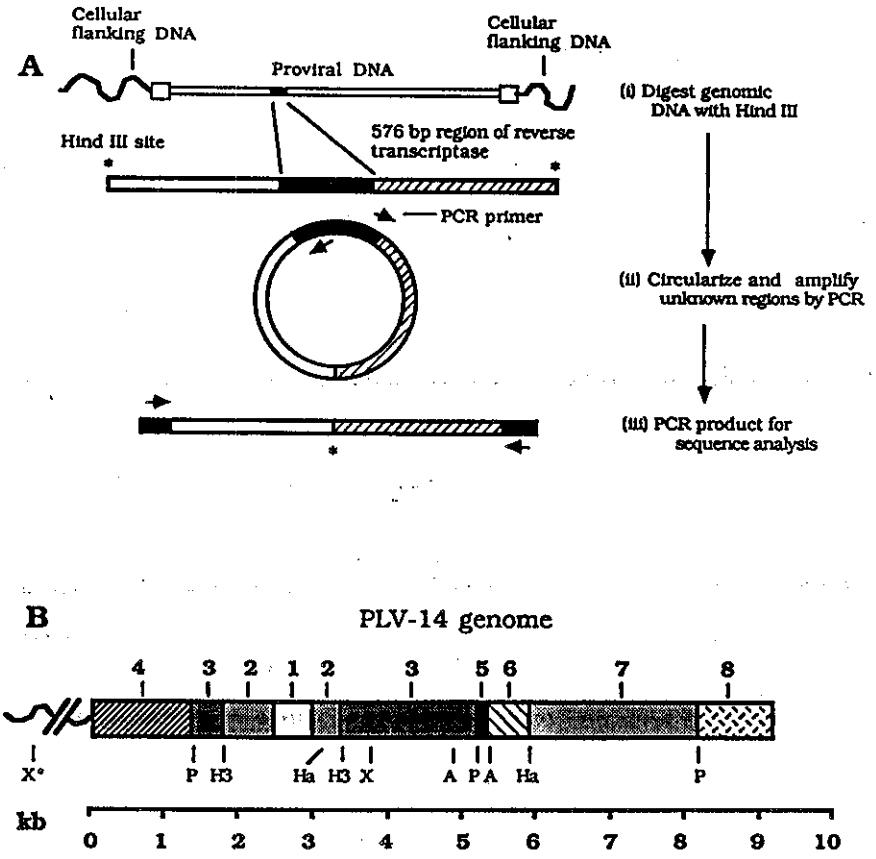


FIG. 1. Strategy for obtaining proviral DNA sequence of PLV-14 using I-PCR. (A) Diagram showing the use of *Hin*dIII sites, as an example, flanking a known region of reverse transcriptase and the manner in which further sequence was generated. (B) Schematic diagram of the PLV genome showing the approximate positions of the enzyme sites used, and the regions obtained using the I-PCR technique. Regions with the same shading were obtained as one fragment. The numbers above the regions represent the order in which they were generated. Restriction enzyme site abbreviations: (A) *Ava*II; (Ha) *Hae*III; (H3) *Hin*dIII; (P) *Pst*I; (X) *Xba*I; (X*) *Xba*I site located in the flanking cellular DNA. For details of the strategies used see Materials and Methods.

sis and two unsuccessful lambda phage library constructions, I-PCR (Ochman *et al.*, 1989; Innis *et al.*, 1990) and conventional PCR methods were utilized to amplify regions of the proviral genome for cloning and sequencing. The I-PCR technique provides a means to amplify, clone, and sequence DNA flanking a region of known sequence. Briefly, the genomic DNA was restriction endonuclease (RE)-digested at sites in the unknown sequence 5′ and 3′ to the known region. The digestion products were then sufficiently diluted in ligation reactions (<2 μg/ml DNA concentration) such that intramolecular ligation was favored to form monomeric circles (Ochman *et al.*, 1989). Upon amplification, PCR primers that hybridize in and near the 5′ and 3′ termini of the known region in the circular template and were designed in opposite orientation to each other extended through the unknown sequence and beyond the regenerated RE site contained within it (see Fig. 1A). Sequence information gained from the PCR products was used for the selection of the next RE to be used and synthesis of primer pairs. To begin the amplification of PLV-14 proviral DNA by I-PCR, Southern blot analysis provided a partial RE map (data not shown) of sites near the 576-bp region of the PLV-14 reverse transcriptase (RT) gene (bp 2482 to 3058; Olmsted *et al.*, 1992). This information was used to generate, by I-PCR, the initial clone composed of nucleotides 3′-2512 to 5′-1694 (*Hin*dIII; located 5′ to the 576-bp RT region) and 5′-2675 to 3′-3277 (*Hin*dIII; located 3′ to the 576-bp region) (Fig. 1B; see below for PCR conditions). The process was repeated to generate overlapping clones comprising the majority of the PLV-14 proviral genome. The following RE sites were used (genome coordinates are in parentheses): *Pst*I (1308, 5108 and 5108, 8085), *Ava*II (4935, 5292) and *Hae*III (3216, 5829) (Fig. 1B). *Xba*I digestion (one site at bp 3684 in the provirus and a site in the 5′ cellular flanking DNA 90 bp upstream to the 5′ LTR) was used to generate a fragment containing the 5′ LTR. The remaining 3′ portion of the genome (bp 8020 to 9054) was generated by standard PCR using sequence data obtained from the 5′ LTR sequence and by amplification across the *Pst*I site at bp 8085 (Fig. 1B; Table 1).

## PCR conditions

Genomic DNA from PLV-14 infected puma PBMC cultures was used as template for both I-PCR and conventional PCR procedures. Sequence examination of the core 576-bp RT region of the PLV-14 genome and Southern blot analysis established the presence of cleavage sites and approximate DNA fragment lengths for use in the I-PCR amplification technique. The REs used and their location within the proviral genome have been described above. Typically, genomic DNA (2 μg) was cleaved by selected REs (2 to 3 U/μg DNA) for 2 hr at 37°. Samples were extracted once with phenol/chloroform and once with chloroform/isoamyl alcohol to inactivate and remove REs. The aqueous phase was retained and the DNA precipitated by addition of 3 *M* ammonium acetate and 2.5 vol of 95% ethanol and left 3 hr to overnight at −20°. Following centrifugation and washing with 70% ethanol the digested DNA was then dried under vacuum and resuspended in 20 μl distilled water. Ten microliters of sample was then diluted in ligation buffer (50 m*M* Tris−HCl (pH 7.4), 10 m*M* $MgCl_2$, 10 m*M* dithiothreitol, 1 m*M* adenosine triphosphate) to a final volume of 1 ml (<2.0 μg DNA/μl). T4 DNA ligase was then added to a concentration of 0.02 Weiss units/μl and incubated overnight at 16°. Ligase was inactivated by incubation at 65° for 15 min and removed by addition of chloroform/

TABLE 1

PRIMERS USED FOR INVERSE PCR AMPLIFICATION OF THE PLV-14 GENOME[a]

| Enzymes and sites (genome coordinates) | Primer sequences (proviral genome coordinates) | | | |
|---|---|---|---|---|
| | Forward | | Reverse | |
| | Outer | Inner | Outer | Inner |
| *Hin*dIII (1694, 3277) | 2664–2688 | 2675–2699 | 2534–2515 | 2512–1491 |
| *Pst*I (1308, 5108) | 3184–3205 | 3184–3205 | 1773–1754 | 1714–1676 |
| *Xba*I (3684)[b] | 3582–3600 | 3615–3635 | 1773–1754 | 1714–1696 |
| *Ava*II (4935, 5292) | 5023–5045 | 5050–5072 | 3276–3256 | 3233–3216 |
| *Hae*III (3216, 5829) | 5023–5045 | 5050–5072 | 3276–3256 | 3233–3216 |
| *Pst*I (5108, 8085) | 5785–5804 | 5812–5831 | 5210–5187 | 5180–5161 |
| *Pst*I/LTR[c] (8085) | 7991–8012 | 8020–8041 | 9079–9055 | 9053–9031 |

[a] Primers are listed such that their sequence runs 5′ to 3′ (see Materials and Methods).

[b] In addition to the *Xba*I site at the indicated location in the provirus, two additional sites were used in the cellular flanking DNA, 90 and 239 base pairs distant from the start of the 5′ LTR.

[c] This portion of the PLV-14 genome was generated by standard PCR using primers that extended across the *Pst*I site indicated, and reverse primers obtained from the 5′ LTR sequence.

isoamyl alcohol. The now circularized DNA was precipitated by addition of salt and ethanol, washed, and resuspended to a final volume of 10 μl in distilled water. The PCR conditions used (for both standard and I-PCR amplifications) were as follows; 94°, 1 min; 55°, 1.5 min; and 72° for 1 min per 1000 bp of expected product. Samples were amplified for 30 cycles using an outer set of primers and 10 μl of this first round reaction was then inoculated into a fresh set of reagents with an inner (nested) set of primers and amplified a further 30 cycles. Lists of the primers used for inverse and standard PCR are given in Tables 1 and 2, respectively. Reagents and equipment used were supplied by Perkin Elmer Cetus and all of the manufacturers instructions were followed. On completion of the second round amplifications with the inner set of primers, 10 μl of the reaction product was analyzed on a 0.9% agarose gel and the remainder was gel-purified, end-flushed, and blunt-end ligated into the plasmid vector pGem7Zf(+) (Promega).

## Sequence determination and verification

Plasmid clones and PCR products were sequenced by the chain termination method with T7 DNA polymerase (United States Biochemical). Both strands of all DNA clones and fragments were sequenced. Those regions of the PLV-14 genome amplified by I-PCR were reamplified using standard PCR, cloned, and sequenced in order to verify the initial sequence data. The sets of primers used for this stage of the study are listed in Table 2. In general, 10 clones were selected and 2 were sequenced simultaneously and their sequences compared. Occasionally a nucleotide residue differed between the 2 clones. When this occurred the sequence of a third (confirmatory) clone was then obtained in the region in question to enable us to assign an identity to the queried nucleotide by matching the nucleotide with one or another of the original clones (in practice the sequence of the confirmatory clone did not differ from both of the original clones for any one position).

TABLE 2

PRIMERS USED IN CONVENTIONAL PCR AMPLIFICATION OF PLV-14 PROVIRAL DNA FOR VERIFICATION OF INVERSE-PCR GENERATED SEQUENCE DATA

| Region of genome amplified | Genome coordinates of primers[a] | | Fragment size in base pairs |
|---|---|---|---|
| | Outer | Inner | |
| 5′LTR-*gag* | 1–22 | 1–22 | |
| | 1773–1753 | 1714–1695 | 1714 |
| *gag-pol* | 1700–1721 | 1724–1743 | |
| | 3276–3255 | 3233–3215 | 1509 |
| *pol* | 3582–3600 | 3615–3635 | |
| | 5105–5084 | 5082–5060 | 1467 |
| *pol-vif* | 5023–5046 | 5050–5073 | |
| | 5831–5809 | 5804–5784 | 754 |
| *vif-env* | 5785–5804 | 5785–5804 | |
| | 6471–6451 | 6441–6420 | 656 |
| *env*-3′LTR[b] | 6420–6440 | 6451–6472 | |
| | 8846–8821 | 8817–8793 | 2365 |
| 3′LTR | 8572–8595 | Not done | |
| | 9100–9075 | | 528 |

[a] All primer coordinates, forward and reverse, are listed such that the sequence runs 5′ to 3′.

[b] These primers were also used to amplify the envelope region of proviral DNA from Puma No. 21 (sequence designated PLV-21).

FIV has a genome organization which encodes a small open reading frame (*orf* 2, Olmsted *et al.*, 1989b; *orf* A, Talbott *et al.*, 1989) directly following the stop codon of the *vif* homolog. Because PLV-14 did not apparently possess such a region in a similar position with respect to *vif* (Fig. 2A) we decided to amplify this region and sequence the PCR product directly to rule out possible plasmid deletions which might have occurred in the cloning processes. Therefore, a section of the genome spanning bp 5785 to 6441 (3′ end of *vif* to the start of the *env* gene) was amplified by standard PCR (primer locations are given in Table 2) and sequenced directly using the sequencing reagents and protocol described above with slight modifications. Briefly, 1 μg of the agarose gel purified PCR product (656 bp) was resuspended to 10 μl in distilled water and mixed with 2 μl of sequencing primer (10 ng/μl), heated to 94° for 3 min, and immediately chilled on ice. Two microliters of the labeling buffer (5× concentrate; 200 m*M* Tris–HCl (pH 7.5), 100 m*M* $MgCl_2$, 250 m*M* NaCl, United States Biochemicals) was added and the remainder of the procedure followed the manufacturer's recommendations for labeling and sequencing. The entire 656-bp fragment was sequenced on both strands in this manner.

Further, because FIV has been shown to demonstrate great variation in envelope sequences (Phillips *et al.*, 1990, Rigby *et al.*, 1993) and also to control for cloning artifacts/rearrangements, we PCR amplified, cloned, and sequenced the majority of the proviral *env* gene DNA from PLV-21 infected PBMC. PLV-21 was isolated from a seropositive daughter of puma 14 (Roelke *et al.*, 1993). Accordingly, an equivalent region of PLV-21 corresponding to bp 6451–8817 of the PLV-14 genome was cloned and sequenced as described above for comparison and confirmation purposes (primers used are listed in Table 2).

## Sequence analyses

Nucleotide sequence alignments and comparisons were performed with the programs Genalign and NUCALN (Wilbur and Lipman, 1983; Needleman and Wunsch, 1970); amino acid sequence alignments were run using Genalign and gaps were given a weight of a single residue regardless of their length (Intelligenetics, Mountainview, CA). Percentage identity of compared se-

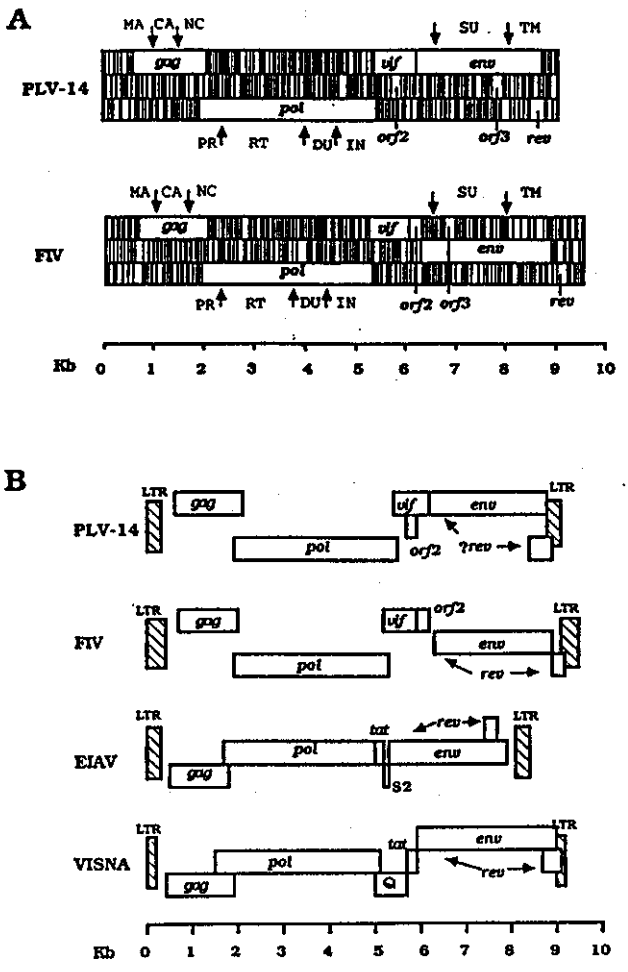


FIG. 2. Genome organization of PLV-14 compared to other lentiviruses. (A) Comparison of potential protein coding regions (open bars) of PLV-14 to described orfs from FIV-14 (Petaluma strain, Olmsted *et al.*, 1989b). Stop codons are denoted by vertical lines, and arrows signify the approximate location of presumed boundaries of functional domains from large genes. MA, matrix; CA, capsid; NC, nucleocapsid; PR, protease; RT, reverse transcriptase/RNase H; DU, deoxyuridine triphosphatase; IN, integrase; SU, surface envelope glycoprotein; TM, transmembrane envelope glycoprotein; (B) PLV-14 genome organization compared to other nonprimate lentiviruses. Open bars denote potential coding regions.

quences was calculated by dividing the number of matches by the length of the shorter of the two sequences, excluding gaps.

### Nucleotide sequence accession numbers

The nucleotide sequences reported in this work for PLV-14 and PLV-21 have been deposited in the Genbank sequence library and assigned the Accession No. U03982 and U03983, respectively.

## RESULTS

### Sequence determination and genome organization

The complete nucleotide sequence of the proviral genome of the PLV isolate, PLV-14 (9100 bp), was determined and analyzed. Due to the low proviral copy number in PLV-14-infected cell genomic DNA, two amplification procedures were used to generate sufficient quantities of viral specific DNA for subsequent cloning and sequencing. I-PCR, as outlined under Materials and Methods and shown diagrammatically in Fig. 1B, was used initially to amplify, in several segments, the majority of the PLV-14 proviral genome for sequence analysis. Verification of the I-PCR generated data was done by sequence analysis of several overlapping regions of the proviral genome amplified by conventional PCR (Table 2). As expected, three large orfs were evident in the PLV-14 genome and represent the characteristic retroviral structural genes: the overlapping *gag* and *pol* coding sequences and *env* (Fig. 2A). Located in the *pol-env* intergenic region are two small orfs (*vif* and *orf* 2). Similar to FIV-14 (Petaluma strain; Olmsted *et al.*, 1989a,b) the *env*-3′ long terminal repeat (LTR) region contains a minimum of two small orfs that are discernible. Thus, PLV-14 shares a similar complexity of genomic organization with other nonprimate lentiviruses (Figs. 2A, 2B). The analyses of the PLV-14 coding sequences are discussed below.

### LTR

The nucleotide sequence of the proviral PLV-14 5′ LTR is shown in Fig. 3. The LTRs are 311 bp in length, which is 44 bp shorter than those reported for the FIV-Petaluma strain (Talbott *et al.*, 1989; Olmsted *et al.*, 1989a). Their termini are demarcated by the inverted repeats 5′-TG and CA-3′. A conserved primer binding site, essential for the initiation of minus strand DNA synthesis, begins 2 nucleotides after the end of the 5′ LTR (Fig. 3). This sequence is identical to that of FIV and is very similar to the primer binding sites of equine infectious anemia virus (EIAV) and HIV-1. A typical polypurine tract (17 bp) ends 2 nucleotides immediately upstream of the 3′ LTR. A

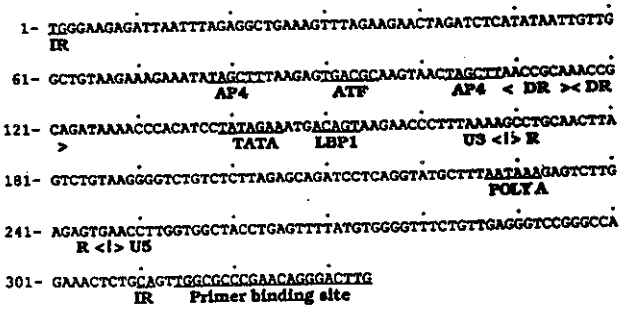


FIG. 3. Nucleotide sequence of the PLV-14 5′-LTR. The 5′ and 3′ LTRs were PCR amplified separately from proviral DNA, cloned, and sequenced as described under Materials and Methods. Identifiable features are underlined and include IR, inverted repeat; DR, direct repeat region. Potential enhancer/promoter elements in U3: AP4, ATF and LBP1 binding sites; TATA element. In R: POLY A, polyadenylation signal. The putative boundaries of the U3, R and U5 regions are indicated by vertical lines.

TABLE 3

PLV-14 GENOME COORDINATES OF PROTEIN CODING REGIONS AND THEIR ESTIMATED MOLECULAR MASS COMPARED TO FIV[a]

| Open reading frame | Coordinates[b] (PLV-14) | Molecular mass (kDa) | | Number of amino acids | |
|---|---|---|---|---|---|
| | | PLV-14 | FIV | PLV-14 | FIV |
| *gag* | 616–2052 | 52.6 | 49.2 | 479 | 450 |
| *pol* | 1929–5456 | 134.6 | 127.6 | 1176 | 1124 |
| *vif* | 5419–6246 | 33.4 | 29.1 | 276 | 251 |
| *orf 2* | 5759–5935 | 6.8 | 9.6 | 59 | 78 |
| *orf 3* | 7778–7999 | 8.8 | 7.7 | 74 | 68 |
| *rev* | 8571–8841 | 11.1 | 8.8 | 90 | 71 |
| *env* | 6250–8769 | 96.8 | 98.1 | 840 | 856 |

[a] FIV-14 (Petaluma strain) data taken from Olmsted *et al.* (1989b).
[b] Coordinates are calculated from the start of the initiating methionine to the first base of the stop codon, except for *pol*, PLV-14 *orf* 2, and PLV-14 *rev*; the entire orf coordinates are given for these.

polyadenylation signal is located between nucleotides 227 and 232 and, by analogy to the FIV LTR, the estimated site for polyadenylation addition is either the G or A nucleotides located at base 246 or 247 from the LTR start. Enhancer–promoter elements within the U3 region of the PLV-14 LTR are tentatively identifiable. Two possible AP-4 binding sites bearing a close similarity to those of FIV are present (Phillips *et al.*, 1990). A TATA element (bp 139 to 145), an ATF site (bp 90 to 95) (Lin and Green, 1988), and a putative LBP-1 binding site (bp 149 to 153) (Jones *et al.*, 1988) are also located within the U3 region (Fig. 3). An NF-kB consensus sequence is not discernible; however, the absence of this motif has been noted in other lentiviruses (Phillips *et al.*, 1990; Hirsch *et al.*, 1993). Direct repeat regions can function as enhancer elements (Phillips *et al.*, 1990) and one such region spanning nucleotides 109–115 and 116–122 is shown in Fig. 3. Nucleotide sequence alignments of the PLV-14 LTR with the FIV and EIAV LTRs revealed only 36 and 32% identities, respectively, while alignments with LTR sequences from other nonprimate and primate lentiviruses did not demonstrate any similarities of significance (data not shown). It should be noted that a single nucleotide difference at base position 56 from the start of the LTR was detected upon comparison of the 5′ and 3′ LTR sequences (T versus C). This is likely due to the independent PCR amplifications performed to derive these separate regions of the proviral genome.

### *Gag* orf

The *gag* orf begins at base 616 and as Table 3 and Fig. 4 show, this region could encode a protein slightly larger than that of FIV (52.6 kDa, PLV; 49.2 kDa, FIV-14; Olmsted *et al.*, 1989b). These estimates are based on translation initiating at the first methionine codon which is in a favored context (Kozak, 1991). Based on amino acid sequence alignments with the *gag* region of FIV and utilizing the cleavage junction analyses of the gag polyprotein (Elder *et al.*, 1993) we have tentatively defined the boundaries of the matrix (MA), capsid (CA), and nucleocapsid (NC) protein domains within the PLV-14 gag polyprotein (Fig. 4; Table 4). Overall the gag protein domains appear to be of a similar size to those of FIV. Interestingly, a 39-amino acid stretch near the amino terminus of the PLV NC protein contains a high percentage of glycine and arginine residues that bears little resemblance to the analogous region in the FIV NC protein (Fig. 4). This sequence was also present in PLV-21 proviral DNA, a closely related isolate of PLV-14 (Olmsted *et al.*, 1992; Roelke *et al.*, 1993). Located downstream of this region is a highly conserved double Cys-His motif, a feature of many retroviral nucleic acid binding proteins including HIV-1 (Fig. 4) (Berg, 1986; Gorelick *et al.*, 1993).

Pairwise alignments of *gag* proteins from other lentiviruses showed that PLV-14 was related more closely to FIV-14 (55% amino acid identity) than to any of the other lentiviruses examined (12 to 22% identity) (Table 5). As expected, based upon shared epitopes observed in Western blot analyses, the capsid proteins of PLV-14 and FIV showed the highest percentage identity among the gag proteins (Olmsted *et al.*, 1992).

### *Pol* orf

The largest open reading frame, *pol*, extends for 1176 amino acids (Table 3). The *gag* and *pol* orfs overlap by 123 base pairs at the 5′ end of the genome and a 7 nucleotide frameshift sequence (AAAAAAC) identical to that noted for EIAV (Jacks *et al.*, 1988) is evident 3 nucleotides downstream from the start of the *pol* orf. As with the PLV-14 *gag* polyprotein, we have tentatively defined the *pol* protein domain boundaries for protease, reverse transcriptase, RNase-H, dUTPase (previously called "protease-like" domain), and integrase (Table 4). These protein domains showed good identity with those of FIV (Table 4) and not surprisingly, the *pol* polyprotein alignments revealed that PLV is more closely related to FIV in this region (61% identity) than to the other lentiviruses (Table 5). Outside of the conserved RT region shared between PLV and FIV (84% amino acid identity) the sequence identities decrease significantly (53–69%; Tables 4 and 5).

Recent work has shown that FIV and other nonprimate lentiviruses, in contrast to the primate lentiviruses, encode a functional deoxyuridine triphosphatase (dUTPase) enzyme (Elder *et al.*, 1992; Wagaman *et al.*, 1993). This gene is located between the RNase-H and integrase regions. A dUTPase enzyme appears to be encoded in the same region in PLV-14 *pol* and the inferred amino acid sequence of the putative PLV dUTPase shares 62% identity with its FIV counterpart (Table 4).

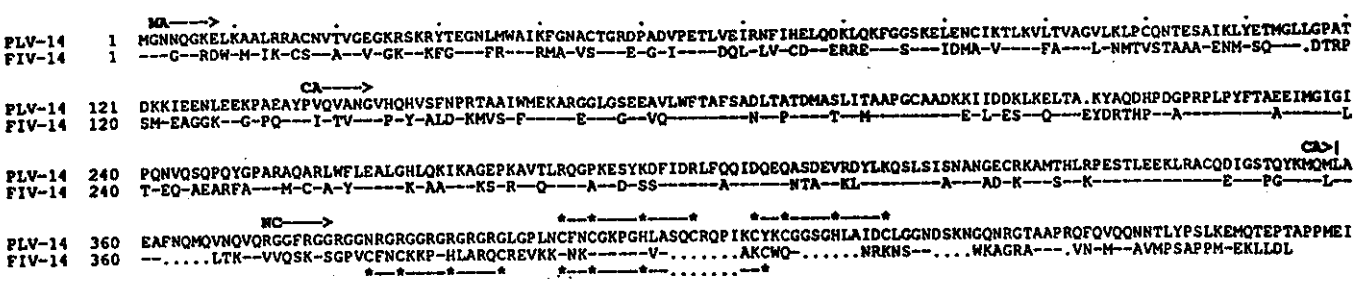


FIG. 4. Alignment of the predicted amino acid sequence of the PLV-14 Gag polyprotein with that of FIV-14 (Petaluma strain, Olmsted *et al.*, 1989b). Dashes represent identity with the amino acid immediately above, and dots signify gaps that were inserted to maximize the alignment. Cleavage junctions within the polyproteins (as defined by Elder *et al.*, 1993) are indicated by vertical lines. The amino acid below the first letter of the two letter abbreviations represents the putative amino terminal residue for each domain. MA, matrix; CA, capsid; and NC, nucleocapsid protein. The symbols *–* denote the location of tandem Cys-His motifs characteristic of nucleic acid binding proteins. Amino acid alignments were performed as described under Materials and Methods.

## *Env* orf

Structural features, characteristic of FIV and ungulate lentiviral *env* glycoproteins, are evident in the deduced amino acid sequence of PLV-14 *env*. Three main hydrophobic domains were identified by hydrophobicity analysis and likely comprise the surface (SU) glycoprotein signal sequence, the N-terminal fusion peptide of the transmembrane (TM) glycoprotein, and the C-terminal membrane anchor of the envelope protein (Fig. 5). The presence of a putative internal signal sequence for PLV *env* in SU would be consistent with its closer phylogenetic relationship to the ungulate lentiviruses than to the primate lentiviruses. Recently it has been observed for FIV that cleavage at the internal signal sequence yields a 20-kDa presequence (leader) which parallels the processing events for the *env* gene products of several

TABLE 4

SEQUENCE COMPARISONS OF PLV-14 GENES WITH FIV-14[a]

| Gene and region | Percentage identity | | Coordinates aligned | |
|---|---|---|---|---|
| | Nucleotide | Amino acid | FIV-14 | PLV-14 |
| *gag* | 59 | 55 | 628–1977 | 616–2052 |
| Matrix | 58 | 43 | 628–1032 | 616–1023 |
| Capsid | 69 | 66 | 1033–1697 | 1024–1686 |
| Nucleocapsid | 49 | 40 | 1726–1977 | 1729–2052 |
| *pol* | 64 | 61 | 1869–5240 | 1929–5456 |
| Protease | 62 | 53 | 1983–2330 | 2043–2381 |
| Reverse transcriptase | 74 | 69 | 2331–3662 | 2382–3830 |
| RNase-H | 67 | 58 | 3663–3998 | 3831–4166 |
| dUTPase | 71 | 62 | 3999–4397 | 4167–4577 |
| Integrase | 63 | 58 | 4398–5240 | 4578–5456 |
| *env* | 37 | 8 | 6266–8834 | 6250–8769 |
| Surface | 38 | 9 | 6266–8098 | 6250–8013 |
| Transmembrane | 35 | 5 | 8099–8834 | 8014–8769 |
| *vif* | 45 | 27 | 5236–5989 | 5419–6246 |
| *orf* 2 | 39 | 10 | 5992–6226 | 5759–5935 |
| *orf* 3 | 23 | 10 | 6712–6916 | 7778–7999 |
| *rev* | 40 | 17 | 8955–9167 | 8571–8841 |

[a] FIV-14 (Petaluma strain) data taken from Olmsted *et al.* (1989b). Regions were aligned as detailed under Materials and Methods. The boundaries of the putative PLV-14 gag and pol polyprotein cleavage products were estimated according to Elder *et al.* (1993). Alignments were performed using the programs Nucaln and Genalign (Intelligenetics, Mountainview, CA) as described under Materials and Methods and gaps were given the weight of a single residue regardless of length.

TABLE 5

AMINO ACID SEQUENCE COMPARISONS OF THE PLV-14 *gag* AND *pol* GENES WITH OTHER LENTIVIRUSES[a]

| Virus comparison | Percentage identity | | |
|---|---|---|---|
| | Gag | Pol | Conserved RT region[b] |
| PLV-14 compared to | | | |
| FIV-14[c] | 55 | 60 | 84 |
| Visna-1514 | 12 | 32 | 61 |
| EIAV-1369 | 22 | 17 | 63 |
| HIV-1 HXB2 | 16 | 33 | 61 |
| SIVsm-H4 | 15 | 18 | 61 |
| FIV-14 compared to | | | |
| FIV PPR[d] | 96 | 95 | 97 |
| Visna-1514 | 43 | 59 | 61 |
| EIAV-1369 | 49 | 61 | 63 |
| HIV-1 HXB2 | 40 | 60 | 61 |
| SIVsm-H4 | 25 | 47 | 59 |

[a] Viruses and regions compared were as follows: PLV-14 *gag* Met-1 to Ile-479, *pol* Phe-1 to Ala-1176; FIV-14 *gag* Met-1 to Leu-456, *pol* Lys-1 to Glu-1124 (Olmsted *et al.*, 1989b); FIV PPR *gag* Met-1 to Leu-456, *pol* Lys-1 to Glu-1124 (Phillips *et al.*, 1990); Visna-1514 *gag* Met-1 to Arg-420, *pol* Asp-1 to Arg-1021 (Sonigo *et al.*, 1985); EIAV-1369 *gag* Met-1 to Asn-480, *pol* Thr-1 to Lys-1139 (Kawakami *et al.*, 1987); HIV-1 HXB2 *gag* Met-1 to Arg-490, *pol* Met-1 to Ala-980 (Wong-Staal *et al.*, 1985); SIVsmH-4 *gag* Met-1 to Glu 507, *pol* Phe-1 to Ala 1019 (Hirsch *et al.*, 1989). Alignments were performed as described under Materials and Methods and the percentage identity was calculated by dividing the number of matches by the length of the shorter of the two sequences, excluding gaps which were assigned the weight of one residue.

[b] Data taken from Olmsted *et al.* (1992) in which a 576-bp fragment (PLV-14 coordinates 2482–3058) of the N-terminal reverse transcriptase domain was compared to the homologous region of the indicated viruses.

[c] FIV-14 (Petaluma isolate) data from Olmsted *et al.* (1989b).

[d] FIV PPR (San Diego isolate) Phillips *et al.* (1990).

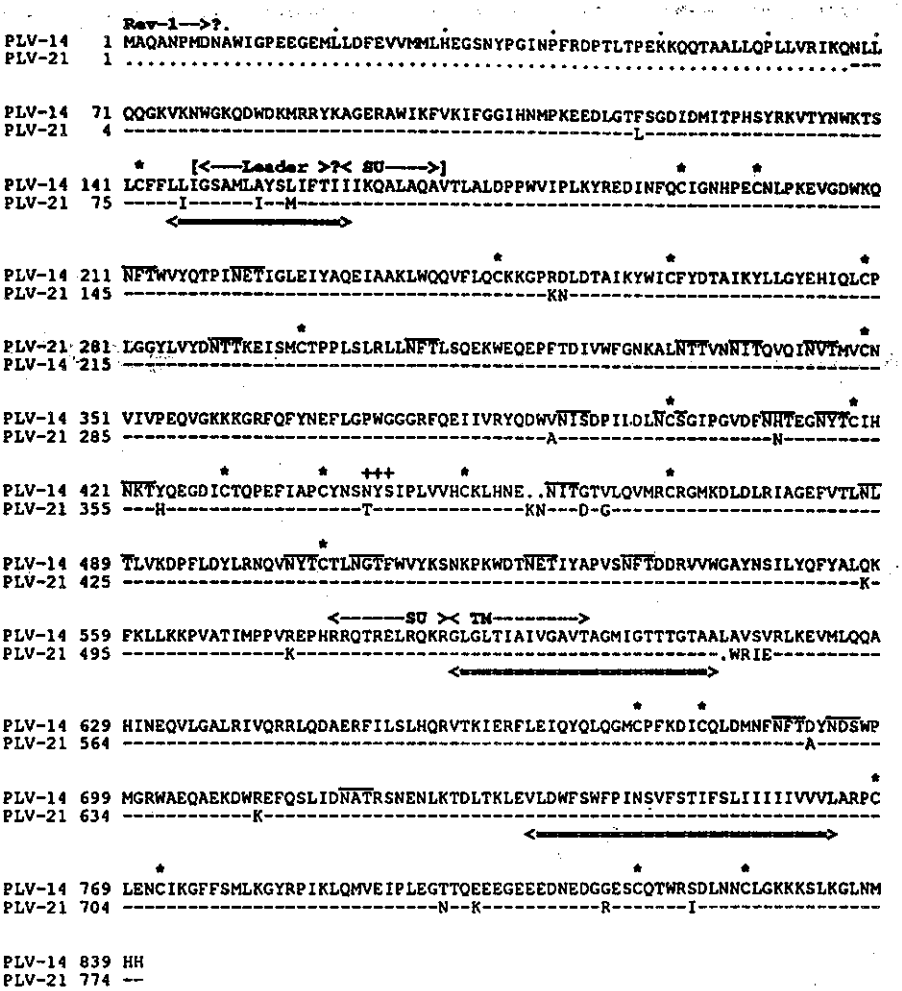


FIG. 5. Alignment of the predicted amino acid sequences of the envelope glycoproteins of PLV-14 and PLV-21. Cysteine residues are marked with an asterisk, potential N-linked glycosylation sites shared by both sequences are indicated by a horizontal line spanning the tripeptide sequence, and one unique site for PLV-14 alone is marked (+++). The predicted proteolytic cleavage site in the envelope glycoprotein precursor molecule is indicated at the junction of the surface (SU) and transmembrane (TM) glycoprotein. Hydrophobic regions are labeled (<==>). Dots within the amino acid sequence represent gaps introduced to maximize the alignment. Dashes in the PLV-21 sequence appearing under PLV-14 residues indicate identity. Sequences were aligned as described under Materials and Methods.

ungulate lentiviruses (Verschoor *et al.*, 1993). The location of the PLV *env* signal sequence would suggest a similar processing pattern for the PLV glycoprotein. The second hydrophobic domain follows the predicted site for the proteolytic cleavage of the *env* precursor into the SU and TM glycoprotein subunits and would serve as the N-terminal fusion peptide of TM. The third hydrophobic peptide presumably represents the C-terminal transmembrane anchor.

Potential N-linked glycosylation sites and cysteine residues, invariably found in lentiviral envelope glycoproteins, were also identified in the predicted amino acid sequence (Fig. 5). Twenty-two potential N-linked glycosylation sites were identified by the tripeptide sequence Asn-X-Ser/Thr which suggests that the level of carbohydrate addition to PLV *env* is similar to that of FIV. However, computer-assisted comparisons with FIV and other lentiviral envelope sequences did not reveal positional conservation of these sites or cysteine residues.

A Genbank search revealed that the PLV-14 envelope sequence was more closely related to the envelope precursor of FIV-Petaluma (Talbott *et al.*, 1989; Olmsted *et al.*, 1989b) than to any other protein. Alignment of the PLV-14 envelope sequence with known FIV sequences (Talbott *et al.*, 1989; Olmsted *et al.*, 1989b; Phillips *et al.*, 1990; Miyazawa *et al.*, 1991; Pancino *et al.*, 1993) revealed negligible nucleotide and amino acid sequence identities (37 and 8% nucleotide and amino acid sequence identities, respectively, with the analogous FIV-14 gene, Table 4). However, manual comparison of the TM sequences of PLV-14 with FIV-14 (data not shown) revealed the potential conservation of the first two cysteine residues in their respective TM proteins (Fig. 5). For PLV-14 and FIV-14 these cysteine residues are 88 and 94, and 88 and 95 residues down stream of their SU/TM cleavage sites, respectively. Despite the lack of significant sequence similarities between the TM sequences of these two feline lentiviruses this apparent

conservation suggests functional and structural constraints in this region.

We also determined the major portion (92%) of the nucleotide sequence of the envelope gene of the closely related isolate, PLV-21 (Fig. 5). Alignments revealed 98 and 92% identities at the nucleotide and amino acid levels, respectively. All cysteine residues and 21 of 22 N-glycosylation sites were conserved (Fig. 5). Thus the portion of the envelope sequence from PLV-21 proviral DNA was in good agreement with that of PLV-14.

### Remaining open reading frames

The characteristic small open reading frames of the lentiviruses were evident in the PLV-14 genome organization (Figs. 2A, 2B). The first large open reading frame following the polymerase gene in PLV-14 has significant similarities based on amino acid alignments and location to the analogous orfs of FIV 34TF10 (Talbott *et al.*, 1989) and FIV-14 (formerly *orf* 1; Olmsted *et al.*, 1989b) which is probably the *vif* homolog in these viruses (Figs. 2A, 2B). The PLV-14 deduced amino acid sequence of this region is 27% similar to FIV-14 and FIV-34TF10 (Table 4). It is also of a comparable size, 33.4 kDa and 276 amino acids, PLV-14 (Table 3), to 29.1 kDa and 251 amino acids FIV-14 (Olmsted *et al.*, 1989b). By positioning, this *orf* may also be analogous to Q of Visna virus (Fig. 2B). Further evidence that this region is the *vif* homolog of PLV-14 is the presence of the conserved hexapeptide motif SLQXLA, which has been noted in the *vif* coding sequences of 34 of 38 lentiviruses (Oberste and Gonda, 1992). The *vif* hexapeptide sequence of PLV-14, SLQRLC (amino acids 221–226, bp 6079–6096), matches 5 of 6 residues of the consensus sequence mentioned above.

Amino acid alignments of *orf* 2 from PLV-14 (Figs. 2A, 2B) showed no similarity to any known *orf*s from other viruses. It is in the general location of *orf* 2 from FIV-14 (Olmsted *et al.*, 1989b) and *orf* A (Talbott *et al.*, 1989) but is positioned in a different reading frame (Figs. 2A, 2B). In addition, a methionine is situated 17 codons downstream from the start of the open reading frame, whereas the initiating methionine follows immediately after the stop codon of *vif* in FIV (Olmsted *et al.*, 1989b; Talbott *et al.*, 1989). This region was only 10.2% similar to FIV *orf* 2 at the amino acid level (Table 4). In view of these facts and the absence of a small open reading frame immediately preceding the deduced envelope gene in PLV-14, we were concerned that sequence deletions had occurred during the cloning experiments. However, PCR-amplified DNA spanning this region resulted in a product of the correct predicted size according to our cloning/sequencing data and was also identical to our clones when the PCR product was sequenced directly and compared (data not shown; see Materials and Methods). Further, the biologically active clone FIV 34TF10 potentially encodes 3 short overlapping reading frames following the *vif* orf and the authors speculate that the proteins encoded by these regions are not critical for viral infection and replication or that they are nonfunctional (Talbott *et al.*, 1989). Similarly, PLV-14 *orf* 2 described here may also be nonfunctional.

*Orf* 3 codes for a protein of a potential 74 amino acids (FIV-14 has 68 amino acids; Olmsted *et al.*, 1989b) beginning with an initiating methionine (Fig. 2A, Table 3). This region is 10.2% similar in amino acid composition, and is located further along the genome and in a different reading frame than *orf* 3 in FIV-14 (Fig. 2A, Table 4). However, this region may not encode a functional gene product in PLV-14 and PLV-21 because the latter sequence has a stop codon at bp 1472, aa 46, (bp 7916, aa 46; PLV-14 genome). Similarly, whether *orf* D/*orf* 3 (Talbott *et al.*, 1989; Olmsted *et al.*, 1989b) in FIV is an actual gene remains open to question as there is some evidence that sequence conservation occurs between isolates (Rigby *et al.*, 1993); however, Miyazawa *et al.* (1991) have shown that a biologically active clone contains stop codons in this region.

*Rev*-2 overlaps the 3′ region of *env* by 56 amino acids (this does not occur in FIV) and extends into the U3 region of the 3′ LTR (Figs. 2A, 2B). It potentially encodes for a protein of 11.1 kDa (Table 3). Our computer search revealed it was 25.0% similar to HIV-1 at the amino acid level. A sequence of positively charged amino acids is encoded for as RRRRRRRRQRRWRR, beginning at aa 27. This is similar to arginine-rich domains in FIV-14 *orf* 4 (Olmsted *et al.*, 1989b) and the second coding *rev* exons in the primate lentiviruses. Further, a typical splice acceptor site is situated at the start of the PLV-14 *rev*-2 potential coding region (bp 8567–8573) and thus may have a role as a 3′ exon for a spliced transcript. Recently this area in the FIV genome has been shown to contribute to a *rev*-like function (Kiyomasu *et al.*, 1991; Phillips *et al.*, 1992). In the present study *rev*-2 was 17% similar to the amino acid sequence of *orf* 4/*rev*-2 from FIV (Table 4).

### Phylogenetic relationships of PLV-14

A computer analysis of aligned sequences of *gag* and *pol* genes indicates that PLV is more closely related to FIV than to other lentivirus sequences (Table 5). FIV and PLV share a common lentivirus ancestor from felidae species which is distinct from other lentiviruses (Olmsted *et al.*, 1992). These results have been interpreted in a phylogenetic context based on analyses of *pol* sequences of FIVs, PLVs, and representative lentiviruses (Olmsted *et al.*, 1992).

## DISCUSSION

The molecular characterization of PLV-14 has contributed valuable information on the genetic makeup of lentiviruses in general and feline lentiviruses in particular.

Previous sequence analysis of the reverse transcriptase region of *pol* showed that the PLVs formed their own distinct clade within the feline lentiviruses and this study serves to accentuate and underscore these observed differences as being even greater than first thought (Olmsted *et al.*, 1992). Thus, the unique position of PLV-14 within the nonprimate group of lentiviruses is emphasized because of the similarities observed within the *gag* and *pol* genes as a whole. This demonstrates that our previous estimations based on a highly conserved region of RT, a domain which is relatively stable in a genetic sense and therefore tends to evolve more slowly (Doolittle *et al.*, 1989; Johnson *et al.*, 1990), can lead to conservative estimations in genetic relatedness. In fact, genetic comparisons of PLV-14 complete gene sequences to FIV and other lentiviruses would almost suggest that PLV-14 is as different from FIV as FIV is to the other lentiviruses, perhaps indicating that the common ancestor of these two feline lentiviruses is very old in a phylogenetic sense.

The complex genome organization of PLV-14 is typical of the lentiviral group. The LTR sequence is poorly conserved, which is a tendency noted among other lentiviruses (Johnson *et al.*, 1990). The presence of a putative dUTPase coding sequence in PLV-14 *pol*, as is seen in FIV (Elder *et al.*, 1992; Wagaman *et al.*, 1993), EIAV, and Visna virus, argues for a common but nonrecent origin for these viruses (McClure *et al.*, 1987). The envelope open reading frame lacks an obvious leader sequence, a feature shared with FIV and Visna virus and other nonprimate lentiviruses. This region of *env*, however, has been shown to encode the first exon of *rev* for FIV (Phillips *et al.*, 1992). It is also interesting to note the changes in the organization, size, and positioning of the smaller orfs (orf 2 and orf 3) of PLV-14 in relation to the other lentiviruses. Their similarities with other viral sequences are quite divergent and there is a possibility that they are nonfunctional. The intergenic regions of the lentiviruses can be quite variable; for example, the primate lentiviruses, bovine immunodeficiency virus, and EIAV all utilize a *tat*-like protein. However, CAEV, Visna virus, and FIV (and perhaps PLV-14) do not encode a structurally similar compound (Derse *et al.*, 1993). The latter viruses may have adapted to make use of stronger elements upstream of the *pol/env* intergenic region or may have dispensed with such regions entirely in order to use a lower level of transcription (Derse *et al.*, 1993). Further examination of the smaller coding regions of PLV-14 can aid in stimulating a reappraisal of the role and functions of these orfs for feline and some of the nonprimate lentiviruses and their relationship to the overall genome organization.

The nucleotide sequence of PLV-14 has been obtained mainly through the use of the inverse PCR technique and then confirmed by using standard PCR technology. Both of these PCR techniques utilized nested primers by necessity, indicating that the copy number of the provirus DNA in the cell genome was very low. This would provide a ready explanation as to why repeated attempts to produce a lambda library of the virus were unsuccessful. This powerful inverse PCR technique will no doubt have application to similar situations in the future. However, the nature of this technique and the amount of virus available in the infected cell genome as used in the present study has, so far, precluded the production of an infectious clone. Thus, the sequences reported here are a compilation or an average of all "quasi-species" present in the target genomic DNA, and their biologic relevance remains to be demonstrated.

This report adds depth to the phylogenetic knowledge of the lentiviral group and further demonstrates that viruses of economic and medical importance can have closely related but distinct forms in nature with obvious implications for disease reservoir considerations. Hirsch *et al.* (1989) and Gao *et al.* (1992) mention the possibility that primate lentiviruses from Old World monkeys may have been the origin of the current HIV-2 lentivirus. Lentiviruses from wild felids and FIV may have a similar relationship. Elucidation of the natural history of lentiviruses and how they can be spread should be of concern, particularly with regard to their mutation potential.

The viruses that comprise the African green monkey/SIV complex have many related but distinct viral cohorts and can differ greatly in their amino acid similarities. They make up the most diverse group of the primate lentiviruses, range over a large area of Africa, and have many clearly distinguishable but related viral subgroups (Hirsch *et al.*, 1993). A similar phenomenon may be true with regard to the feline lentiviruses (Olmsted *et al.*, 1992) and it is predictable that wild feline species will also yield a family of closely related lentiviruses that bear a broad similarity to the prototype feline virus for this subgroup, FIV.

The Florida puma population that this virus and related viruses (Olmsted *et al.*, 1992) were originally isolated from display no obvious clinical disease associated with viral infection (Roelke *et al.*, 1993). Similarly, SIV infection of free-ranging African green and sooty mangabey monkeys are also apparently asymptomatic (Fultz *et al.*, 1986; Lowenstine *et al.*, 1986; Murphey-Corb *et al.*, 1986; Hirsch *et al.*, 1989). It is tempting to speculate that this virus/host connection may have evolved into a symbiotic rather than a seriously pathogenic type of relationship. Further study into the working of this viral–host genome interaction may give some insight into the cause of the pathogenic process in lentiviruses. The fact that PLV-14 infection of pumas and FIV infections of domestic cats does not lead to a rapid, progressive immunodeficiency-like syndrome may offer biological clues for identifying characteristics within the viral–host interaction which facilitate rapid progression to an immunodeficiency state caused by lentivirus infection in other species.

To summarize, analysis of the PLV-14 genome is an

important addition to our knowledge of the feline and nonprimate family of lentiviruses. Although considerably more information has been garnered from the many molecular descriptions detailed so far of FIV, significant insights into the genomic nature and evolutionary aspects of feline lentiviruses have nonetheless been supplied by this study. Comparisons of sequence information from PLV-14 and FIV, considered together with the nature of the conditions that result from infection of pumas with PLV and domestic cats with FIV (Olmsted *et al.*, 1992; Gardner, 1991), furnish an opportunity to establish a potential set of biological and genomic characteristics for the natural history of the feline lentiviruses. Finally, sequence analysis of PLV-14 shows that it occupies a highly distinctive clade within a feline subset of nonprimate lentiviruses and adds emphasis to the hypothesis that the common origin of these viruses, which appear to be specific for large and small cats, is likely to be very ancient.

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## REFERENCES

Ackley, C. D., Yamamoto, J. K., Levy, N., Pedersen, N. C., and Cooper, M. D. (1990). Immunologic abnormalities in pathogen-free cats infected with feline immunodeficiency virus. *J. Virol.* 64, 5652–5655.

Barlough, J. E., Ackley, C. D., George, J. W., Levy, N., Acevedo, R., Moore, P. F., Rideout, P. A., Cooper, M. D., and Pederson, M. C. (1990). Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: Comparison of short-term and long-term infections. *J.A.I.D.S.* 4, 219–227.

Berg, J. (1986). Potential metal binding domains in nucleic acid binding proteins. *Science* 232, 485–487.

Brown, E. W., Olmsted, R. A., Martenson, J. S., and O'Brien, S. J. (1993). Exposure to FIV and FIPV in wild and captive cheetahs. *Zoo Biol.* 12, 135–142.

Derse, D., Carroll, R., and Carvalho, M. (1993). Transcriptional regulation of equine infectious anemia virus. *Sem. Virol.* 4, 61–68.

Doolittle, R. F., Feng, D. F., Johnson, M. S., and McClure, M. A. (1989). Origins and evolutionary relationships of retroviruses. *Q. Rev. Biol.* 64, 1–30.

Elder, J. H., Lerner, D. L., Hasselkus-Light, C. S., Fontenot, D. J., Hunter, E., Luciw, P. A., Montelaro, R. C., and Phillips, T. R. (1992). Distinct subsets of retroviruses encode dUTPase. *J. Virol.* 66, 1791–1794.

Elder, J. H., Schnolzer, M., Hasselkus-Light, C. S., Henson, M., Lerner, D. A., Phillips, T. R., Wagaman, P. C., and Kent, B. H. (1993). Identification of proteolytic processing sites within the gag and pol polyproteins of Feline Immunodeficiency virus. *J. Virol.* 67, 1869–1876.

Fultz, P. N., McClure, H. M., Anderson, D. C., Swenson, R. B., Anand, R., and Srinivasan, A. (1986). Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). *Proc. Natl. Acad. Sci. USA* 83, 5286–5290.

Gao, F. L., Yue, L., White, A. T., Pappas, P. G., Barchue, J., Hanson, A. P., Greene, B. M., Sharp, P. M., Shaw, G. M., and Hahn, B. H. (1992). Human infection by genetically diverse SIVsm-related HIV-2 in West Africa. *Nature* 358, 495–499.

Gardner, M. B. (1991). Simian and feline immunodeficiency viruses: Animal lentivirus models for evaluation of AIDS vaccines and antiviral agents. *Antiviral Res.* 15, 257–286.

Gorelick, R. J., Chabot, D. J., Rein, L., Henderson, L. E., and Arthur, L. O. (1993). The two zinc fingers in the human immunodeficiency virus type 1 nucleocapsid protein are not functionally equivalent. *J. Virol.* 67, 4027–4036.

Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H., and Johnson, P. R. (1989). An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature (London)* 339, 389–392.

Hirsch, V. M., Dapolito, G. A., Goldstein, S., McClure, H., Emau, P., Fultz, P. N., Isahakia, M., Lenroot, R., Myers, G., and Johnson, P. R. (1993). A distinct African lentivirus from Sykes' monkeys. *J. Virol.* 67, 1517–1528.

Huet, T., Cheynier, R., Meyerhans, A., Roelants, G., and Wain-Hobson, S. (1990). Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature (London)* 345, 356–359.

Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J. (1990). "PCR Protocols: A Guide to Methods and Applications." Academic Press, San Diego, CA.

Jacks, T., Madhani, H. D., Masiarz, F. R., and Varmus, H. E. (1988). Signals for ribosomal frameshifting in the Rous sarcoma virus *gag-pol* region. *Cell* 56, 447–458.

Johnson, P. R., Fomsgaard, A., Allan, J., Gravell, M., London, W. T., Olmsted, R. A., and Hirsch, V. M. (1990). Simian immunodeficiency viruses from African green monkeys display unusual genetic diversity. *J. Virol.* 64, 1086–1092.

Jones, K. A., Luciw, P. A., and Duchange, N. (1988). Structural arrangements of transcription control domains within the 5′-untranslated leader regions of the HIV-1 and HIV-2 promoters. *Genes Dev.* 2, 1101–1114.

Kawakami, T., Sherman, L., Dahlberg, J., Gazit, A., Yaniv, A., Tronick, S. R., and Aaronson, S. A. (1987). Nucleotide sequence analysis of equine infectious anemia virus proviral DNA. *Virology* 158, 300–312.

Kiyomasu, T., Miyazawa, T., Furuya, T., Shibata, R., Sakai, H., Sakuragi, J. I., Fukasawa, M., Maki, N., Hasegawa, A., Mikami, T., and Adachi, A. (1991). Identification of feline immunodeficiency virus *rev* gene activity. *J. Virol.* 65, 4539–4542.

Kozak, M. (1991). Structural features in eukaryotic mRNAs that modulate the initiation of translation. *J. Biol. Chem.* 266, 19867–19870.

Lin, Y. S., and Green, M. R. (1988). Interaction of a common cellular transcription factor, ATF, with regulatory elements in both Ela- and cyclic AMP-inducible promoters. *Proc. Natl. Acad. Sci. USA* 85, 3396–3400.

Lowenstine, L. J., Pedersen, N. C., Higgins, J., Pallis, K. C., Uyeda, A., Marx, P., Lerche, N. W., Munn, R. J., and Gardner, M. B. (1986). Seroepidemiologic survey of captive Old-World primates for antibodies to human and simian retroviruses and isolation of a lentivirus from sooty mangabeys (*Cercocebus atys*). *Int. J. Cancer* 38, 563–574.

Lutz, H., Isenbugel, E., Lehmann, R., Sabaparra, R. H., and Wolfensberger, C. (1992). Retrovirus infections in non-domestic felids: Serological studies and attempts to isolate a lentivirus. *Vet. Immunol. Immunopathol.* 35, 215–224.

McClure, M. A., Johnson, M. S., and Doolittle, R. F. (1987). Relocation of a protease-like gene segment between two retroviruses. *Proc. Natl. Acad. Sci. USA* 84, 2693–2697.

McCune, J. M. (1991). HIV-1: The infective process *in vivo*. *Cell* 64, 351–363.

Miyazawa, T., Fukasawa, M., Hasegawa, N., Maki, N., Ikuta, K., Takahashi, E., Hayami, M., and Mikami, T. (1991). Molecular cloning of a novel isolate of feline immunodeficiency virus biologically and genetically different from the original U.S. isolate. *J. Virol.* 65, 1572–1577.

Murphey-Corb, M. L., Martin, L. N., Rangan, S. R., Baskin, G. B., Gormus, B. J., Wolf, R. H., Andes, W. A., West, M., and Montelaro,

R. C. (1986). Isolation of an HTLV-III related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. *Nature (London)* 321, 435–437.

NARAYAN, O., and CLEMENTS, J. E. (1989). Biology and pathogenesis of lentiviruses. *J. Gen. Virol.* 70, 1617–1639.

NEEDLEMAN, S. B., and WUNSCH, C. D. (1970). A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J. Mol. Biol.* 48, 443–453.

OBERSTE, M. S., and GONDA, M. A. (1992). Conservation of amino acid sequence motifs in lentivirus vif proteins. *Virus Genes* 6, 95–102.

OCHMAN, H., AJIOKA, J. W., GARZA, D., and HUNT, D. (1989). Inverse polymerase chain reaction. *In* PCR Technology: Principles and Applications for DNA Amplification (H. Erlich, Ed.), pp. 105–118. Stockton Press, New York.

OHTA, Y. T., MASUDA, T., TSUJIMOTO, H., ISHIKAWA, K., KODAMA, T., MORIKAWA, M., NAKAI, M., HONJO, S., and HAYAMI, M. (1988). Isolation of simian immunodeficiency virus from African green monkeys and seroepidemiologic survey of the virus in various non-human primates. *Int. J. Cancer* 41, 115–122.

OLMSTED, R. A., BARNES, A. K., YAMAMOTO, J. K., HIRSCH, V. M., PURCELL, R. H., and JOHNSON, P. R. (1989a). Molecular cloning of feline immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* 86, 2448–2452.

OLMSTED, R. A., HIRSCH, V. M., PURCELL, R. H., and JOHNSON, P. R. (1989b). Nucleotide sequence analysis of feline immunodeficiency virus: Genome organization and relationship to other lentiviruses. *Proc. Natl. Acad. Sci. USA* 86, 8088–8092.

OLMSTED, R. A., LANGLEY, R., ROELKE, M. E., GOEKEN, R. M., ADGER-JOHNSON, D., GOFF, J. P., ALBERT, J. P., PACKER, C., LAURENSON, M. K., CARO, T. M., SCHEEPERS, L., WILDT, D. E., BUSH, M., MARTENSON, J. S., and O'BRIEN, S. J. (1992). Worldwide prevalence of lentivirus infection in wild feline species: Epidemiologic and phylogenetic aspects. *J. Virol.* 66, 6008–6018.

OROSZLAN, S., and TOZER, J. (1990). The retroviral proteinases. *Seminars Virol.* 1, 369–378.

PANCINO, G., FOSSATI, I., COLOMBE, C., CASTELOT, S., HURTREL, B., MORAILLON, A., KLATZMANN, D., and SONIGO, P. (1993). Structure and variations of feline immunodeficiency virus envelope glycoproteins. *Virology* 192, 659–662.

PEDERSEN, N. C., HO, E. W., BROWN, M. L., and YAMAMOTO, J. K. (1987). Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 235, 790–793.

PEETERS, M., HONORE, C., HUET, T., BEDJABAGA, L., OSSARI, S., HUSSI, P., COOPER, R. W., and DELAPORTE, E. (1989). Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. *AIDS* 3, 625–630.

PHILLIPS, T. R., TALBOTT, R. L., LAMONT, C., MUIR, S., LOVELACE, K., and ELDER, J. H. (1990). Comparison of two host cell range variants of feline immunodeficiency virus. *J. Virol.* 64, 4605–4613.

PHILLIPS, T. R., LAMONT, C., KONINGS, D. A. M., SHACKLETT, B. L., HAMSON, C. A., LUCIW, P. A., and ELDER, J. H. (1992). Identification of the Rev transactivation and Rev-responsive elements of feline immunodeficiency virus. *J. Virol.* 66, 5464–5471.

RIGBY, M. A., HOLMES, E. C., PISTELLO, M., MACKAY, A., LEIGH BROWN, A. J., and NEILL, J. C. (1993). Evolution of structural proteins of feline immunodeficiency virus: Molecular epidemiology and evidence of selection for change. *J. Gen. Virol.* 74, 425–436.

ROELKE, M. E., MARTENSON, J. S., and O'BRIEN, S. J. (1993). The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Curr. Biol.* 3, 340–350.

SONIGO, P., ALIZON, M., STASKUS, K., KLATZMANN, D., COLE, S., DANOS, O., RETZEL, E., TIOLLAS, P., HAASE, A., and WAIN-HOBSON, S. (1985). Nucleotide sequence of the visna lentivirus: Relationship to the AIDS virus. *Cell* 42, 369–382.

TALBOTT, R. L., SPARGER, E. E., LOVELACE, K. M., FITCH, W. M., PEDERSON, N. C., LUCIW, P. A., and ELDER, J. H. (1989). Nucleotide sequence and genomic organization of feline immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* 86, 5743–5747.

TOMPKINS, M. B., NELSON, P. D., ENGLISH, R. V., and NOVOTNEY, R. C. (1991). Early events in the immunopathogenesis of feline retrovirus infections. *J. Am. Vet. Med. Assoc.* 199, 1311–1315.

VERSCHOOR, E. J., HULSKOTTE, E. G. J., EDERVEEN, J., KOOLEN, M. J. M., HORZINEK, M. C., and ROTTIER, P. J. M. (1993). Post-transcriptional processing of the feline immunodeficiency virus envelope precursor protein. *Virology* 193, 433–438.

WAGAMAN, P. C., HASSELKUS-LIGHT, C. S., HENSON, M., LERNER, D. L., PHILLIPS, T. R., and ELDER, J. H. (1993). Molecular cloning and characterization of deoxyuridine triphosphatase from feline immunodeficiency virus (FIV). *Virology* 196, 451–457.

WILBUR, W. J., and LIPMAN, D. J. (1983). Rapid similarity searches of nucleic acid and protein sequence data banks. *Proc. Natl. Acad. Sci. USA* 80, 726–730.

WONG-STAAL, F., GALLO, R. C., CHANG, N. T., GHRAYEB, J., PAPAS, T. S., LAUTENBERGER, J. A., PEARSON, M. L., PETTEWAY, S. R. JR., IVANOFF, L., BAUMEISTER, K., WHITEHORN, E. A., RAFALSKI, J. A., DORAN, E. R., JOSEPHS, S. J., STARCICH, B., LIVAK, K. J., PATARCA, R., HASELTINE, W. A., and RATNER, L. (1985). Complete nucleotide sequence of the AIDS virus, HTLV-III. *Nature* 313, 277–284.

YAMAMOTO, J. K., SPARGER, E., HO, E. W., ANDERSON, P. R., O'CONNOR, T. P., MANDELL, C. P., LOWENSTINE, L., MUNN, R., and PEDERSON, N. C. (1988). Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. *Am. J. Vet. Res.* 8, 1246–1258.