Available online at www.sciencedirect.com
ScienceDirect
Virology 370 (2008) 55–62
VIROLOGY
www.elsevier.com/locate/yviro

Evolution of the long terminal repeat and accessory genes of feline immunodeficiency virus genomes from naturally infected cougars

Mary Poss [a,*], Howard Ross [b]

[a] Department of Biology, Center for Infectious Disease Dynamics, 208 Mueller Lab, The Pennsylvania State University, University Park, PA 16802, USA
[b] New Zealand Bioinformatics Institute, University of Auckland, Auckland, New Zealand

Received 18 May 2007; returned to author for revision 29 June 2007; accepted 21 August 2007
Available online 29 September 2007

Abstract

FIVpco is a member of the feline immunodeficiency virus family that is endemic in wild cougar populations. Virus replication is robust in FIVpco-infected cougars but there are no consequences of infection to cougar survival, fecundity or susceptibility to other infections. Unlike pathogenic lentiviruses, there is no evidence for positive selection on FIVpco *gag* or *env*. To better understand how lentivirus genomes evolve in natural infections, we evaluated the regulatory region and accessory genes from fourteen full-length FIVpco genomes, which represent the FIVpco diversity in the Northern Rockies Ecosystem. Our data demonstrate that the two sister groups of FIVpco have each acquired binding sites for different interferon response factors (IRF). The most variable gene in the FIVpco genome encodes OrfA, although there is no indication that it, or any other accessory gene, is under positive selection. There is a single-splice acceptor site for *vif* expression, which is conserved among all FIVpco genomes. However, there are several putative means to express *rev* and *orfA*, which differ between the phylogenetic groups of FIVpco. Our comparative study on divergent FIVpco genomes indicates that variation in potential gene regulation mechanisms, not changes in structural proteins, characterize the evolution of FIVpco in natural infections.

Keywords: Evolution; FIVpco; LTR; Accessory genes; Gene regulation

Introduction

The family *Retroviridae* contains seven groups of viruses, which share many aspects of viral life history and the function and genomic organization of structural genes. However, there are differences among the retroviruses in the number of accessory genes that are encoded. Accessory genes typically have regulatory roles in the virus life cycle and may interact with host proteins. Lentiviruses and spumaviruses have the largest genomes in the Retrovirus family and encode up to six accessory genes.

The expression of virus genes is under the control of the lentivirus long terminal repeat (LTR), which contains binding sites for both viral and host transcription factors. There is substantial variation in the LTR of different lentiviruses (Thompson et al., 1994; Yamada et al., 1995) and evidence that differences in regulatory regions can affect phenotype. For example, acquisition of a transcription regulatory site can change tissue tropism and affect the level of virus production in a stimulated cell (Carvalho et al., 1993; Maury, 1998; Tsichlis and Lazo, 1991). Further, there is subtype-specific variation in the HIV-1 LTR that affects viral replication in vitro (Jeeninga et al., 2000; Naghavi et al., 1999; van Opijnen et al., 2004).

There are three accessory proteins that share similar functions among members of the lentivirus family (Strebel, 2003). The accessory proteins are located between *pol* and *env* or at the 3′ end of the viral genome and are produced on subgenomic messages. All lentiviruses encode a protein that enhances transcription of the viral genome. In some lentiviruses, this transactivating protein interacts with a specific stem–loop structure in the LTR but in feline immunodeficiency virus (FIV) and the two small ruminant lentiviruses (visna virus and caprine arthritis–encephalitis virus, CAEV) enhancement of viral transcription by the transactivating protein is mediated through interaction with host proteins (Miller et al., 2000). A

* Corresponding author. Fax: +1 814 865 9131.
E-mail address: mposs@bx.psu.edu (M. Poss).

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.08.024

second viral protein found in all lentiviruses except equine infectious anemia virus (EIAV) is the viral infectivity factor, Vif. Recently, research on HIV-1 has shown that Vif can counter the DNA editing by host cytidine deaminase APOBEC3G on the viral genome that occurs during reverse transcription (Harris et al., 2003; Sheehy et al., 2003; Yu et al., 2004; Zhang et al., 2003). Editing by this cellular deaminase causes a high frequency of G to A substitutions and, consequently, defective proviruses (Bishop et al., 2004; Esnault et al., 2005; Kieffer et al., 2005; Poss et al., 2006). Vif depletes APOBEC3G by binding to the N-terminal domain of the deaminase and to an ubiquitin–ligase complex composed of cullin-5 and elongins B and C through the conserved SLQ(Y/F)LA motif in the C-terminal portion of Vif (Mehle et al., 2006; Yu et al., 2003). This motif is similar to that encoded by suppressors of cytokine signaling (SOCS) proteins, which recruit a multi-subunit ubiquitin–ligase complex and target activated cytokine-associated kinases for degradation (Kile et al., 2002). Although the role of Vif in countering host innate defenses is critical to viral infectivity, Vif, like other lentivirus accessory proteins, is clearly a multifunctional protein and it also contributes to the normal maturation and stability of the virus particle (Akari et al., 2004; Dettenhofer et al., 2000; Goncalves et al., 1996; Simon and Malim, 1996).

The third of the common lentivirus accessory proteins is Rev. Rev is essential for export of unspliced or partially spliced viral transcripts to the cytoplasm. The viral Gag proteins and enzymes are produced from a full-length unspliced transcript while the envelope proteins and accessory genes are expressed from subgenomic mRNA. Rev itself is produced from a fully spliced mRNA in primate lentiviruses. In EIAV, Rev is produced with the transactivating protein (Tat) from a bicistronic mRNA, which is comprised of four exons. Exclusion of the third exon reportedly downregulates Rev activity while maintaining Tat levels (Belshan et al., 1998). Rev is expressed as both a bicistronic and a multiply spliced, one- and two-exon mRNA in FIV (de Parseval and Elder, 1999; Zou et al., 1997). Thus, there are different mechanisms of expressing some of the lentiviral accessory genes both within and between species. The use of alternative splicing increases the number of functionally distinct proteins that can be encoded in the genome in both viruses and eukaryotic organisms and provides additional means to regulate gene expression in different tissues and at different stages of cell differentiation (Garcia-Blanco et al., 2004; Stamm et al., 2005).

Although FIV causes an acquired immunodeficiency syndrome in domestic cats, feline lentiviruses are endemic in populations of cougars (*Puma concolor*) and lions (*Panthera leo*) and infection does not result in disease (Biek et al., 2006a,b). We recently demonstrated that recombination is discernable in the genomes of FIVpco as this virus evolves in an expanding cougar population (Bruen and Poss, 2007). The regions involved in recombination are almost exclusively between the end of *pol* and the start of *env*; the region of the lentiviral genome which encodes viral accessory genes. Our previous studies indicated that there was no evidence of positive selection on viral *env* or *pol* fragments either within or between infected cougars (Biek et al., 2003). Further, a detailed analyses of all feline lentiviral Gag proteins indicated that positive selection is not shaping this viral protein as the virus evolves in cougars or other feline host species (Burkala and Poss, 2007). However, there is evidence that viral lineages circulating in the Northern Rockies Ecosystem differ in phenotype (Blake et al., 2006). Viruses that are endemic and minimally pathogenic in their host populations may evolve under different pressures than pathogenic viruses, which can elicit a strong immune response that drives virus intrahost evolution. In this report, we evaluated the complete genomes of fourteen full-length FIVpco genomes, which represent the viral diversity throughout the study region, to determine the consequences of virus evolution on viral regulatory regions and accessory genes in this population of naturally infected cougars.

Results and discussion

LTR

The LTR of the FIVpco genomes varies in length from 333 to 357 bp. The majority of gaps occur in the first 70 bp in the aligned sequences. We evaluated the LTR for the presence of binding sites that were less than 1% different from those of established mammalian transcription factors. We further restricted our search to those binding sites that would be expected to occur by chance less than 0.05 times in a randomized 1 kb sequence. Similar to FIV (Chatterji et al., 2002; Thompson et al., 1994), all FIVpco have a conserved AP1 site located in the first 65 bp of the LTR (Fig. 1). However, the two sister groups of FIVpco have recognition sites for different interferon response factors (IRF). IRF-1 enhances transcription of genes regulated by interferon alpha and gamma. There is a site for this transcription factor at position 16–24 in the LTR of sequences that group in the lineage containing JM01, JF6, YM137 and SR631 (Fig. 1). We previously demonstrated that these sequences are recombinant and are derived from parental sequences ancestral to YF125 and YM29 (Bruen and Poss, in press). However, neither of these parental viruses contains the IRF-1 site, suggesting that it arose by spontaneous mutation after the recombination event occurred. The recognition sequence for IRF-1 in the FIVpco LTR is 99.3% similar to the site identified in humans and it is expected to occur by chance 1.9 times in 10^5 bases. In contrast, all members of group 1 except CoLV contain a binding site for IRF-3. IRF-3 is induced by virus infection and regulated by phosphorylation. The binding site reported for IRF-3 is an exact match to that in the LTR in this FIVpco group with the exception of Mc100, to which it is 99.3% identical. This site could be expected to occur once in a 10-kb genome by chance alone, although it is unlikely that it would arise by chance in each of the phylogenetically related genomes. Thus, IRF sites, which may enhance virus replication consequent to an innate interferon response, evolved independently in the two main FIVpco groups. Neither IRF-1 nor IRF-3 binding sites have been reported to occur in FIV. The absence of the IRF-3 site in CoLV may be because this isolate has been maintained in tissue culture for over 15 years. Under