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The Ability of Primate Lentiviruses to Degrade the Monocyte Restriction Factor SAMHD1 Preceded the Birth of the Viral Accessory Protein Vpx

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SUMMARY

The human SAMHD1 protein potently restricts lentiviral infection in dendritic cells and monocyte/macrophages but is antagonized by the primate lentiviral protein Vpx, which targets SAMHD1 for degradation. However, only two of eight primate lentivirus lineages encode Vpx, whereas its paralog, Vpr, is conserved across all extant primate lentiviruses. We find that not only multiple Vpx but also some Vpr proteins are able to degrade SAMHD1, and such antagonism led to dramatic positive selection of SAMHD1 in the primate subfamily *Cercopithecinae*. Residues that have evolved under positive selection precisely determine sensitivity to Vpx/Vpr degradation and alter binding specificity. By overlaying these functional analyses on a phylogenetic framework of Vpr and Vpx evolution, we can decipher the chronology of acquisition of SAMHD1-degrading abilities in lentiviruses. We conclude that *vpr* neofunctionalized to degrade SAMHD1 even prior to the birth of a separate *vpx* gene, thereby initiating an evolutionary arms race with SAMHD1.

INTRODUCTION

HIV-1 and other primate lentiviruses encode accessory genes that serve to enhance virus replication and counteract host immune factors (Malim and Emerman, 2008). Studies of these accessory proteins have led to the identification of important restriction factors encoded by host genomes (Kirchhoff, 2010). The accessory protein Vpx was previously shown to be critical for the ability of primate lentiviruses to efficiently infect monocytes, dendritic cells, and mature macrophages (Ayinde et al., 2010; Sharova et al., 2008). Recently, the target of Vpx has been identified as the restriction factor SAMHD1 (Hrecka et al., 2011; Laguette et al., 2011), where the binding of Vpx to SAMHD1 leads to the proteasomal degradation of SAMHD1.

Humans with missense mutations in SAMHD1 are associated with Aicardi-Goutieres syndrome (AGS), an encephalitis syndrome which mimics a state of viral infection leading to interferon production and an autoimmune syndrome (Rice et al., 2009; Stetson et al., 2008). SAMHD1 has recently been shown to be a deoxynucleotide triphosphohydrolase enzyme which could suppress cellular dNTP pools to inhibit retrovirus reverse transcription (Goldstone et al., 2011; Powell et al., 2011). Thus, by targeting this host protein for degradation, Vpx allows primate lentiviruses to infect key immunomodulatory cells types.

Despite this important function, only two of the eight major lineages of primate lentiviruses (reviewed in Peeters and Courgnaud, 2002) encode Vpx: HIV-2/SIVsm-related viruses and a lineage represented by SIV from red-capped mangabeys (SIVrcm). On the other hand, all extant primate lentivirus lineages encode a paralogous gene called Vpr that causes cell-cycle arrest (Sharp et al., 1996; Tristem et al., 1990, 1998). Both Vpr and Vpx are incorporated into the core of budding viruses (Yu et al., 1988; Yuan et al., 1990), and both bind to the Cul4 complex through interactions with DDB1 and DCAF1 (reviewed in Ayinde et al., 2010). Despite its limited representation in primate lentiviruses, Vpx appears to be more critical than Vpr for replication of SIV in monkeys (Gibbs et al., 1995; Hirsch et al., 1998). The important role played by Vpx has led to a conundrum as to why this protein is missing in lentiviruses like HIV-1. The recent identification of SAMHD1 as the target of Vpx allows us to characterize Vpx function from diverse lentiviruses with SAMHD1 from different hosts. Such an analysis can distinguish between the possibility that SAMHD1 degradation had an ancient origin and was subsequently lost in some lineages due to lack of selective pressure from SAMHD1, or that it was a recent adaptation of some viruses.

The evolutionary analysis of both host and viral proteins combined with functional analysis can reveal the evolutionary dynamics of this arms race, both in terms of its birth and its more recent adaptations. Host defense genes like *SAMHD1* that are involved in antagonistic virus-host interactions often display strong signatures of diversifying selection as a result of repeated episodes of selection by viral antagonists (Emerman and Malik, 2010; Meyerson and Sawyer, 2011). This methodology can be used to pinpoint the exact amino acid residues involved in the viral-host interaction (Lim et al., 2010; McNatt

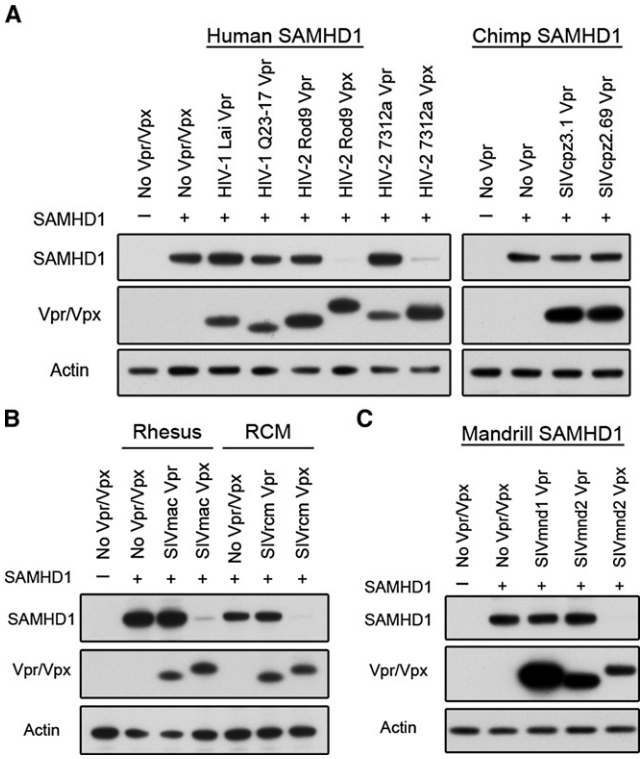


Figure 1. Vpx from Diverse Primate Lentiviruses Degrades SAMHD1

(A) The ability of Vpx and Vpr to degrade SAMHD1 was assayed by western blot analysis of HA-epitope-tagged SAMHD1 from respective primate species cotransfected with 3xFLAG-epitope-tagged Vpr or Vpx constructs as indicated. Actin was probed as a loading control. Indicated Vpr and Vpx constructs were expressed in the presence of human SAMHD1 (left) or chimpanzee SAMHD1 (right).

(B) Similar western blots as in (A) are shown, analyzing rhesus macaque SAMHD1 (left) and red-capped mangabey (RCM) SAMHD1 (right) expression in the presence of indicated Vpr and Vpx constructs.

(C) Similar western blots as in (A) are shown, analyzing mandrill SAMHD1 expression in the presence of indicated Vpr and Vpx from SIVmnd1 or SIVmnd2.

et al., 2009; Sawyer et al., 2005), and when applied to a phylogenetic tree, can provide a temporal context for when these interactions have taken place (Emerman and Malik, 2010).

Our functional analyses reveal that multiple Vpx proteins share the ability to degrade SAMHD1 but that this ability is often host specific. Furthermore, we find that some Vpr proteins from Vpx-lacking lentiviruses also can potently degrade SAMHD1. Moreover, our evolutionary analyses reveal a burst of diversifying selection that shaped SAMHD1 in the *Cercopithecinae* subfamily of old world monkeys (OWMs) which was driven by its antagonism with Vpr/Vpx proteins. By tracing the evolution of Vpr and Vpx function on a phylogenetic framework, we show that the ability to degrade SAMHD1 is the result of neofunctionalization of Vpr that preceded the acquisition of Vpx in primate lentiviruses. We conclude that *vpr* gained a new function to degrade SAMHD1 once during viral evolution, thereby initiating an evolutionary arms race with SAMHD1. However, many lentiviral lineages, including those leading to HIV-1, never acquired this function.

RESULTS

Species-Specific Antagonism of SAMHD1 by Diverse Vpx Proteins

A recent study found that SIVrcm Vpx could not degrade human SAMHD1 (Laguette et al., 2011), suggesting that this function might be very limited among primate lentiviruses. We first wished to test if the ability of Vpx to degrade SAMHD1 is conserved, and if there is species specificity to the interaction. Thus, we cloned SAMHD1 from a panel of primates and assayed for Vpx-mediated SAMHD1 degradation by western blot analysis after transient cotransfection of epitope-tagged SAMHD1 proteins with *vpr* or *vpx* from different lentiviruses.

Consistent with previous reports, we found that HIV-2 (Rod9) Vpx degraded human SAMHD1 and that SIVmac Vpx degraded rhesus SAMHD1 (Hrecka et al., 2011; Laguette et al., 2011) (Figures 1A and 1B). We also found that Vpx from a primary strain of HIV-2, 7312a, degraded SAMHD1 (Figure 1A). Surprisingly, we found that while the HIV-2 (Rod9) had a relatively narrow specificity, only degrading SAMHD1 from human and De Brazza's monkeys among a broader panel of primate SAMHD1 proteins (Table 1), HIV-2 (7312a) Vpx could degrade SAMHD1 from humans and all of the OWMs tested (Table 1). The corresponding Vpr proteins of both viruses were unable to degrade human SAMHD1 (Figure 1A), similar to Vpr proteins from HIV-1 and SIVcpz strains (Figure 1A).

SIVrcm also encodes both Vpx and Vpr. However, SIVrcm Vpx is only 42% identical to HIV-2/SIVsm Vpx at the amino acid level. Nonetheless, we found that SIVrcm Vpx potently degraded SAMHD1 from the host species it naturally infects—the red-capped mangabey (RCM) (Figure 1B). Furthermore, we found that SIVrcm Vpx can degrade SAMHD1 from other OWMs, but not from sooty mangabeys or humans (Table 1). The corresponding Vpr protein of SIVrcm did not have this activity (Figure 1B). Thus, our findings not only suggest that the ability to degrade SAMHD1 is conserved in other clades of Vpx, but also shows species specificity (Table 1).

HIV-1 encodes only *vpr*, while HIV-2 encodes both *vpr* and *vpx*, yet both infect humans. There is an analogous situation in mandrills which are naturally infected by two highly divergent lentiviruses, SIVmnd1, which encodes only *vpr*, and SIVmnd2, which encodes both *vpr* and *vpx* (Souquière et al., 2001; Takemura and Hayami, 2004; Tsujimoto et al., 1988). We found that the Vpx protein from SIVmnd2 was able to degrade mandrill SAMHD1, but neither SIVmnd2 Vpr nor SIVmnd1 Vpr could degrade mandrill SAMHD1 (Figure 1C). Thus, even within a given host, some lentiviruses have a protein with the ability to degrade SAMHD1, while others do not.

Some Vpr Proteins Also Antagonize SAMHD1

Thus far, analyses suggest a clear separation of function between the *vpr* and *vpx* genes examined (reviewed in Ayinde et al., 2010), implying that *vpx* alone evolved the ability to degrade SAMHD1. However, the evolutionary history of these genes is far from clear, in part due to the high diversity of sequences (Sharp et al., 1996; Tristem et al., 1990; Tristem et al., 1998). This raises the possibility that at least some divergent lentiviral *vpr* genes might share the property of degrading SAMHD1. We used 115 *vpr* and *vpx* gene sequences from

Table 1. Species-Specific SAMHD1 Degradation by Vpr and Vpx

SAMHD1	HIV-2 Rod9 Vpx	HIV-2 7312a Vpx	SIVmac Vpx	SIVdeb Vpr	SIVmus Vpr	SIVagm.Gri Vpr	SIVagm.Ver Vpr	SIVrcm Vpx	SIVmnd2 Vpx
Human	+	+	+	+	+	–	–	–	–
Rhesus	–	+	+	+	+	–	+	+	+
Red-capped mangabey	–	+	+	+	+	–	+	+	+
Mandrill	–	+	+	+	+	–	+	+	+
African green monkey	–	+	+	+	+	+	+	+	–
De Brazza's	+	+	+	+	+	–	+	+	+
Sooty mangabey	–	+	+	+	+	–	+	–	+

The table summarizes results of western blot analyses of SAMHD1 degradation phenotype by indicated Vpr and Vpx across a panel of primate SAMHD1. The host species SAMHD1 is listed in the left column, and the Vpx/Vpr proteins tested against SAMHD1 are listed in the top row. "+" indicates combinations that resulted in a greater than 90% decrease in SAMHD1 levels. "–" indicates combinations that had no significant changes in SAMHD1 levels. The following Vpr proteins from HIV-1 Lai, HIV-1 Q23-17, HIV-2 Rod9, HIV-2 7312a, SIVcpz 3.1, SIVcpz 2.69, SIVmac239, SIVrcm, SIVmnd1, SIVmnd2, and SIVolc—which are inactive against their host species (Figure 1 and Figure 2)—were also unable to degrade the panel of primate species' SAMHD1 (data not shown). The AGM SAMHD1 tested is from the Vervet subspecies matching the SIVagmVer 9648 host strain; SAMHD1 from the Tantalus subspecies was found to be heterozygous for a second allele that was resistant to all HIV/SIV Vpr and Vpx tested (data not shown).

diverse HIV and SIV isolates to construct phylogenies using both maximum likelihood (ML) and Bayesian (BI) methods; both methods yielded congruent unrooted topologies (Figure 2A, see Figure S1A available online). The primate lentivirus *vpr* and *vpx* sequences grouped into seven phylogenetic clusters (cutoff at ML bootstrap >75, BI posterior probability >0.8; shaded by colors in Figure 2A). Phylogenies obtained from an application of the Fast Statistical Alignment (FSA) algorithm that is more conservative in terms of homology assignment (Bradley et al., 2009), or trimmed to the minimal 44 shared amino acid positions from the FSA alignment, yielded the similar seven phylogenetic clades (Figure S1B). In all cases, a subset of the *vpr* genes clustered closer to the *vpx* genes than they did to other *vpr* genes (the yellow and green groups in Figure 2A). Thus, we tested the *vpr* genes from each of the diverse primate lineages against their own host SAMHD1 as well as other primate SAMHD1 genes.

The Vpr protein from SIVolc (from the gray color group in Figure 2A), which infects olive colobus monkeys (OLC) and does not carry Vpx, cannot degrade colobus SAMHD1, similar to HIV-1/SIVcpz Vpr and SIVmnd1 Vpr. On the other hand, however, we found that Vpr from SIVdeb that infects De Brazza's monkeys (from the green color group in Figure 2A) not only degraded De Brazza's monkey SAMHD1 (Figure 2B) but also potently degraded SAMHD1 from all primate species including humans (Table 1). Vpr from SIVmus, in the same group as SIVdeb, also had a broad specificity against primate SAMHD1 proteins (Table 1). Extending these analyses further, we found that Vpr proteins from both SIVagm Grivet (677 strain) and SIVagm Vervet (9648 strain) (yellow group in Figure 2A) also degraded SAMHD1 from their African green monkey (AGM) host (Figure 2B). SIVagmGri Vpr had a narrow specificity only capable of degrading AGM SAMHD1, whereas SIVagmVer Vpr had a broader specificity (Table 1). These data reveal that phylogenetically distinct Vpr proteins functionally degrade SAMHD1, at times with striking species specificity.

We overlaid the functional analysis of Vpr and Vpx proteins that do and do not degrade SAMHD1 on the unrooted phlyogenetic tree. Notably, all of the Vpr/Vpx proteins that do degrade SAMHD1 are found on one side of the tree (Figure 2A, blue stars), while all of the Vpr proteins that do not degrade SAMHD1 are found on the other side (Figure 2A, red stars). There is strong bootstrap support for the separation of these two subtrees (bootstrap support [BS] = 90.3, posterior probability [PP] = 1), which argues that there was a single gain/loss event for the function of degrading SAMHD1 in Vpr/Vpx evolution, and this function is not only confined to the previously classified "*vpx*" genes but is also observed in "*vpr*" genes from diverse lentiviral lineages.

Binding of Diverse Vprs to SAMHD1 Correlates with Degradation

Previous studies have shown that Vpx from SIVsm and HIV-2 are able to bind SAMHD1 directly in order to promote its degradation (Hrecka et al., 2011; Laguette et al., 2011). To determine if the diverse Vpr proteins that degrade SAMHD1 also antagonize through protein-protein interactions, we performed coimmunoprecipitations. As the immunoprecipitation was directed against SAMHD1, the proteasome inhibitor MG132 was added to the cells in an attempt to prevent degradation of SAMHD1. Consistent with our degradation results, we found that SIVdeb Vpr coimmunoprecipitates with SAMHD1 from De Brazza's monkeys (Figure 2C). Similarly, we found that SIVagm Vpr binds AGM SAMHD1. The AGM-SAMHD1 Vpr complexes, but not the SIVdeb Vpr, also interacted with the Cul4 ubiquitin ligase complex protein, DDB1 (Figure 2C). In contrast, SIVmnd1 Vpr did not bind to Mandrill SAMHD1 (Figure 2C), indicating that only Vprs that degrade SAMHD1 are able to bind SAMHD1. As the degradation of De Brazza's SAMHD1 was not rescued by MG132, and SIVdeb Vpr was not found to interact with DDB1 by SAMHD1 coimmunoprecipitation, this may suggest an alternate means of degradation by SIVdeb Vpr. However, this is most likely not representative of this clade of *vpr*, as we found that degradation by the related SIVmus Vpr to be rescued by MG132. Furthermore, we found that SIVmus Vpr interacts with DDB1 in a SAMHD1 complex (data not shown). Thus, the ability of Vpr to cause degradation of SAMHD1 correlates with its ability to bind SAMHD1 and is conserved with the known mechanism of Vpx interaction with SAMHD1.

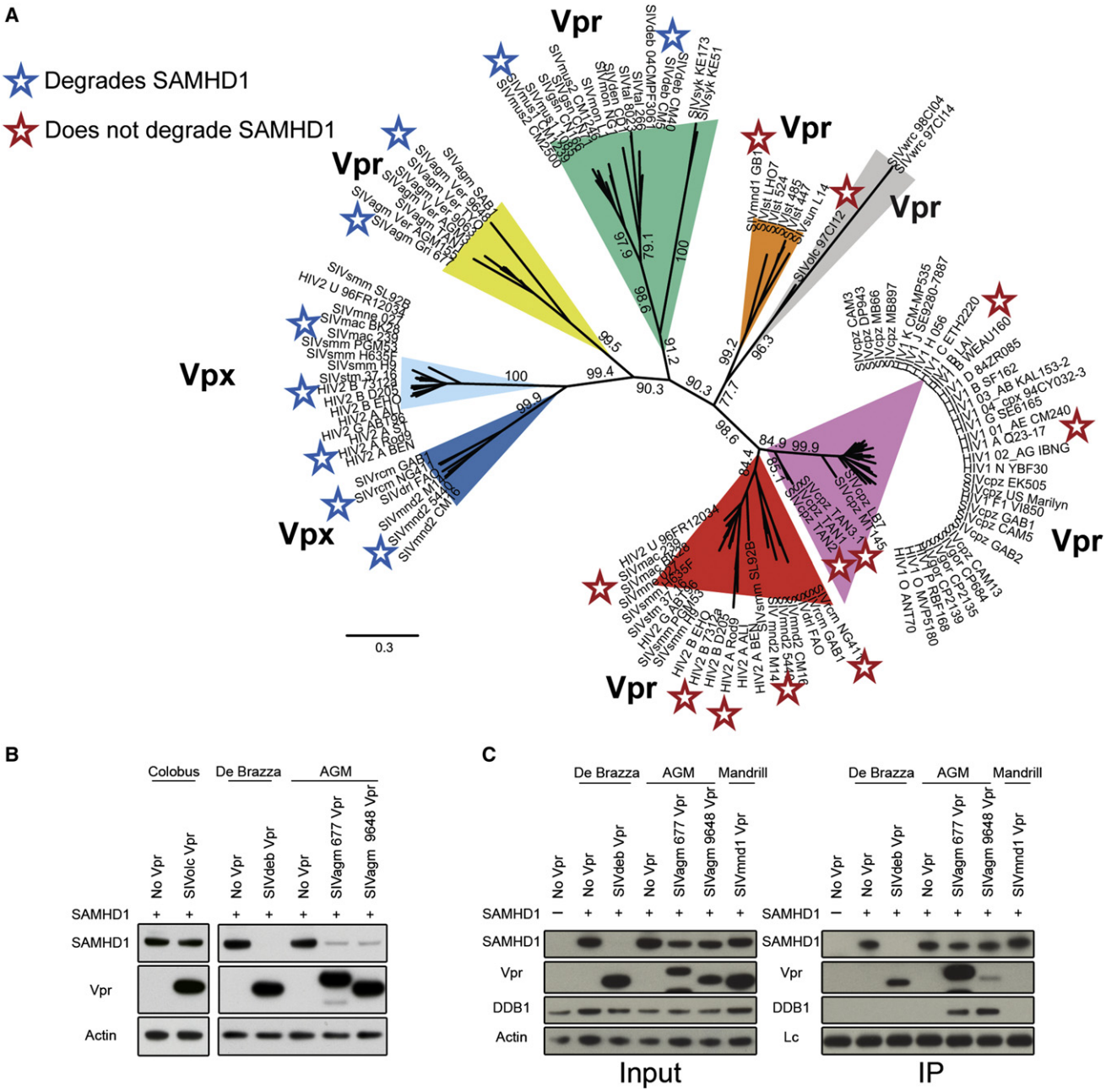


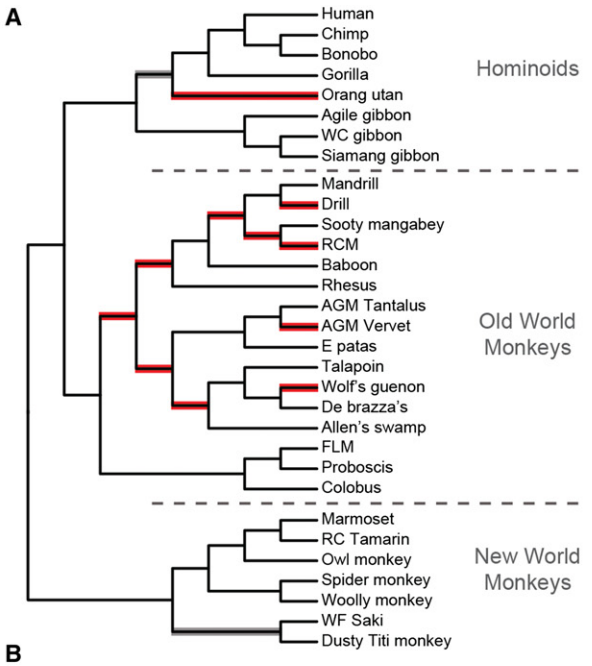
Figure 2. Some Vpr Proteins Degrade SAMHD1

(A) Unrooted phylogeny of 115 *vpr* and *vpx* sequences among diverse primate lentiviruses. Bootstrap values indicate maximum likelihood proportions that are highly supported by Bayesian inference (Figure S1A). Seven phylogenetic clusters are shaded in colors (cutoff at ML bootstrap >75, Bayesian posterior probability >0.87). *Vpx* sequences form two clades (shaded in light blue and dark blue) that have strong support of monophyly from all other *vpr* sequences. Functional phenotypes of Vpr and Vpx (Table 1) that degrade SAMHD1 (Blue stars) or do not degrade SAMHD1 (Red stars) are overlaid on the phylogeny. See also Figure S1B.

(B) Western blot analysis of Colobus monkey, De Brazza's monkey, and African green monkey (AGM) SAMHD1 in the presence of indicated Vpr constructs. The AGM SAMHD1 tested is from the Vervet subspecies matching the SIVagmVer 9648 host strain; the Colobus SAMHD1 tested is from the Colobus guereza subspecies.

(C) Association of SAMHD1 with Vpr and DDB1 by coimmunoprecipitation was detected by western blot analysis of HA-immunoprecipitated SAMHD1 for FLAG-epitope-tagged Vpr and DDB1 association (IP), or input expression (Input). After transfection, cells were treated with 25 μM MG-132 for 12 hr prior to immunoprecipitation. SIVmnd1 Vpr, which fails to degrade mandrill SAMHD1, was assayed as a negative control. Actin and the antibody light chain (Lc) are shown as loading controls.

A



B

Evolutionary analysis for signatures of positive selection in primate SAMHD1

	2lnλ	df	*P*-value
All primates	**52.18**	**2**	**< 0.001**
New world monkeys (NWM)	3.25	2	> 0.19 (NS)
Old world monkeys (OWM)	**22.28**	**2**	**< 0.001**
Hominoids (HOM)	2.04	2	> 0.35 (NS)

C

Analysis of sites under positive selection in Old World monkeys' SAMHD1

	2lnλ	df	*P*-value
Full gene	**22.28**	**2**	**< 0.001**
Full gene (- SAM domain)	**9.21**	**2**	**< 0.01**
Full gene Δ32, 36, 46, 69, 107, 486	5.22	2	> 0.11 (NS)
N Term domain (aa 1 - 43)	3.20	2	> 0.20 (NS)
SAM domain (aa 44 - 110)	**14.75**	**2**	**< 0.001**
Middle domain (aa 111 - 163)	2.47	2	> 0.29 (NS)
HD domain (aa 164 - 319)	1.11	2	> 0.57(NS)
C Term domain (aa 320 - 627)	3.08	2	> 0.21 (NS)

Figure 3. Primate SAMHD1 Has Been Evolving under Positive Selection

(A) Cladogram of 31 primate *SAMHD1* genes sequenced for the evolutionary analyses. The panel of primates was comprised of 8 hominoids, 16 OWMs, and 7 NWMs. No evidence of recombination was detected by a GARD analysis (Kosakovsky Pond et al., 2006). Values of ω (dN/dS) along each branch were calculated by a free ratio analysis using PAML (Figure S2B). Branches with statistically significant ω values >1 are highlighted in red; branches highlighted in gray indicate lineages that show ω values >1 but are not statistically significant based on two-ratio likelihood tests (Figure S2). Note positive selection in the *Cercopithecinae* subfamily represented by the clade of OWMs containing Mandrills through Allen's swamp monkeys in the phylogenetic tree, but not in the *Colobinae* subfamily of OWM represented by FLM, proboscis, and colubus.

(B) Likelihood ratio test statistics were used to determine if *SAMHD1* evolution across various primate lineages was associated with dN/dS ratios significantly greater than 1 (hence under positive selection). Neutral models (M7) were compared to selection models (M8) under the F61 model of codon substitution. Similar results were obtained in a comparison of M1 (neutral) versus M2 (selection) (data not shown). See also Figure S2.

(C) Six positively selected codons were identified (32, 36, 46, 69, 107, 486) with significant posterior probability (Figure S3A) using PAML. The analysis was performed on *SAMHD1* sequences from the panel of 16 OWMs, which showed the strongest burst of positive selection in primates (Figure 3B). Likelihood ratio tests were performed between the M7 (neutral) and M8 (selection) models for the full *SAMHD1* gene, without the SAM domain or with amino acids 32, 36, 46, 67, 107, and 486 omitted from the alignment. Domains were analyzed for signatures of positive selection, with the strongest signals located in the SAM domain. See also Figure S3.

Vpr/Vpx Antagonism Drove Positive Selection of *SAMHD1* in Old World Monkeys

One key to the question of whether antagonism of a host protein by a viral protein is ancient or recent is to determine the selective pressures that have shaped the host protein evolution. Therefore, we investigated whether SAMHD1 is under positive selection by sequencing the coding region of *SAMHD1* from 31 primate species representing approximately 40 million years of evolutionary divergence (Figure 3A). The phylogeny constructed from the primate SAMHD1 sequences was congruent with the generally accepted primate species phylogeny (Perelman et al., 2011), confirming that the sequences are orthologous. We found that there was strong evidence of recurrent positive selection on SAMHD1 during primate evolution (Figure 3B, $p < 0.001$), and this conclusion was corroborated with other methods (Figure S2A). This signature of positive selection clearly stemmed from the OWM clade ($p < 0.001$), as neither new world monkey (NWM) ($p > 0.19$) nor hominoid ($p > 0.35$) clades showed significant evidence of positive selection (Figure 3B). The lack of positive selection in NWM or hominoids was not a result of low statistical power from limited evolutionary depth, as the tree length (number of substitutions per codon) of the NWM clade (0.20) and hominoid clade (0.15) was greater than that of the OWM clade (0.13).

In order to investigate the selective pressures across the different primate lineages, we calculated the omega ratio (dN/dS) along each branch by performing a free ratio analysis using PAML, where omega (dN/dS) ratios >1 are indicative of positive selection (Figure 3A and Figure S2B). Aside from OWMs, only the branch leading to orangutans had statistically significant dN/dS >1 (Figure S2C). Strikingly, SAMHD1 has evolved by positive selection in multiple branches of the OWM subfamily *Cercopithecinae* (Figure 3A, Figure S2D). This suggests that the most dramatic signatures of recurrent positive selection are exhibited by members of the *Cercopithecinae* primate subfamily (the top clade of OWM in Figure 3A) and occurred after this lineage split from the *Colobinae* subfamily (the bottom clade of OWM in Figure 3A).

Positive selection analysis identified six amino acid residues (aa 32 and 36 in the N-terminal domain; aa 46, 69, and 107 in the SAM domain; and aa 486 in the C-terminal domain) as having evolved under recurrent positive selection with strong confidence (posterior probability >0.95) (Figure 3C, Figure S3). Furthermore, if we removed all six residue positions from the primate SAMHD1 alignment, the bulk of the gene-wide signature of positive selection was lost ($p > 0.11$), indicating that these

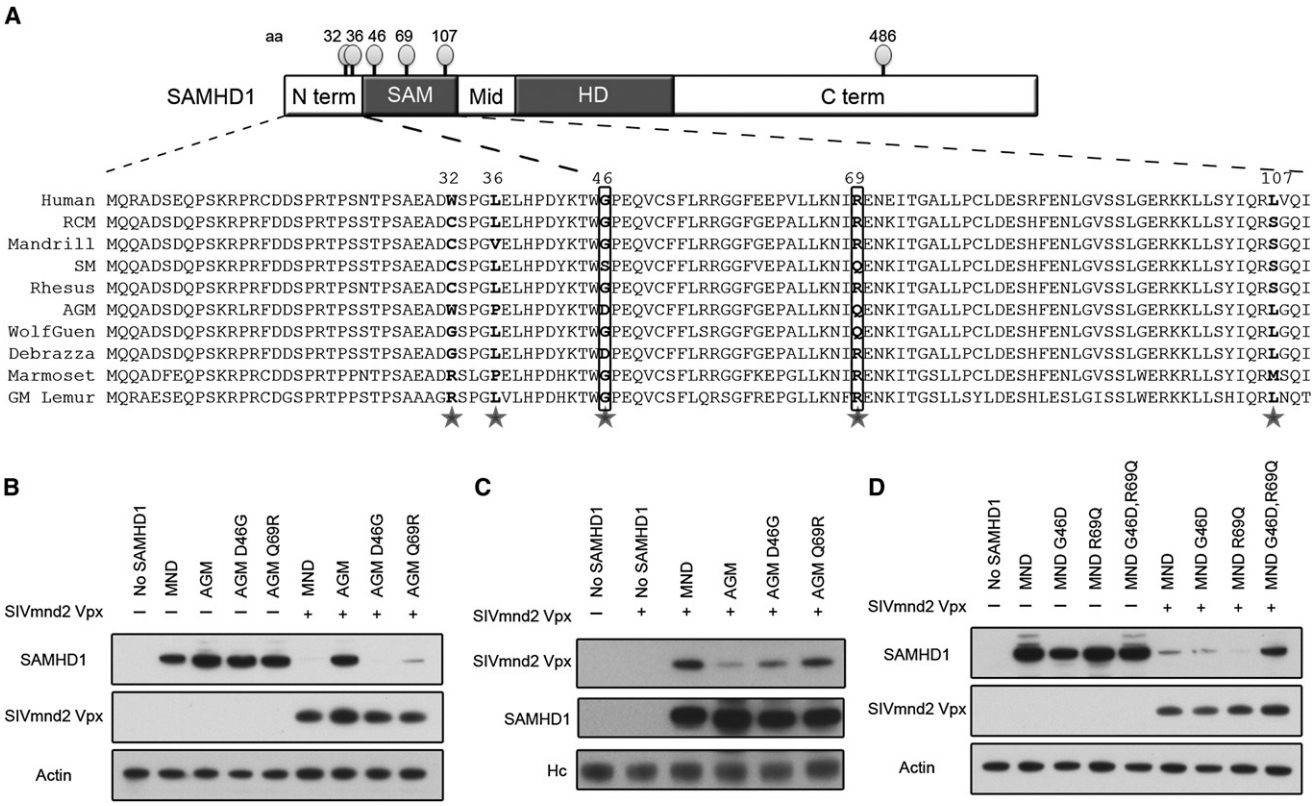


Figure 4. SAMHD1 Positive Selection Residues Map to Vpx Sensitivity

(A) Alignment of N-terminal and SAM domain regions from indicated primates. Symbols (circle on a stick) represent the positively selected residues marked on the SAMHD1 domains. Sites 46 and 69, which displayed highly significant signals of positive selection, are boxed in the alignment. Stars represent the codons under positive selection with strong support (Figures S3A–S3C). The N-terminal region of SAMHD1 from gray mouse lemur is included to represent amino acid residues encoded by a distantly related prosimian primate, showing that the G is the ancestral state at amino acid 46 and the R is the ancestral state at position 69.

(B) Expression of mandrill, AGM, and AGM point mutants (AGM D46G and AGM Q69R) were analyzed by western blot, in the presence or absence of SIVmnd2 Vpx expression.

(C) Western blot analysis of HA-immunoprecipitated SAMHD1 for FLAG-epitope-tagged SIVmnd2 Vpx association. Cells were treated with 25 μM MG-132 for 12 hr prior to immunoprecipitation. Heavy chain (Hc) is shown as a loading control.

(D) Expression of SAMHD1 from mandrill and mandrill-derived mutations (Mnd G46D, Mnd R69Q, and Mnd G46D, R69Q) in the presence or absence of SIVmnd2 Vpx.

amino acids are largely responsible for the signal across the entire gene (Figure 3C).

If amino acid residues under positive selection determine sensitivity to Vpx antagonism, this would strongly argue that a Vpx-like factor was responsible for the signature of positive selection acting on SAMHD1. Alternatively, if the sites under recurrent positive selection did not affect SAMHD1's susceptibility to Vpx antagonism, this would strongly suggest that Vpx and Vpx-like factors are too recent to have significantly affected SAMHD1 evolution. Of the six sites identified under strong positive selection, residues 46 and 69 in the SAM domain showed unmistakably strong signals of recurrent positive selection (Figure 4A and Figure S3). These residues also differ in certain primate species' SAMHD1 that show opposite susceptibility to Vpx. In particular, AGM and mandrill SAMHD1 differ at positions 46 and 69, with mandrill encoding the "ancestral state" at both sites, while AGM encodes the "derived" state (Figure 4A).

To determine if the changes at positions 46 and 69 are responsible for the species specificity of SAMHD1 antagonism by Vpx, we investigated SAMHD1 degradation by SIVmnd2 Vpx, which can degrade mandrill but not AGM SAMHD1 (Table 1). We made D46G and Q69R mutations in the AGM-"resistant" SAMHD1 backbone, reverting these two positions to their ancestral state. We found that the introduction of either mutation resulted in increased susceptibility to degradation by SIVmnd2 Vpx (Figure 4B, see AGM D46G, AGM Q69R). This increased sensitivity of SAMHD1 correlated with increased binding to SIVmnd2 Vpx, since SIVmnd2 Vpx strongly coimmunoprecipitated with mandrill SAMHD1, but its interaction with AGM SAMHD1 was much weaker (Figure 4C). However, either single reversion point mutation (AGM D46G, AGM Q69R) resulted in a stronger interaction with SIVmnd2 Vpx (Figure 4C; compare last three lanes). Thus, changes at the positively selected residues 46 and 69 in SAMHD1 determine both binding and susceptibility to Vpx.

We also tested the reciprocal G46D and R69Q mutations in the "sensitive" Mandrill SAMHD1. We found that while neither mutation alone was sufficient to confer resistance to SIVmnd2 Vpx

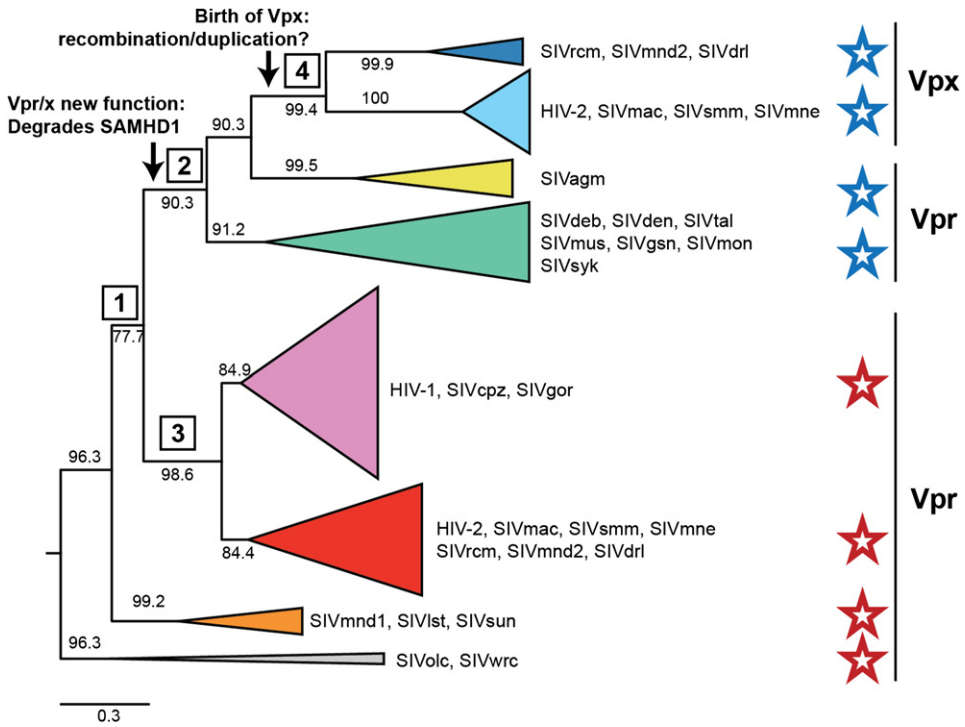


Figure 5. SAMHD1 Degradation by Some Vpr Proteins Preceded the Birth of Vpx

The phylogeny shown in Figure 2A was rooted to a common ancestor of SIVolc/SIVwrc, as determined by the phylogenetic positioning of the flanking *pol* and *env* genes in relation to pSIVgml (Figure S4), and is consistent with previous reports that the *Colobinae* SIVs are outgroup to the *Cercopithecinae* SIVs (Gifford et al., 2008; Gilbert et al., 2009; Liégeois et al., 2009). Important nodes that infer ancestral traits are boxed in numbers.

degradation (Figure 4D, Mnd R69Q and Mnd G46D), a combination of both mutations together resulted in the gain of resistance against degradation by SIVmnd2 Vpx (Figure 4D, Mnd R69Q G46D). Thus, these results demonstrate that changes in amino acids evolving under positive selection in SAMHD1 are necessary and sufficient to determine specificity of Vpx antagonism. This strongly suggests that a Vpx-like factor was responsible for the recurrent positive selection on SAMHD1 during primate evolution.

The Ability to Degrade SAMHD1 Preceded the Birth of Vpx in Primate Lentiviruses

We wished to determine whether the ability to degrade SAMHD1 was an ancestral trait common to all Vpr/Vpx proteins, and that function was subsequently lost by some Vpr lineages across evolution; or alternatively, whether the ancestral Vpr/Vpx lacked the ability to degrade SAMHD1, but the trait was gained (neofunctionalized) over the course of primate lentivirus evolution. However, in order to interpret whether there was a gain or a loss of the ability of Vpr/Vpx to degrade SAMHD1, it was necessary to root the *vpr/vpx* phylogenetic tree from Figure 2A. Previous studies demonstrated that the endogenous lentivirus in the genomes of lemurs, pSIVgml, is 2–6 million years old and unambiguously forms an outgroup to all extant primate lentiviruses (Gifford et al., 2008; Gilbert et al., 2009; Liégeois et al., 2009). However, pSIVgml does not encode a *vpr* or *vpx* gene. Therefore, we performed a phylogenetic analysis of *pol* sequences and found that SIVolc and SIVwrc, which infect the primate species of the *Colobinae* subfamily of OWM and contain an existing *vpr/vpx* gene, are the closest relatives to SIVgml (Figure S4A). This result is consistent with previous studies (Gifford et al., 2008; Gilbert et al., 2009). Analysis of *env* sequences (which are 3′ of *vpr* and *pol*) showed that the pSIVgml nests with the similar cluster of sequences (Figure S4B). Therefore, we used SIVolc/SIVwrc *vpr* sequences to root the *vpr/vpx* tree, reflecting the high likelihood that this clade represented the earliest branching event of extant primate lentiviruses.

Using this rooted tree, we overlaid the SAMHD1 degradation phenotype onto the phylogeny and found that the *vpr* genes that lacked SAMHD1-degrading ability (Figure 5, red stars) were clearly separable from the SAMHD1 degrading *vpr* and *vpx* genes (Figure 5, blue stars). Strikingly, all *vpr* and *vpx* genes that shared the ability to degrade SAMHD1 nest within the same monophyletic clade with high confidence (Figure 5, BS, 90.3; Figure S1A, PP, 0.98). Since Vpr from HIV-1, HIV-2, SIVmac, SIVrcm, and SIVmnd2 was unable to degrade SAMHD1 (Figure 5, red stars), the most parsimonious explanation is that the Vpr of their common ancestor (Figure 5, node 3) lacked the SAMHD1 degradation capability. Given that the outgrouping SIVmnd1 Vpr and SIVolc Vpr proteins were incapable of degrading SAMHD1 (Figures 2 and 3), this strongly supports the hypothesis that the ancestral Vpr was ''inactive'' against SAMHD1 (Figure 5, node 1) and that the ability to degrade SAMHD1 subsequently arose only once during *vpr* and *vpx* evolution.

Based on the phylogeny, we can clearly pinpoint that the neofunctionalization of Vpr to degrade SAMHD1 occurred on the

branch leading up to the split of SIVagm, SIVdeb/mus/mon lineages (Figure 5, node 2). Importantly, based on phylogeny, our results suggest that the birth of the *vpx* recombination/duplication dated after the neofunctionalization occurred (Figure 5, node 4). Thus, the combined phylogenetic and functional study presented here strongly supports a scenario in which the degradation of SAMHD1 by Vpx was preceded by the neofunctionalization of Vpr in a transitional SIV lineage. Furthermore, this phylogenetic framework argues against a subsequent loss of SAMHD1-degrading ability in any lentiviral Vpr protein; that is, those Vpr proteins that currently lack this ability including HIV-1 Vpr likely never possessed it.

DISCUSSION

Here we show that diverse Vpx proteins as well as some Vpr proteins have the ability to target their host species' SAMHD1 for degradation. Both Vpx and Vpr antagonists display species-specific degradation of SAMHD1 which, in some cases, is quite specific to the virus's extant host. Such species specificity is a hallmark of an antagonistic "arms race" between host and virus, in which both sides rapidly evolve to gain an advantage. Indeed, we show that SAMHD1 has been evolving under positive selection in primates. We demonstrate that the residues under positive selection in the SAM domain of SAMHD1 determine the specificity of degradation by Vpx, directly implicating Vpr/Vpx antagonism as the source of the remarkable signature of positive selection detected in SAMHD1, which is most pronounced in the *Cercopithecina* subfamily of OWMs. By combining our functional results with phylogenetic analyses, we show that the ability to degrade SAMHD1 is a neofunctionalization of Vpr which preceded the birth of Vpx by recombination/duplication.

Based on our combined phylogenetic and functional analyses, the common ancestor of SIV viruses most likely encoded a single Vpr that was inactive against SAMHD1. The ability to recruit a protein degradation complex is important for Vpr-mediated cell-cycle arrest (reviewed in Dehart and Planelles, 2008) and thus may represent the ancestral function of Vpr/Vpx. Interestingly, although cell-cycle arrest and SAMHD1 degradation functions are segregated into two separate proteins in those viruses that encode Vpr and a Vpx (Ayinde et al., 2010), SIVagm Vpr is able to cause both cell-cycle arrest (Planelles et al., 1996; Stivahtis et al., 1997) and SAMHD1 degradation (Figure 2A). This indicates that the two functions are not mutually exclusive. Furthermore, since cell-cycle arrest by Vpr has some species specificity (Stivahtis et al., 1997), it is likely that the substrate used by Vpr to cause cell-cycle arrest will, like SAMHD1, have evolved under positive selection.

While the cellular protein targeted by Vpr to cause cycle-cell arrest is not yet known, the adaptive evolution of SAMHD1 might provide a clue as to why some viruses evolved to encode a separate Vpx and Vpr gene. One scenario we propose is that the neofunctionalization of the ancestral Vpr/x to target SAMHD1 exerted a strong selective pressure on OWMs' SAMHD1. As a result, variants of SAMHD1 that conferred protection from Vpr/x antagonism were selected for, leading to the signatures of rapid evolution in SAMHD1, especially localized within the SAM domain. This posed a unique challenge to the ancestral Vpr/x that had to recognize both the cell-cycle arrest factor and multiple rapidly evolving variants of SAMHD1. In order to maintain both functional capabilities, a recombination/duplication of Vpr might have given rise to Vpx. This subsequently allowed the subfunctionalization of Vpx to maximize its SAMHD1-targeting capability, while preserving the cell-cycle arrest phenotype in Vpr. This model might explain the complicated evolutionary history of *vpr* and *vpx* (Sharp et al., 1996; Tristem et al., 1990, 1998). Thus, we speculate that the "birth" of a new gene in some lineages leading to both *vpr* and *vpx* in the same viral genome, a more modern event compared to the neofunctionalization of Vpr, may have been directly driven by the rapid evolution of the SAMHD1 protein.

HIV-1 lacks the capability of degrading SAMHD1, since its Vpr protein is unable to degrade SAMHD1 and it does not encode Vpx. Since SIVcpz Vpr also lacks SAMHD1-degrading ability (Figure 2A), this function was missing in HIV-1 even prior to its cross-species transmission from chimpanzees into humans. Moreover, human SAMHD1 is not special in terms of its resistance to Vpr antagonism, as it is readily degraded by HIV-2 Vpx. This situation is directly analogous to the two lentiviruses that infect mandrills. SIVmnd1 contains only a Vpr gene that has no activity against mandrill SAMHD1 (Figure 1C), whereas SIVmnd2 has both Vpr and Vpx, the latter of which is capable of degrading mandrill SAMHD1 (Figure 1C). Intriguingly, SIVmnd1 appears more pathogenic than SIVmnd2, similar to the higher pathogenicity of HIV-1 relative to HIV-2 (Souquière et al., 2009). One possible explanation is that both HIV-1 and viruses like SIVmnd1 evolved unique antagonistic functions (or more effective countermeasures) that collectively allow HIV-1 to achieve sufficient replicative potential in target cells (including SAMHD1-expressing monocytes) even in the absence of SAMHD1-degrading abilities. On the other hand, Vpx-encoding viruses may have become more dependent on the ability to counteract SAMHD1 to achieve successful replication in target cells and have relaxed selection on alternate measures used by viruses like HIV-1.

Most of the signatures of positive selection in primate *SAMHD1* appear to originate from the OWM lineages, specifically the subfamily *Cercopithecinae* after its split from *Colobinae*. This highly localized positive selection on the primate phylogeny is unusual. Most previously analyzed host immune genes, such as TRIM5alpha, Tetherin, PKR, and APOBEC3G, display signatures of positive selection throughout many primate lineages including hominoids and NWMs (Elde et al., 2009; Lim et al., 2010; McNatt et al., 2009; Meyerson and Sawyer, 2011; Sawyer et al., 2004, 2005), while others have been restricted to hominoids and OWMs alone (TRIM22). Such a localized signature of positive selection might signal the advent of a highly specialized and unique antagonist. Orangutan SAMHD1 is the only primate species outside of the *Cercopithecinae* subfamily that also has strong signals of positive selection. However, while there is no evidence of SIVs infecting orangutans to date, there have been reports of simian T-lymphotropic virus (STLV) and simian type D retrovirus (SRV) infecting orangutans (Verschoor et al., 2004; Warren et al., 1998).

Intriguingly, our phylogenetic framework (Figure 5) strongly argues that the Vpr/Vpx proteins' ability to degrade SAMHD1 arose within the primate lentiviruses, and specifically among

lentiviruses that infect *Cercopithecinae* and jumped into *Hominidae*, but not from viruses isolated from *Colobinae.* Together with our findings that residues in SAMHD1 under positive selection directly determine Vpx sensitivity, this suggests that the birth of the SAMHD1-degrading ability within primate lentiviruses initiated the evolutionary arms race that led to such a highly localized signature of positive selection within *Cercopithecinae.* Thus, both the positive selection of SAMHD1 and consequently the birth of Vpx may have been driven by the neofunctionalization of Vpr to antagonize SAMHD1.

EXPERIMENTAL PROCEDURES

Sequencing of Primate SAMHD1 Genes

The SAMHD1 genes from the following primates were amplified from RNA isolated from cell lines obtained from Coriell Cell Repositories (Camden, NJ): chimpanzee (Pan troglodytes), bonobo (Pan panisucus), gorilla (Gorilla gorilla), Sumatran orangutan (Pongo pygmaeus), white-cheeked gibbon (Nomascus leucogenys), agile gibbon (Hylobates agilis), Siamang gibbon (Hylobates syndactylus), Rhesus macaque (Macaca mulatta), patas monkey (Erythrocebus patas), talapoin monkey (Miopithecus talapoin), greater white-nosed monkey (Cercopithecus nictitans), De Brazza's monkey (Cercopithecus neglectus), Wolf's guenon (Cercopithecus wolfi), Allen's swamp monkey (Allenopithecus nigroviridis), sooty mangabey (Cercocebus atys), red-capped mangabey (Cercocebus torquatus), mandrill (Mandrillus sphinx), drill (Mandrillus leucophaeus), Kikuyu colobus (Colobus guereza kikuyuensis), Francois' leaf monkey (FLM) (Trachypithecus francoisi), proboscis monkey (Nasalis larvatus), tamarin (Saguinus labiatus), pygmy marmoset (Callithrix pygmaea), white-faced saki (Pithecia pithecia), spider monkey (Ateles geoffroyi), owl monkey (Aotus trivirgatus), dusky titi monkey (Callicebus moloch), and woolly monkey (Lagothrix lagotricha). Human, African green monkey (Chlorocebus pygerythrus), and baboon (Papio anubis) SAMHD1 was amplified by reverse transcriptase-PCR (RT-PCR) from an RNA extract of 293T cells, Vero cell (AGM Vervet subspecies), COS-7 cells (AGM Sabaeus subspecies), and B-LCL cells. SAMHD1 was amplified by RT-PCR with SuperScript III One-Step RT-PCR (QIAGEN), and the cDNA derived was directly sequenced. SAMHD1 was amplified with forward primer SAMHD1-Hominoid-F (5′-ATGCAGCGAGCCGATTCCGAGCAGCC-3′), SAMHD1-OWM-F (5′-ATGCAGCAAGCCGACTCCGACCAGCC-3′) or SAMHD1-NWM-F (5′-ATGCAGCAAGCCGACTTCGAGCAGCC-3′) in combination with reverse primer SAMHD1-Hominoids-r (5′-TCACATTGGGTCATCTTTAAAAAGCTG-3′), SAMHD1-OWM-r (5′-TCACTTTGGGTCATCTTTAAAAAGCTG-3′) or SAMHD1-NWM-r (5′-TCACACCGGGTCATCCTTAAAAAGCTG-3′).

SAMHD1 Sequence Analysis

SAMHD1 DNA sequences were aligned by ClustalX (42) and were edited by hand based on amino acid sequences or with PhyML (10) by the ML method. The two methods yielded trees with identical topologies. ML analysis was performed with CODEML from the PAML suite of programs (55) as previously described (17). Briefly, *SAMHD1* sequences were fitted to NSsites models that disallowed (NSsites model 1 and 7) or permitted (NSsites model 2 and 8) positive selection. Likelihood ratio tests were performed to evaluate whether permitting codons to evolve under positive selection gave a better fit to the data. Data were fitted with an F61 model of codon frequency, and consistent results were obtained when the data were fitted with an F3 × 4 model of codon frequency. These analyses (M8) identified amino acid residues with high posterior probability ($p > 0.95$) of having evolved under positive selection. Analyses were also validated with PARRIS and REL from the HyPhy package (Pond et al., 2005). Free ratio analysis in PAML was used to calculate the ω (dN/dS) ratios of individual branches.

Plasmids

Primate SAMHD1 was cloned from cDNA from the respective species and ligated into pLPCX construct, with a hemagglutinin (HA) epitope tag fused to the C termini. Vpr and Vpx constructs ligated into a pCDNA3.1 expression vector, with a 3xFLAG epitope tag fused to the N termini. The following genes were cloned from provirus plasmids: HIVLai Vpr, SIVagmGri677 Vpr, SIVagmVer 9648 Vpr, HIV-2 Rod9 Vpr and Vpx, HIV-2 7312a Vpr and Vpx (as previously described [Stivahtis et al., 1997]); HIV-1 Q23-17 Vpr (provirus was a gift from Julie Overbaugh [Poss and Overbaugh, 1999]); SIVmac239 Vpr and Vpx (provirus plasmid, obtained from NIH AIDS Research and Reference Reagent Program [Regier and Desrosiers, 1990]); and SIVcpzTan2.69 Vpr and SIVcpzTan3.1 Vpr (proviral plasmid, obtained from the NIH AIDS Research and Reference Reagent Program [Takehisa et al., 2007]). The following genes were codon optimized and synthesized (Genscript): SIVolc 97CI12 Vpr (FM165200), SIVmnd1 GB1 Vpr (M27470), SIVrcm NG411 Vpr and Vpx (AF349680), SIVmnd2 5440 Vpr and Vpx (AY159322), SIVdeb CM5 Vpr (AY523866), and SIVmus1 CM1239 Vpr (EF070330).

Transfection

293T cells were transfected with 100 ng of SAMHD1 (in LPCX expression vector, C-terminal HA-epitope tag) with or without 100 ng of Vpr/Vpx constructs (in pCDNA3.1 expression vector, N-terminal 3xFLAG epitope tag) using TransIT-LT1 (Mirus Bio). The amount of codon-optimized Vpr/Vpx was reduced to normalize for similar levels of protein expression. The total amount of DNA in all transfections was maintained constant with appropriate empty vectors. Forty-eight hours posttransfection, cells were harvested for western blot analysis.

Western Blotting

Western blot analysis was performed as described previously (Lim and Emerman, 2009; Lim et al., 2010) with the following antibodies: HA-specific antibody (Babco), anti-FLAG M2 antibody (Sigma-Aldrich), anti-actin (Sigma-Aldrich), and DDB1 antibody (Cell Signaling). Primary antibodies were detected with a corresponding horseradish peroxidase-conjugated secondary antibody.

Coimmunoprecipitations

293T cells were transfected by TransIT-LT1 (Mirus Bio) with the appropriate plasmids 36 hr prior to lysis, and were treated with 25 μM MG-132 (Calbiochem) for 12 hr. Cells were washed twice with PBS and lysed with IP lysis buffer (50 mM Tris [pH 7.4], 250 mM NaCl, 0.4% [v/v] NP-40, 1 mM DTT, plus Protease Inhibitor Cocktail [Roche]). Lysates were cleared at 15,500 g for 15 min, and immunoprecipitations were performed for 1 hr at 4°C with EZview Red anti-HA affinity gel (Sigma-Aldrich). Following immunoprecipitation, affinity gel was washed four times with IP lysis buffer; proteins were eluted in 2× Laemmli sample buffer and analyzed by western blotting.

Phylogenetic Analysis

Phylogenetic trees were constructed from amino acid alignments of *vpr* and *vpx* sequences obtained from the Los Alamos HIV Sequence Database (Los Alamos HIV Sequence Database, 2011). Alignments were performed by using ClustalX (Thompson et al., 1997) and edited manually or by using FSA (Bradley et al., 2009) for a more conservative alignment that maximizes accuracy. Phylogenies were constructed with PhyML (10) by the ML method, and MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) and BEAST v1.6.2 (Drummond and Rambaut, 2007) using a Bayesian MCMC inference. Support for ML trees was assessed by 1,000 nonparametric bootstraps. MrBayes analyses were run for 10,000,000 steps with a sample frequency set to 1,000 and burn-in length of 1,000,000. BEAST analyses were run until convergence with a minimum of 1,000,000 generations, sampling every 1,000 and discarding the initial 10% as burn-in. Convergence and mixing for both MrBayes and BEAST were assessed using Tracer v1.5 (Drummond and Andrew, 2009). Analyses from both Bayesian methods were performed at least twice.

ACCESSION NUMBERS

The sequences of the 31 *SAMHD1* genes have been entered into the GenBank database under accession numbers NP_056289 and JQ231123–JQ231152.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at doi:10.1016/j.chom.2012.01.004.

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REFERENCES

Ayinde, D., Maudet, C., Transy, C., and Margottin-Goguet, F. (2010). Limelight on two HIV/SIV accessory proteins in macrophage infection: is Vpx overshadowing Vpr? Retrovirology *7*, 35.

Bradley, R.K., Roberts, A., Smoot, M., Juvekar, S., Do, J., Dewey, C., Holmes, I., and Pachter, L. (2009). Fast statistical alignment. PLoS Comput. Biol. *5*, e1000392. 10.1371/journal.pcbi.1000392.

Dehart, J.L., and Planelles, V. (2008). Human immunodeficiency virus type 1 Vpr links proteasomal degradation and checkpoint activation. J. Virol. *82*, 1066–1072.

Drummond, A.J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. *7*, 214.

Drummond, A.J., and Andrew, R. (2009). Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/).

Elde, N., Child, S., Geballe, A., and Malik, H. (2009). Protein kinase R reveals an evolutionary model for defeating viral mimicry. Nature *457*, 485–489.

Emerman, M., and Malik, H. (2010). Paleovirology—modern consequences of ancient viruses. PLoS Biol. *8*, e1000301. 10.1371/journal.pbio.1000301.

Gibbs, J.S., Lackner, A.A., Lang, S.M., Simon, M.A., Sehgal, P.K., Daniel, M.D., and Desrosiers, R.C. (1995). Progression to AIDS in the absence of a gene for vpr or vpx. J. Virol. *69*, 2378–2383.

Gifford, R., Katzourakis, A., Tristem, M., Pybus, O., Winters, M., and Shafer, R. (2008). A transitional endogenous lentivirus from the genome of a basal primate and implications for lentivirus evolution. Proc. Natl. Acad. Sci. USA *105*, 20362–20367.

Gilbert, C., Maxfield, D., Goodman, S., and Feschotte, C. (2009). Parallel germline infiltration of a lentivirus in two Malagasy lemurs. PLoS Genet. *5*, e1000425. 10.1371/journal.pgen.1000425.

Goldstone, D.C., Ennis-Adeniran, V., Hedden, J.J., Groom, H.C., Rice, G.I., Christodoulou, E., Walker, P.A., Kelly, G., Haire, L.F., Yap, M.W., et al. (2011). HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature *480*, 379–382.

Hirsch, V.M., Sharkey, M.E., Brown, C.R., Brichacek, B., Goldstein, S., Wakefield, J., Byrum, R., Elkins, W.R., Hahn, B.H., Lifson, J.D., et al. (1998). Vpx is required for dissemination and pathogenesis of SIV(SM) PBj: evidence of macrophage-dependent viral amplification. Nat. Med. *4*, 1401–1408.

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S.K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M.P., and Skowronski, J. (2011). Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature *474*, 658–661.

Huelsenbeck, J.P., and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics *17*, 754–755.

Kirchhoff, F. (2010). Immune evasion and counteraction of restriction factors by HIV-1 and other primate lentiviruses. Cell Host Microbe *8*, 55–67.

Kosakovsky Pond, S., Posada, D., Gravenor, M., Woelk, C., and Frost, S. (2006). GARD: a genetic algorithm for recombination detection. Bioinformatics *22*, 3096–3098.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature *474*, 654–657.

Liégeois, F., Lafay, B., Formenty, P., Locatelli, S., Courgnaud, V., Delaporte, E., and Peeters, M. (2009). Full-length genome characterization of a novel simian immunodeficiency virus lineage (SIVolc) from olive Colobus (Procolobus verus) and new SIVwrcPbb strains from Western Red Colobus (Piliocolobus badius badius) from the Tai Forest in Ivory Coast. J. Virol. *83*, 428–439.

Lim, E., and Emerman, M. (2009). Simian immunodeficiency virus SIVagm from African green monkeys does not antagonize endogenous levels of African green monkey tetherin/BST-2. J. Virol. *83*, 11673–11681.

Lim, E., Malik, H., and Emerman, M. (2010). Ancient adaptive evolution of tetherin shaped the functions of vpu and nef in human immunodeficiency virus and primate lentiviruses. J. Virol. *84*, 7124–7134.

Los Alamos HIV Sequence Database (2011). http://www.hiv.lanl.gov/.

Malim, M., and Emerman, M. (2008). HIV-1 accessory proteins—ensuring viral survival in a hostile environment. Cell Host Microbe *3*, 388–398.

McNatt, M., Zang, T., Hatziioannou, T., Bartlett, M., Fofana, I., Johnson, W., Neil, S., and Bieniasz, P. (2009). Species-specific activity of HIV-1 Vpu and positive selection of tetherin transmembrane domain variants. PLoS Pathog. *5*, e1000300. 10.1371/journal.ppat.1000300.

Meyerson, N.R., and Sawyer, S.L. (2011). Two-stepping through time: mammals and viruses. Trends Microbiol. *19*, 286–294.

Peeters, M., and Courgnaud, V. (2002). Overview of primate lentiviruses and their evolution in non-human primates in Africa. In HIV Sequence Compendium, C. Kuiken, B. Foley, E. Freed, B. Hahn, B. Korber, P.A. Marx, F. McCutchan, J.W. Mellors, and S. Wolinsky, eds. (Los Alamos, NM), 2–23.

Perelman, P., Johnson, W.E., Roos, C., Seuánez, H.N., Horvath, J.E., Moreira, M.A., Kessing, B., Pontius, J., Roelke, M., Rumpler, Y., et al. (2011). A molecular phylogeny of living primates. PLoS Genet. *7*, e1001342. 10.1371/journal.pgen.1001342.

Planelles, V., Jowett, J.B., Li, Q.X., Xie, Y., Hahn, B., and Chen, I.S. (1996). Vpr-induced cell cycle arrest is conserved among primate lentiviruses. J. Virol. *70*, 2516–2524.

Pond, S.L., Frost, S.D., and Muse, S.V. (2005). HyPhy: hypothesis testing using phylogenies. Bioinformatics *21*, 676–679.

Poss, M., and Overbaugh, J. (1999). Variants from the diverse virus population identified at seroconversion of a clade A human immunodeficiency virus type 1-infected woman have distinct biological properties. J. Virol. *73*, 5255–5264.

Powell, R.D., Holland, P.J., Hollis, T., and Perrino, F.W. (2011). The Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. J. Biol. Chem. *286*, 43596–43600.

Regier, D.A., and Desrosiers, R.C. (1990). The complete nucleotide sequence of a pathogenic molecular clone of simian immunodeficiency virus. AIDS Res. Hum. Retroviruses *6*, 1221–1231.

Rice, G.I., Bond, J., Asipu, A., Brunette, R.L., Manfield, I.W., Carr, I.M., Fuller, J.C., Jackson, R.M., Lamb, T., Briggs, T.A., et al. (2009). Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. Nat. Genet. *41*, 829–832.

Sawyer, S., Emerman, M., and Malik, H. (2004). Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. PLoS Biol. *2*, E275. 10.1371/journal.pbio.0020275.

Sawyer, S., Wu, L., Emerman, M., and Malik, H. (2005). Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. Proc. Natl. Acad. Sci. USA *102*, 2832–2837.

Sharova, N., Wu, Y., Zhu, X., Stranska, R., Kaushik, R., Sharkey, M., and Stevenson, M. (2008). Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. PLoS Pathog. *4*, e1000057. 10.1371/journal.ppat.1000057.

Sharp, P.M., Bailes, E., Stevenson, M., Emerman, M., and Hahn, B.H. (1996). Gene acquisition in HIV and SIV. Nature *383*, 586–587.

Souquière, S., Bibollet-Ruche, F., Robertson, D.L., Makuwa, M., Apetrei, C., Onanga, R., Kornfeld, C., Plantier, J.C., Gao, F., Abernethy, K., et al. (2001). Wild Mandrillus sphinx are carriers of two types of lentivirus. J. Virol. *75*, 7086–7096.

Souquière, S., Onanga, R., Makuwa, M., Pandrea, I., Ngari, P., Rouquet, P., Bourry, O., Kazanji, M., Apetrei, C., Simon, F., et al. (2009). Simian immunodeficiency virus types 1 and 2 (SIV mnd 1 and 2) have different pathogenic potentials in rhesus macaques upon experimental cross-species transmission. J. Gen. Virol. *90*, 488–499.

Stetson, D.B., Ko, J.S., Heidmann, T., and Medzhitov, R. (2008). Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell *134*, 587–598.

Stivahtis, G.L., Soares, M.A., Vodicka, M.A., Hahn, B.H., and Emerman, M. (1997). Conservation and host specificity of Vpr-mediated cell cycle arrest suggest a fundamental role in primate lentivirus evolution and biology. J. Virol. *71*, 4331–4338.

Takehisa, J., Kraus, M., Decker, J., Li, Y., Keele, B., Bibollet-Ruche, F., Zammit, K., Weng, Z., Santiago, M., Kamenya, S., et al. (2007). Generation of infectious molecular clones of simian immunodeficiency virus from fecal consensus sequences of wild chimpanzees. J. Virol. *81*, 7463–7475.

Takemura, T., and Hayami, M. (2004). Phylogenetic analysis of SIV derived from mandrill and drill. Front. Biosci. *9*, 513–520.

Thompson, J., Gibson, T., Plewniak, F., Jeanmougin, F., and Higgins, D. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. *25*, 4876–4882.

Tristem, M., Marshall, C., Karpas, A., Petrik, J., and Hill, F. (1990). Origin of vpx in lentiviruses. Nature *347*, 341–342.

Tristem, M., Purvis, A., and Quicke, D.L. (1998). Complex evolutionary history of primate lentiviral vpr genes. Virology *240*, 232–237.

Tsujimoto, H., Cooper, R.W., Kodama, T., Fukasawa, M., Miura, T., Ohta, Y., Ishikawa, K., Nakai, M., Frost, E., and Roelants, G.E. (1988). Isolation and characterization of simian immunodeficiency virus from mandrills in Africa and its relationship to other human and simian immunodeficiency viruses. J. Virol. *62*, 4044–4050.

Verschoor, E.J., Langenhuijzen, S., Bontjer, I., Fagrouch, Z., Niphuis, H., Warren, K.S., Eulenberger, K., and Heeney, J.L. (2004). The phylogeography of orangutan foamy viruses supports the theory of ancient repopulation of Sumatra. J. Virol. *78*, 12712–12716.

Warren, K.S., Niphuis, H., Heriyanto, Verschoor, E.J., Swan, R.A., and Heeney, J.L. (1998). Seroprevalence of specific viral infections in confiscated orangutans (Pongo pygmaeus). J. Med. Primatol. *27*, 33–37.

Yu, X.F., Ito, S., Essex, M., and Lee, T.H. (1988). A naturally immunogenic virion-associated protein specific for HIV-2 and SIV. Nature *335*, 262–265.

Yuan, X., Matsuda, Z., Matsuda, M., Essx, M., and Lee, T.H. (1990). Human immunodeficiency virus vpr gene encodes a virion-associated protein. AIDS Res. Hum. Retroviruses *6*, 1265–1271.