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The Restriction Factors of Human Immunodeficiency Virus*

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Cellular proteins called "restriction factors" can serve as powerful blockades to HIV replication, but the virus possesses elaborate strategies to circumvent these barriers. First, we discuss general hallmarks of a restriction factor. Second, we review how the viral Vif protein protects the viral genome from lethal levels of cDNA deamination by promoting APOBEC3 protein degradation; how the viral Vpu, Env, and Nef proteins facilitate internalization and degradation of the virus-tethering protein BST-2/tetherin; and how the viral Vpx protein prevents the premature termination of reverse transcription by degrading the dNTPase SAMHD1. These HIV restriction and counter-restriction mechanisms suggest strategies for new therapeutic interventions.

Restriction Factor Hallmarks

Restriction factors have at least four defining characteristics (see Fig. 1*A*). First and foremost, a restriction factor must directly and dominantly cause a significant decrease in HIV infectivity. This is often determined by cotransfecting cell lines such as HEK293 and HeLa with a molecular clone of the virus, with or without a plasmid expressing the restriction factor, and measuring the amount and infectivity of virus recovered in the cell culture medium after 1–2 days of incubation. Such assays are ideally done over a range of restriction factor expression, with the highest levels often imposing log-scale drops in viral infectivity (see Fig. 1*B*). This "single-cycle" assay for viral replication is useful for testing the impact of viral mutations and/or restriction factor variations.

Second, if a restriction factor is a true threat to viral replication, then the predecessors of HIV invariably evolved an equally potent counter-restriction mechanism that still exists in the present day virus. For instance, titrating a counter-restriction factor into the aforementioned single-cycle infectivity experiment can result in a full recovery of viral infectivity despite the presence of an active restriction factor (Fig. 1*B*). Viruses lacking these various countermeasures are able to replicate in some, but not all, cell types depending on the expression level of the relevant restriction factor. Cell lines that support replication are termed "permissive," and those that do not are termed "nonpermissive." This life/death dichotomy has been elegantly exploited to identify several restriction factors and their corresponding viral antagonists.

Third, because the interactions between restriction and counter-restriction factors occur through direct protein-protein interactions, the restriction factor often shows signatures of rapid evolution. In general, mutations are maintained in a population only if they confer a selective advantage. If a host species experiences iterative rounds of pathogenic pressure, altered variants of host restriction factors that are no longer susceptible to the pathogen's counteraction mechanism are selected. Over evolutionary time, this results in an overabundance of amino acid substitution mutations in these genes relative to non-amino acid changing, or silent, mutations. These positive selection signatures become apparent by comparing restriction factor gene sequences between the host and evolutionarily related species. It is important to note that each amino acid substitution could have been selected by an independent pathogen conflict and that the ancestral pathogen may not have even resembled present day HIV. Thus, a major corollary to the hallmark of positive selection is the strong likelihood that each present day restriction factor has emerged from many ancient host-pathogen conflicts and has thus been fine-tuned to protect against a formidable number of parasites (*i.e.* restriction factors elicit broad activity). These hyper-evolved restriction factor protein sequences will undoubtedly continue to change as a result of ongoing interactions with modern pathogens.

Fourth, the expression of each restriction factor is often hard-wired to the innate immune response. For instance, restriction factor expression is often strongly induced by interferon and is thus tied directly to the host's innate immune response. Many other genes are also induced by interferons (as well as the interferon genes themselves), with each restriction factor or group of restriction factors comprising a relatively small portion of the much larger immune response. Because the overall composition of the innate immune regulon is shaped in part by the pathogens to which each species and its ancestors were exposed, innate immune effector proteins can vary between species (*i.e.* with one type proving more important for one species in comparison with another). Thus, this membership to the larger innate immune regulon helps rationalize why the number of each type of restriction factor often varies between mammalian lineages.

It is also worth noting that restriction factors are relatively rare in contrast to other host proteins that impact viral replication (dependency factors). Following the criteria established above, human cells probably possess only a few HIV restriction factors compared with hundreds of dependency factors and

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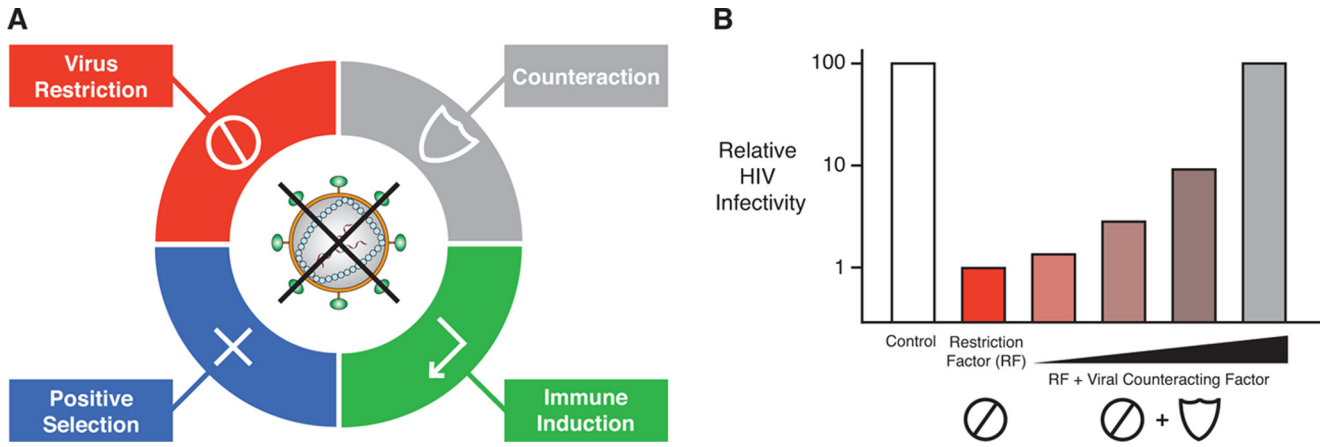

FIGURE 1. **Hallmarks of a restriction factor.** *A*, four defining hallmarks of an HIV restriction factor include dominant restriction of viral replication (*no-go sign*; clockwise from top left), a virus-encoded counteraction mechanism (*shield sign*), interferon responsiveness (*promoter sign*), and positive selection signatures (*plus sign*). *B*, histogram depicting viral infectivity in the presence of a restriction factor and a dose response of the relevant counter-restriction mechanism.

thousands of proteins that are not particularly relevant to HIV replication. *Bona fide* restriction factors result in log-scale differences in viral infectivity and interact directly with at least one viral component. Here, we discuss three such HIV restriction factors: the APOBEC3 DNA deaminase subfamily, the BST-2 (bone stromal tumor protein 2)/tetherin integral membrane protein, and the SAMHD1 dNTP hydrolase. We also encourage readers to see recent reviews on the TRIM5 proteins, which represent an important barrier to HIV replication in other species but not in humans (*e.g.* Ref. 1).

Restriction by Hypermutation: APOBEC3 DNA Deaminases

Classic studies showed that the HIV Vif (viral infectivity factor) protein is dispensable for viral replication in some T cell lines but not others (2, 3). Hybrid cells constructed by fusing permissive and nonpermissive cell lines inherit the nonpermissive phenotype, suggesting the existence of a dominant-acting restriction factor (4). This difference led to the molecular identification of APOBEC3G in nonpermissive cell types (5). Importantly, the expression of APOBEC3G in permissive T cells was sufficient to render them nonpermissive for Vif-deficient HIV replication (5).

At the same time, APOBEC3G was independently identified and shown to be part of a larger subfamily of seven APOBEC3 proteins that each have the capacity to catalyze DNA C-to-U deamination (6). This biochemical activity suggested not only a mechanism for APOBEC3G-mediated HIV restriction but also an explanation for the phenomenon of G-to-A hypermutation noted previously in HIV sequences from clinical samples (*e.g.* Refs. 7 and 8). Indeed, three groups demonstrated that APOBEC3G-dependent HIV restriction is characterized by massive levels of viral genomic strand G-to-A hypermutation, which can only be explained by a cDNA C-to-U deamination mechanism (Fig. 2) (9–11). This conclusion is now fortified by biochemical and mutagenesis studies, with the most notable showing that near-physiologic levels of APOBEC3G suppress spreading Vif-deficient viral replication in permissive T cell lines and induce G-to-A hypermutations, whereas physiologic levels of a catalytically inactive variant fail to do so (12–14).

APOBEC3G has a strong intrinsic preference for deaminating the second cytosine of a 5′-CC dinucleotide motif, which results in 5′-GG-to-AG hypermutations (9, 15, 16). HIV sequences from clinical samples frequently bear this pattern, but at near-equal levels they also often contain a 5′-GA-to-AA pattern (*e.g.* Refs. 7 and 8). Many studies have been dedicated to deducing which of the six other APOBEC3 proteins is responsible for inflicting this additional signature, with widely varying results (reviewed in Refs. 17 and 18). However, recent studies have brought clarity to this area by strongly implicating APOBEC3D, APOBEC3F, and APOBEC3H in HIV restriction (and simultaneously excluding APOBEC3A, APOBEC3B, and APOBEC3C) (19, 20).

The four HIV-relevant APOBEC3 proteins share the following properties: (i) expression in nonpermissive $CD4^+$ T cells (the primary HIV target cell), (ii) capacity to package into the nucleic acid-containing viral core (*i.e.* encapsidate), (iii) potent virus restriction activity, (iv) ability to inflict G-to-A mutations, (v) susceptibility to HIV Vif, and (vi) functional conservation with the homologous proteins of rhesus macaque (Fig. 2) (19). These observations are supported by systematic *APOBEC3* knock-out and knockdown studies in nonpermissive cells that demonstrate the importance of APOBEC3G in causing 5′-GG-to-AG hypermutations and that reveal overlapping roles for both APOBEC3D and APOBEC3F in Vif-deficient HIV restriction and 5′-GA-to-AA hypermutation (20). The contribution of APOBEC3H to Vif-deficient HIV restriction and 5′-GA-to-AA hypermutation may vary depending on the stability of the expressed protein, as multiple stable and unstable haplotypes are circulating in the human gene pool (21, 22).

HIV is a "successful" pathogen in part because its Vif protein mediates the polyubiquitination and subsequent degradation of the four restrictive APOBEC3 proteins by the 26 S proteasome (19). This is an ancient and conserved counter-restriction mechanism because all related lentiviruses, except equine infectious anemia virus, express a Vif protein that neutralizes the relevant APOBEC3 proteins of their host species (*e.g.* Ref. 23). An excellent recent example is provided by studies on nat-

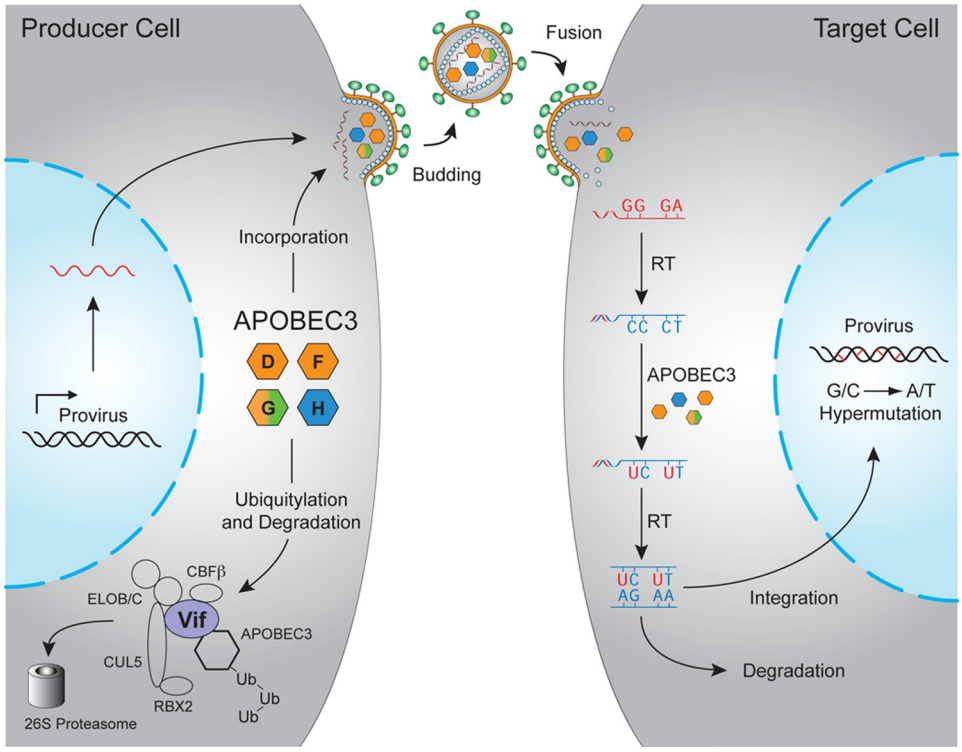

FIGURE 2. **HIV restriction by APOBEC3 proteins.** APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H can encapsidate into HIV virions and result in the deamination of cytosines to uracils in viral cDNA upon initiation of reverse transcription (*RT*) in target cells. Uracil templates adenine upon second-strand synthesis, resulting in a guanine-to-adenine mutation. These proviral cDNAs are subsequently degraded or integrated (although many are rendered nonfunctional). HIV-1 Vif overcomes the APOBEC3 restriction block in the producer cell by binding CBFβ and recruiting an E3 ubiquitin (*Ub*) ligase complex to polyubiquitinate the APOBEC3 proteins and target them for degradation by the 26 S proteasome. The figure was adapted from Ref. 19 and reproduced with permission.

ural simian immunodeficiency virus (SIV)[4] infection of four African green monkey (SIV_{agm}) subspecies (24). As expected, the Vif protein of each SIV_{agm} strain degrades the APOBEC3G protein of its respective host. However, only a few of these Vif proteins are able to degrade the APOBEC3G proteins of other African green monkey subspecies. Genetic studies deduced that Vif resistance/susceptibility maps to only a few amino acids in APOBEC3G. These studies suggest that SIV may have been transmitted into animals with Vif-resistant APOBEC3G proteins and subsequently altered its Vif protein to regain the capacity to degrade the new host's APOBEC3G protein.

Original biochemical and genetic studies combined to show that Vif recruits an E3 ligase complex consisting of CUL5, ELOB, ELOC, and RBX to mediate APOBEC3G degradation (Fig. 2) (25, 26). Despite this knowledge, Vif has resisted purification and biochemical studies, suggesting that a cofactor might be missing. Recent proteomic studies identified the transcription factor Core Binding Factor b (CBFβ) as a Vif-associated protein that also associates with the E3 ligase but only in the presence of Vif (27, 28). Knockdown studies demonstrated that CBFβ is essential for Vif stability and APOBEC3 degradation in human cells (27, 28). Importantly, CBFβ permitted the biochemical purification of the tetrameric complex CBFβ-Vif-ELOC-ELOB, which can be combined with a CUL5/RBX2 dimer to form a hexameric complex with APOBEC3G polyubiquitination activity (27). It is likely that this breakthrough will soon lead to the first atomic structures of Vif, the Vif-APOBEC3 interface, and perhaps an entire Vif-E3-APOBEC3 complex. Such information will undoubtedly expedite the development of drugs to disrupt the Vif-APOBEC3 interaction and possibly extinguish HIV replication by lethal mutagenesis. This strategy may lead to a new class of therapeutic agents to suppress viral loads and may have the potential to be curative if combined with ongoing work in the field to purge cells of latently integrated viruses.

The fact that the APOBEC3 proteins are promutagenic raises another provocative possibility. It is conceivable that lentiviruses, including HIV, use Vif as a mutational rheostat to regulate the overall load and impact of APOBEC3-driven mutations (29, 30). In the extreme, it is possible that HIV has become dependent upon the APOBEC3 proteins to achieve the overall high degree of genetic diversity required for the virus to "outrun" antibody and T cell responses. Thus, therapeutic strategies to inhibit APOBEC3 activity and to starve HIV of the fuel driving much of its genetic variability may be worth investigating as a means to constrain immune evasion.

Restriction by Particle Tethering: BST-2/Tetherin Integral Membrane Protein

Tetherin, also known as BST-2 or CD317, was identified as the cellular protein that accounts for a late-stage defect in the release of *vpu* (viral protein U)-deleted HIV-1 from restrictive cells (31, 32). This discovery traces back to original observations that deletion of the HIV-1 *vpu* gene results in a 5–10-fold

[4] The abbreviation used is: SIV, simian immunodeficiency virus.

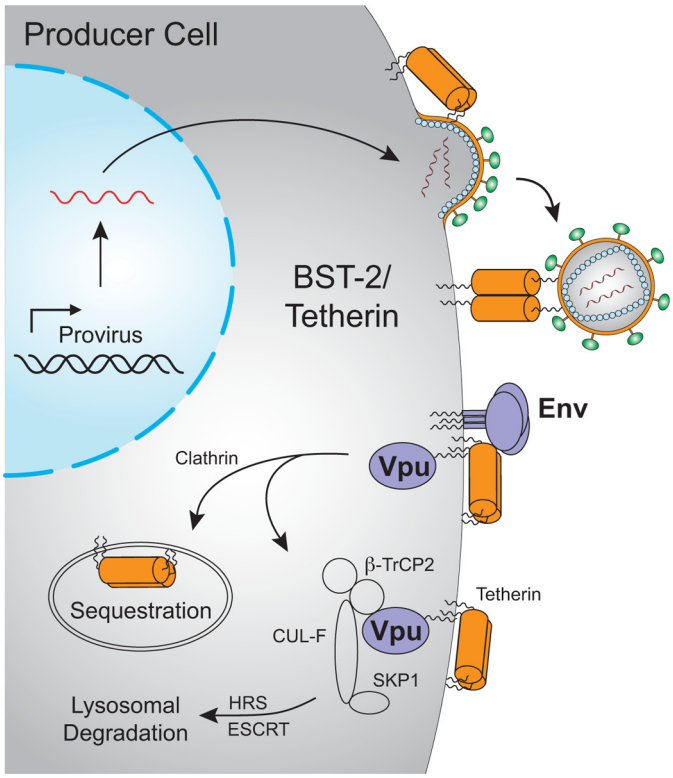

FIGURE 3. **HIV restriction by tetherin.** Tetherin acts to physically tether budding virions to the cell surface of productively infected T cells. HIV-1 Vpu or HIV-2 Env overcomes tetherin restriction by internalizing and sequestering tetherin in compartments away from sites of viral budding. HIV-1 Vpu can also recruit an E3 ubiquitin ligase complex that ubiquitinates tetherin and targets it for degradation in lysosomes. SIV Nef (not shown) can also counteract tetherin.

decrease in virus release from infected T cells without impairing the expression of other viral proteins (33). This defect in virus release was later shown to be cell type-dependent and to result in a particularly dramatic phenotype observable by electron microscopy, whereby mature viral particles are unable to detach from infected cells in the absence of Vpu and consequently accumulate on the plasma membrane and within intracellular compartments (34). The explanation for this phenomenon remained enigmatic for several years until heterokaryon fusions of permissive and nonpermissive cells revealed a dominant block to the release of *vpu*-deleted HIV-1 (35). Subsequent work demonstrated that the restriction factor is expressed on the cell surface and is interferon-inducible (36, 37), features that ultimately led to the identification of BST-2 (named "tetherin") as the cellular protein responsible for inhibiting the release of Vpu-deficient HIV-1 (31, 32).

The topology of tetherin, which includes an N-terminal cytoplasmic domain followed by a single-pass transmembrane domain, an extracellular coiled-coil domain, and a C-terminal glycosylphosphatidylinositol anchor (38), accounts for its broad antiviral activity not only against HIV-1 but also against many other enveloped viruses (Fig. 3). By virtue of having a membrane-spanning domain and a glycosylphosphatidylinositol anchor at opposite ends of the molecule, tetherin can simultaneously associate with both viral and cellular membranes. As viruses attempt to bud from infected cells, tetherin becomes incorporated into the viral envelope and physically bridges nascent virions to the cell (39–41). Evidence suggests that tetherin forms a parallel homodimer, and although either the N- or C-terminal domain can be oriented in the cell (39), at least some of the dimers need to have their N-terminal domains in the cell to interact with the cellular endocytosis machinery. Captured virions are subsequently internalized for degradation in lysosomes (39, 42).

There is a general consensus that the mechanism of tetherin counteraction by HIV-1 Vpu begins with a direct physical interaction between the antiparallel membrane-spanning helices of Vpu and tetherin (Fig. 3) (43–45). However, the relative importance of alternative cellular pathways leading to the removal of tetherin from sites of virus release is less clear. A number of studies have shown that casein kinase II phosphorylation of a pair of conserved serine residues (Ser-52 and Ser-56) in the cytoplasmic tail of Vpu recruits β-TrCP2, a component of the SKP1-CUL-F box E3 ubiquitin ligase complex, which leads to the down-regulation and degradation of tetherin (44, 46–48). β-TrCP2-dependent degradation involves non-lysine ubiquitination of residues in the cytoplasmic domain of tetherin (49, 50), which serves as a signal for HRS binding and ESCRT-mediated trafficking to lysosomal compartments (51). Vpu-mediated targeting of tetherin to lysosomes also requires Rab7A (52), a small GTPase essential for the maturation of late endosomes and lysosomal fusion. Other studies have revealed a β-TrCP2-independent mechanism of tetherin antagonism by Vpu, which leads to the sequestration of tetherin in a perinuclear compartment without degradation (48, 53, 54). This could occur either by trapping newly synthesized tetherin or by blocking the recycling of tetherin to the plasma membrane (55, 56). The internalization and trafficking of tetherin by Vpu, whether for sequestration or degradation, depend in part on dynamin-2, a GTPase important for the scission of vesicular membranes (57). Although the relative contribution of pathways leading to the sequestration *versus* degradation of tetherin is presently unclear, it is important to recognize that these are not mutually exclusive mechanisms, and both may contribute to the optimal resistance to tetherin afforded by Vpu.

Most primate lentiviruses do not have Vpu and therefore depend on other viral proteins to counteract tetherin. In the case of certain HIV-2 and SIV isolates, this activity has been acquired by the viral envelope glycoprotein (58–61). Indeed, prior to the identification of tetherin, the envelope glycoproteins of certain HIV-2 isolates were shown to have "Vpu-like" activity that could rescue the release of Vpu-deficient HIV-1 from restrictive cells (62). Tetherin antagonism by Env depends on physical interaction between Env and tetherin and on a conserved tyrosine-based endocytosis motif ($YXX\phi$) in the cytoplasmic tail of the Env transmembrane protein gp41 (59, 60, 63). The residues that contribute to Env-tetherin interactions are not well defined but appear to be located in the extracellular domains of both proteins based on analyses of recombinant forms of Env and tetherin (60, 64) and on the identification of defined amino acid changes in the ectodomains of gp41 and tetherin that disrupt anti-tetherin activity (64–66). Interaction with Env does not result in the degradation of tetherin but instead leads to internalization and sequestration of tetherin

away from sites of virus release at the plasma membrane by a clathrin-dependent pathway (57, 60, 63).

The majority of primate lentiviruses, including phylogenetically diverse SIVs endemic to chimpanzees (SIV_{cpz}), sooty mangabeys (SIV_{smm}), and African green monkeys (SIV_{agm}), use Nef (negative regulatory factor) to counteract the tetherin proteins of their non-human primate hosts (61, 67, 68). In fact, the anti-tetherin activities of HIV-1 Vpu and HIV-2 Env appear to have been acquired after the respective cross-species transmission of SIV_{cpz} and SIV_{smm} into humans due to the absence of a five-amino acid sequence in human tetherin required for susceptibility to Nef (61, 68). SIV Nef counteracts the tetherin proteins of apes and Old World monkeys but not humans through the recognition of residues in the N-terminal cytoplasmic domain ($(G/D^{14})DIWK^{18}$ in rhesus macaque, sooty mangabey, and chimpanzee tetherin) that are missing in human tetherin (61, 68). SIV_{cpz}, SIV_{mac}, and SIV_{agm} Nef proteins down-modulate the tetherin proteins of their respective hosts from the cell surface by AP-2-dependent endocytosis (69). The nature of the molecular interactions between Nef and tetherin and the ultimate fate of tetherin in SIV-infected cells remain to be defined. Nevertheless, current evidence suggests that, similar to HIV-1 Vpu and HIV-2 Env, the anti-tetherin activity of SIV Nef reflects its ability to remove tetherin from sites of virus release at the plasma membrane.

In a remarkable instance of convergent evolution, the envelope glycoprotein of a *nef*-deleted strain of SIV also acquired the ability to counteract tetherin during serial passage in rhesus macaques. Similar to HIV-2 Env, the anti-tetherin activity of this Env is dependent on a physical interaction with tetherin and on the $YXX\phi$ motif in gp41 (58). However, in this case, compensatory changes in the cytoplasmic domain of gp41, rather than in the ectodomain, stabilize a physical interaction with rhesus macaque tetherin (58). These observations imply that tetherin antagonism is important for efficient viral replication *in vivo* and ultimately for pathogenesis.

There is now growing interest in the development of novel therapeutic agents to enhance the antiviral activity of tetherin as a treatment for HIV-1 infection. NMR structural data and computational modeling have provided a high-resolution picture of the transmembrane interface between Vpu and tetherin that represents a promising target for drug design (70, 71). Pharmaceutical disruption of this interface would, in principle, render HIV-1 susceptible to restriction by tetherin, significantly attenuating viral replication *in vivo*. However, features unique to the biology of tetherin suggest that this may not be so straightforward. Unlike other restriction factors, tetherin does not impose an absolute block to viral replication; *vpu*-deleted HIV-1 and *nef*-deleted SIV still replicate, albeit at significantly reduced rates, in primary lymphocytes and infected animals (37, 58). Comparisons of the mechanisms of tetherin antagonism by HIV-1, HIV-2, and SIV also suggest that the primate lentiviruses have unusual latitude in adapting to the tetherin proteins of their respective hosts, having evolved to use at least three different proteins to counteract this restriction. Thus, although the development of drugs to interfere with tetherin antagonism by Vpu certainly represents a worthwhile and potentially fruitful avenue of investigation, these considerations suggest that such drugs probably would not fully suppress HIV-1 replication on their own and might rapidly select for viral resistance.

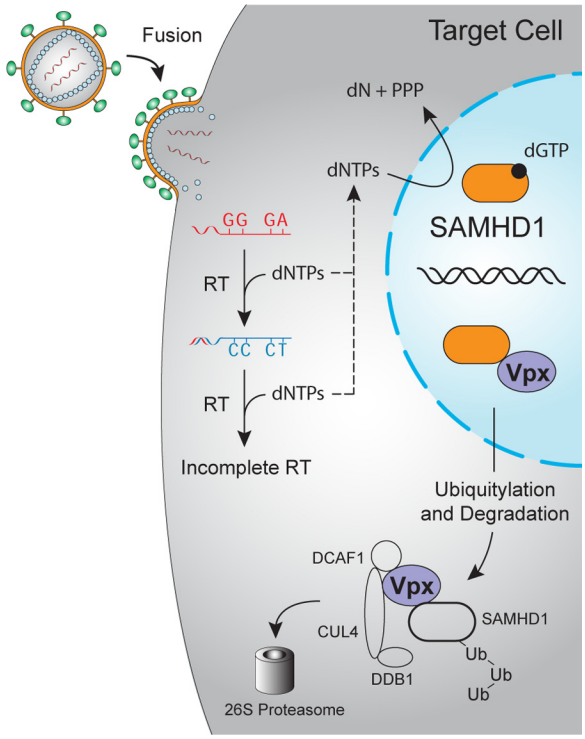

FIGURE 4. **HIV restriction by SAMHD1.** SAMHD1 acts to block HIV-1 reverse transcription (*RT*) by depletion of cellular dNTPs in myeloid target cells. HIV-2/SIV Vpx and some Vpr variants can overcome the SAMHD1 restriction block by acting as an adaptor to an E3 ubiquitin (*Ub*) ligase complex that polyubiquitinates SAMHD1 and targets it for degradation by the 26 S proteasome.

Restriction by Starving Reverse Transcriptase: SAMHD1 dNTP Hydrolase

Myeloid cell types such as macrophages and dendritic cells have long been known to be more resistant to HIV-1 infection than $CD4^+$ T lymphocytes (72–74). Major clues to understanding this large phenotypic difference were obtained in experiments showing that the Vpx (viral protein X) protein of HIV-2 or SIV, naturally absent in HIV-1, improved susceptibility of these cells to HIV-1 infection when delivered by preinfection with Vpx-containing virus-like particles (74–78). Affinity purification and mass spectrometry studies identified SAMHD1 as a Vpx-interacting protein, and supporting functional studies established that SAMHD1 knockdown renders myeloid cell types more permissive to HIV-1 infection (79, 80).

SAMHD1 is composed of a putative protein-interacting sterile alpha motif (SAM) and a C-terminal dNTP phosphohydrolase domain containing conserved histidine and aspartate residues (HD domain). Biochemical activity was predicted based on homology to the EF1143 protein of *Enterococcus faecalis*, which was shown previously to elicit dNTPase activity (81, 82). This mechanistic possibility was tested and confirmed by biochemical studies with the human and mouse enzymes, demonstrating dNTPase activity and a requirement for dGTP as a cofactor (82, 83). A high-resolution crystal structure of the HD domain confirmed strong structural similarities with the *Enterococcus* enzyme (83).

Current evidence supports a model for HIV-1 restriction in which SAMHD1 causes a diminution of cellular dNTP levels that effectively starves reverse transcriptase of essential building blocks needed for viral cDNA synthesis (Fig. 4) (82–85). A failure to complete cDNA synthesis in a timely manner (4–8 h) eventually leads to particle disintegration and component degradation by various cellular proteases and nucleases. HIV infection is thought to trigger an innate immune response, which results in interferon production that feeds forward to elevate cellular SAMHD1 levels. It is not clear, however, whether a single incoming viral particle can trigger an interferon response that elevates SAMHD1 to restrictive levels or whether SAMHD1 up-regulation is a manifestation of a broader interferon-dependent mechanism that serves to protect nearby uninfected cells. Presumably, SAMHD1 up-regulation is not harmful to the cell, as macrophages and dendritic cells are terminally differentiated and do not require high levels of dNTPs.

The HIV-2 and SIV Vpx proteins counteract restriction by triggering the degradation of SAMHD1 (Fig. 4) (79, 80). Vpx serves as a scaffold for the formation of an E3 ubiquitin ligase complex consisting of DCAF1, DDB1, and CUL4, which combine to promote the polyubiquitination and degradation of SAMHD1 (79, 80, 86). Mutagenesis and structural studies are still at early stages, but several reports have already mapped the Vpx-interacting domain to the C terminus of SAMHD1 and identified residues in Vpx required for functional interaction (87, 88). Although Vpx is a relatively new primate lentiviral protein thought to have originated through duplication of the *vpr* (viral protein R) gene, SAMHD1 degradation function predates this duplication. Almost all present day primate Vpx proteins and many (but not all) Vpr proteins share SAMHD1 counter-restriction activity (89, 90). This evolutionary link is supported by mutagenesis studies that have revealed a conserved hydrophobic motif in Vpx/Vpr proteins required for a functional interaction with SAMHD1 (87). Clusters of positive selection map throughout SAMHD1, including to regions outside of the implicated Vpx/Vpr-interacting domain, strongly suggesting past and likely ongoing interactions with a variety of distinct pathogens (88–90).

HIV-1 does not have a Vpx protein, nor does it appear to have a Vpr protein capable of interacting with SAMHD1. This deficiency provides a molecular explanation for the resistance of myeloid cell types to HIV-1 infection. It also suggests a provocative hypothesis that could explain why HIV-2 is less pathogenic than HIV-1 and why many SIV strains are non-pathogenic in monkey populations. Greater infection of myeloid cells may shift the balance of antigen presentation to favor reduced immune activation and $CD4^+$ T cell turnover (91). Thus, small-molecule inhibitors of SAMHD1 may provide a means to induce better immune responses and natural control of HIV-1 infection.

Additional Restriction Factors and the Bigger Picture

Undoubtedly, additional HIV restriction factors await discovery. For instance, $CD4^+$ T cells are much more susceptible to infection by Nef-expressing compared with Nef-deficient viruses, suggesting that this accessory protein may serve to counteract other cellular restriction factors (92–94). The cellular target(s) of Vpr and its associated E3 ligase complex has also yet to be identified (17, 95). Other cellular proteins such as human TRIM5α do not inhibit HIV-1, although they are clearly *bona fide* retrovirus restriction factors (1, 96). In fact, a single amino acid substitution in human TRIM5α can endow strong HIV-1 restriction activity, suggesting a strategy for gene therapy and/or small-molecule mimicry (97, 98).

Although the examples of restriction and counter-restriction described here are largely binary and involve viral proteins that are dispensable under some growth conditions, it is probable that a subset of viral counterdefense mechanisms could be complicated by overlapping function. For instance, a counterdefense measure might be embedded within an essential retroviral protein such as Gag, reverse transcriptase, or Env. In these instances, identifying the salient viral protein domain(s) and the relevant host restriction factor may require a combination of biochemical, proteomic, and genetic methods to clearly distinguish between counter-restriction activities and essential viral functions.

Another theme, perhaps best illustrated by tetherin, is the likelihood that the virus may have multiple ways to block restriction (*i.e.* functional redundancy). If a restriction factor poses a great enough threat, the virus could very well employ parallel counter-restriction mechanisms. Moreover, there may be major and minor counter-restriction mechanisms, such that if a major pathway is inactivated or nonfunctional, the minor pathway may become more prominent. Of potential concern is the possibility that the activation of a minor pathway may only require the virus to make a small number of amino acid changes. Thus, not only should obvious therapeutic possibilities be explored (such as small molecules that promote virus restriction by antagonizing major counter-restriction processes), but less obvious pathways of counter-restriction should be investigated to ensure the virus does not have easy escape routes (*e.g.* Refs. 58, 99, and 100).

As with the first decade of HIV restriction factors, beginning roughly with the discovery of APOBEC3G in 2002, the second decade is likely to be equally as informative and exciting. One should look forward to the discovery of additional restriction mechanisms and to the translation of existing knowledge into new therapeutics. HIV is likely to remain at pandemic proportions until a cure is found and applied at the population level. Given the extreme hypervariability of HIV, it would be naïve to assume that one strategy such as vaccination or mono-drug therapy will be curative. Like current combination drug therapies, which simultaneously block multiple viral enzymes, a curative therapy may need to exploit multiple mechanisms. It is conceivable that leveraging a natural restriction factor against the virus, in combination with other methods such as eliminating latent cells and/or boosting antibody responses, may be necessary to extinguish HIV from the human population.

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