inhibitors results in increased levels of BST-2 and loss of Vpu-mediated viral release, suggesting that the anti-BST-2 effect of Vpu involves proteasomal degradation [87, 88, 93]. However, prolonged cell exposure to proteosomal inhibitors was shown to deplete the cellular pool of free ubiquitin, thus affecting not only the proteasomal, but also ubiquitin-dependent lysosomal degradation. These data therefore support the hypothesis that the Vpu-induced downregulation of BST-2 is at least in part ubiquitin-dependent [97, 106].

In an alternative mechanism, treatment with inhibitors of the endolysosomal pathway has been shown to prevent the Vpu-mediated degradation of BST-2 [36, 84-86], and result in the visible co-localization of the restriction factor to endolysosomal compartments [85, 102] suggesting a potent mechanism of lysosome-dependent BST-2 degradation by Vpu. Janvier *et al.* reported that Vpu accelerates BST-2 degradation *via* an interaction with HRS, a component of the ESCRT-0 machinery that sorts ubiquitinated proteins to lysosomes for degradation [86]. Importantly, they showed that inhibition of HRS led to accumulation of BST-2 at the PM, the TGN, and in endolysosomal compartments. This suggests that while cell-surface BST-2 internalized by Vpu is transiently sequestered in intracellular compartments, a significant fraction is ultimately re-routed to lysosomes for degradation

CONCLUSIONS

The ability of HIV-1 Vpu to antagonize the antiviral activity of BST-2 is an important step in the effective release of infectious viral particles. While much knowledge has been gained recently about the molecular mechanisms and structural constraints of the Vpu–BST-2 interaction (reviewed in [107]), both the actual cellular compartment of interaction and the subsequent trafficking pathways remain controversial. As discussed extensively in this review, the most likely putative sites of action of Vpu in BST-downregulation are; 1) the biosynthetic/secretory pathway, where BST-2 is potentially sequestered by Vpu, blocking anterograde membrane transport of the restriction factor [35, 37, 103]; 2) postendocytic compartments (i.e., TGN, recycling endosomes), where the sequestration of BST-2 by Vpu, preventing the recycling step after endocytosis [35-37] has been proposed; 3) the PM, from where, consistent with our observations and those of others, BST-2 is internalized directly by Vpu, resulting in enhanced endolysosomal trafficking and subsequent BST-2 degradation [85, 86, 96, 104].

These models are not mutually exclusive; each one explains the mechanisms of Vpu-induced downregulation of BST-2 to varying degrees in different cellular contexts; e.g., 1) blocking the membrane transport of BST-2 might be more important when viral infection induces a strong IFN response; 2) the clearance of cell-surface BST-2 might be a major mechanism in cells expressing the protein at high levels. Overall, newly synthesized, directly internalized, or recycled BST-2 could be relocated to the endolysosomal pathway after its temporary sequestration in intracellular compartments, or subjected to ERAD-like proteasomal degradation. Thus, the antagonistic activity of Vpu on BST-2 downregulation would depend on a combination of sequestration in various cellular compartments, altered cellular trafficking leading to degradation, and direct internalization. Further investigations will likely provide insights into the exact steps of Vpu-mediated BST-2 downregulation and thus to the development of novel therapeutic agents targeting viral antagonism of this host restriction factor.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Health, Labor and Welfare of Japan (Research on H24-AIDS-ippan-005 and -008), and from the Ministry of Education, Science, Technology, Sports and Culture of Japan (22590428).

CONFLICT OF INTEREST

Declared none.

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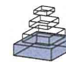
Received: March 9, 2012 **Revised: March 29, 2012** **Accepted: April 2, 2012**

PMID: 22524176

frontiers in
MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE
published: 30 August 2012
doi: 10.3389/fmicb.2012.00314



Allele frequency of antiretroviral host factor TRIMCyp in wild-caught cynomolgus macaques (*Macaca fascicularis*)

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A recent study showed that the frequency of an antiretroviral factor *TRIM5* gene-derived isoform, TRIMCyp, in cynomolgus macaques (*Macaca fascicularis*) varies widely according to the particular habitat examined. However, whether the findings actually reflect the prevalence of TRIMCyp in wild cynomolgus macaques is still uncertain because the previous data were obtained with captive monkeys in breeding and rearing facilities. Here, we characterized the *TRIM5* gene in cynomolgus macaques captured in the wild, and found that the frequency of the TRIMCyp allele was comparable to those in captive monkeys. This suggests that the previous results with captive monkeys do indeed reflect the natural allele frequency and that breeding and rearing facilities may not affect the frequency of *TRIM5* alleles. Interestingly, the prevalence of a minor haplotype of TRIMCyp in wild macaques from the Philippines was significantly lower than in captive ones, suggesting that it is advantageous for wild monkeys to possess the major haplotype of TRIMCyp. Overall, our results add to our understanding of the geographic and genetic prevalence of cynomolgus macaque TRIMCyp.

Keywords: cynomolgus monkey, TRIM5α, TRIMCyp, genetic diversity, host factor

INTRODUCTION

In 2004, TRIM5-Cyclophilin A (CypA) chimeric protein, referred to as TRIMCyp, was first identified in owl monkeys (*Aotus trivirgatus*), which belongs to New World monkeys (NWMs) (Sayah et al., 2004). The discovery of TRIMCyp in owl monkeys explains the novel post-entry restriction of human immunodeficiency virus type 1 (HIV-1), which is uniquely seen in owl monkey-derived cells but not in other NWM-derived cells. Owl monkey TRIMCyp is derived from LINE-1-mediated retrotransposition of CypA cDNA into the region between *TRIM5* exons 7 and 8. On the other hand, the strong post-entry restriction of HIV-1 in Old World monkey (OWM)-derived cells was thought to be dependent on a TRIM5α-mediated mechanism (Stremlau et al., 2004; Nakayama and Shioda, 2010). Interestingly, among OWMs, pig-tailed macaques (*Macaca nemestrina*; hereafter denoted as PMs) uniquely show higher susceptibility to HIV-1 infection when compared with other OWMs (Agy et al., 1992). However, the mechanism underlying this higher susceptibility was unclear. Thereafter, it was found that PMs exclusively have the TRIMCyp genotype, which is a strong genetic determinant of their susceptibility to HIV-1 infection (Liao et al., 2007; Brennan et al., 2008; Virgen et al., 2008). Subsequently, TRIMCyp was also discovered in rhesus macaques (*Macaca mulatta*; hereafter denoted as RMs) and cynomolgus macaques (*Macaca fascicularis*; hereafter denoted as CMs) (Brennan et al., 2008; Newman et al., 2008; Wilson et al., 2008).

TRIMCyp is an alternatively spliced isoform of the *TRIM5* gene in which the PRYSPRY domain of TRIM5α is replaced with a retrotransposed *CypA* gene. Unlike owl monkey TRIMCyp, the *CypA* gene in OWM TRIMCyp, is inserted in the 3′-untranslated region (UTR) of the *TRIM5* gene. The retrotransposition of the CypA sequence is concomitant with a single nucleotide polymorphism (SNP) at the exon 7 splice acceptor site; this leads to skipping of exons 7 and 8 encoding the PRYSPRY domain and splicing to the inserted *CypA* gene (Johnson and Sawyer, 2009). Thus, the presence or absence of the CypA sequence in the 3′ UTR leads to expression of TRIMCyp or TRIM5α (Nakayama and Shioda, 2012).

Current data suggest that PMs exclusively express TRIMCyp and not TRIM5α. In the case of RMs, the frequency of TRIMCyp in Indian RM was approximately 25%, while it was not found in the Chinese RM population (Wilson et al., 2008). In addition, we observed that the frequency of TRIMCyp in Burmese RM was approximately 10% (unpublished data), suggesting a geographical deviation in the frequency of RM TRIMCyp. In the case of CM, we and other groups reported that TRIMCyp is present at higher frequency when compared with RM (De Groot et al., 2011; Dietrich et al., 2011; Saito et al., 2012). Interestingly, we and other groups found a geographical deviation in the frequency of TRIMCyp in CM (Dietrich et al., 2011; Berry et al., 2012; Saito et al., 2012). In particular, we showed that the frequency of TRIMCyp in the Philippine population was higher than that in Indonesian and Malaysian populations. Dietrich et al. also reported that the frequency of TRIMCyp in the Philippine population was higher than that in Indonesia, Indochina, and Mauritian populations (Dietrich et al., 2011). Moreover, they

claimed that the frequency of TRIMCyp in Indonesian CMs was higher than that of Indochina and Mauritian populations. However, all these analyses were performed with captive monkeys in breeding and rearing facilities. Therefore, these results may not reflect the natural gene frequencies. For instance, a small number of animals of a certain genotype introduced into facilities may affect the frequency of TRIMCyp via the founder effect. Furthermore, breeding policies may lead to a deviation of specific genotype. Hence, in order to understand the prevalence of TRIMCyp in CM precisely, it is necessary to analyze the frequency of TRIMCyp in wild CM. Therefore, in the present study, we sought to determine the geographic and genetic diversity of the *TRIM5* gene in wild-caught CM.

MATERIALS AND METHODS

SAMPLE COLLECTION

Blood samples from the wild-caught CMs, which had been cryopreserved for veterinary and microbiological examination as quarantine, were used in this study. These animals had been imported in the 1970's from the Philippines, Malaysia, and Indonesia to Japan as the founders of a breeding colony. These animals were directly sent to Japan without breeding in these countries.

DETERMINATION OF *TRIM5* GENOTYPE

The genotyping of *TRIM5* gene was performed as described previously with slight modifications (Saito et al., 2012). Briefly, the genomic DNA was extracted from frozen blood samples of 88 CMs with a QIAamp DNA Blood Mini kit (*Qiagen, Tokyo, Japan*). The genomic DNA was amplified by PCR using Ex Taq HS (TaKaRa, *Otsu, Japan*) with TC forward (5′-TGA CTC TGT GCT CAC CAA GCT CTT G-3′) and TC reverse (5′-ACC CTA CTA TGC AAT AAA ACA TTA G-3′) primers as described by Wilson et al. (2008). After amplification, PCR products were visualized on a 1% agarose gel stained with ethidium bromide.

SEQUENCING OF THE CypA DOMAIN OF TRIMCyp

Amplified products of the CypA domain from 44 TRIMCyp homozygotes and 21 TRIMCyp/TRIM5α heterozygotes were purified using Wizard SV Gel and PCR Clean-Up System (*Promega, Tokyo, Japan*) and then subjected to direct sequencing using primer pairs of MfasCypA_F (5′-CAA CCC TAC CGT GTT CTT CG-3′) and MfasCypA_R (5′-TCG AGT TGT CCA CAG TCA GC-3′). Sequencing products were analyzed on a 3130xl Genetic Analyzer (*Applied Biosystems, Tokyo, Japan*).

RESULTS

HIGHER FREQUENCY OF TRIMCyp IN A WILD PHILIPPINE POPULATION AS COMPARED TO INDONESIAN AND MALAYSIAN POPULATIONS

We first analyzed the frequency of TRIMCyp in these wild-caught animals. The PCR-based assay performed here was designed to differentiate between the presence and absence of the CypA insertion (**Figure 1A**). The electrophoretic pattern of PCR products is shown in **Figure 1B**. The upper bands indicate TRIMCyp, while the lower bands indicate TRIM5α. A heterozygote is expected to possess both bands. As summarized in **Table 1**, we found that the 35 of the 49 Philippine CMs were homozygous for TRIMCyp, 11 were heterozygous, and 3 were homozygous for TRIM5α. In the case of Malaysian CM, 11 of the 29 animals were homozygous for TRIMCyp, 8 were heterozygous, and 10 were homozygous for TRIM5α. Finally, in the case of Indonesian CMs, none of the 10 animals were homozygous for TRIMCyp, 3 were heterozygous, and 7 were homozygous for TRIM5α. The calculated frequency of TRIMCyp in these populations was 82.7%, 48.3%, and 15.0%, respectively. Statistical analysis revealed that the frequency of TRIMCyp in the Philippine population was significantly higher than that in the Indonesian and Malaysian populations.

DIFFERENCE IN THE HAPLOTYPE FREQUENCY OF TRIMCyp BETWEEN WILD AND CAPTIVE PHILIPPINE POPULATIONS

Others and we have recently demonstrated the presence of several haplotypes in TRIMCyp of captive CM (Dietrich et al., 2011; Saito et al., 2012). Specifically, the major haplotype in which amino acid residues at positions 369 (Cyp66) and 446 (Cyp143) are aspartic acid (D) and lysine (K) is abundant in captive-CM TRIMCyp alleles [denoted as TRIMCyp-major (DK)]. In addition, the minor haplotype encoding asparagine (N) and glutamic acid (E) at positions 369 (Cyp66) and 446 (Cyp143) is also present [denoted as TRIMCyp-minor (NE)].

In this study, we further investigated the haplotypes of TRIMCyp in the wild-caught CM and compared the frequency of each haplotype in these animals with those reared in captivity. We found that although both haplotypes were present in wild-caught CM, the frequency of TRIMCyp-minor (NE) in wild Filipino CM was much less than that in captive Filipino CM (1.2% versus 14.3% of TRIMCyp; $p < 0.01$) (**Table 2**). By contrast, the frequency of TRIMCyp-minor (NE) in wild Malaysian CM was comparable to that in captive Malaysian CM (10.7% versus 11.1% of TRIMCyp; $p > 0.05$). In the case of wild Indonesian CM, all animals analyzed here possess TRIMCyp-major (DK), although the size of samples was too small to determine whether this was significant. Thus, the precise frequency of each haplotype in wild Indonesian CM is unclear.

DISCUSSION

In the present study, we analyzed the incidence of TRIMCyp in wild-caught animals and found that its frequency was comparable to that in captive animals (**Table 1**). Although blood samples from other regions were unavailable, it is reasonable to assume that the equivalence in the frequency of TRIMCyp between captive and wild-caught CM in other regions may have a similar tendency. Interestingly, we also found that the frequency of the TRIMCyp-minor (NE) haplotype in wild CM was lower than that in captive CM in the case of the Filipino population, but not in the case of the Malaysian population (**Table 2**). Although the reason for this discrepancy remains to be elucidated, we speculate that it might be hazardous for wild Filipino CM to possess TRIMCyp-minor (NE), as it may render them susceptible to TRIMCyp-minor (NE)-resistant pathogens present in the Philippines, but not in Malaysia. Based on this hypothesis, wild Filipino CM might be forced to expand TRIMCyp-major (DK) in order to counteract invasions from such pathogens. Conversely, weaker attacks, if any, from these pathogens in the breeding and rearing facilities might allow captive Filipino CM to expand TRIMCyp-minor (NE)

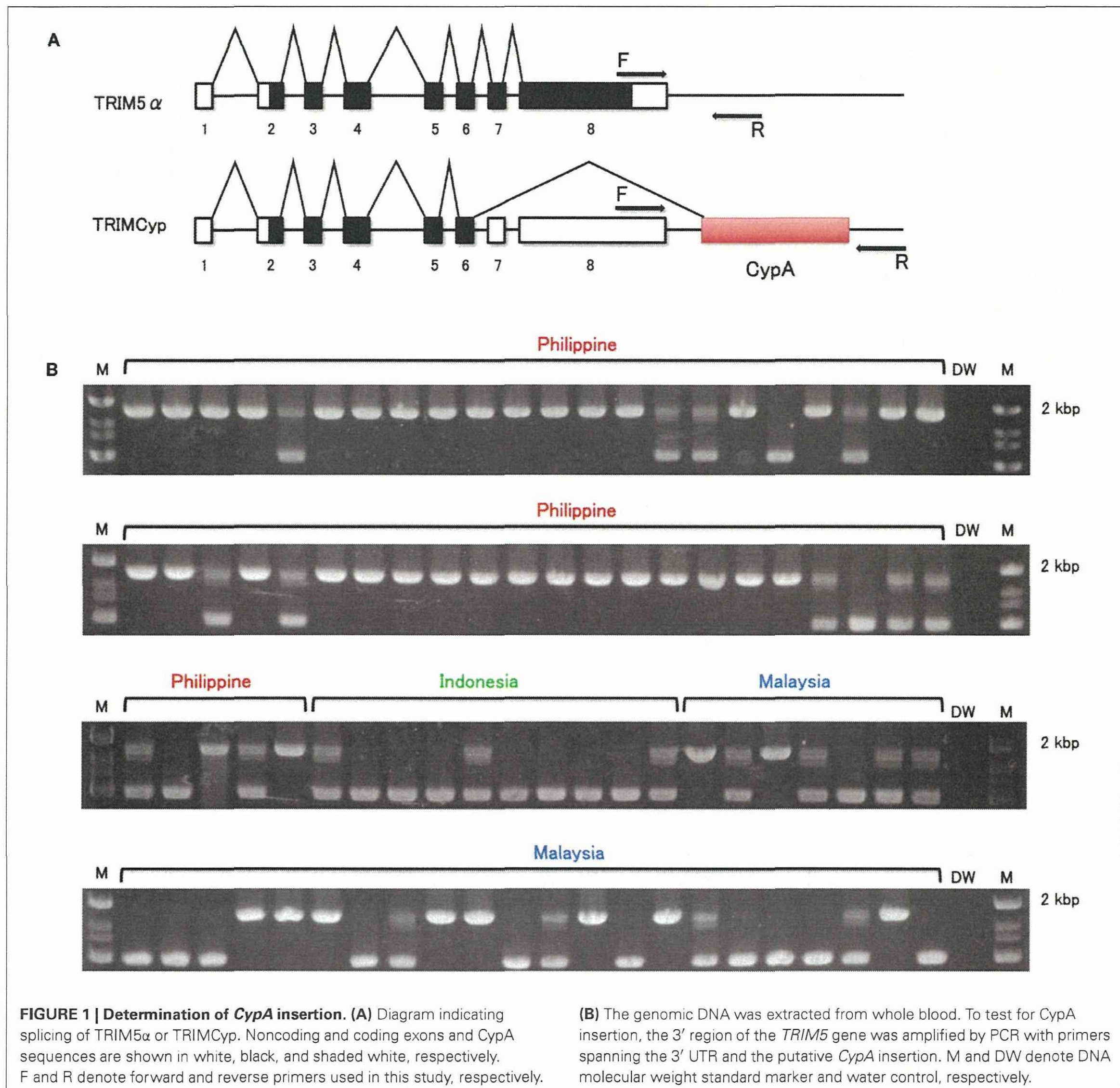

FIGURE 1 | Determination of *CypA* insertion. (A) Diagram indicating splicing of TRIM5α or TRIMCyp. Noncoding and coding exons and CypA sequences are shown in white, black, and shaded white, respectively. F and R denote forward and reverse primers used in this study, respectively. **(B)** The genomic DNA was extracted from whole blood. To test for CypA insertion, the 3′ region of the *TRIM5* gene was amplified by PCR with primers spanning the 3′ UTR and the putative *CypA* insertion. M and DW denote DNA molecular weight standard marker and water control, respectively.

haplotype in their population. Although it might also be hypothesized that the difference in the frequency of these haplotypes between wild-caught and captive animals was a consequence of the founder effect, the fact that more than 100 animals were introduced from wild (wild-caught animals) to breeding and rearing facility (captive animals) by dividing into several times suggests that the difference in the frequency of TRIMCyp haplotype may not be due to founder effect.

Since it is assumed that Filipino CM originated from Indonesian CM stocks (Thierry and Abegg, 2002), the fact that Malaysian and Indonesian CMs also possess TRIMCyp-major (DK) implies that this haplotype arose earlier than the migration of Indonesian CM stocks to the Philippine islands. Probably, TRIMCyp-major (DK) appeared in the ancestor of these CMs for some reason. Since only CM but neither PM nor RM possess TRIMCyp (DK) as one of the TRIMCyp haplotypes, it is reasonable to speculate that some pathogen(s) exerted a strong selection pressure on CM during their evolution. After the appearance of TRIMCyp-major (DK), Malaysian CM continued to maintain TRIMCyp-minor (NE) at a frequency of approximately 10% of total TRIMCyp alleles, while Filipino CM might exclude this haplotype. Alternatively, since Filipino CMs are thought to have originated from a small group of Indonesian CMs (Blancher et al., 2008), the limited prevalence of TRIMCyp-minor (NE) in wild

Table 1 | Frequency of TRIMCyp alleles in wild Philippine, Malaysian, and Indonesian populations.

Country	Origin of sample	#animals	Genotype (# animals)			Allele frequency		Citation
			TRIM5α homozygote	heterozygote	TRIMCyp homozygote	% TRIM5α	% TRIMCyp	
Philippines	Wild-caught	49	3	11	35	17.3	82.7	This study
Philippines	Captive	46	1	10	35	13.0	87.0	Saito et al., 2012
Philippines	Captive	4	0	0	4	0	100	Dietrich et al., 2011
Malaysia	Wild-caught	29	11	8	10	51.7	48.3	This study
Malaysia	Captive	47	11	26	10	51.1	48.9	Saito et al., 2012
Indonesia	Wild-caught	10	7	3	0	85.0	15.0	This study
Indonesia	Captive	33	13	17	3	65.2	34.8	Saito et al., 2012
Indonesia	Captive	18	3	10	5	44.4	55.6	Dietrich et al., 2011

Table 2 | Frequencies of DK and NE haplotypes in TRIMCyps of wild CM.

Country	Origin of sample	#animals	Genotype (# chromosomes)				Frequency		Citation
			TRIM5α/TRIMCyp heterozygote[a]		TRIMCyp homozygote[b]		% DK	% NE	
			DK	NE	DK	NE			
Philippines	Wild-caught	46	10	1	70	0	98.8	1.2	This study
Philippines	Captive	28	6	1	36	6	85.7	14.3	Saito et al., 2012
Malaysia	Wild-caught	18	7	1	18	2	89.3	10.7	This study
Malaysia	Captive	21	14	1	10	2	88.9	11.1	Saito et al., 2012
Indonesia	Wild-caught	3	3	0	0	0	100	0	This study
Indonesia	Captive	15	12	0	4	2	88.9	11.1	Saito et al., 2012

[a] *Haplotypes were determined by direct sequencing of the PCR products.*

[b] *Haplotypes were inferred by the Maximum-Likelihood estimation using the results of direct sequencing of the PCR products.*

Filipino CMs might be due to a founder effect. Unfortunately, we were unable to place a statistically meaningful value on the prevalence of the TRIMCyp-minor (NE) allele in wild Indonesian CM, since the sample size was too small. In the case of Malaysian CM TRIMCyp, the high frequency of the TRIMCyp-major (DK) allele suggests that it is preferable to posses this haplotype in their habitat. From this point of view, it will be of interest to consider why TRIMCyps of PMs and RMs are NE rather than DK type. In particular, the habitats of PM partially overlap with those of CM, except for the Java and Philippine islands (Thierry and Abegg, 2002). As Dietrich et al. proposed (Dietrich et al., 2010), it is likely that TRIMCyp evolved in the common ancestor of Asian macaques since TRIMCyp is present in both the silenus group, which includes PM, and the fascicularis group, which includes RM and CM. Furthermore, Ylinen et al. speculated that although the CypA sequence that has been retrotransposed into the macaque *TRIM5* locus is expected to be identical to the inherent CypA sequence, an arginine-to-histidine substitution at amino acid 69 may have occurred early in a common ancestor of Asian macaques. This may have been advantageous in that it helped to expand the spectrum of antiviral activity (Ylinen et al., 2010). This group further speculated that TRIMCyp (NE) arose in PMs and RMs independently; however, it is possible that TRIMCyp (NE) arose in the common ancestor of Asian macaques, since TRIMCyp (NE) is also present in CMs (**Table 2**). It is reasonable to imagine that the ancestors of PMs and RMs might fix TRIMCyp (NE) in order to protect themselves from invasion by TRIMCyp (NE)-sensitive pathogens. Specifically, the fact that PMs exclusively possess TRIMCyp (NE) instead of TRIM5α or TRIMCyp (DK) implies the importance of maintaining this *TRIM5* genotype in their habitat. Otherwise, the founder or bottleneck effect might affect the prevalence of TRIMCyp haplotypes in these macaque species. As an alternative hypothesis, TRIMCyp-minor (NE) in CM might be a vestige of an introgression between CMs and RMs with TRIMCyp (NE). In any case, future studies should analyze the prevalence of TRIMCyp in wild CMs by using samples from many regions to verify the correlation of genetic prevalence between wild and captive CMs.

More importantly, these two haplotypes in CM TRIMCyp are reported to show different antiviral activity (Ylinen et al., 2010; Dietrich et al., 2011; Saito et al., 2012). We and other groups reported that TRIMCyp-major (DK) suppresses the replication of HIV-1, but not that of HIV-2. Conversely, it was shown that TRIMCyp-minor (NE) suppresses the replication of HIV-2, but not that of HIV-1. Thus, these haplotypes of TRIMCyp present in CM are expected to show different antiviral activity in nature.

It will be of great interest to investigate the pathogens that acted as a selective pressure to alter the prevalence of TRIMCyp haplotypes.

Taken together, we analyzed the geographic and genetic characteristics of TRIMCyp in wild-caught CM for the first time and found (1) a higher frequency of TRIMCyp in the Philippine population as compared to those in other populations; (2) a similar tendency in the frequency of TRIMCyp between wild-caught and captive CM, and (3) a significant difference in the frequency of TRIMCyp-minor (NE) haplotype between captive and wild Filipino CM. These results provide important insights into the prevalence of CM TRIMCyp and increase our understanding of the evolution of antiretroviral host factors in Asian macaques.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Health, Labor, and Welfare in Japan, and Environment Research and Technology Development Fund (D-1007) of the Ministry of the Environment, Japan.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 June 2012; paper pending published: 08 July 2012; accepted: 13 August 2012; published online: 30 August 2012.

Citation: Saito A, Kawamoto Y, Higashino A, Yoshida T, Ikoma T, Suzaki Y, Ami Y, Shioda T, Nakayama EE and Akari H (2012) Allele frequency of antiretroviral host factor TRIMCyp in wild-caught cynomolgus macaques (Macaca fascicularis). Front. Microbio. 3:314. doi: 10.3389/fmicb.2012.00314

This article was submitted to Frontiers in Virology, a specialty of Frontiers in Microbiology.

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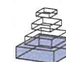
frontiers in
MICROBIOLOGY

REVIEW ARTICLE
published: 10 August 2012
doi: 10.3389/fmicb.2012.00297



SAMHD1-dependent and -independent functions of HIV-2/SIV Vpx protein

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Both human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) encode a unique set of accessory proteins that enhance viral replication in the host. Two similar accessory proteins, Vpx and Vpr, are encoded by HIV-2. In contrast, HIV-1 encodes Vpr but not Vpx. Recent studies have indicated that Vpx counteracts a particular host restriction factor, thereby facilitating reverse transcription in myeloid cells such as monocyte-derived macrophages and monocyte-derived dendritic cells. This mechanism of counteraction is similar to that of the accessory proteins Vif and Vpu which antagonize other host factors. In 2011, the protein SAMHD1 was identified as the restriction factor counteracted by Vpx. Studies have since revealed that SAMHD1 degrades deoxynucleoside triphosphates (dNTPs), which are components of viral genomic cDNA, in order to deprive viruses of dNTPs. Although interactions between SAMHD1 and Vpx continue to be a major research focus, Vpx has also been shown to have an apparent ability to enhance nuclear import of the viral genome in T lymphocytes. This review summarizes the current knowledge regarding SAMHD1-dependent and -independent functions of Vpx, and discusses possible reasons why HIV-2 encodes both Vpx and Vpr, unlike HIV-1.

Keywords: Vpx, HIV-2, SIV, SAMHD1, reverse transcription, dNTP, nuclear import, Vpr

INTRODUCTION

Human and simian immunodeficiency viruses (HIV/SIVs) carry a unique set of accessory proteins, Vif, Vpx, Vpr, Vpu, and Nef, which enhance viral replication in the host. Of these accessory proteins, Vpx is unique to HIV-2-type viruses, defined in this paper as the HIV/SIVs carrying both Vpr and Vpx, such as HIV-2, SIVsmm (Sooty mangabey), and SIVmac (Rhesus monkey) (Fujita et al., 2010). Vpr and Vpx are small proteins of approximately 100 amino acids and similar sequence (approximately 20–25% similarity). Both Vpr and Vpx are predicted to have a similar structure consisting of three major helices (Khamsri et al., 2006). In contrast, while HIV-1 carries Vpr, it does not carry Vpx. The answer to the question why HIV-2 viruses encode these two similar proteins while HIV-1 carries only one remains elusive, and must await the determination of their functional details.

Extensive research over the past decade has revealed that lentiviruses carry genes for accessory proteins that overcome host antiviral factors. The first such accessory protein identified was Vif, which inactivates APOBEC3 proteins, cellular cytidine deaminases that restrict the replication of retroviruses by hypermutating viral cDNA and/or inhibiting reverse transcription (Sheehy et al., 2002; Goila-Gaur and Strebel, 2008; Kitamura et al., 2011). Vif reduces the amount of APOBEC3 through proteasome-mediated degradation and other degradation-independent mechanisms. The second major finding in this area was that the viral protein Vpu counteracts host BST-2/tetherin, which normally blocks the release of virions by directly tethering viral particles to the membranes of infected cells (Neil et al., 2008; Van Damme et al., 2008; Arias et al., 2011). The mechanism through which Vpu antagonizes the function of BST-2/tetherin may be proteasome/lysosome degradation or relocalization from the cell surface.

Recently, it was reported that the viral accessory protein Vpx inhibits the host restriction factor SAMHD1 in monocyte-derived macrophages (MDMs) and monocyte-derived dendritic cells (MDDCs) (Hrecka et al., 2011; Laguette et al., 2011), stimulating interest in SAMHD1 and Vpx. In addition to inhibiting SAMHD1 in MDMs and MDDCs, Vpx is also capable of enhancing viral replication in T lymphocytes (Guyader et al., 1989; Kappes et al., 1991; Yu et al., 1991; Akari et al., 1992; Gibbs et al., 1994; Kawamura et al., 1994; Tokunaga et al., 1997; Ueno et al., 2003; Doi et al., 2011). In this review, we summarize current research into SAMHD1-dependent and -independent functions of Vpx and discuss the virological significance of this protein.

SAMHD1-DEPENDENT FUNCTIONS OF Vpx

Several studies have shown that while wild-type HIV-2-type viruses grow well in MDMs, growth of these Vpx-deletion mutants is completely suppressed, demonstrating that Vpx is essential for viral replication in MDMs (Ueno et al., 2003; Fujita et al., 2008a). It is known that Vpx is packaged in virions and functions in the target cell. Independent work in our laboratory and that of another group revealed that Vpx is critical for reverse transcription of the viral RNA genome in MDMs (Fujita et al., 2008a; Srivastava et al., 2008), correcting the long-held misconception that Vpx contributes to nuclear import of the viral genome but does not play a role in reverse transcription. Furthermore, Vpx was shown to