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Intrinsic host restrictions to HIV-1 and mechanisms of viral escape

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Abstract

To replicate in their hosts, viruses have to navigate the complexities of the mammalian cell, co-opting mechanisms of cellular physiology while defeating restriction factors that are dedicated to halting their progression. Primate lentiviruses devote a relatively large portion of their coding capacity to counteracting restriction factors by encoding accessory proteins dedicated to neutralizing the antiviral function of these intracellular inhibitors. Research into the roles of the accessory proteins has revealed the existence of previously undetected intrinsic defenses, provided insight into the evolution of primate lentiviruses as they adapt to new species and uncovered new targets for the development of therapeutics. This Review discusses the biology of the restriction factors APOBEC3, SAMHD1 and tetherin and the viral accessory proteins that counteract them.

When the nucleotide sequence of HIV-1 was determined in 1983, the complexity of its genome as compared to those of the murine and avian retroviruses became immediately apparent. In addition to the *gag*, *pol* and *env* genes that encode the structural proteins in all retroviruses, there are open reading frames in the 3′ portion of the genome that have no homologs in the genomes of the simpler animal retroviruses. In the years since, research into the regulatory and accessory proteins encoded by these open reading frames has provided fascinating insight not only into virus replication but into cell biology and immunology as well. The Tat and Rev regulatory proteins were found to be required for the replication of the virus in all cells, serving to induce transcription of the proviral DNA and to transport the unspliced and partially spliced viral RNA transcripts from the nucleus to cytoplasm, respectively. More mysterious were the accessory proteins that were found to be required for the virus to replicate in some, but not all, cell types (Vif, Vpx, Vpu, Nef and Vpr). As the roles of accessory proteins in virus replication were unraveled, the theme that has repeatedly emerged is that they serve as a means to counteract host antiviral defense mechanisms. It was through efforts to understand the roles of accessory proteins in virus replication that previously unimagined antiviral defense mechanisms and the ‘restriction factors’ that mediate them were identified. Viruses go to great lengths to counteract these restriction

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factors, devoting a substantial portion of their coding capacity to the task and devising complex splicing signals and overlapping reading frames to express accessory proteins. We review here the APOBEC3, SAMHD1 and tetherin (also known as BST2) restriction factors and the accessory proteins that the virus encodes to counteract them (Fig. 1).

Vif and the APOBEC3 cytidine deaminases

The virion infectivity factor (Vif) is encoded by all primate lentiviruses except equine infectious anemia virus[1]. Vif is required for lentiviral replication in nonpermissive cells, such as $CD4^+$ T cells and monocyte-derived macrophages (MDMs)[2], whereas in permissive T cell lines, Vif-deleted (*Δvif*) HIV-1 viruses replicate to high levels[3,4]. Moreover, *Δvif* HIV-1 virions produced in permissive cells are fully infectious and infect both permissive and nonpermissive cells[4]. Progress in understanding the role of Vif was stalled for several years until the elusive dominant inhibitor was identified as apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (A3G)[5].

A3G belongs to a family of single-stranded DNA deaminases[6,7]. The *APOBEC3* gene locus expanded from a single gene in rodents to seven in primates (encoding three single-domain deaminases, A3A, A3C, A3H and four double-domain deaminases, A3B, A3D, A3F, A3G)[7]. *APOBEC3* genes have been subject to strong selective pressure resulting from mutations that provided resistance to lentivirus infection[8,9]. APOBEC3 proteins are expressed in many cell types, including $CD4^+$ T cells and MDMs[10]. Work from many laboratories has elucidated the mode of action of APOBEC3 proteins and the mechanism by which they are degraded by Vif[11,12]. Initial studies focused on A3G, but A3B, A3D, A3F and A3H are also active against HIV-1.

APOBEC3 proteins, if not degraded by Vif, potently inhibit lentivirus replication through cytidine deamination of the viral genome. The antiviral activity requires that the proteins are packaged into the virion and is manifested only in the next cycle of infection (Fig. 2a)[13,14]. The reverse transcription products generated by the *Δvif* HIV-1 virions produced in cells that express APOBEC3 is characterized by the presence of numerous G→A mutations. These result from APOBEC3-mediated C→U deamination of the minus strand of the virus DNA[14]. Because U is read as T by the polymerase, synthesis of the plus strand results in G→A mutation. Depending on the degree of deamination, the reverse transcripts either are recognized as aberrant by the cell and degraded or become integrated into the genomic DNA but, because they contain translational termination codons and missense mutations, fail to produce infectious progeny[14,15] (Fig. 2a).

APOBEC3 proteins are packaged into virions as they assemble at the plasma membrane through interactions with several types of RNA (viral RNA, small noncoding RNAs, cellular mRNA) in a nucleocapsid-dependent manner[16,17]. Although virion packaging of APOBEC3 proteins is required for antiviral activity, it may not be sufficient. For example, A3A is a potent cytidine deaminase, but when packaged in virions it has little effect on infectivity. Packaging A3A in virions by fusing it to Vpr activates the ability of the deaminase to inhibit infectivity by altering its localization within the virion[18].

APOBEC3-driven mutagenesis occurs over the entire viral genome, with a gradient of higher mutational frequency toward the 3′ end, an effect that is likely to result from this region of the viral genome remaining single-stranded for an extended time during reverse transcription[19,20]. Although many cytosine nucleotides are targeted for deamination, APOBEC3 proteins have preferences for specific dinucleotides in single-stranded DNA (minus strand: 5′-CC, 5′-TC[11,20,21]). The target-site preferences of the APOBEC3 proteins provide clues to the identity of the deaminase that left its footprint on a provirus: GGD (A3G, A3D), GAG (A3G) or GAD and GGA (A3F, A3H), where D is A, G or T[11,21]. Mutations in the A3G- or A3D-favored context (GG→GA) frequently produce translation termination codons (for example, TGG→TAG), enhancing the lethality of these deaminases[11,12,21].

Although cytidine deamination is the primary mechanism by which APOBEC3 proteins restrict lentiviruses, there is evidence that they also exert deaminase-independent antiviral activity. Virion-packaged APOBEC3 can interfere with reverse transcription by preventing tRNA binding to the primer-binding site on the viral genomic RNA or by causing termination of minus-strand synthesis[22,23]. The contribution of deaminase-independent restriction remains to be determined in light of reports that catalytically inactive A3G mutants lack antiviral activity[24,25].

To counteract the antiviral activity of APOBEC3 proteins, Vif induces their degradation before virion packaging (Fig. 2a). Vif binds to APOBEC3 molecules in the producer cell and recruits a cullin 5–based E3 ubiquitin ligase complex consisting of elongin B, elongin C and Rbx-1 (refs. 26,27). Vif expression is further stabilized by CBF-b, a transcription factor[28–31]. The complex ubiquitinates the bound APOBEC3 proteins, which are then rapidly degraded by proteasomes[13,32]. The crystal structure of the Vif-CBF-b E3 ligase pentameric complex[33] shows Vif in an elongated cone-like shape with a larger and a smaller domain separated by a zinc-binding region[33]. The structure of Vif bound to APOBEC3 has not been determined but extensive evidence indicates that the N terminus of Vif interacts with the different APOBEC3 (refs. 11,12). The presence of A3G-, A3F- and A3H-specific amino acid motifs within Vif suggests that its activity against individual APOBEC3 proteins can adapt for optimal virus replication[11,34–36].

Although lentiviruses go to great lengths to exclude APOBEC3 proteins from their virions, the packaging of the proteins in small amounts may provide a benefit to the virus. Nonlethal mutagenesis could provide a means by which the virus can generate sequence diversity to accelerate its evolution (Fig. 2d). The inability of Vif to fully prevent deamination is demonstrated by the hypermutated proviruses found in acutely and chronically infected patients and in vertically infected infants[12,21]. Several factors could contribute to incomplete counteraction of APOBEC3 by Vif. Interferon induction may cause the production of an amount of APOBEC3 sufficient to overwhelm the Vif produced by the virus; and viruses may encode Vif variants with reduced affinity for specific APOBEC3. The possibility that sublethal deamination may lead to beneficial genetic diversity has been demonstrated in cell culture by the appearance of antiretroviral drug–resistant virus as a result of APOBEC3-generated G→A mutation[37,38]. In support of this possibility *in vivo*, antiretroviral treatment failure has been found in some cases to be associated with viruses that bear partially

defective Vif alleles[39]. In humanized mouse models, APOBEC3 increases the diversity of viral T cell epitopes, allowing escape from cytotoxic T lymphocytes[40–42]. APOBEC3 may also facilitate co-receptor switching by generating G→A mutations in *env* that encode amino acids in the V3 loop of gp120 (ref. 40).

Pandemic HIV-1 strains are resistant to multiple APOBEC3 proteins. Although these circulating viruses generally counteract A3G and A3F efficiently[43–45], not all viral strains can target A3H for degradation[45]. The explanation for the differential susceptibility of HIV to A3H is found in the fact that not every individual expresses an active A3H haplotype[46]. Indeed, single-nucleotide polymorphisms in the human A3H coding region result either in stable variants that display potent antiviral activity or in unstable variants that are inactive[46–50] (Fig. 2b). As the virus is transmitted and successfully spreads in the new host, its Vif has to evolve to counteract the recipient's A3H haplotype[36] (Fig. 2c). The failure of Vif to adapt could prevent transmission or attenuate HIV disease presentation in the case of an A3H-sensitive virus that encounters a host with stable A3H[36,50] (Fig. 2c)—a scenario that is reminiscent of the role played by A3G in limiting the zoonotic transmission of lentiviruses from nonhuman primates. Because Vif-APOBEC3 interactions are species specific, they may determine whether a virus will be transmitted across species. A single charged amino acid at position 128 of A3G determines whether Vif will bind to human or nonhuman primate APOBEC3 proteins[51–53]. A successful jump across species requires that the nonhuman primate Vif be able to counteract the APOBEC3 proteins of the new host[53,54]. The APOBEC3-Vif axis is a key element of viral pathogenicity. It will be important in future studies to determine the degree of sensitivity of circulating viral strains to each APOBEC3 protein as a means of understanding how Vif diversity modulates AIDS progression.

SAMHD1 restriction of HIV-1 in non-dividing cells

The Vpr and Vpx accessory proteins arose by gene duplication during evolution and remain about 30% similar in amino acid sequence[55,56]. Both are virion packaged, a unique feature among the nonstructural HIV proteins. Both localize to the nucleus of the cell, yet lack a consensus nuclear localization sequence (NLS). Deletion of Vpx from SIVmac has no effect on the ability of the virus to infect activated $CD4^+$ T cells, but renders the virus unable to infect myeloid cells such as dendritic cells (DCs) and MDMs[57,58]. Upon infection, few reverse transcripts are produced, suggesting a failure of reverse transcriptase to synthesize viral DNA. Although Vpx has no effect on the infection of activated T cells, it increases the ability of the virus to infect resting T cells[59,60]. Resting T cells do not support productive virus replication because of several blocks in the virus life-cycle. By relieving the block to reverse transcription in resting T cells, Vpx could allow the establishment of latently infected cells which upon subsequent activation would become producers of infectious virus. For HIV-2 and SIV, this effect could contribute to the pool of latently infected T cells that sustains chronic infection.

Vpr is encoded by all HIV and SIV lineages, whereas Vpx is present only in HIV-2 and some SIVs, notably those from macaques (SIVmac) and from red-capped (SIVrcm) and sooty mangabeys (SIVsm). Although HIV-1 does not encode Vpx, the virus is susceptible to the infectivity enhancement provided by the SIV accessory protein. Introducing Vpx from

SIV into MDMs via Vpx-containing virus-like particles (VLPs) increases their susceptibility to HIV-1 by two orders of magnitude[61]. Moreover, HIV-1 that has been engineered to package SIV Vpx infects human DCs and MDMs with a similar increase in titer[62,63], a feature that has been exploited as a means of producing lentiviral vectors that efficiently transduce primary myeloid cells[64,65]. Thus, it would appear advantageous for HIV-1 to encode Vpx, yet, curiously, it does not.

In cells, Vpx forms a complex with a cullin 4A–based E3 ubiquitin ligase, the components of which include DCAF1 and DDB1 (refs. 66,67). The complex regulates the degradation of a large number of cellular DNA repair proteins, replication enzymes and transcription factors. Its association with Vpx provided the first clue that Vpx might serve to neutralize a host restriction factor. Using a mass spectrometry–pull-down approach, the restriction factor was identified as SAM domain–and HD domain–containing protein 1 (SAMHD1)[68,69]. Vpx in virions or VLPs induces the degradation of SAMHD1, and knockdown of SAMHD1 in DCs relieves the block to infection by HIV-1 (refs. 68,69). To study the role of SAMHD1 *in vivo*, two groups generated SAMHD1-knockout mice[70,71]. MDMs from these SAMHD1-deficient mice are more susceptible to infection by HIV-1 (ref. 70) or an attenuated HIV-1 with a mutation in reverse transcriptase[71]. The deficiency did not increase susceptibility to mouse retroviruses, most likely because of additional blocks to retrovirus infection of myeloid cells[70,71].

SAMHD1 consists of an N-terminal SAM (sterile alpha motif) domain and a C-terminal HD domain, the latter of which is characteristic of a class of enzymes with phosphodiesterase, phosphatase and nuclease activities. The enzyme contains an N-terminal consensus NLS and localizes to the nucleus[72,73]. Upon infection of MDM with SIV, HIV-2 or Vpx-containing engineered HIV-1, SAMHD1 is rapidly degraded. Its abundance starts to fall about an hour after infection and remains low for several days[74,75]. The deletion of amino acids 13–17 removes the NLS of SAMHD1, localizing it to the cytoplasm[72,73]. NLS-deficient SAMHD1 retains antiviral activity but cannot be degraded by Vpx, suggesting that Vpx seeks out SAMHD1 in the nucleus[72,73]. The need for Vpx to target SAMHD1 in the nucleus may explain its use of the DCAF1-DDB1 E3 ubiquitin ligase, a complex that is specialized in the degradation of nuclear proteins.

Recombinant SAMHD1 produced in *Escherichia coli* has dGTP-regulated phosphohydrolase activity that removes the triphosphate from deoxynucleotide triphosphates[76–78]. When expressed by lentiviral vector transduction in differentiated U937 cells, SAMHD1 causes a dramatic drop in the concentration of dNTPs[78], suggesting a straightforward mechanism of restriction. After virus entry, reverse transcriptase generates a double-stranded DNA using the cellular pool of dNTPs. SAMHD1 could block HIV-1 infection by causing the concentration of dNTPs to fall to a level insufficient to support reverse transcription. In the case of HIV-2 or SIV infection, Vpx would be released from the virion, inducing the degradation of SAMHD1. As dNTPs levels were increased, reverse transcription would then proceed. In support of this mechanism, SAMHD1-mediated restriction is relieved by the addition of exogenous deoxynucleosides (dN) to DCs[78]. Moreover, such a mechanism could act more broadly. SAMHD1 blocks a wide range of retroviruses yet, interestingly, is inactive

against foamy viruses, a class of retrovirus that is unusual for its ability to complete reverse transcription in the virion, before entry[79].

Although this model made sense, recent findings have called into question whether dNTP pool depletion fully accounts for SAMHD1-mediated restriction. In activated cells, SAMHD1 is phosphorylated by specific cyclin-dependent kinases at amino acid Thr592. SAMHD1 with a T592E mutation that mimics the phosphorylated protein shows loss of antiviral activity[80–83] yet, surprisingly, appears to retain dNTPase activity[82]. This finding would suggest that dNTP depletion is either not sufficient for antiviral activity or not required. If that is the case, then how does SAMHD1 restrict virus replication? Perhaps rather than preventing reverse transcription, it attacks the viral RNA or the newly synthesized viral DNA. In fact, *E. coli*–produced recombinant SAMHD1 was reported to have RNA and single-strand DNA exonuclease activity on synthetic substrates[84]. In an analysis of the relative contributions of the two catalytic activities to restriction, the dNTPase and RNase activities of SAMHD1 were genetically separated by point mutations[85]. A D137N mutation disabled the dNTPase but not the RNase activity yet had no effect on antiviral activity. Conversely, a Q548A mutation that inactivated the RNase but not the dNTPase activity caused the loss of antiviral activity, implicating the RNase and not the dNTPase activity as the mediator of virus restriction (Fig. 3). In spite of these findings, it is difficult to understand how SAMHD1 could degrade the viral genomic RNA or reverse transcription intermediates given that reverse transcription is generally thought to at least initiate in the cytoplasm and SAMHD1 is largely nuclear. In addition, Vpx-containing VLPs relieve the block to infection in DCs when added to cells several hours after infection[86,87]. Similarly, dNs added to DCs several hours after infection relieve the block to infection, and a temporary pharmacologic block to E3 ubiquitin ligase function stalls but does not prevent infection[86]. The reversibility of SAMHD1-mediated restriction is consistent with a lack of nucleotides, but hard to reconcile with a nucleolytic attack of the viral RNA.

Another piece of the puzzle comes from an entirely different field of research. Aicardi-Goutières syndrome (AGS), a rare autosomal recessive neurological condition with early onset, is characterized by high expression of type I interferon (type I IFN) in the central nervous system and upregulation of IFN-stimulated genes. The disease can be caused by defects in the genes encoding SAMHD1, TREX1, RNase H2A, RNase H2B, RNase H2C, ADAR or IFIH1 (ref. 88), all of which are enzymes involved in nucleotide or nucleic acid metabolism. The $3'\rightarrow5'$ cytoplasmic single-strand DNA exonuclease TREX1 is a negative regulator of the interferon-stimulatory DNA response[89] that digests endogenous cytoplasmic retroelement DNA, thereby preventing the activation of DNA sensors such as cGAS[90]. Such activation would trigger innate immune response pathways, leading to chronic inflammation similar to what is observed in patients with AGS. TREX1 is also thought to digest newly synthesized HIV-1 DNA, thereby limiting the activation of host antiviral defenses[90,91]. Similarly, SAMHD1 could control endogenous elements by degrading them or preventing their synthesis[92], further blunting the innate immune response. In the absence of SAMHD1, the accumulation of cytoplasmic DNA would result in chronic inflammation. Consistent with this possibility, in SAMHD1-null mice, some interferon-stimulated genes are constitutively induced[70,71]. Such a model would offer a unified explanation as to how SAMHD1 restricts HIV-1 and causes AGS. However, evidence for increased amounts of

endogenous retroelement RNA or DNA in SAMHD1-deficient mice or AGS patients is lacking.

How does HIV-1 survive without Vpx?

Although Vpx is the accessory protein most closely associated with inducing SAMHD1 degradation, it appears that it was Vpr that initially evolved for this purpose[93]. Consistent with this model, the SIV of African green monkeys (SIVagm) encodes Vpr but not Vpx, and its Vpr degrades SAMHD1. As the primate species evolved, mutations in the *SAMHD1* gene that allowed for escape from SIV Vpx were selected An analysis of primate SAMHD1 sequences suggests that precisely those amino acids that are contacted by Vpx were under selective pressure[93]. The duplication of the Vpr open reading frame in the evolution of SIV allowed the two genes to specialize their functions, allowing Vpx the freedom to better adapt to amino acid sequence changes in SAMHD1. As the virus jumped from Old World monkeys to chimpanzees, before its transfer into humans, it deleted *vpx*, giving rise to the current SIV of chimpanzees (SIVcpz)[94]. Deletion of *vpx* disabled the ability of the virus to degrade SAMHD1 but freed *vif*, which overlaps with *vpx*, to alter its sequence, allowing it to evolve to better counteract chimpanzee APOBEC3.

The absence of Vpx in HIV-1 raises the question of how the virus replicates and induces pathogenesis without the benefit of an important accessory protein. One possibility is that the virus has assigned the role of Vpx to another viral protein. For example, HIV-1 reverse transcriptase has a higher affinity for dNTPs than that of HIV-2 or SIV, allowing it to synthesize DNA in low dNTP concentrations[95]. It has also been suggested that HIV-1 does induce SAMHD1 degradation, not through an accessory protein but by activating host cyclin L2 (ref. 96). Another possibility is simply that the virus, unlike HIV-2 or SIV, survives without the need to counteract the restriction. That HIV-1 largely fails to escape SAMHD1-mediated restriction is nicely demonstrated by the ability of Vpx to enhance the infectivity of the virus on DCs. HIV-1 could survive with little need to infect myeloid cells, replicating largely by T cell–to–T cell rather than MDM–to–T cell transmission. In contrast, HIV-2 and SIV may be dependent on MDM–to–T cell transfer of virus, an effective means of transmission in a setting in which there are few activated T cells, as is the case in the natural primate hosts. HIV-1 infection in humans may cause generalized T cell activation, providing a sufficient number of activated target cells to sustain the infection without the need for MDM–to–T cell transfer. In support of this model, rhesus macaques infected with *Δvpx* SIVmac have few infected myeloid lineage cells and low T cell viral burdens[97]. A lesson to be learned here is that even though HIV-1 has no Vpx, understanding this accessory protein has provided insight into AIDS pathogenesis.

The role of Vpu and tetherin in virus release

The Vpu accessory protein is a type I transmembrane protein that is expressed from a bicistronic viral mRNA and localizes to the ER. It is encoded by HIV-1 and some SIVs (those of macaques, mona monkeys and greater spot-nosed monkeys) but not by HIV-2 or most other SIVs. *Δvpu* HIV-1 has two distinct phenotypic traits that distinguish it from the wild-type virus: the virions have fewer envelope glycoprotein spikes, and they accumulate in

massive clusters on the cell surface because they fail to detach from the producer cell[98–100]. Early studies showed that the decreased number of envelope glycoprotein spikes results from the interaction between CD4 and gp160 in the Golgi[101], which prevents the transit of gp160 to the plasma membrane. Vpu acts on CD4 to prevent its interaction with gp160, and as such it frees gp160 to transit to the plasma membrane. The failure of *Δvpu* HIV-1 to release virions was more difficult to understand. The effects of Vpu on CD4 and on virion release were genetically separable by point mutations in Vpu that affected one function or the other. Moreover, the block to virion release was active only in certain cell types[102,103] and could be enhanced by type I interferon[104]. The analysis of heterokaryons formed between permissive and nonpermissive cell types showed that the block in virion release was dominant, suggesting that it was caused by an inhibitor of this step[105]. A microarray screen to identify membrane-associated, interferon-inducible proteins specific to nonpermissive cell types identified the bone marrow stromal cell antigen BST2 (also known as CD317) (ref. 104). Transfection of BST2 into permissive cells blocked virion release by *Δvpu* HIV-1 but not wild-type HIV-1, confirming that BST2 is the Vpu target[106,107]. The protein was re-christened ‘tetherin’ for its role in tethering virus to the plasma membrane.

The interesting topology of tetherin immediately suggested how it might prevent the release of enveloped viruses. The protein is a disulfide-bonded homodimer with an extended coiled-coil alpha-helical extracellular domain flanked by a short cytoplasmic tail, an N-terminal transmembrane anchor and a C-terminal glycosyl-phosphatidylinositol (GPI) linkage[108,109]. If it were to insert one end into the viral lipid bilayer and the other into the cell plasma membrane, the virus would be unable to leave from the cell surface. In support of this model, the amino acid sequence of the central domain of tetherin is not critical for its function. Remarkably, an artificial tetherin could be constructed from the functional domains of unrelated proteins, demonstrating that it is the topology of the protein and not its sequence that endows it with antiviral activity[110]. Tetherin could presumably insert in either orientation in the viral and cell membranes. However, proteolytic treatment of tethered virions leaves an N-terminal fragment of tetherin in the plasma membrane, suggesting that this orientation is preferred[102]. To relieve the block to virus release, Vpu interacts with tetherin, sequestering it in the perinuclear region and causing its endocytosis and proteasomal degradation.

In addition to its role in preventing virion release, tetherin has an additional function as a sensor of viral infection (Fig. 4). As tetherin grabs onto budding virions, it clusters at the plasma membrane, becoming unlinked from the underlying cortical actin cytoskeleton and exposing dual tyrosines in the cytoplasmic domain that become targets for phosphorylation by the tyrosine kinase Syk[105,111–113]. The phosphorylated tetherin molecules recruit the signaling adaptors TRAF2, TRAF6 and the mitogen-activated protein kinase TAK-1; activates the transcription factor NF-kB[111,113]; and induces the expression of the proinflammatory cytokines CXCL10, IL-6 and type I IFN[111]. Tetherin activates NF-κB in HIV-1-infected cells, leading to the production of interferon, and this is prevented by Vpu[114]. Thus, Vpu antagonizes both the effect of tetherin on viral release and its role in viral sensing. During the course of chronic HIV-1 infection, as viral sequence variants accumulate, the ability of tetherin to downregulate CD4, sequester tetherin and prevent

innate immune responses is preserved, indicating the importance of these functions *in vivo*[115].

How do HIV-2 and SIVs that lack Vpu escape restriction by tetherin? In SIVcpz SIVmac, SIVagm and SIVsm, this function is reassigned to Nef[116–118]. In these viruses, Nef binds to the cytoplasmic N terminus of tetherin, inducing its endocytosis in an AP-2-dependent mechanism. Human tetherin is not subject to antagonism by Nef because it has a deletion of the Nef binding site at amino acids 14–18 (ref. 119). Thus, zoonosis of SIVcpz into humans forced the virus to find another means to counteract tetherin. With just a few amino acid sequence changes, SIVcpz Vpu acquires the ability to target human tetherin[120]. The ease with which the species-specificity of Vpu could be altered is thought to have facilitated the ability of the virus to establish itself in humans. HIV-2 antagonizes tetherin with yet another viral protein. The virus, which is derived from SIVsm, lacks Vpu and instead uses its envelope glycoprotein protein for this purpose[121,122]. This evolutionary adaptation was recreated experimentally in *Δnef* SIV–infected rhesus macaques. During *in vivo* passage, the virus generated mutations in gp41 that allowed for escape from tetherin, mimicking HIV-2. Taken together, these findings again demonstrate the plasticity with which viral proteins mold to acquire different functions as they adapt to new species[123].

Concluding remarks

This Review has focused on the three restriction factors that lentivirus have evolved accessory proteins to escape, but cells express other antiviral factors, some that have been identified and, most likely, others that remain to be discovered. In the case of antiviral proteins that are not counteracted by accessory proteins, the virus may have found an alternative means of escape. HIV-1 escapes TRIM5α by altering its capsid protein, for example. For antiviral proteins such as MxB, an interferon-induced protein that targets the viral capsid, the virus may simply live with a decreased ability to replicate.

Despite a plethora of sophisticated host antiviral defense mechanisms, viruses continue to ravage the human species. Viruses like HIV-1 are evolutionarily nimble, replicating small genomes with a generation time on the order of hours to produce huge numbers of genetically diverse progeny. HIV-1 has a remarkable degree of genetic plasticity that allows its proteins to interact with the altered host proteins it encounters during species transmission and that gives it the ability to swap the functionality of viral proteins when point mutations are insufficient. Although the virus may have found ways around antiviral host defenses, the restriction factors and viral accessory proteins provide targets for the development of new strategies by which the virus will be ultimately defeated. The study of the lentiviral accessory proteins has led to the discovery of new mechanisms of intrinsic immunity, continuing a long history of viruses teaching us about the inner working of cells.

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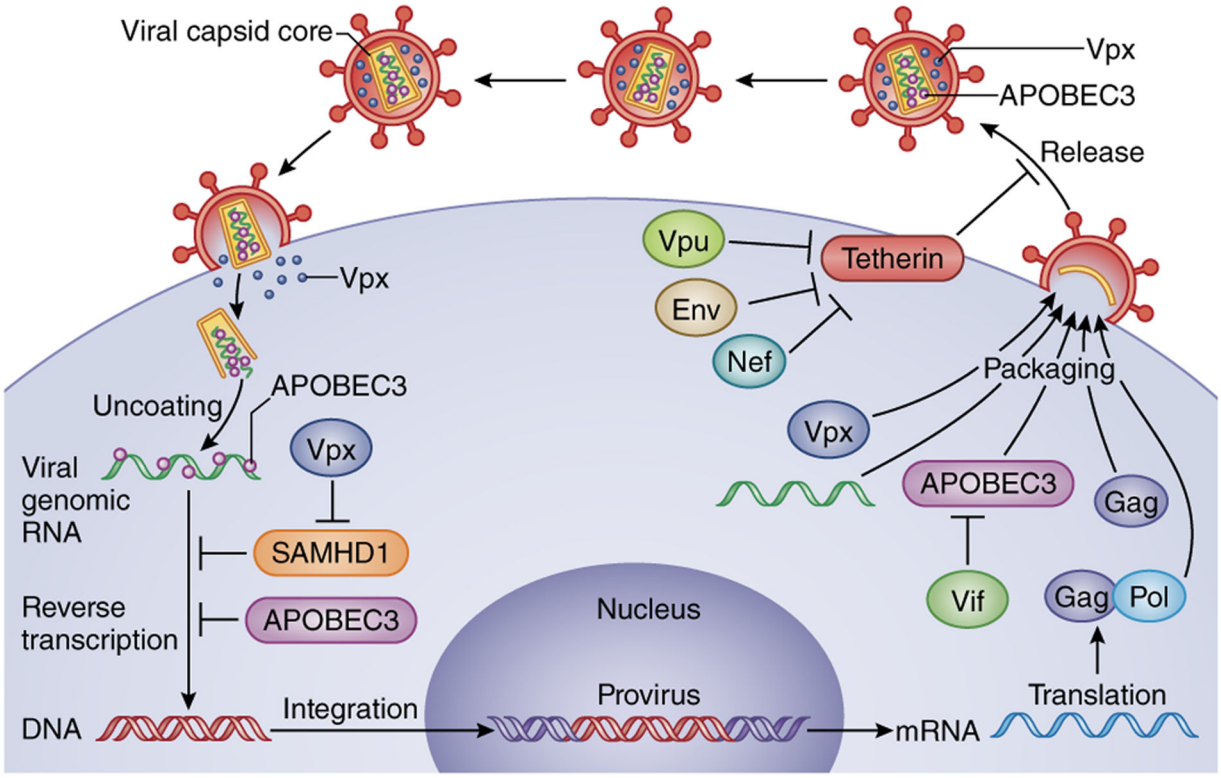

Figure 1.

The host restriction factors SAMHD1, APOBEC3 and tetherin, and the lentiviral accessory proteins that counteract them in the context of virus replication. After virus entry, SAMHD1 and APOBEC3 interfere with reverse transcription (SAMHD1) or modify the reverse-transcribed viral DNA (APOBEC3). Tetherin acts late in the replication cycle to prevent the release of virions. Vif counteracts APOBEC3-driven mutagenesis of the viral genome, by preventing its packaging into virions. Vpx relieves the SAMHD1-mediated block to reverse transcription. Vpu prevents tetherin from holding on to the virus at the plasma membrane. HIV-2 and SIVs that lack Vpu use Env and Nef, respectively, to counteract tetherin by sequestering it intracellularly.

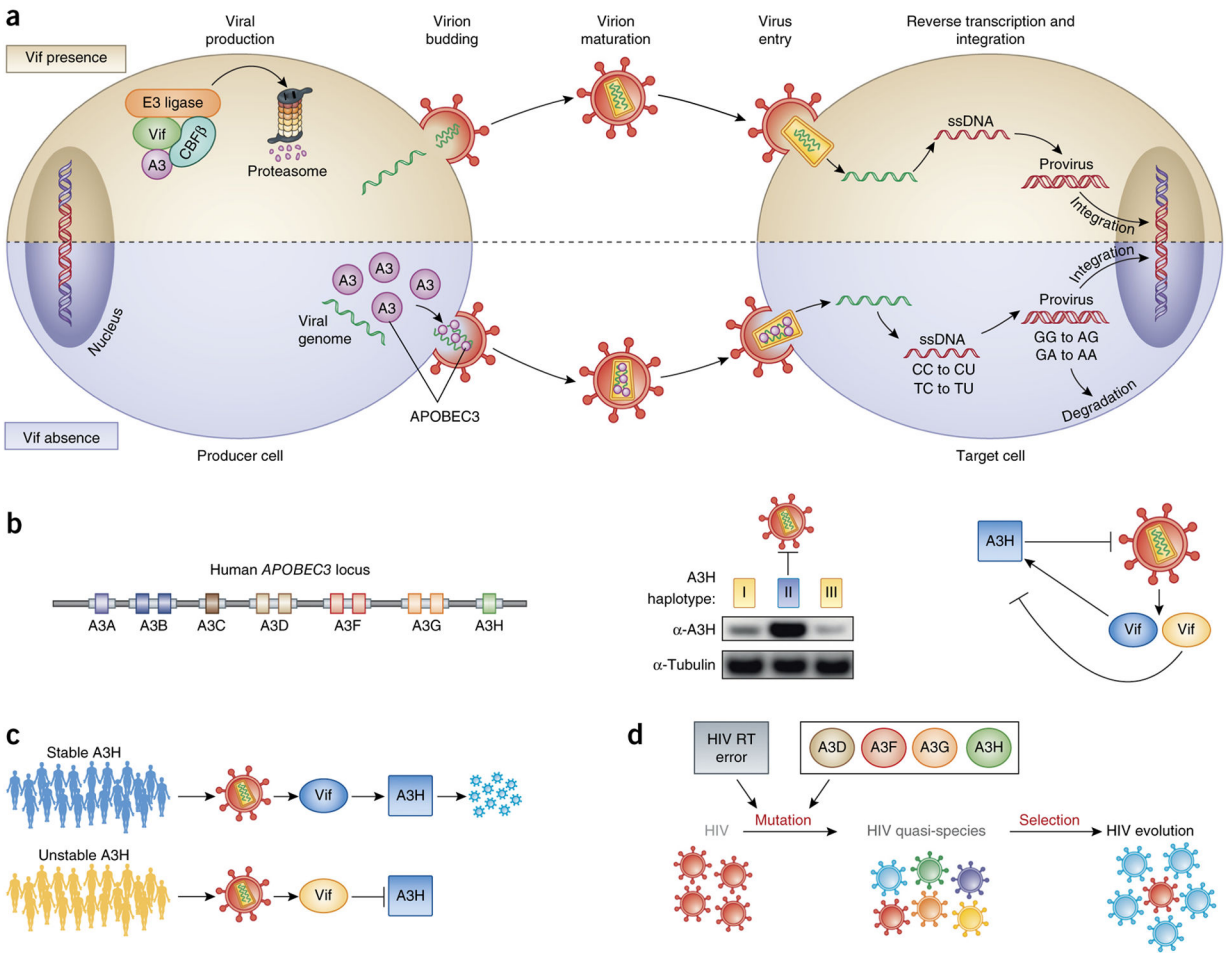

Figure 2.

Overview of the mode of APOBEC3 (A3) restriction and the implications of suboptimal Vif activity on HIV transmission and diversification. (**a**) APOBEC3 proteins are degraded in the producer cell in the presence of Vif, but in the absence of Vif they are packaged into the budding viral particle. In the next cycle of infection, the APOBEC3 proteins mutagenize the viral genome during reverse transcription by deaminating cytosines to uracils in the minus-strand DNA. Mutated viral DNA may be degraded by DNA repair enzymes or integrated into the host cell genome. (**b**) Left, the human *APOBEC3* locus encodes seven different deaminases that carry either one (A3A, A3C, A3H) or two deaminase domains (A3B, A3D, A3F, A3G). Middle, A3H stands out among the APOBEC3 proteins because its haplotypes differ in protein stability and antiviral activity: haplotypes I and III (yellow) are unstable, whereas haplotype II (blue) is stable. Right, HIV Vif alleles differ in their ability to counteract the stable A3H haplotype II. (**c**) HIV Vif adapts to A3H haplotypes *in vivo*. A3H-resistant HIV (blue Vif) efficiently replicates in patients carrying an active A3H (blue individual). A3H-sensitive HIV (yellow Vif) replicates well in patients with unstable A3H (yellow individuals), but when it is transmitted to a new host who encodes stable A3H, its spread is limited. (**d**) HIV sequence diversification can be caused by reverse transcriptase (RT) errors and mutagenesis by different APOBEC3 proteins (A3D, A3F, A3G, A3H).

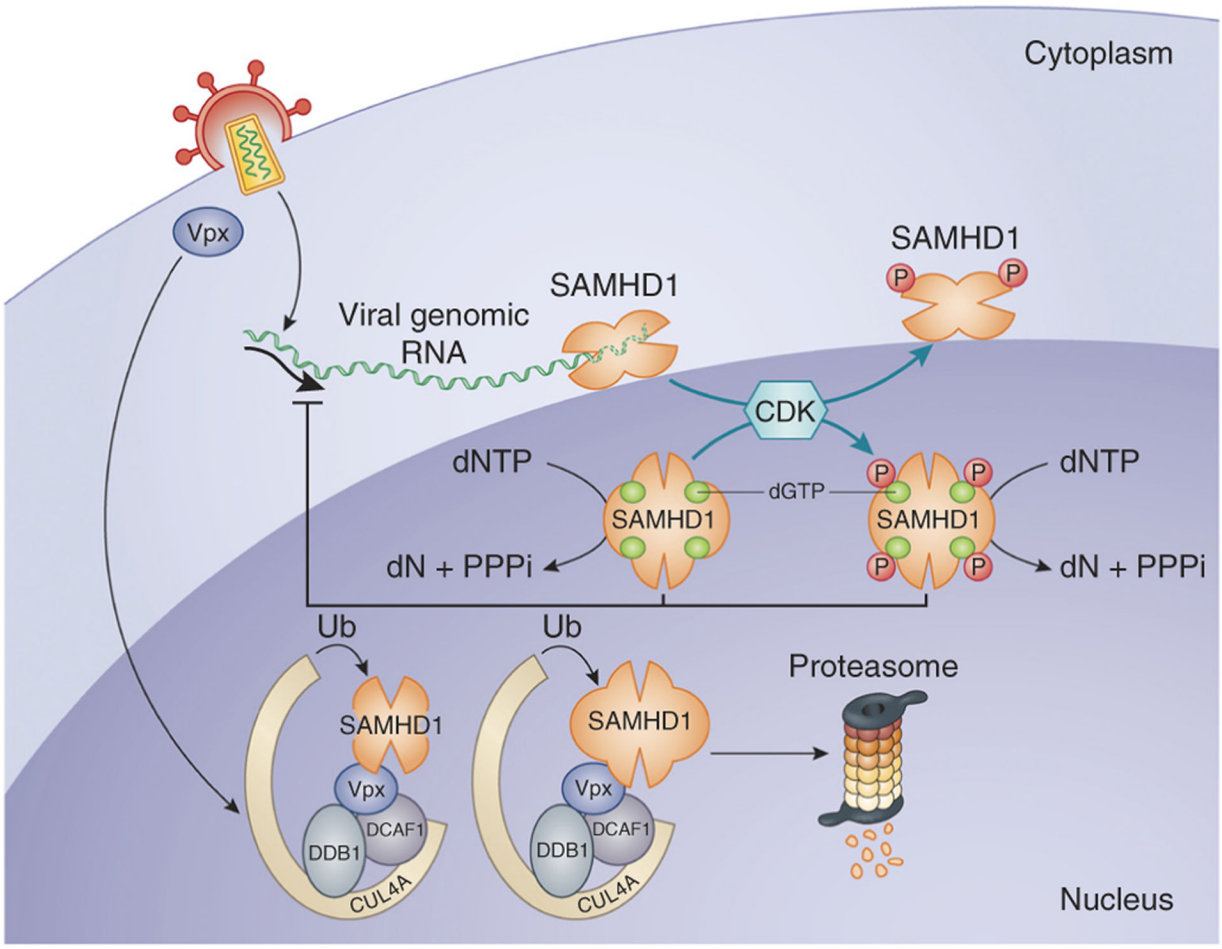

Figure 3.

Proposed models for SAMHD1-mediated restriction. HIV-2, SIV or engineered HIV-1 containing Vpx enters a myeloid cell. Vpx transits to the nucleus and binds to the DCAF1-DDB1-CUL4A E3 ubiquitin ligase complex and to SAMHD1, which is then degraded by proteasomes in the nucleus. If the virus lacks Vpx, SAMHD1 depletes the pool of dNTPs, blocking reverse transcription of the bound tRNA primer. An alternative model calls for SAMHD1 to degrade the viral genomic RNA as it is reverse transcribed in the cytoplasm. This activity is regulated by the phosphorylation of amino acid Thr592 by CDK-1, CDK-2 and CDK-6.

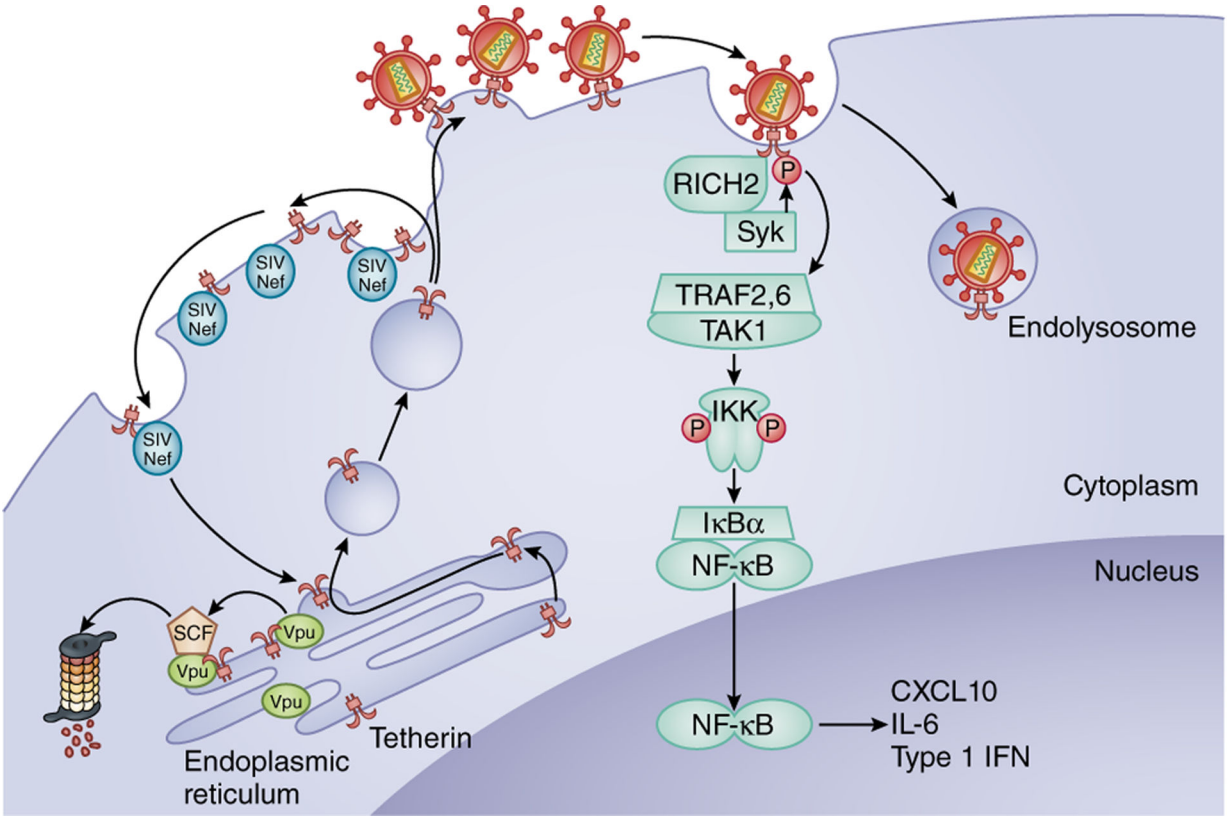

Figure 4.

Tetherin blocks virus release, activates an innate immune response and is counteracted by Vpu or Nef. Tetherin (BST2 or CD317) prevents virus release by inserting its N-terminal transmembrane domain in the plasma membrane and its GPI-linked C terminus in the virus envelope lipid bilayer. The tethered virus is then endocytosed. Tetherin contacts cortical actin through an interaction with RICH2. Tetherin is phosphorylated on tyrosines near its N terminus by Syk. Phosphorylated tetherin activates TRAF2, TRAF6 and TAK1. The complex phosphorylates IKK, causing the degradation of IκBα and thereby activating NF-κB, which induces the transcription of the proinflammatory cytokines CXCL10, IL-6 and IFN-β. Sequestration of tetherin by HIV-1 Vpu in the ER prevents its transit to the plasma membrane. Tetherin proteasomal degradation is induced by its interaction with the SCF-β-TRCP complex. In most SIVs, Nef binds to tetherin at the plasma membrane to induce its endocytosis through an AP2-dependent pathway.