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Extensive sequence variation of feline immunodeficiency virus *env* genes in isolates from naturally infected cats

W. K. Greene[1], Joanne Meers[1], Gloria del Fierro[1], P. R. Carnegie[2], and W. F. Robinson[1]

[1] School of Veterinary Studies and [2] School of Biological and Environmental Sciences, Murdoch University, Perth, Australia

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Summary. In an investigation of the evolution of feline immunodeficiency virus (FIV) in vivo, sequential isolates from a persistently infected cat were examined by direct sequencing following amplification of selected subgenomic regions by polymerase chain reaction (PCR). Three isolates, T 90, T 91, and T 92, obtained over a three-year period revealed no changes to regions known to be conserved within *gag* and *pol* genes. Additionally, no change occurred within *gag* and *pol* in an isolate recovered from a second cat which was experimentally infected with T 90. Changes were detected within an N-terminal region of the envelope glycoprotein gp 120 (*env*). These consisted of point mutations, some of which would result in amino acid substitutions and the predicted amino acid changes tended to cluster within variable domains. Inoculation of T 90 into a second cat resulted in a different pattern of mutations than that observed for the three isolates from the first cat. In all cases, virus isolates derived from the same cat were much more highly related to each other (extent of *env* variation was 0.5–1.5%) than to isolates from other cats (10–12% *env* variation). The rate of change of FIV was estimated to be 3.4×10^{-3} nucleotide substitutions per site per year for the *env* gene and less than 10^{-4} nucleotide substitutions per site per year for the *gag* and *pol* genes, values concordant with that found for human immunodeficiency virus 1. Both nucleotide and amino acid changes in the gp 120 region were found to be directional, suggesting that selective pressures influence FIV envelope gene sequences.

Introduction

Feline immunodeficiency virus (FIV) is a T-lymphotropic lentivirus with worldwide prevalence [35]. Because of its similar genetic structure and disease pathogenesis, FIV infection is now well accepted as a valuable small animal model

for human immunodeficiency virus (HIV) infection [8, 13, 19, 35], particularly in the areas of drug testing and vaccine development.

One major difficulty in the development of lentiviral vaccines is their extreme genomic plasticity. Nucleic acid sequencing of HIV-1 has revealed extensive genomic variability, both between patients and within individual patients at any one point in time [1, 5, 7, 18]. Furthermore, rapid sequence evolution of HIV-1 and generation of sequence diversity of viruses isolated from individual patients over time has been well documented [3, 11, 12, 17, 24, 33, 39, 47]. Genomic variation during the course of persistent infection has also been reported for simian immunodeficiency virus (SIV) [2, 14] and the non-primate lentiviruses equine infectious anaemia virus (EIAV) [34, 40] and visna virus [4, 41]. The variation was unevenly distributed across the genome with little change occurring in genes such as *gag* or *pol* which code for the major capsid protein (p 24) and reverse transcriptase enzyme. This is in marked contrast to the highly mutable variable regions of the surface glycoprotein encoded by the *env* gene.

Although there has been a rapid increase in FIV sequence data particularly for the *env* region [20, 26, 28, 30, 31, 36, 38, 42, 46], compared with HIV-1, little is known about the rate and nature of sequence evolution in FIV. Here we describe the in vivo evolution of FIV isolates obtained sequentially from a persistently infected cat over a three-year period. By analysing the consensus sequence of various viral genomes present at each point in time, our findings show that the FIV *env* gene is capable of relatively rapid and extensive variation in vivo in the order of 10^{-3} nucleotide substitutions/site/year. By contrast, the major capsid (p 24) and reverse transcriptase genes were highly stable when compared with envelope glycoprotein gene. These results indicate that genetic variation in FIV is similar to the pattern and rate of variation found in other lentiviruses including HIV-1.

Materials and methods

Virus isolates

FIV isolates T 90, T 91, and T 92 were obtained at yearly intervals from a cat with naturally acquired infection. Isolates N 91, DC 91 (Perth, Western Australia) and S 90 (Melbourne, Victoria) were derived from FIV-infected cats [10]. An isolate (4.3/92) was obtained by inoculating subcutaneously a 22-week-old cat, seronegative for FIV and antigen-negative for feline leukaemia virus (FeLV) with 1 ml of T 90 culture supernatant containing approximately $10^{5.5}$ $TCID_{50}$. Isolate 4.3/92 was obtained from this cat at 74 weeks postinfection. Virus was isolated as previously described [22, 23]. Briefly, peripheral blood mononuclear cells (PBMCs) from infected cats were purified over Ficoll-Hypaque and virus isolated either by co-cultivation of PBMCs with MYA-1 cells, a feline interleukin-2 dependent T-lymphoblastoid cell line [25] or co-cultivation of PBMCs with Concanavalin-A stimulated PBMCs from FIV-negative cats. Sequential isolates were stored as MYA-1 cell supernatants at − 70 °C. Sequences from other FIV *env* genes were obtained from published sequences deposited into GenBank which comprise two clones (34TF10 and FIV-14) from the U.S. Petaluma isolate [30, 46], FIV PPR also from the U.S.A. [36], FIV TM 1 and TM 2 from Japan [20, 26], two clones 19 k 1/k 32 from the Netherlands [42],

FIV Z1 and Z2 from Switzerland [28], FIV Wo from France [31], and FIV UK2 and UK8 from the U.K. [38].

Oligonucleotide primers

Three oligonucleotide primer pairs were used to amplify subgenomic regions within *gag*, *pol*, and *env* genes, respectively. Primer pairs L 928-R 1394 which amplified a 467 bp segment of *gag* and L 2402-R 3039 which amplified a 638 bp segment of *pol* have been described previously [10]. The primer pair L 6299-R 6866 amplified a fragment of predicted size of 568 bp. L 6299 is located at positions 6299-6319 of *env* (gp 120) and has the sequence 5′ AGGACCAGAAGAAGCTGAAGA 3′, while R 6866 is located at positions 6866-6846 of *env* (gp 120) and has the sequence 5′ TTCTGGTGCCCAACAATCCCA 3′.

Polymerase chain reaction (PCR)

Genomic DNA containing FIV proviral sequences for use in PCR reactions was isolated as described [10] according to the method of Kellogg and Kwok [16]. PCR was performed using 10 pmol of each primer, 50 ng DNA, 0.2 mM each dNTP, 2 mM $MgCl_2$, and 2 U *Taq* polymerase (Biotech International, Perth, W.A.) in a 25 µl total reaction volume. The reaction was overlayed with paraffin oil and cycled on a Hybaid thermoreactor (Hybaid, Teddington, U.K.) for 35 cycles with 30 sec at 94 °C, 1 min at 55 °C and 2 min at 72 °C with the exception of 5 min at 94 °C, on the first cycle and 10 min at 72 °C on the final cycle. PCR products were resolved on 1.5% agarose gels stained with 0.1 µg/ml ethidium bromide.

DNA sequencing

FIV proviral DNA amplified by PCR was purified using Prep-a-Gene (Bio-Rad, Richmond, CA) to remove excess deoxynucleotides and primers. Sequencing reactions were performed by the dideoxynucleotide chain termination methods using a *Taq* DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The sequence was resolved on an Applied Biosystems model 373 A automated DNA sequencer.

Rates of evolution

Rates of evolution, expressed as nucleotide substitutions per site per year, were calculated using the method of Gojobori and Yokoyama [9] where R = D/2T and R = subs/site/year, D = $-3/4 \ln(1-4/3P)$, T = time since divergence, and P = the proportion of different nucleotides.

Results

Sequence variation in FIV gag *and* pol *genes*

Subgenomic regions of *gag* (p 15/p 24) and *pol* (RT) amplified by PCR from T90, T91, and T92, shown in Fig. 1 which represent sequential virus isolates obtained at yearly intervals from a persistently infected cat with naturally acquired FIV, were directly sequenced. For each isolate, the nucleotide sequence was identical with no changes over the 3036 nucleotides analysed (Fig. 2). This was consistent with a mutation rate of less than 1.7×10^{-4} substitutions/site/year (assuming one change over the three-year period). Inoculation of isolate T90 into a separate cat followed by virus isolation (isolate 4.3/92) and se-

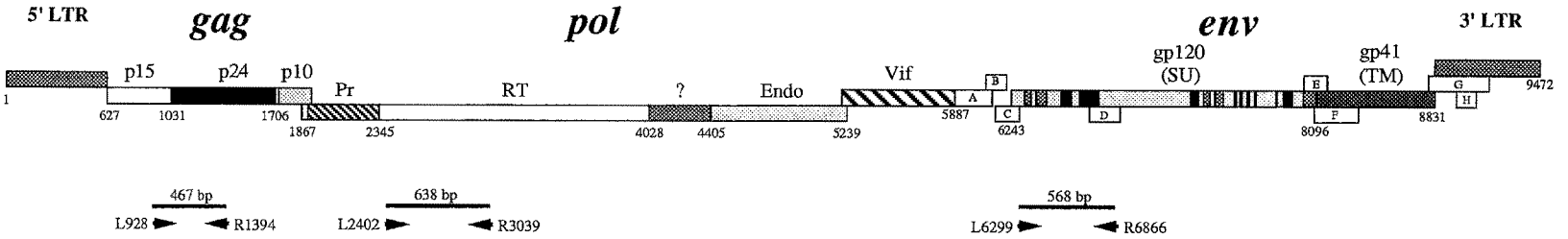

Fig. 1. Schematic representation of FIV proviral genome. The locations of the oligonucleotide primers used for PCR amplification and sequencing are indicated below. Arrowheads indicate the polarity of the primers and bars the position of the resultant PCR products with sizes indicated. Shaded boxes within the gp 120 gene represent the variable domains according to Pancino et al. [31] with those in black denoting hypervariable regions

quencing 74 weeks later also revealed 100% nucleotide sequence identity (Fig. 2 a, b).

Sequence variation in FIV env *genes*

A 5′ segment of the *env* gene coding for an N-terminal region of gp 120 spanning the first and second variable domains (see Fig. 1), was also amplified by PCR and sequenced for each of the sequential isolates; T 90, T 91, and T 92. The nucleotide sequence for each isolate, representing a different time point, was found to be unique (Fig. 2 c), with nucleotide sequence homologies with the original isolate (T 90) being 99.4% for T 91 and 98.6% for T 92. T 91 and T 92 isolates had a sequence identity of 99.2%. This represents an average of 3.4×10^{-3} substitutions/site/year for *env* which is at least 10-fold higher than that found in highly conserved domains of *gag* and *pol*.

T 90 was injected into a FIV-negative cat and 74 weeks later a new isolate (4.3/92) was obtained. This isolate had an *env* sequence different from T 90, T 91, or T 92 with 98.6%, 98.4%, and 98.1% sequence identity with each of these isolates, respectively. Both unique and shared base changes were observed with 2 of the 7 differences between isolates T 90 and 4.3/92 being shared with either T 91 or T 92. The nucleotide sequence variation between isolates from different cats was greater than that found between sequential isolates from the same cat. Isolate T 91 differed from the three other Australian isolates, DC 91, N 91, and S 90 with 90.3%, 88.6%, and 87.8% nucleotide sequence homology in this *env* region, respectively.

The sequence variation in *env* was due to simple base substitutions with many of these also causing amino acid changes. These tended to cluster within the variable regions (Fig. 3) which had a high proportion of mutations leading to amino acid changes (non-synonymous mutations) than the conserved regions. Significantly, of nine nucleotide substitutions in or very near variable regions of the T 90–T 92 and 4.3/92 isolates (Fig. 2) seven (78%) were non-synonymous. By contrast, only two of the five (40%) were non-synonymous in the conserved framework regions of gp 120.

The base changes in T 90–T 92 were found to be directional with no nucleotide reversion to that of the original isolate. Thus all changes, once acquired, were retained and this was also evident at the amino acid level (Fig. 3).

As was found for the nucleotide sequence, the amino acid homology was much higher between virus isolated from the same cat at different times than between virus isolated from different cats. T 90 showed 99% amino acid homology with T 91, 98% with T 92, and 97% with 4.3/92. By contrast, the amino acid sequence homology was 81–84% between the four Australian isolates (DC 91, N 91, T 91, and S 90) shown in alignment with eleven other published FIV *env* sequences (Fig. 4). The pattern of *env* amino acid variability found in the Australian isolates was consistent with the recently proposed nomenclature for FIV *env* variable regions [31]. The *env* region of the Australian isolates was most closely related to the U.S. Petaluma isolate, U.K. 2 and 8 isolates, and

a) *gag* Region (948-1370)

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        948                                                                                                                    1034
T90     AAT ATG TAT ACT CAG ATG GGA TTA GAC ACT AGA CCA TCT ATG AAG GAA GCA GGG GGA AAA GAG GAA GGC CCC CCA CAG GCA TAT CCT
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               1121
T90     ATT CAA ACA GTA AAT GGA GCA CCG CAA TAT GTA GCA CTT GAC CCA AAA ATG GTG TCC ATT TTT ATG GAA AAG GCA AGA GAA GGA TTA
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               1208
T90     GGA GGT GAG GAA GTT CAA TTA TGG TTT ACA GCC TTC TCT GCA AAT TTA ACA CCT ACT GAC ATG GCC ACA TTA ATA ATG GCC GCG CCA
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               1295
T90     GGA TGC GCT GCA GAT AAA GAA ATA TTG GAT GAA AGC TTA AAG CAA TTG ACA GCA GAA TAT GAT CGT ACA CAT CCC CCT GAT GGG CCT
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                       1370
T90     AGA CCA TTA CCC TAT TTT ACT GCA GCA GAA ATT ATG GGT ATA GGG CTA ACT CAA GAA CAA CAA GCA GAG GCA AGG
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
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b) *pol* Region (2425-3013)

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        2425                                                                                                                   2509
T90        A ATT GAA GCT TTA ACA GAA ATA GTA GAA AGA CTA GAA AGA GAA GGG AAA GTA AGA AGA GCA AAT TCA AAT AAT CCA TGG AAT ACA
T91        * *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92        * *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92     * *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               2596
T90     CCA GTA TTT GCT ATA AAA AAG AAA AGT GGA AAA TGG AGA ATG CTC ATA GAT TTT AGA GAA TTG AAT AAA CTA ACT GAG AAA GGA GCA
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               2683
T90     GAG GTC CAG TTG GGA CTA CCT CAT CCT GCT GGT TTA CAA ATG AAA AAA CAA GTA ACA GTA TTA GAT ATA GGG GAT GCA TAT TTC ACC
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               2770
T90     ATT CCC CTT GAT CCA GAT TAT GCT CCT TAC ACA GCA TTT ACT TTA CCT AGG AAG AAT AAT GCG GGA CCA GGA AGA AGA TAT GTG TGG
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               2857
T90     TGT AGC CTA CCA CAA GGC TGG ATT TTA AGC CCA TTG ATA TAT CAA AGT ACA TTA GAC AAT ATA ATA CAA CCT TTC ATT AGA CAA AAC
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               2944
T90     CCT CAA TTA GAT ATT TAC CAA TAT ATG GAT GAC ATT TAT ATA GGA TCA AAC TTA AGT AAA AAG GAG CAT AAA GAA AAA GTA GAA GAC
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                               3013
T90     TTA AGA AAA TTA CTA TTA TGG TGG GGA TTT GAG ACT CCA GAA GAT AAA TTA CAG GAA GAA CCC CCA TAT
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
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c) *env* Region (6327-6842)

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        6327                                                                                                                   6413
T90     GAT TTC GAT ATA GCA ACA CAA TTA AGT GAA GAG GGG CCA CTA AAT CCA GGG GTA AAC CCA TTT AGG GTA CCT GGA ATA ACA GAA AAA
T91     *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** **T *** *** *** *** *** *** *** *** **A *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

                                                                                                                               6500
T90     GAA AAG CAA GGA TAT TGT TCC ATA TTA CAA CCC AGG TTA CAG GAC CTA AGG AAT GAA ATT CAA GAG GTA AAA TTG GAA GAA GGA AAT
T91     *** *** *** *** *** *** *** *** *** *** *** *A* *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
T92     *** *** *** *** *** *** *** *** *** *** *** *A* *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

4.3/92  *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
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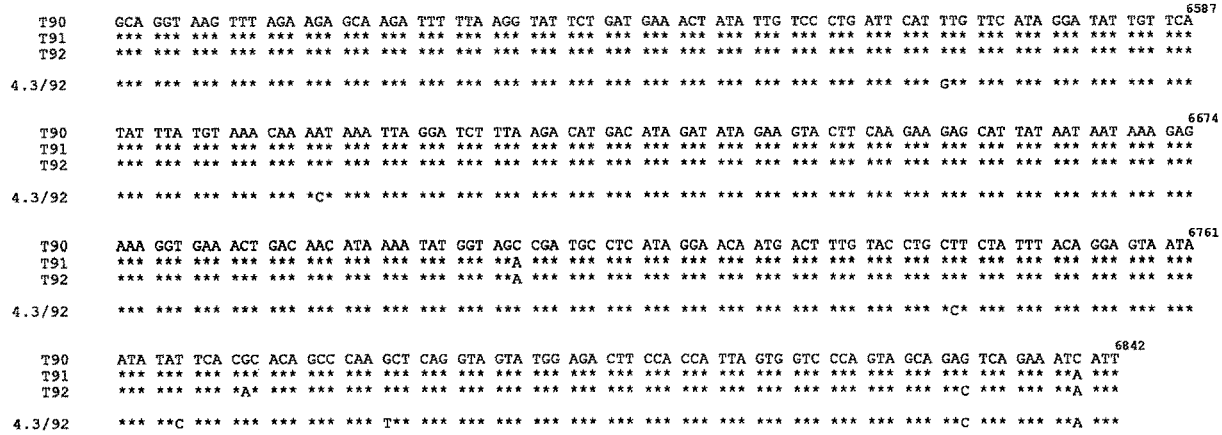

Fig. 2. Nucleotide sequence alignment of **a** *gag* p 24, **b** *pol* RT, and **c** *env* gp 120 gene segments from sequential isolates of FIV (T 90, T 91, T 92) taken at yearly intervals from a naturally infected cat. Also shown is 4.3/92 obtained 74 weeks postinfection of a naive cat with the T 90 isolate. Asterisks indicate sequence identity with T 90

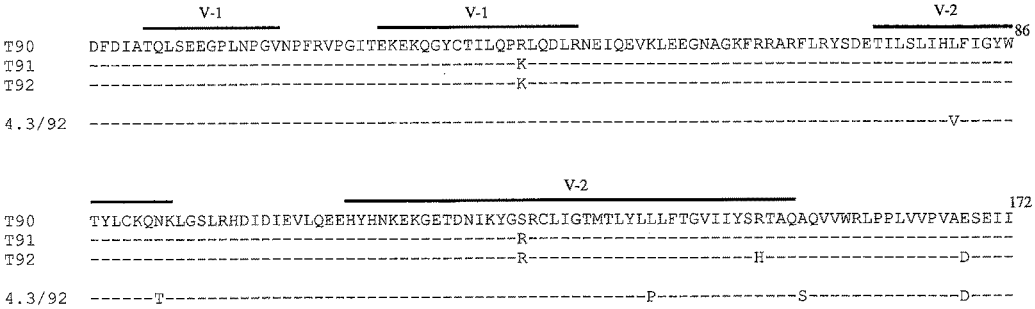

Fig. 3. Comparison of deduced amino acid sequences of an N-terminal region of *env* gp 120 from sequential isolates of FIV. Dashes indicate sequence identity with FIV isolate T 90. Bars denote variable domains according to [31]

the Swiss isolate Z 1 (80–85% amino acid homologies) and most distant from the Japanese isolates TM 1 and TM 2 (69–71% homology).

Discussion

We have examined the extent of genetic variation of FIV over time by directly sequencing three regions of the genome: conserved domains of the *gag* and *pol* genes and an N-terminal region of gp 120 in the *env* gene. These regions were chosen because previous studies of lentiviral genomic variation have shown that the major capsid protein and reverse transcriptase enzyme of *gag* and *pol*, respectively, are highly conserved, whereas the *env* gene, and in particular the hyper-variable regions of gp 120 are significantly more variable [5, 11, 14, 44]. A direct sequencing approach was chosen because as recently pointed out by

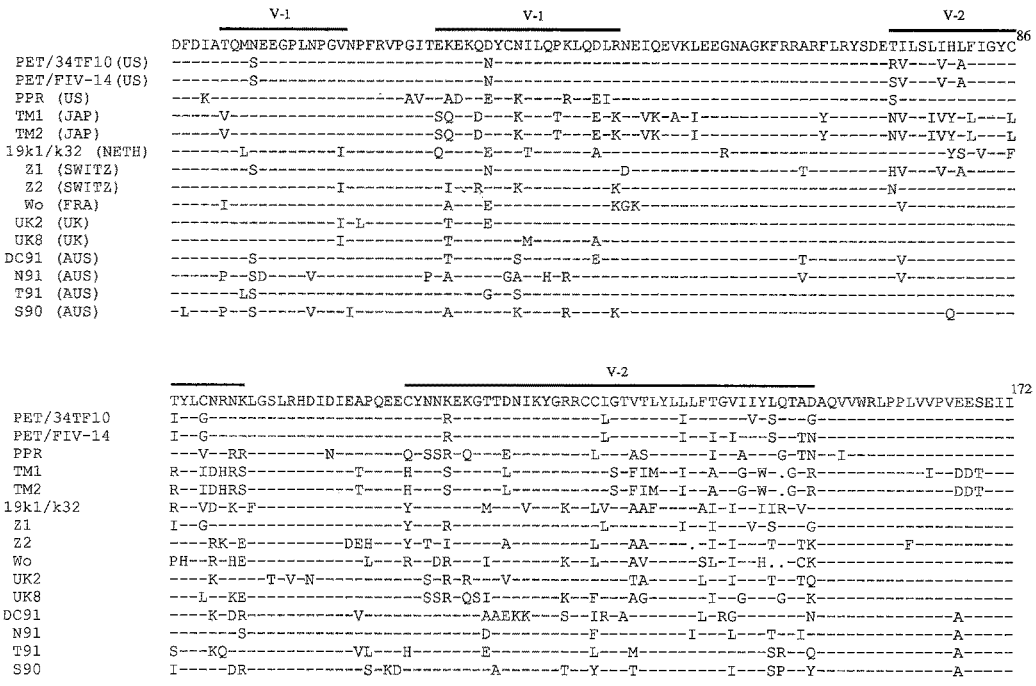

Fig. 4. Comparison of deduced amino acid sequences of an N-terminal region of gp 120 from Australian isolates together with all published *env* sequences of FIV. Dashes indicate identity with the consensus sequence and dots represent deletions. Bars denote variable domains according to [31]. The geographic origins of the virus isolates are indicated in parentheses

Uhlen and colleagues [49], studies of lentiviral quasispecies using PCR have involved the time-consuming sequencing of many individual virus clones which may be subject to errors introduced by *Taq* polymerase.

As found for other lentiviruses, the internal structural genes of *gag* and *pol* appeared to incur substitutions at a much lower frequency than the *env* gene, with a difference of at least an order of magnitude ($\sim 10^{-3}$ substitutions/site/year for *env* versus less than $\sim 10^{-4}$ for *gag* and *pol*). Nevertheless, although the sequential isolates from the same cat differed from one another in the order of 0.5–1.5% at the nucleotide level in the *env* region, this difference was much less than isolates from different cats (10–12% difference between Australian isolates), which is similar to the situation found for HIV-1 [11]. Additionally, within the *env* gene of FIV the mutations were generally confined to the variable domains recently proposed by Pancino et al. [31].

Experimental infection of a second cat with the original viral isolate led to a different pattern of nucleotide and hence amino acid sequence changes in the *env* gene. This finding, along with the fact that both nucleotide and amino acid substitutions appeared to be directional, provided evidence for host selective pressures influencing the evolution of FIV *env* genes. Variation within the variable domains of *env* is most probably the result of selection imposed by

the immune response. Genetic variation thus appears to provide for antigenic variation, as first indicated by studies with other non-primate lentiviruses such as visna [4, 41], caprine arthritis-encephalitis virus (CAEV) [6] and EIAV [27, 40]; and more recently by HIV-1 [21, 29, 37, 48].

The intrinsic mutation rates between *gag, pol*, and *env* genes are relatively high because lentiviruses, like all retroviruses, replicate via an error-prone reverse transcriptase enzyme which has either limited or no proof-reading function [45]. This, however, is not reflected in a rapid mutation rate in *gag* or *pol* of progeny virus since many mutations in these critical genes are strongly disfavoured. By contrast, the envelope glycoproteins which are targeted by immune response, appear to be subject to a positive selection for change. Such a phenomenon has been well documented for HIV-1 and SIV *env* genes [2, 43]. In this study, mutations in the variable domains of gp 120 resulted in an amino acid substitution 78% (7 of 9) of the time compared with only 40% (2 of 5) for the conserved domains. Thus as well as undergoing a greater number of nucleotide changes, the variable regions were selectively subject to non-synonymous mutations, presumably as a result of intense pressure by the immune system. In support of this, a recent study has identified two immunogenic epitopes within variable domains of FIV *env* glycoproteins [32].

Although the immune system appears to be an important selective mechanism in lentiviral *env* variation, it may not be the only selective force. For example Johnson et al. [14] found significant variation in SIV *env* genes in regions normally not exposed to the immune system. Moreover, only a transient humoral response was observed. This is not surprising, however, since apart from critical domains such as that responsible for CD4-binding, the *env* glycoproteins appear to be pliable structures built upon a conserved structural framework. They are, therefore, permissive to amino acid sequence changes in regions (loops) which play little or no role in the structure of these molecules. Another role for genetic variation in lentiviral *env* genes may be to allow rapid adaptation to different environments by altering host or tissue tropisms. Indeed, *env* variants of EIAV have been shown to home to different tissues, most likely as a result of selection of tissue-specific determinants [15].

Collectively, the data in this paper reveals FIV to resemble other known lentiviruses in terms of its rate and nature of sequence evolution, which suggests that these viruses share a common mechanism(s) for genomic variation. This together with the similar morphology, genomic organisation, cell tropism, and disease pathogenesis between FIV and HIV-1 strengthens the usefulness of FIV infection as a model for human AIDS.

Acknowledgements

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Authors' address: Dr. W. F. Robinson, Department of Veterinary Pathology, University of Queensland, Brisbane, Queensland 4027, Australia.

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