An overview of the molecular phylogeny of lentiviruses

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Introduction

Lentiviruses are one of several groups of retroviruses. In early studies of the molecular phylogenetic analysis of endogenous and exogenous retroviruses it was suggested that the retroviruses could be divided into four groups, two complex with several accessory or regulatory genes (lentiviruses; and the bovine and primate leukemia virus group now known as deltaretrovirus) and two simple, with just the *gag*, *pol* and *env* genes and few or no accessory genes (a group including spumaretroviruses, C-type endogenous retroviruses and MMLV; the other group including Rous Sarcoma virus, HERV-K Simian Retrovirus 1 and MMTV) [1–5]. A more recent study, including many more recently discovered exogenous and endogenous retroviruses, illustrates the diversity of retroviruses [6]. The retroviridae are currently officially classified into seven different genera, according to the Seventh Report of the International Committee on Taxonomy of Viruses, 2000 (`http://www.ncbi.nlm.nih.gov/ICTV`). The seven genera are named alpha through epsilon retroviruses plus lentiviruses and spumaviruses. Phylogenetic trees based on *pol* gene sequences can be found in several recent papers[6–8]. None of the retroviruses in either group of complex retroviruses have been found in an endogenous state to date.

Within the Lentiviruses, the primate lentiviruses discovered to date form a monophyletic cluster (Figure 1). One notable difference between the primate lentiviruses and the non-primate lentiviruses is that all nonprimate lentiviruses, except the BIV/JDV lineage, contain a region of the *pol* gene encoding a dUTPase, whereas all primate lentiviruses lack this region of *pol*. In the BIV/JDV lineage, an insert is still present in this region of *pol*, but the sequence similarity is low, and the region seems to be no longer capable of encoding a functional dUTPase. This lack of dUTPase, coupled with the distances between primate lentiviruses as compared to the distances between other mammalian lentiviruses, suggests that the lentiviruses originated in a non-primate mammal. A single transfer of virus from a non-primate mammal into primates was followed by spread of the virus into many different primate species. If the primate lentiviruses were older than the non-primate lentiviruses, one would expect greater diversity within the primate clade than between nonprimate clades. Feline immunodeficiency viruses have been studied from wild African lions [9], as well as North American wildcats [10], the Kazakstan Pallas cat [11] and housecats from around the world [12]. The global diversity of feline immunodeficiency viruses is similar to the diversity of the primate lentiviruses. The global diversity of housecat FIVs is roughly twice as great as the diversity of the HIV-1 M group in humans. These facts indicate that domestic cats have been spreading FIV around the world for a longer period of time than humans have been spreading the HIV-1 M group, assuming that the two viral lineages evolve at close to equal rates.

Within the primate lentivirus group, each species and subspecies of primate seems to carry its own monophyletic lineage of lentivirus or to be free from lentiviral infection. Humans seem to not have their own lineage, but instead have acquired two very different lineages, named HIV-1 and HIV-2, from chimpanzees and sooty mangabeys, respectively. Baboons and macaques, like humans, seem only to acquire lentiviruses from other species and not have their own lineage[13–17]. The intra-species diversity in each lineage is not yet fully understood, because only one or a relatively small number of virus isolates from each species of primate have been sequenced to date. Primates and felines captured in distant geographic locations tend to have more diverse viral genotypes [12], whereas animals of the same species captured in proximity to one another tend to share similar genotypes of virus. Cross-species transmission of virus appears to be rare in comparison to within-species transmission, as evidenced by different primate

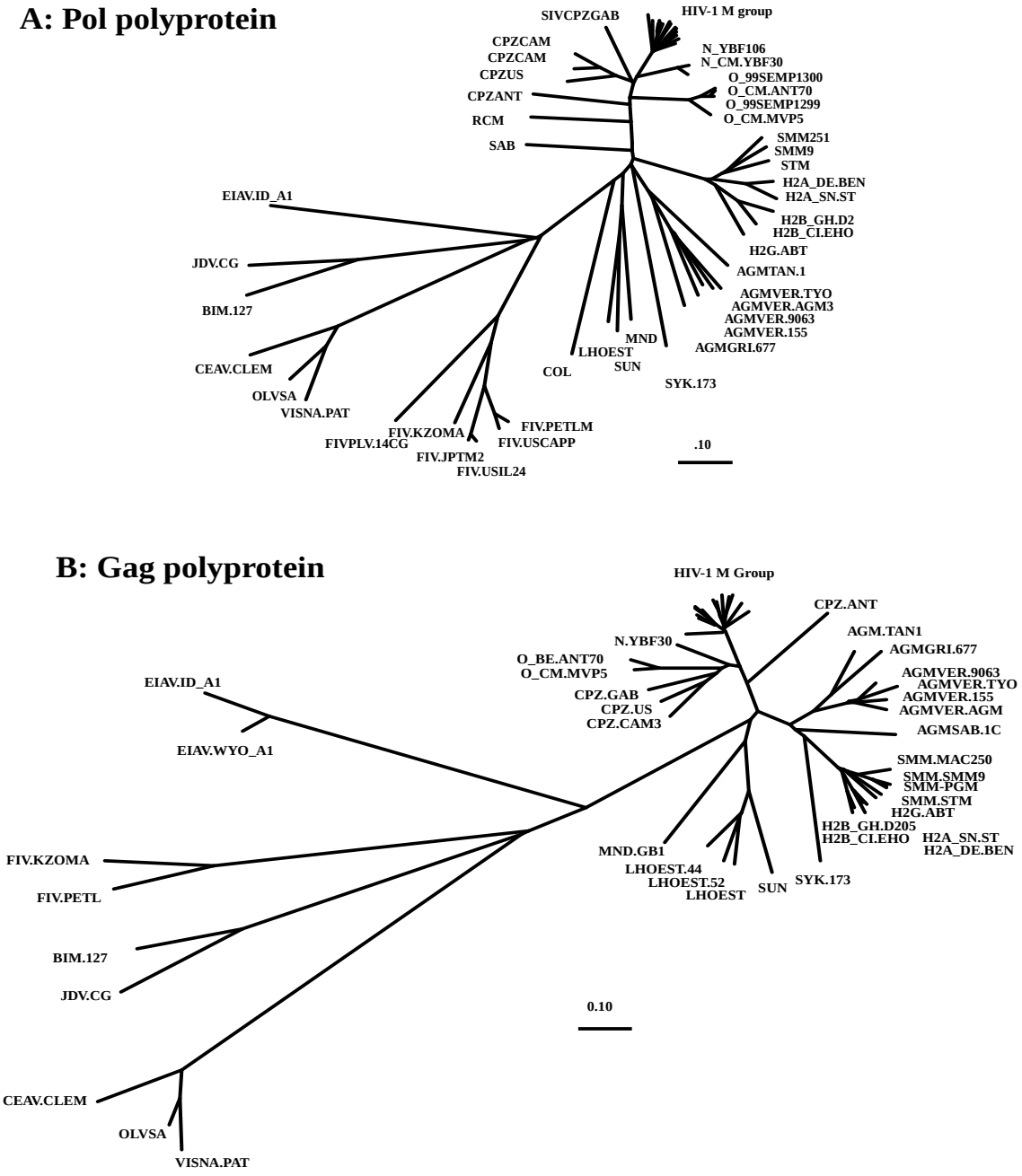

Figure 1. *Phylogenetic Tree of Lentiviral pol and gag Genes.*
A: *The complete pol genes, including protease, reverse transcriptase, RNAse H and integrase coding regions of lentiviruses were aligned.*
B: *Nearly complete gag genes, from start codon to the end of the EIAV sequences near the Gag-Pol ribosomal slip site, were aligned. For both trees, columns containing gaps were removed (thus removing the dUTPase region from the Pol data) and the DNAdist and NEIGHBOR programs in the PHYLIP package were used. In DNAdist, the F84 (also called maximum likelihood) model of evolution was used with a transition/transversion ratio of 1.7. The trees were re-sized in TREETOOL so that they both have the same distance scale. The alignments used to build the trees are available from btf@t10.lanl.gov.*

species which share overlapping habitats and feline species which share overlapping habitats but each species carries its own lineage of virus. For example chimpanzee habitat overlaps African green monkey habitat, but there is no evidence of exchange of virus between chimpanzees and African green monkeys. Both interactions between the host animals and species restrictions of the viruses can contribute to cross-species transfer or lack of transfer. Likewise housecats all over the world appear to share a domestic cat FIV, rather than picking up FIVs from local wildcats. Examples of the relatively rare cross-species transmission events are noted in the literature. A Peruvian wildcat sampled in a zoo appeared to have picked up a domestic cat FIV in captivity [10]. A Japanese wildcat was infected with domestic cat FIV in the wild [18]. Humans are another notable example, having apparently picked up three lineages of virus from chimpanzees to create the HIV-1 M, N and O groups, and as many as seven lineages of virus from sooty mangabeys to create HIV-2 subtypes A though G [19–21]. Baboons, which are noted to lack their own strain of simian immunodeficiency virus, have repeatedly become infected from the vervet subspecies of African green monkeys with which they share habitats[13, 14]. Cross-species transmissions of visna and Caprine Arthritis-Encephalitis Virus (CAEV) between domestic goats and sheep are reported to be rather common [18, 22–26]. The SIVs from sooty mangabeys were also transferred into several species of macaques in captivity in the USA [15, 17, 27].

Molecular evolution of the lentiviruses

Lentiviruses have been estimated to mutate at a rate as much as 106 to 107 times the rate of mutations found in eukaryotic germ-line DNA [28, 29]. The rate of evolution of any region of a genome, viral or cellular, is a function of the spontaneous mutation rate, positive and negative selection pressures, and numbers of generations per unit of time upon which selection can act. Factors such as codon usage bias, methylation of CpG dinucleotides and nucleotide composition bias are examples of selective pressures that can contribute to the evolution rate. Lentiviruses not only mutate faster than eukaryotes, but also have vastly higher numbers of generations per unit time, and thus they are expected to, and are observed to, evolve at a much faster rate than eukaryotes [29]. One of the most recent estimates of the evolution rate of members of the HIV-1 M group is 0.0024 (0.0018 to 0.0028) substitutions per base pair per year in the *env* gp160 gene region and 0.0019 (0.0009 to 0.0027) substitutions per base pair per year in the *gag* gene region [30]. The deltaretroviruses, also known as T-cell leukemia viruses (TCLVs) [31], have many parallels to the lentiviruses. Both TCLVs and lentiviruses infect white blood cells. Both are complex retroviruses with regulatory genes in addition to the *gag*, *pol* and *env* genes found in all retroviruses. Both have made numerous cross species jumps from non-human primates into humans, and between non-human primate species [14, 32–36]. Both are found in non-primate mammals as well as in primates, with inter-primate distances less than inter-mammalian distances [6]. Neither has ever been found in an endogenous state in any mammal. It has therefore been of interest to compare and contrast these two classes of complex retroviruses.

Two recent papers have compared the molecular evolution of TCLVs and primate lentiviruses [29, 37]. The Wodarz paper [37] suggests that the large difference in evolution rate between the two viruses is primarily due to the ability of HIV-1, but not HTLV-I, to infect macrophages in addition to CD4+ T-helper cells. The Sala paper [29] suggests that the difference in rates of evolution between HIV-1 and HTLV-I is due to clonal expansion of HTLV-infected cells, versus active replication of HIV-1. The Wodarz paper specifically disagrees with the explanation offered by Sala. Neither paper addressed the issue of the difference in nucleotide composition between the TCLVs and lentiviruses. The lentiviruses are all A-rich and C-poor, with nucleic acid compositions of 36% A, 18% C, 24% G and 22% T. The A-richness and C-poorness is roughly evenly distributed across the lentiviral genomes, with the exception of bovine immunodeficiency-like virus and Jembrana disease virus, which both show a dramatic difference in nucleotide composition between the 5' and 3' halves of their genomes (Figure 2 panels A and B). The T-cell leukemia viruses are C-rich and G-poor with nucleic acid compositions of 23% A, 33% C, 18% G and 23% T, again with a relatively constant composition bias across the genome (Figure 2 panels C and D). Although Figure 2 uses a 500 base window to smooth out the nucleotide composition plots,

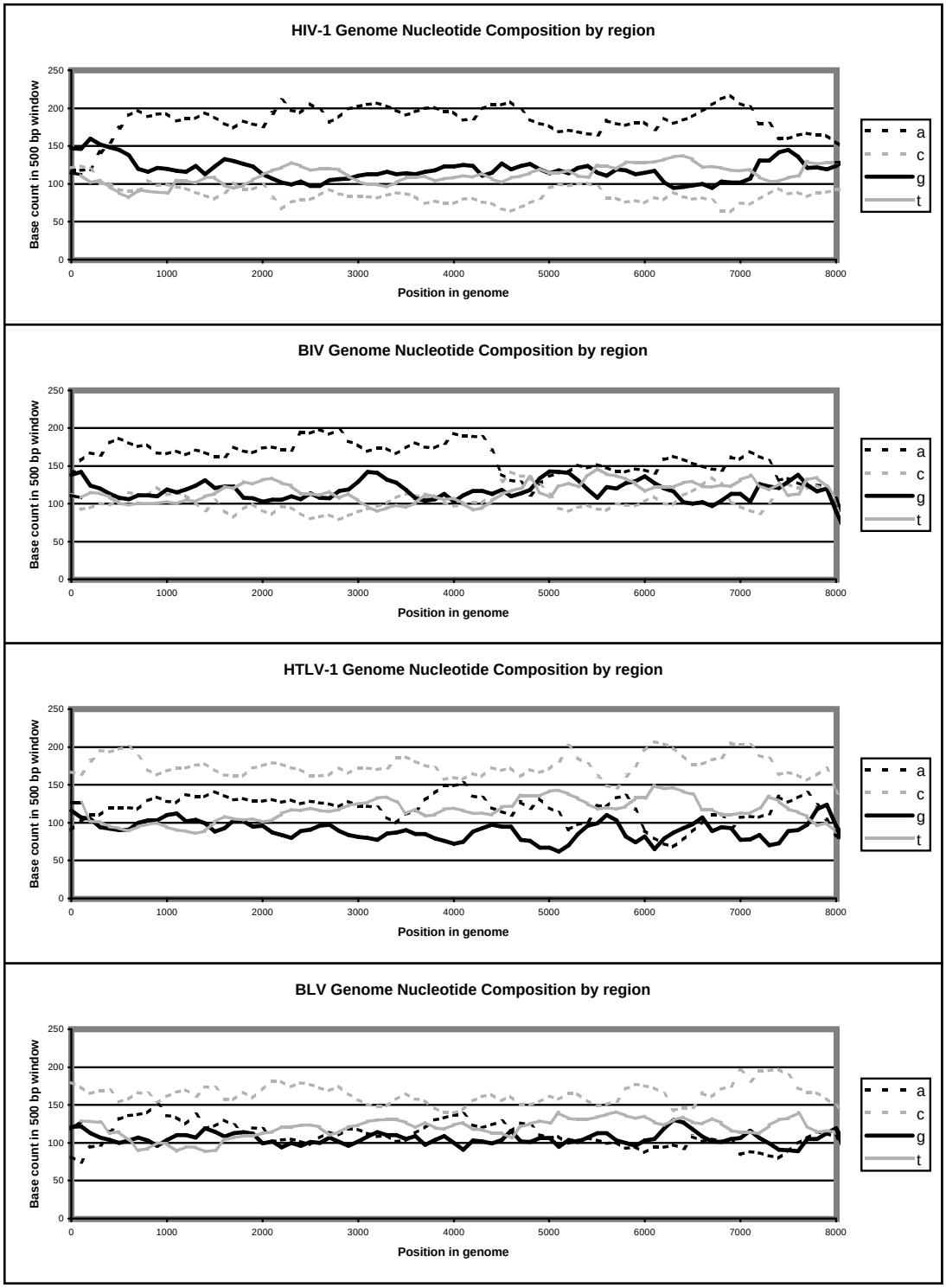

Figure 2. *Nucleotide Composition of Lentiviruses and Deltaretroviruses. A Microsoft EXCEL spreadsheet was used to count the number of each base within a 500 base sliding window across each genome. In the top two panels, HIV-1 isolate B-HXB2 (K03455) is compared to bovine immunodeficiency virus isolate HXB3 (M32690) to illustrate the marked change in composition in the BLV genome. All other lentiviruses examined (FIV, Visna, CAEV, HIV-2, SIV-SMM, SIV-AGM) were A-rich across the genome, similar to HIV-1 (data not shown). In the lower panels, HTLV-I isolate CAR (D13784) and BLV isolate B19 (AF257515) are plotted for comparison to each other and to the lentiviral genomes.*

smaller window sizes can be used to detect regions of the genome, such as the TAR element and the RRE, where RNA secondary structures require a more G+C-rich nucleotide composition bias. No biological reason for the difference in nucleotide base composition bias between the T-cell leukemia viruses and lentiviruses is apparent at this time. The fact that there is a striking difference suggests differences in the pattern of evolution as well as differences in rates of evolution between these two types of virus. Within the lentiviruses, the bovine viruses were exceptional in their nucleotide composition pattern across their genome (Figure 2), and also displayed nucleotide composition within the *pol* gene that differed from the other lentiviruses in being less A-rich (Figure 3).

The lentiviruses are extremely diverse in the DNA sequences of their genomes and the proteins encoded by them. Sala and Wain-Hobson point out that the amino acid sequence distance between the Pol protease peptides of HIV-1 and HIV-2 is roughly equivalent to the distance observed in homologous proteins from eubacteria and eukaryotes which last shared a common ancestor some 2 billion years ago [29]. The 10^6-fold faster rate of evolution in lentiviruses compared to those DNA genomes indicates that this diversity could have accumulated in closer to two thousand years than to two billion years. Any such speculations about the date of the most recent common ancestor of the primate lentiviruses are highly uncertain at this time, for numerous reasons. Korber *et al*. have shown that there is some uncertainty in the slope of the DNA sequence distances vs. time line even within the relatively closely related HIV-1 M group of primate lentiviruses [30]. They estimated that the HIV-1 M group of viruses last shared a common ancestor between 1915 and 1941 with a 95% confidence interval on the slope. Salemi *et al*. used a different method and arrived at a similar date for the origin of the HIV-1 M group. They further calculated that the common ancestor of the HIV-1 M group and the SIV-CPZs isolated from *Pan troglodytes troglodytes* dated to the late 17th century with a 99% confidence interval for a date between 1591 and 1761 [38]. The epidemiology of the AIDS pandemic tends to agree with a 20th century origin of the HIV-1 M group. The epidemic apparently went unnoticed in central sub-Saharan Africa for several decades [39]. Both the epidemiology and the molecular phylogenies of the HIV-1 O group and the HIV-2 viruses are less well understood than those of the HIV-1 M group. The nucleotide and amino acid sequence diversity of HIV-2 viruses is greater than the diversity of the HIV-1 M group viruses, but roughly equivalent to the diversity within the HIV-1/SIV-CPZ clade (Figure 1). The diverse HIV-2 lineages are thought to have arisen in sooty mangabeys, with several cross-species transfers from sooty mangabeys into humans, one for each subtype of HIV-2. The subtypes of HIV-2 are thus analogous to the groups (M, N and O) of HIV-1, both in terms of sequence diversity and in terms of the cross-species transfer events thought to have created them.

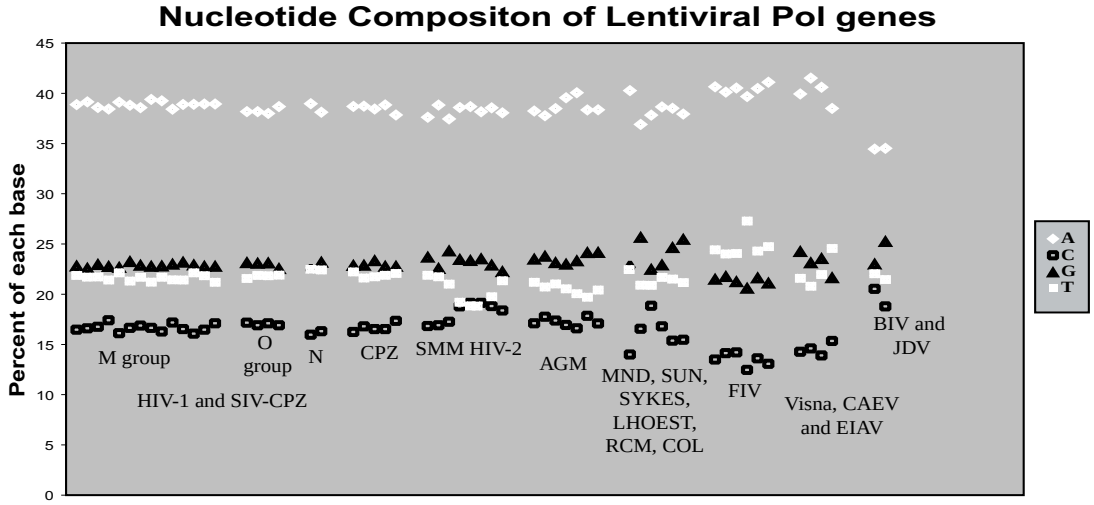

Figure 3. *Nucleotide Composition of Lentivirus pol genes. The pol gene alignment used to produce the pol tree in figure 1 was analyzed for nucleotide composition. Each set of points (A, C, G and T) is for one viral sequence.*

In any estimation of rates of evolution and times of divergence from common ancestors, an assumption that different lineages of the same organism evolve at similar rates greatly simplifies the calculations. A recent paper by Salemi *et al*. estimated that lineages of the HTLV-II virus evolves at a rate 150 to 350 times faster when the virus is transmitted between IV drug users, than lineages of the same virus propagated by mother-to-infant transmission [40]. Many studies of HIV-1 evolution in IV drug users and other communities have not turned up any such discrepancy in the rate of evolution of HIV-1 [41–44]. One study found that the rate of mutation of the *env* gene was 62% higher in frequent drug injectors, compared to those who had not injected drugs in the last 6 months [45]. Salemi *et al*. postulated that the increase in viral transmission rate between IV drug users, which can be many transmissions per year, accounted for the increase in HTLV-II evolution rate. HTLV-II mother to infant transmission would be expected to occur just once every 14 to 30 years for a given viral lineage. With HIV-1, both sexual and IV drug user transmissions can occur several times per year, and long-term chains of mother to infant transmission are never expected due to the lethality of HIV-infection in infants. In the rapidly expanding IV drug user epidemics that have been studied, no increase in HIV-1 evolution rate of needle-spread HIV-1 compared to sexually transmitted HIV-1, has been noted [46–48].

Conserved elements in the lentiviral genome

Despite the rapid evolution rate of lentiviruses, many elements in the lentiviral genome have been conserved over time. One of the most conserved regions of the lentiviral genome is the Lys-tRNA primer binding site (PBS), where the host Lysine transfer RNA hybridizes to the viral RNA genome to serve as a primer for reverse transcription. The PBS is short, just 15 bases (GAACAGGGACUUGAA), but nearly perfectly conserved in all lentiviruses. It exists within a secondary structure element that is conserved in structure, but not sequence [49]. The polypurine tract, Rev-responsive element, Phi element, and other elements involved in replication and packaging of the viral genome are also conserved to varying degrees between all lentiviruses. The protein-coding regions of the genome are also conserved to varying degrees, with *gag* and *pol* being more conserved overall, than *tat* or *env*. Within the proteins, catalytic and/or functional domains are highly conserved. One such element is the C-X2-C-X4-H-X4-C zinc knuckle domain of the Gag p7 nucleocapsid peptide that binds to the psi element to specifically package the viral genome into budding virions [50]. Whenever two regions of the genome need to coevolve to retain the interaction between the two elements, such as Tat protein binding to the TAR element or Rev protein binding to the RRE element, the evolution rate of both regions is slowed.

The *gag* and *pol* genes of lentiviruses are conserved well enough that multiple sequence alignments of this region can be built with confidence. The *env* gene is much more variable, including many insertions and deletions, and even if the cysteines that form disulfide bonds to create the loop structures of the Env protein are aligned, one cannot be sure that the alignment is phylogenetically correct. Although the Env protein of the bovine immunodeficiency and Jembrana disease viruses display an aberrant nucleotide composition bias in comparison to the other lentiviruses, there is no evidence that this is due to recombination. A BLAST search of their *env* genes against the databases produces other lentiviral *env* genes as the most significant matches (Figure 2 and data not shown).

Although the linear sequence of nucleotides in the *env* genes or amino acids in the Env proteins are not highly conserved, the coiled-coil structure of the fusion domains of lentiviruses are conserved and share structural similarity with the fusion domains of other viral and host proteins [51–54]. Likewise, the structures of polymerases and proteases are conserved, as are key residues in catalytic sites [55–66]. A review of HIV protein structures was published in 1998 [67]. Likewise, although the lentiviruses display remarkable diversity in linear DNA sequences, the nucleotide composition bias appears to be constrained as shown in Figures 2 and 3.

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