occurs in vivo and is a real behavior of the virus in human biology awaits further investigation. Furthermore, v-FLIP likely antagonizes host autophagy not only to evade OIS but also to favor viral persistence. From a viral perspective, during long-term persistent infection wherein the viral genome is replicated in tight conjunction with host chromosomal DNA, reshaping cellular autophagy may have an active role in antagonizing host antiviral immune responses, such as antigen presentation, to allow persistence. From a host perspective, since autophagy has been implicated in patrolling genomic stability, blunting autophagy may also render virally infected cells error prone, an environment more favorable for viral fitness and survival. Despite our growing understanding of the molecular nature of autophagy, how autophagy enables cells' self-disabling process remains a question that is currently unanswered and is certainly a future challenge. Nonetheless, the Leidal et al. (2012) work suggests that evasion of autophagy may be a shared value for oncogenic viruses and that technologies that interfere with viral undermining of host autophagy could have considerable promise in treating virally associated malignancies.

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SAMHD1 Joins the Red Queen's Court

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The host restriction factor SAMHD1 hinders lentiviral infection of myeloid cells, a function counteracted by the viral protein Vpx. Two papers in this issue of *Cell Host & Microbe* document the genetic conflict between SAMHD1 and the Vpr/Vpx proteins, which has subjected SAMHD1 to intense periods of diversifying selection through primate evolution.

In Lewis Carroll's book *Through the Looking-Glass*, the Red Queen says, "It takes all the running you can do, to keep in the same place." Evolutionary biologists have often used the Red Queen's race as a metaphor for the never-ending evolutionary race between a host and a pathogen. Cellular proteins that fight viral infection are subject to constant attack by their viral counterparts, and they must continue to evolve and escape in an iterative process leading to coevolution. In the lentivirus literature a number of innate immune defense proteins have been documented, commonly referred to as "restriction factors" (reviewed in Malim and Emerman, 2008). The sterile alpha motif (SAM) domain and histidine/aspartic acid domain (HD)-containing protein 1 (SAMHD1) is the most recent addition to the list of restriction factors that act against lentiviruses (Hrecka et al., 2011; Laguette et al., 2011), thus joining APOBEC3G, TRIM5α, and tetherin (Malim and Emerman, 2008). Correspondingly, lentiviral "accessory" proteins such as Nef, Vpu, and Vpx have been identified to overcome the effect of restriction factors. Specifically, while SAMHD1 effectively restricts HIV-1 replication, Vpx from HIV-2 and related simian immunodeficiency viruses (SIVsmm/mac) counteract the restrictive mechanism by promoting proteasome-dependent degradation of SAMHD1 (Hrecka et al., 2011; Laguette et al., 2011).

SAMHD1 was recently shown to convert deoxynucleoside tryphosphates (dNTP) into deoxynucleosides and inorganic tryphosphate, thus controlling intracellular levels of dNTPs, the substrates for reverse transcription (Goldstone et al., 2011). The levels of dNTPs are limiting in nondividing cells such as macrophages and dendritic cells—approximately 200-fold lower than in activated T cells (Diamond et al., 2004)—in part due to SAMHD1's activity. Low levels of dNTPs fail to support efficient viral reverse transcription.

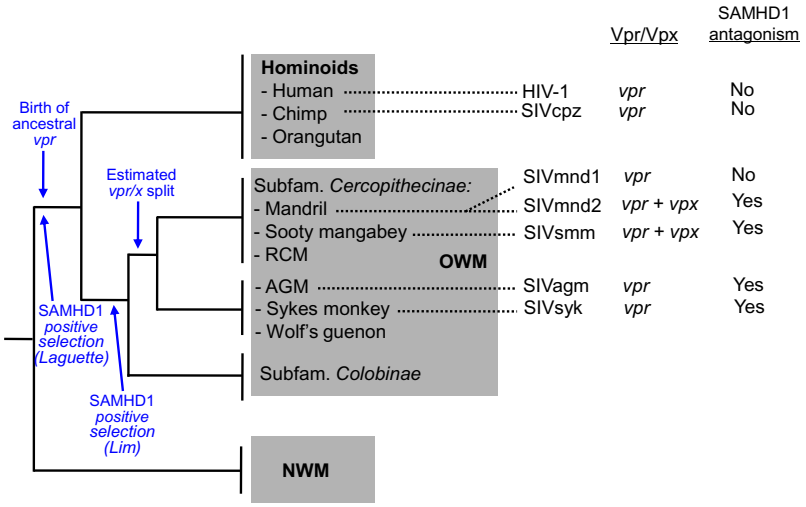

Figure 1. Overview of the Genealogy of Primates and Events in the Evolution of *vpx* and *vpr* as They Relate to SAMHD1 Diversifying Selection
Branch lengths are not drawn to scale. Adapted from Laguette et al. (2012) and Lim et al. (2012).

Now, two groups have independently investigated the evolutionary relationships of SAMHD1 orthologous genes through primate species, and their potential sensitivities to degradation in the presence of *vpx* and *vpr* alleles from the cognate simian immunodeficiency viruses (Laguette et al., 2012; Lim et al., 2012). The overarching conclusion from these studies is that SAMHD1, indeed, has been subject to periods of strong positive selection through its evolution in primates, specifically in monkey lineages that are infected by *vpx*-expressing lentiviruses. These studies provide us with many new and exciting notions about the biology of SAMDH1, the Vpx/Vpr viral proteins, and their coevolution.

Both groups employed a fairly extensive panel of SAMHD1 orthologs and used in silico analysis to derive inferences regarding the evolutionary fate of SAMHD1. Lim et al., in addition, assembled a genealogic tree for the *vpr* and *vpx* alleles in many species of primate lentiviruses and "superimposed" it on the SAMHD1 tree. The basic tool in these analyses is the ϖ factor, which is the ratio of nonsynonymous to synonymous substitutions (dN/dS). Both studies also pursued structure-function analyses on SAMHD1, which helped validate some of the evolutionary inferences.

According to the analysis by Lim and colleagues, strong positive selection on SAMHD1 could first be detected as a new function of Vpr at the tree branch that represents the most immediate common ancestor of the *Cercopithecinae* and the *Colobinae* subfamilies (Figure 1). *Cercopithecinae* and *Colobinae* are the two subfamilies that make up the Old World monkeys (OWMs). Since viruses that infect hominoids encode *vpr* but do not appear to antagonize SAMHD1, Lim et al. infer that the ancestral *vpr* (present in the ancestor of hominoids and OWMs) was initially devoid of the ability to degrade SAMHD1 and instead performed a different function. *Vpr* acquired SAMHD1 antagonism at a later time ("neofunctionalization"), initiating an arms race with SAMHD1 (Figure 1). The second important notion stemming from Lim and colleagues' analysis is that the duplication or recombination event leading to viruses encoding *vpr* and its paralog, *vpx*, was subsequent to the acquisition of SAMHD1 antagonism (Figure 1).

If the ability to antagonize SAMHD1 is a new function of *vpr* as suggested by Lim et al., did this new SAMHD1 antagonism replace Vpr's ancestral function? Or, alternatively, did both functions coexist in the same protein over some time during evolution? The obvious way to address these questions would be to examine the ancestral proteins and their activities in the ancestral primates. But that would be rather difficult. Instead, one may look at *vpr* and *vpx* alleles in present-day viruses and then infer what their evolution pathway might have been. Finding extant viruses that preserve SAMHD1 antagonism together with Vpr's ancestral function would support that the coexistence of both functions, at least in those viral lineages. The other presumed function of Vpr/Vpx proteins is their ability to degrade an unknown cellular protein leading to cell cycle arrest in G_2 (reviewed in Dehart and Planelles, 2008). Degradation of this putative cellular factor occurs via recruitment of a ubiquitin ligase of identical composition as the one targeted by Vpx: $Cul4^{DDB1/DCAF1}$ (reviewed in Dehart and Planelles, 2008). If we consider induction of G_2 arrest as a proxy for the unknown function of Vpr, it is clear that this function is present in virtually all primate lentiviruses. Thus, it is tempting to speculate that the ancestral function of Vpr was related to $Cul4^{DDB1/DCAF1}$ and G_2 arrest induction.

As Lim and colleagues now show (Lim et al., 2012), Vpr proteins that can induce both SAMHD1 antagonism and G_2 arrest do exist in the simian viruses, SIVagm and SIVsyk (Figure 1). Therefore, Lim and colleagues speculate that before the *vpr/vpx* split, it may have become too difficult for Vpr to simultaneously compete in two different arms races with different primate host proteins, thereby leading to the gene duplication in lineages such as SIVrcm, SIVmnd2, SIVdrl, and viruses in the HIV-2/SIVsmm group (Figure 1). In the lineage leading to AGM and sykes monkeys, Vpr remained bifunctional. The case of Vpr from AGM and sykes monkeys is reminiscent of the ability of HIV-1 Vif to target two different proteins for ubiquitination, APOBEC3G and APOBEC3F (Russell and Pathak, 2007). A graphic model for the evolution of Vpx and Vpr proteins is presented in Figure 2.

The unusually high level of diversifying selection observed in SAMHD1 in the OWM lineage is not unexpected because OWMs are the only hosts known to harbor infections by lentiviruses that can antagonize SAMHD1. In contrast, the closest relatives to OWMs, the hominids, are infected by lentiviruses that do not antagonize SAMHD1, such as SIVcpz. Little diversifying selection is observed among hominoid SAMHD1s, with one notable exception: the orangutan (note that both studies found strong diversifying selection in this species [Laguette et al., 2012;

Lim et al., 2012]). At present, no lentivirus has yet been identified in orangutans that could explain this observation. SAMHD1 diversifying selection could well be in response to an as-yet unidentified lentivirus or perhaps an STLV-like retrovirus or a variant of the hepatitis B virus, which also use reverse transcriptase in their replication cycles.

A similar evolutionary analysis by Laguette and colleagues produced slightly different conclusions (Laguette et al., 2012). In the Laguette study, strong positive selection was detected at the node representing a common ancestor for hominoids and OWMs (Figure 1), and this would be one node earlier than was proposed by Lim et al. (2012). According to the scenario proposed by Laguette et al., SAMHD1 antagonism would have appeared simultaneously with or close to the birth of *vpr*. The reasons for this discrepancy with the Lim study are likely related to the different sampling and analysis methods that either study utilized. A future, broader analysis, perhaps including yet-to-be identified simian viruses very likely will resolve this issue.

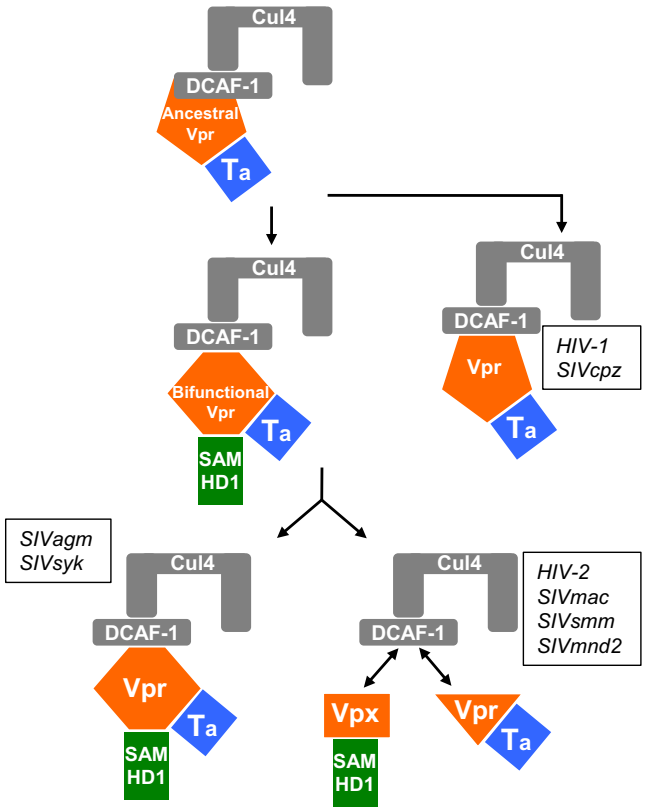

Figure 2. Model for the Evolution of Vpx and Vpr Proteins
Possible evolution of the ancestral *vpr* (orange pentagon), including neofunctionalization to acquire SAMHD1 (green rectangle) antagonism and the predicted *vpr*/*vpx* split, are shown. Ancestral Vpr was thought to induce degradation of an ancestral target, T_a (blue square). Virus names in boxes represent modern-day isolates. Cul4 and DCAF-1 (in gray) are components of the host ubiquitin ligase known as $Cul4^{DDB1/DCAF1}$. Based on the model proposed by Lim and colleagues (Lim et al., 2012).

Both Lim et al. and Laguette et al. performed evolutionary analyses focusing on specific amino acids and found a handful of residues that, at high stringency, appeared to be under positive selection. Surprisingly, the residues identified by either group did not overlap, as Lim et al. found the most positively selected residues in the amino-terminal domain of SAMHD1, whereas Laguette et al. identified mostly carboxy-terminal residues. It is important to note that structure-function analyses confirmed the importance of the corresponding residues in each case. For example, mutation of methionine 626 to alanine in SIVmac251 Vpx abrogated its ability to interact with and induce degradation of human SAMHD1 (Laguette et al., 2012). As another example, SIVmnd2 Vpx can degrade mandrill but not AGM SAMHD1. AGM and mandrill SAMHD1 differ at amino acid positions 46 and 69, which are subject to very high positive selection (Lim et al., 2012). When Lim et al. mutated aspartic acid at 46 to glycine and glutamine at 69 to arginine to make AGM SAMHD1 resemble the mandrill counterpart, the resulting mutant then became sensitive to degradation by SIVmnd2 Vpx. The general conclusions from these structure-function studies are that (1) the ability of Vpx to antagonize SAMHD1 correlates with binding, (2) positively selected residues in SAMHD1 regulate sensitivity to degradation, and (3) residues on both the N-terminal and C-terminal domains of SAMHD1 are important for interaction with Vpx.

The functional interactions between the viral proteins Vpx and Vpr are only beginning to surface. Beyond the arms races, it is reasonable to anticipate that these proteins have broader effects on immune escape and disease induction. And there is also the exciting prospect of therapeutically targeting viral accessory protein activities as they are revealed, as is now the case for Vpx.

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