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Adaptive evolution of primate TRIM5α, a gene restricting HIV-1 infection ☆

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Abstract

Recent studies showed that nonhuman primate TRIM5α can efficiently block HIV-1 infection in human cell lines. It can also restrict other retroviruses, therefore, suggested as a general defender against retrovirus infection. Here, we present an evolutionary analysis of TRIM5α in primates. Our results demonstrated that TRIM5α has been evolving rapidly in primates, which is likely caused by Darwinian positive selection. The SPRY domain of TRIM5α, which may be responsible for recognition of incoming viral capsids showed higher nonsynonymous/synonymous substitution ratios than the non-SPRY domain, indicating that the adaptive evolution of TRIM5α in primates might be an innate strategy developed in defending retrovirus infection during primate evolution. In addition, the comparative protein sequence analysis suggested that the amino acid substitution pattern at a single site (344R/Q/P) located in the SPRY domain may explain the differences in susceptibilities of HIV-1 infection in diverse primate species.

Keywords: TRIM5α; Primate; Darwinian positive selection; HIV-1 restriction

1. Introduction

Virus infection is one of the major challenges throughout the history of primate evolution. The fossil record of endogenous retrovirus (Boeke and Stoye, 1997) provided solid evidence that retrovirus and their vertebrate host have coexisted for tens of million years (Johnson and Coffin, 1999; Belshaw et al., 2004; Goodchild et al., 1993). Endogenous retrovirus elements in the germ lines of mammals have also shown that mammals have been subject to retrovirus infection repeatedly (Boeke and Stoye, 1997; Coffin, 1996; Herniou et al., 1998). In primates, more than a dozen retroviruses have been reported including the well-known simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) (Yin and Liu, 1997). These viruses establish persistent infection with a broad spectrum of pathogenic potential, ranging from highly pathogenic to nonpathogenic, depending on various host and environmental factors (Lerche and Osborn, 2003). The most notorious retrovirus, HIV can cause severe acquired immune deficiency syndrome (AIDS) in humans, but has a different clinical outcome when inoculated into nonhuman primates.

Chimpanzees can be infected by HIV-1 (HIV virus type 1) (Francis et al., 1984; Alter et al., 1984; Nara et al., 1987; Gajdusek et al., 1985; Fultz et al., 1986), and passed from chimpanzee to chimpanzee (Gajdusek et al., 1985). Most chimpanzees infected with HIV-1 do not develop clinical or immunological abnormalities in 2–5 years, but various degrees

Abbreviations: TRIM5α, tripartite motif-containing 5a; SPRY, SPlA knase and RYanodine receptor.

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of lymphadenopathy (Fultz et al., 1986), e.g., the transient decrease in the percentage of CD4 cells and in the CD4/CD8 ratio are detectable (Alter et al., 1984; Fultz et al., 1986). Infection of HIV-1 in other great ape species, i.e., bonobo, gorilla and orangutan has not been reported. Gibbons (lesser apes) could be chronically infected with HIV-1, but the clinical characteristics of HIV-1 infection in humans did not occur in gibbons (Lusso et al., 1988). In Old World monkeys (OWM), HIV-1 can enter the cells, and then encounters an efficient post-entry block (Morrow et al., 1989; McClure et al., 1987; Agy et al., 1992). In New World monkeys, infection with HIV-1 have not been reported, but multiple cell lines derived from squirrel monkeys, a New World monkey species can be infected with HIV-1 (Hofmann et al., 1999; Owens et al., 2003).

During the course of evolution, the hosts had developed various defense mechanisms against virus. Innate immunity plays an important role in resisting virus infection. Many kinds of mammalian cells can express restriction factors inhibiting retrovirus replication in a cell autonomous manner. For example, APOBEC3G and ZAP can act on HIV-1 nucleic acids and inhibit its replication (Sheehy et al., 2002; Gao et al., 2002; Zhang et al., 2003; Mangeat et al., 2003; Harris et al., 2003). Recently, another kind of restriction factors was reported to prevent retrovirus infection by targeting the retroviral capsids (Bieniasz, 2003; Stoye, 1998; Goff, 1996). Among them, TRIM5α was shown to be the most effective block for HIV-1 infection in nonhuman primates (Stremlau et al., 2004). TRIM5α from rhesus monkey can restrict HIV-1's ability to establish an infection when expressed in human *HeLa* cells. In this restriction, TRIM5α is not just necessary but also sufficient. Though TRIM5α from humans also showed restriction effect in *HeLa* cells, it is much less efficient than in rhesus monkeys (Stremlau et al., 2004). In addition, rhesus monkey and African green monkey derived TRIM5α can also restrict replications of N-tropic murine leukemia viruses (N-MLV) and equine lentivirus Equine infectious anemia virus (EIAV) in African green monkey cell lines (Yap et al., 2004; Perron et al., 2004; Hatziioannou et al., 2004; Keckesova et al., 2004). Therefore, TRIM5α seems to act as a general defender against retrovirus infection.

In this study, we delineated the molecular evolution of TRIM5α in diverse primate species by analyzing the nonsynonymous/synonymous substitution patterns. Our results demonstrated that TRIM5α had undergone strong Darwinian positive selection in primates by rapidly accumulating amino acid substitutions in the virus-recognition domain, an innate strategy developed against retrovirus infection.

2. Materials and methods

2.1. DNA samples

We sequenced 8 nonhuman primate species (one individual for each species), including three great ape species (chimpanzees_*Pan troglodytes*, gorillas_*Gorilla gorilla* and orangutan_*Pongo pygmaeus*), two lesser ape species (white-browed gibbon_*Hylobates hoolock* and white-cheeked gibbon_*Hylobates leucogenys*), and three Old World monkey species (rhesus macaque_*Macaca mulatta*, Assamese macaque_*Macaca assamensis* and pig-tailed macaque_*Macaca nemestrina*). We also acquired the published sequences of human (NM_033034) and three other nonhuman primate species (two African green monkey species, *Cercopithecus aethiops*, AY669399 and *Cercopithecus tantalus*, AY593973, and one New World monkey species: owl monkey, *Aotus trivirgatus*, AY684992). The owl monkey sequence was from the V2 splice form of the TRIM5α-cyclophilin A fusion gene, which contains the SPRY domain though it is non-translational for the V2 form (Nisole et al., 2004). Through database search and phylogenetic analysis (data not shown), the homologous sequences of mouse (NM_175677) and rat (XM_219046) were also obtained and used as outgroup in the analysis. The divergence times between humans and the nonhuman primate species are 5–6 million years (myr) for chimpanzee, 6–7 myr for Gorilla, 14 myr for orangutan, 18–20 myr for lesser apes, 23–25 myr for Old World monkeys and 40 myr for New World monkeys (Goodman et al., 1998). All DNA samples were obtained from the collections of Kunming Cell Bank, Chinese Academy of Sciences.

2.2. PCR and sequencing

The complete coding region (1689 bp) of TRIM5α was sequenced in all the nonhuman primate samples. Primers were designed by comparing the published human and mouse sequences in Ensembl (http://www.ensemble.org). There are 8 exons in *TRIM5α* gene and exon 1 is not translational. We designed 5 primer pairs to amplify the coding sequence (exon2–exon 8). The primer sequences are:

F23 5-gagatgatgggtcacacaaagat-3; R23 5-tcacggactcaaaaag-tagcac-3
F4 5-tactctcctgctactatgtcccctc-3; R4 5-ggaaatagccttcacttttctt-taatc-3
F5 5-tctcccacctcatcttccc-3; R5 5-gagtctcactttgtcgccc-3
F67 5-tgtcctatgagtttaagatagacag-3; R67 5-gaaaactgggagaaccttgtt-3
F8 5-tataacttctaaacaaggttcctcc-3; R8 5-cagaattgaagtcattttga-cagta-3.

Sequencing was performed in both directions by forward and reverse primers using the *BigDye* terminator sequencing kit on an ABI 3100 automated sequencer (ABI, Inc.).

2.3. Data analysis

DNA sequences were aligned using *Cluster_W* (Thompson et al., 1994). The phylogenetic relationships among specie were reconstructed based on the neighbor joining method in *MEGA2.0* using only the synonymous substitutions (Kumar et al., 2001). The ancestral sequences (internal nodes) of the phylogenetic tree were inferred by the maximum likelihood method in the *PAML* program developed by Yang (1997). The non-synonymous and synonymous substitution rates (K_a and K_s) were calculated based on Pamilo–Bianchi–Li's method, in

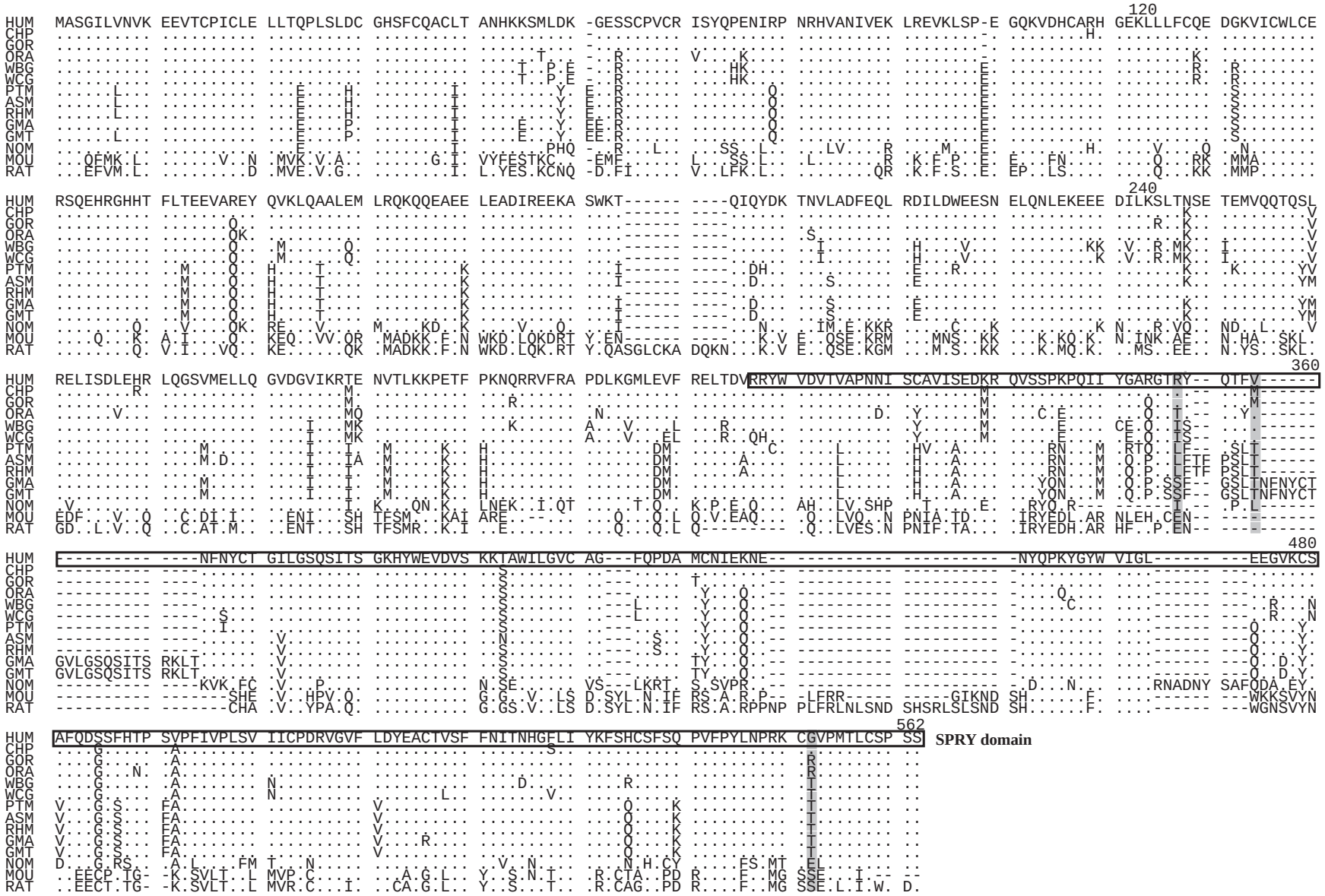

Fig. 1. The aligned protein sequences of TRIM5α in primates and rodents. The shadowed sites are the three identified sites showing positive selection (Yang et al., 2000; Yang, 1997). The framed sequences indicate the SPRY domain. HUM_human, CHP_chimpanzee, GOR_gorilla, ORA_orangutan, WBG_white-browed gibbon, WCG_white-cheeked gibbon, PTM_pig-tailed macaque, ASM_Assamese macaque, RHM_rhesus macaque, GMA_African green monkey (*Cercopithecus aethiops*), GMT_African green monkey(*Cercopithecus tantalus*); NOM_owl monkey, MOU_mouse, RAT_rat.

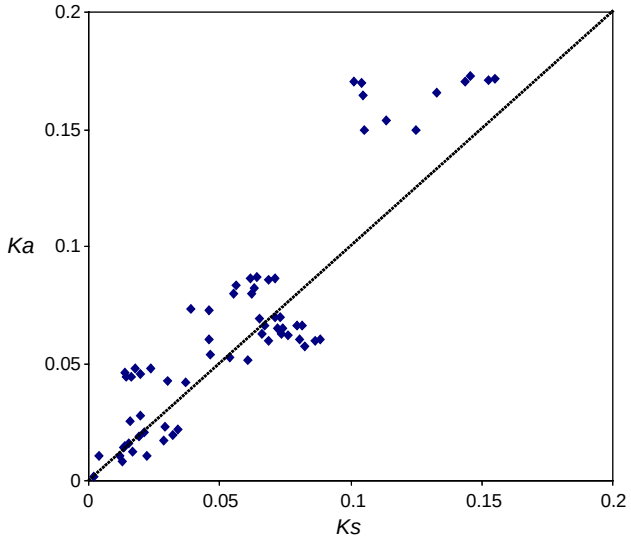

Fig. 2. The plotting of pair-wised K_a/K_s ratios in the primate species tested. The K_a/K_s ratios were estimated following Pamilo–Bianchi–Li's method (Pamilo and Bianchi, 1993; Li, 1993).

which the transitional/transversional substitution bias was taken into account (Pamilo and Bianchi, 1993; Li, 1993). The Z test was used to detect the deviation of the K_a/K_s ratios from neutrality ($K_a/K_s=1$) (Kumar et al., 2001). We conducted the sliding window analysis using the program *DnaSP4.0* to reveal the distribution of K_a/K_s ratios across the functional domains of TRIM5α (Rozas et al., 2003). The window length used is 100 codons with a step size of one codon.

3. Results and discussion

3.1. Sequence substitution patterns of TRIM5α in primates

We sequenced the complete coding region of TRIM5α in 8 primate species plus sequences from human and three other primate species, together covering great apes, lesser apes, Old World monkeys and New World monkeys. The aligned protein sequences revealed high variations in primates (Fig. 1). The pair-wised calculation of nonsynonymous substitution rate (K_a) versus synonymous substitution rate (K_s) in the 12 primate species indicated higher values ($K_a/K_s>1$) in 38 out of the 66 pairs (Fig. 2), an implication of deviation from neutral expectation ($K_a/K_s=1$) and possible positive selection of TRIM5α during primate evolution. We then checked the detailed substitution pattern of each primate lineage in the phylogenetic tree (Fig. 3a). There were 10 out of the 22 primate lineages (including internal branches) showing large K_a/K_s ratios (>1), again an indication of adaptive evolution in almost half of the primate lineages. In contrast, the rodent lineages (mouse and rat) have very small K_a/K_s ratios, the signature of strong functional constraint of TRIM5α in rodents. The significant values were observed in the chimpanzee, orangutan and gibbon lineages ($K_a/K_s>1$, $p<0.05$, one-tailed Z test) (Kumar et al., 2001). We conducted the relative rate test for the gibbon lineage of which a large K_a/K_s ratio was observed ($K_a/K_s=6.85$, $p<0.01$, one-tailed Z test). When compared with the great ape species (human, chimpanzee, gorilla and orangutan; rhesus monkey was used as outgroup), the synonymous substitution rate of the gibbon lineage is similar with those of

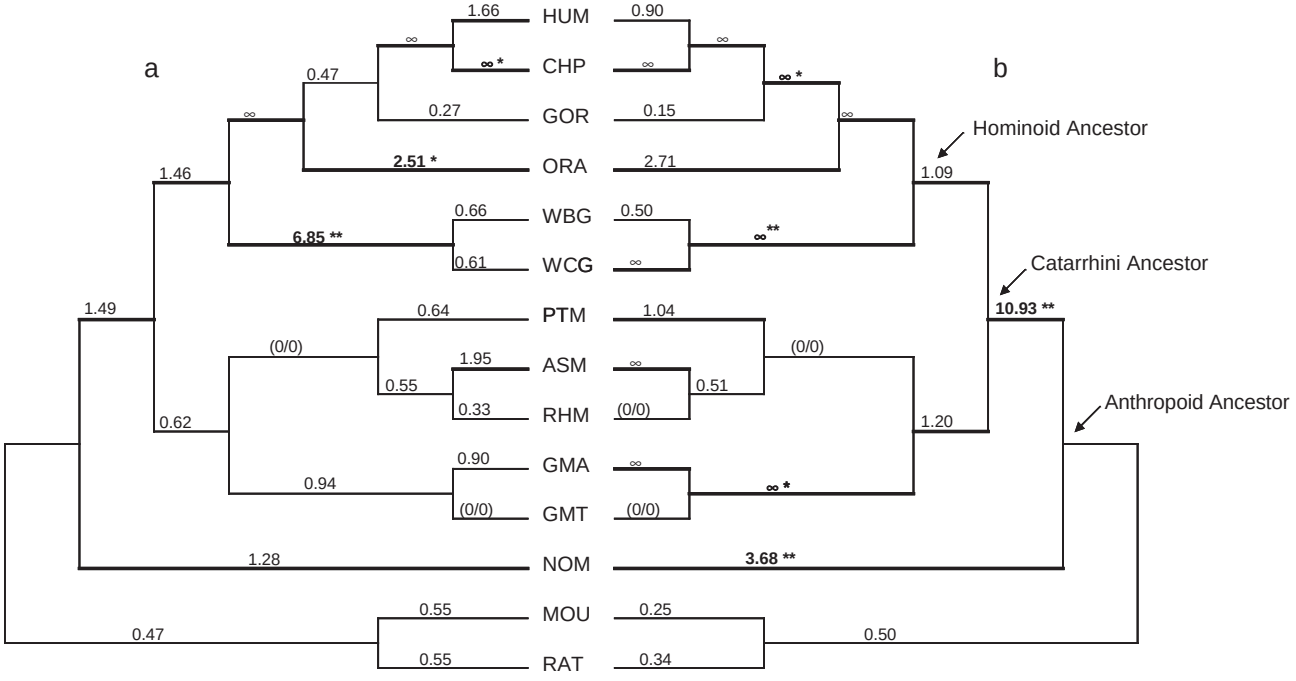

Fig. 3. The K_a/K_s ratios in diverse primate lineages. a) The K_a/K_s ratios based on the complete coding sequences. b) The K_a/K_s ratios based only on the SPRY domain sequences (about 44% of the complete coding region). The K_a/K_s ratios were estimated following Pamilo–Bianchi–Li's method (Pamilo and Bianchi, 1993; Li, 1993). The mouse and rat sequences were used as outgroup data and no positive selection was detected in the rodent lineage. The bolded lines are those with K_a/K_s ratios larger than one. When $K_a>0$, $K_s=0$, the lineages were labeled as "∞".The lineages with no substitutions observed were labeled as "(0/0)". *$p<0.05$; **$p<0.01$.

the great apes, an implication of constant synonymous substitution rates across primate species (data not shown). However, when the amino acid sequences were compared, significant rate differences were observed ($p<0.05$ for orangutan, $p<0.01$ for human, chimp and gorilla), and the gibbon lineage showed a much faster amino acid substitution rate than those of the great apes. Hence, there are rate variations in protein evolution across the primate lineages, an implication of varied selective pressures on different primate species during evolution.

The rapid evolution of primate TRIM5α could also be explained by relaxation of functional constraint which might result in large K_a/K_s ratios. However, considering the critical role of TRIM5α in innate immunity and also the fact that primates have been under constant selective pressure from virus infection, Darwinian positive selection seems a better explanation for the observed accelerated evolution of TRIM5α in primates, which is likely an adaptive strategy developed in defense of virus infection.

The large K_a/K_s ratios in primates became more prominent when the virus recognition domain (SPRY domain) of TRIM5α was under scrutiny (Fig. 3b), in which 15 out of the 22 primate lineages showing $K_a/K_s>1$. The number of $K_a/K_s>1$ lineages for the SPRY domain is significantly higher than that of the non-SPRY domain (15/22 vs. 7/22, $p=0.034$, two-tailed Fisher's exact test) (Fig. 4). It was reported that the SPRY domain of TRIM5α is responsible for recognition of incoming viral capsids (Stremlau et al., 2004; Sayah et al., 2004). It may directly bind and ubiquitimate the capsid of HIV-1, disrupt the proper uncoating of the capsid, and eventually block the transcription of virus RNA into DNA (Cohen, 2004; Lee and KewelRamani, 2004). Hence, the SPRY domain might have been under stronger selective pressure than the non-SPRY domain, which explains the observed faster evolution of the SPRY domain in primates. A recent study showed that a single amino acid substitution in the SPRY domain of the human TRIM5α can confer the ability to restrict HIV-1, a clear indication of the importance of the SPRY domain in virus restriction (Yap et al., 2005). In Fig. 3b, there were five lineages showing significant $K_a/K_s>1$ ($p<0.05$, one-tailed Z test), including the owl monkey (New World monkey), the African green monkey, the Catarrhini ancestor and two great ape lineages. This observation suggested that adaptive evolution of the SPRY domain of TRIM5α is a common phenomenon in diverse primate species, which is consistent with the hypothesized general defender role of TRIM5α in restricting retrovirus infection.

3.2. Sliding window analysis and codon-based neutrality test

We further conducted the sliding window analysis for the Catarrhini ancestor lineage of which a strong positive selection was observed ($K_a/K_s=10.93$, $p<0.01$, one-tailed Z test). The result of the sliding window analysis is consistent with the proposed stronger selection pressure on the SPRY domain of TRIM5α, in which the SPRY domain showed higher K_a/K_s ratios than the non-SPRY domain (Fig. 5a). Similar results were also observed when performing the sliding window analyses for the other lineages with large K_a/K_s values (Fig. 5b, c). If relaxation of functional constraint were the cause of the observed large K_a/K_s ratios in TRIM5α, we would expect an even distribution of K_a/K_s ratios across the coding region of TRIM5α because substitutions would be accumulated by chance. Therefore, the higher K_a/K_s ratios observed in the SPRY domain were a clear signature of positive selection in primates.

It should be noted that in the owl monkey *TRIM5α* gene, there is an insertion of CypA (cyclophilin A) pseudogene in the intron region between exon 7 and exon 8, resulting in a fusion protein lack of most parts of the SPRY domain (Nisole et al., 2004). Therefore, there is a possibility that the SPRY domain in the owl monkey may become intron sequences. However, it was suggested that the CypA sequences could be removed by

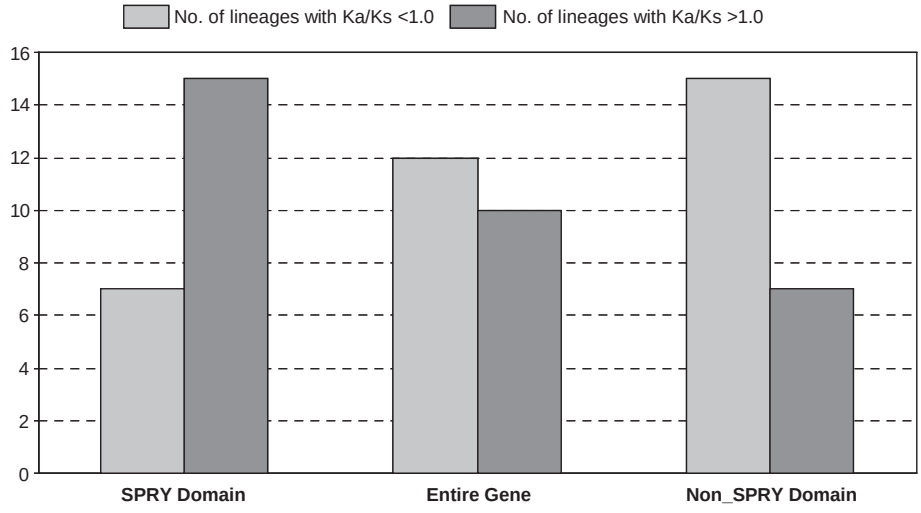

Fig. 4. The comparison of large (>1) and small (<1) K_a/K_s ratios between the SPRY and non-SPRY domains in the 22 primate lineages of the phylogenetic tree shown in Fig. 3. The SPRY domain shows the highest number of large K_a/K_s ratios.

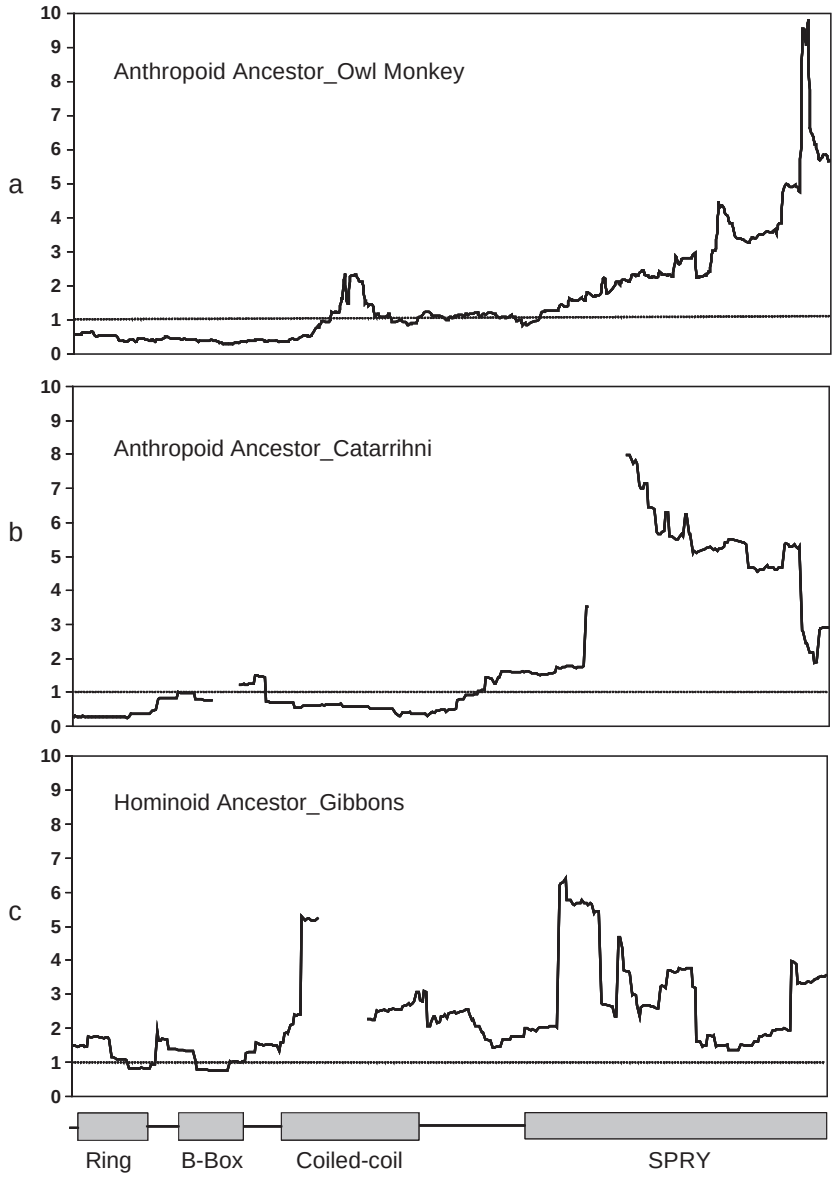

Fig. 5. The sliding window analysis of the K_a/K_s ratios across the coding region of TRIM5α. The *DnaSP4.0* program was used to generate the K_a/K_s curves, in which the Nei and Gojobori's method was employed to estimate the K_a/K_s ratios (Nei and Gojobori, 1986). The window length is 100 codons with a step size of one codon. The domains of TRIM5α were illustrated in the bar underneath, of which the SPRY domain showed the highest K_a/K_s ratios due to strong positive selection on this domain. The K_a/K_s ratios were not shown in part of the curves in "b" and "c", and it is because the K_s is zero in this region.

normal splicing resulting in a splice form with complete TRIM5α protein coding sequences (Nisole et al., 2004). Our sliding window analysis also showed that the SPRY domain of the owl monkey has been under strong positive selection (Fig. 5a), therefore an indication of a functional SPRY domain in the owl monkey TRIM5α. When we removed the owl monkey sequence from the analysis, the same nonsynonymous/synonymous patterns were observed in the other primate lineages (data not shown).

To identify individual amino acid sites potentially undergone positive selection, we performed the codon-based neutrality test following Yang et al. (2000). Assuming different evolutionary rates across primate lineages (model M2 in *PAML*) (Yang, 1997), we detected three potential sites showing the highest probabilities of positive selection (site 347, 354 and 552, $p > 0.95$, Fig. 1) (Nisole et al., 2004). All the three sites are located in the SPRY domain, and site 347 and 354 are within the short region (344–354 in Fig. 1) suggested imparting virus restriction specificity (Yap et al., 2005), again consistent with the proposed stronger positive selection on the SPRY domain in primates. These sites are potentially useful in design of functional assays delineating the molecular mechanism of virus restriction.

3.3. Sequence variations and species-specific HIV-1 restriction

It was demonstrated that an engineered single mutation from arginine to proline (site 344 in Fig. 1 and it was referred as site 332 in the human TRIM5α in reference Yap et al., 2005) in the SPRY domain of the human TRIM5α can confer

HIV-1 restriction (Yap et al., 2005). All the Old World monkeys except for the pig-tailed macaque are proline at this site which explains the HIV-1 resistance of these species (Morrow et al., 1989; McClure et al., 1987; Agy et al., 1992). Human and chimpanzee are arginine at this site, and both of them are HIV-1 susceptible (Francis et al., 1984; Alter et al., 1984; Nara et al., 1987; Gajdusek et al., 1985; Fultz et al., 1986). Gorilla, orangutan, gibbons and pig-tailed macaque are glutamine at this site. Previous reports showed that gibbon can be infected by HIV-1 (Lusso et al., 1988), and pig-tailed macaque can also be infected by HIV-1, the only exception in Old World monkeys (Agy et al., 1992). Therefore, the glutamine at site 344 may not confer the ability of HIV-1 restriction. In addition, neither the pig-tailed macaque nor do gibbons have the eight amino acid segment (LFTFPSLT, site 347–354) which was also shown to be HIV-1 resistant in rhesus macaque, and it is redundant with the proline at site 344 (Yap et al., 2005). Hence, this explains why the pig-tailed macaque is the exception in Old World monkeys because this species are lack of both of the HIV-1 resistant components in its TRIM5α. Since human, apes and New World monkeys do not have the HIV-1 resistant sequences, the two HIV-1 resistant components must have originated within Old World monkeys. Consequently, the amino acid substitution pattern at site 344 may explain the differences in HIV-1 susceptibilities in diverse primate species (Francis et al., 1984; Alter et al., 1984; Nara et al., 1987; Gajdusek et al., 1985; Fultz et al., 1986; Lusso et al., 1988; Morrow et al., 1989; McClure et al., 1987; Agy et al., 1992). Based on the individual codon test, site 344 also has a high probability for positive selection though it is not statistically significant ($p=0.936$).

What is the biological driving force behind the rapid amino acid substitutions of primate TRIM5α? It would be natural to infer that defending virus infection is the most likely cause for the adaptive evolution of TRIM5α in primates. This notion is consistent with our current knowledge about the restrictive effect of TRIM5α on virus infection. As aforementioned, nonhuman primates TRIM5α protein can block the replication of HIV-1 in human cell lines (Stremlau et al., 2004; Yap et al., 2004; Perron et al., 2004; Hatziioannou et al., 2004; Keckesova et al., 2004). It can also restrict replications of N-MLV and EIAV, therefore considered a general defender against retrovirus infection. Since the retrovirus genomes usually evolve rapidly to escape the host immune system, the rapid evolution of TRIM5α in primates might be a coping strategy developed during evolution to survive repeated retrovirus infection. Besides TRIM5α, another antiviral protein (APOBEC3G) was also reported to have undergone rapid evolution in primates (Zhang and Webb, 2004; Sawyer et al., 2004). Hence, the adaptive evolution of antiviral proteins in primates might play a crucial role in building up the innate immunity of primates against virus infection during evolution.

During the preparation of this article, Sawyer et al. (2005) published a similar study on the molecular evolution of TRIM5α in primates, in which they also identified strong positive selection in primates. They suggested that TRIM5α evolution has been driven by antagonistic interactions with a wide variety of viruses and endogenous retroviruses that predate the origin of primate lentiviruses.

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