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EDITORIAL
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Receptor usage and the pathogenesis in acute and chronic virus infections

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In the first phase of the viral life cycle, the virus enters cells using a specific cell surface receptor. Many viruses use multiple receptors: some of which are unique to a certain cell type, whereas others are found in many cell types. After the virus enters into cells, various cellular proteins may interact with it; some support virus replication, while others inhibit it. Once virus succeeds to establish its life cycle in the target cell, the progeny viruses disseminate within the tissues or systemically via viremia. The intrinsic pro- or anti-viral cellular machinery differs among cell types. Thus, depending on the receptors used, the viral cell tropism is determined, resulting in the characteristic distribution of virus-infected cells/tissues and the disease outcome *in vivo*.

How viral cell tropism, determined by the receptor used, can affect the disease outcome in acute and chronic virus infection is a major subject under extensive investigation in Virology. To understand the mechanism which causes human diseases by viruses, that is, viral pathogenesis, we have studied various aspects of virus infection at a cell/tissue level or using animal models. In this context, the recent development of reverse genetics allows us to visualize virus-infected cells/tissues or even the virus itself. By applying such manipulated viruses to animal models, it is also possible to analyze the dynamics of virus infection *in vivo*.

In this Research Topic, we selected on studies connecting virus receptor usage and the pathogenesis of various viruses causing acute or chronic infection. Eventually, it could expand to cover the receptor-pathogenesis relationship in various acute and chronic virus infection. This research topic comprises an original research article on HIV-1, two opinions articles on the hepatitis C virus (HCV) and norovirus, while the remaining review articles on HTVL-1, measles virus (MV), mouse hepatitis virus (MHV), influenza virus, HCV, and enterovirus (EV) provide overviews on various aspects of viral pathogenesis. In all these review/opinion articles, at least one comprehensive table or figure is incorporated so that readers who are unfamiliar with these viruses can get a message at a glance.

With regards to the cell tropism of HIV-1, Terahara et al. (2012) presented his recent study using CCR5-tropic and CXCR4-tropic HIV-1 with distinct fluorescent reporter. These HIV-1s allowed us to detect HIV-infected cells at a different stage of infection and to evaluate the level of virus replication in $CD4^+$ T cells with distinct differentiation phenotype including $CCR5^+$ memory. In contrast, a receptor for HTLV-1 and related pathogenesis is still intriguing issue, which is described by Hoshino (2012) in his extensive review.

The two reviews on the MV (Kato et al., 2012; Takeda et al., 2012) were published at a very appropriate time as a third receptor for MV entry into epithelial cells, nectin 4, had just been discovered (Muhlebach et al., 2011; Noyce et al., 2011). Here, Kato et al. (2012) focused on the receptor usage of MV *in vivo* which may influences the disease outcome using monkey models, while Takeda et al. (2012) discussed about the dual-tropic nature of MV using SLAM and nectin 4 expressed in immune cells and epithelial cells, respectively.

Ito et al. (2012) addressed the importance of B cells as a reservoir for persistent HCV infection. In two reviews on HCV, Moriishi and Matsuura (2012) overviewed a current research focus on lipid components for the HCV pathogenesis, while Shoji et al. (2012) discussed about glucose metabolic disorders associated with HCV infection.

Nishimura and Shimizu (2012) and Yamayoshi et al. (2012), both of whom successfully identified two novel receptors for EV, overviewed the current knowledge about receptor usage and various diseases associated with EV infection. For the coronavirus, Taguchi and Hirai-Yuki (2012) overviewed studies on the receptor and related cellular factors for MHV, which may contribute to the mouse susceptibility to MHV infection.

As regards to the virus recognizing sugar moieties, Ramos and Fernandez-Sesma (2012) provided insights about the interaction of influenza A virus with sialic acid receptors on immune cells with special reference to the innate immune response. Shirato (2012) described about the norovirus with distinct genotypes which recognize a specific structure of sugar chain.

We will learn by these articles the fact that to identify a receptor is the first important step to know a virus, but many questions remain in order to fully understand human diseases caused by viruses. I would like to express my cordial thanks to all the contributors for this topic. I hope readers find the content interesting, but most importantly, that the information will prove very useful for future research.

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Editorial

HIV-1 Vpu and BST-2/Tetherin: Enemies at the Gates

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In the mid-1990s, we and others found that an HIV-1 accessory protein, Vpu, enhances virus production in a cell type-specific manner. For example, COS7 and HeLa cells are permissive and non-permissive, respectively, for the production of Vpu-deficient virions [1, 2]. The question of what determines this cell type specificity has remained unanswered for many years. By contrast, another function of Vpu, i.e. CD4 degradation, has been well-defined [3-5]. In 2003, Spearman's group reported that COS7-HeLa heterokaryons showed the same phenotype as HeLa cells, suggesting the existence of an unknown endogenous restriction factor(s) that could be counteracted by Vpu [6]. A few years later, Neil and Bieniasz showed that the host restriction factor blocked by Vpu was actually an interferon-α-inducible cell surface protein [7]. In 2008, they finally identified this protein and renamed it tetherin [8], a transmembrane protein previously known as BST-2, CD317, or HM1.24 (referred to hereafter as BST-2/tetherin). Over the past four years, intensive studies have been undertaken worldwide to characterize this restriction factor, and substantial progress has been made. It is now known that BST-2/tetherin blocks the production of enveloped viruses by tethering them to the plasma membrane of the virus producing cells; however, HIV-1 Vpu counteracts the inhibitory activity of BST-2/tetherin by downregulating it from the cell surface [8, 9]. Vpu interacts with BST-2/tetherin *via* mutual transmembrane domains [10-13], leading to intracellular sequestration [14, 15], or to proteasomal [16, 17] or lysosomal [11, 18-20] degradation. The intracellular mechanism underlying Vpu-mediated downregulation of BST-2/tetherin requires, at least in part, β-TrCP [11, 17-19], a subunit of the ubiquitin ligase complex, possibly together with an unidentified cellular factor(s) [11]. SIV, which does not encode Vpu, uses Nef as a tetherin antagonist [21-23], suggesting that blocking BST-2/tetherin activity is a viral strategy developed throughout the evolution of primate lentiviruses.

In this hot topic special issue of *Current HIV Research*, experts in the field review recent advances in the molecular, structural, and cell-biological mechanisms underlying this newly discovered host-pathogen interaction, including its species specificity, and discuss how the battle between host and virus has developed through the acquisition of their defensive proteins. This issue contains eight reviews, beginning with a paper by Serra-Moreno and Evans, who describe the extraordinary plasticity of primate lentiviruses in counteracting BST-2/tetherin of their respective hosts, suggesting that the restriction factor represents a key barrier to cross-species transmission of primate lentiviruses. Arias, Iwabu, and Tokunaga discuss three models outlining the sites of action of Vpu in BST-2/tetherin downregulation (i.e., interference with the membrane transport, inhibition of recycling, and direct internalization from the plasma membrane) and the latter's intracellular fate. Guo and Liang review the molecular details of the transmembrane interaction between Vpu and BST-2/tetherin, and the targeting of the latter's cytoplasmic region and ectodomain by other viral antagonists. Weissenhorn and colleagues highlight the dynamic structural properties of the ectodomain of BST-2/tetherin, and the physical tethering of HIV-1 particles mediated by bridging the cellular and viral membranes after the completion of budding. Blanchet, Mitchell, and Piguet focus on Vpu-induced downregulation of cell surface CD4 and BST-2/tetherin through a mechanism involving a β-TrCP-containing E3 ubiquitin ligase complex, which is an important viral strategy used to maintain a productive infection. Janvier, Roy, and Berlioz-Torrent describe the role of the endosomal ESCRT machinery in Vpu-induced lysosomal targeting of BST-2/tetherin. Fujita and colleagues provide an overview of the intracellular logistics of BST-2/tetherin, including transcription/expression, post-translational modification in the endoplasmic reticulum, sorting of BST-2/tetherin at the *trans*-Golgi network, and endocytic/recycling pathways. The final paper by Sandberg and colleagues reviews a recently discovered function of Vpu, i.e., its interference with innate cellular immunity, which is mediated by CD1d on invariant natural killer T cells and NTB-A on natural killer cells.

This issue provides a broad and insightful overview of the current state of research on the intensively studied host-pathogen interaction. As a guest editor of this hot topic special issue on Vpu and BST-2/tetherin, I am indebted to all the authors for their time and effort in providing excellent and professional review articles; this editorial is dedicated to them. I would also like to express my gratitude to the peer-reviewers for their constructive comments that have helped to improve the manuscripts. Finally, I truly hope that the readers will benefit from the collection of reviews in this special issue and gain further insight into the ongoing battle for supremacy between these two powerful enemies.

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CONFLICT OF INTEREST

Declared none.

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Sites of Action of HIV-1 Vpu in BST-2/Tetherin Downregulation

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Abstract: The interferon-inducible host restriction factor bone marrow stromal antigen 2 (BST-2/tetherin) blocks the release of human immunodeficiency virus type 1 (HIV-1) by directly cross-linking virions to the membrane of infected cells. This antiviral effect is counteracted by the HIV-1 accessory protein viral protein U (Vpu) through mechanisms that remain unclear. Accumulating evidence suggests that Vpu antagonizes BST-2 by removing it from the plasma membrane; however, neither the cellular sites of interaction nor the effector mechanisms that result in the downregulation of BST-2 cell-surface expression have been fully determined. Based on current evidence regarding the subcellular localization of Vpu and BST-2 and the latter's trafficking defects induced by their interaction, three models have been proposed. In the first, Vpu is hypothesized to block the traffic of newly synthesized BST-2 towards the cell surface by retaining it in the biosynthetic/secretory compartment. The second model suggests that Vpu sequesters BST-2 within intracellular compartments corresponding to recycling endosomes and the *trans*-Golgi network by blocking its recycling after endocytosis. In the third model, we and others have proposed that Vpu directly internalizes BST-2 from the plasma membrane and induces its enhanced endolysosomal trafficking and degradation. As for its intracellular fate, the viral antagonism of BST-2 is likely dependent on the intracellular sequestration, or the proteasomal/lysosomal degradation of the restriction factor. This review summarizes the current advances in our understanding of the cellular pathways and sites of action of Vpu in the downregulation of cell-surface BST-2.

Keywords: HIV-1, Vpu, BST-2, plasma membrane, downregulation, trafficking, *trans*-Golgi network, recycling endosome.

INTRODUCTION

Interferon (IFN)-α-inducible host restriction factors have evolved in mammals as effectors of intrinsic immune responses, providing resistance to infections by directly interfering with the viral life cycle. Four of these host factors restrict the efficiency of HIV-1 replication: the apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) family of cytidine deaminases [1]; the α-isoform of the tripartite motif-containing protein 5 (TRIM5α) [2]; bone marrow stromal antigen 2 (BST-2), also known as tetherin or CD317 [3, 4]; and, the most recently described restriction factor, SAM domain HD domain-containing protein 1 (SAMHD1) [5, 6]. To effectively establish a productive infection, HIV-1 has acquired a series of *trans*-acting viral accessory proteins, including Vif and Vpu, to overcome the antiviral activity of these restriction factors. Vif blocks APOBEC3-mediated cytosine deamination in single-stranded DNA, which halts HIV replication. Vpu antagonizes the antiviral effect of BST-2 that bridges the budding virions to the cell membrane, thereby blocking the release of progeny viruses. HIV-1 is able to replicate in human cells because the viral capsid is not effectively recognized by human TRIM5α [2]. In HIV-2 and related simian immunodeficiency viruses (SIVs), the Vpx protein antagonizes SAMHD1, which blocks HIV-1 replication in myeloid cells.

BST-2, the focus of this review, is an IFN-inducible type II membrane glycoprotein that exhibits unusual topology, consisting of a short N-terminal cytoplasmic tail (CT) followed by an α-helical transmembrane (TM) domain, an extended coiled-coil extracellular domain (EC), and a C-terminal glycophosphatidylinositol (GPI) component that serves as a second membrane anchor [7, 8], although the presence of the GPI-anchor has been recently challenged [9]. Interestingly, in mammalian cells, this unusual double-anchor topology has thus far been found only in an isoform of the prion protein [10]. BST-2 was initially identified as a surface marker in terminally differentiated B-cells in patients with multiple myeloma [11, 12] and as a target of Kaposi's sarcoma-associated herpesvirus K5 ubiquitin ligase [13]. Subsequently, this host protein was rediscovered as a restriction factor [3, 4]. BST-2 efficiently blocks the release of a wide range of enveloped viruses by directly tethering viral particles to the membranes of infected cells. The list of viruses restricted by BST-2 continues to grow, including retroviruses [14-16], filoviruses, arenaviruses, paramyxo-viruses [17-20], gamma-herpesviruses [21, 22], and rhabdo-viruses [23].

The accessory viral protein U (Vpu), encoded in the genome of HIV-1 and a few SIV strains, is a type I transmembrane protein consisting of a short N-terminal domain, a single TM α-helix domain that is also an uncleaved signal peptide, two cytosolic α-helices separated by a short flexible connector loop, and a C-terminal tail. Vpu mediates the degradation of CD4 receptors [24, 25] and enhances the release of progeny virions from virus-producer cells [26-30] by antagonizing the restriction to viral egress imposed by BST-2 [3, 4, 31]. Furthermore, counteraction of BST-2 by Vpu is important for HIV-1 pathogenesis *in vivo* [32-34], indicating that BST-2 is a functionally active restriction factor whose antagonism is required for the establishment of a successful infection *in vivo*. Here, we summarize the current knowledge of the cellular pathways

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and sites of action of Vpu in downregulation of cell-surface BST-2.

BST-2 LOCALIZATION AND TRANSPORT

In addition to the cell surface, its site of action, BST-2 is found in various cellular compartments, particularly the *trans*-Golgi network (TGN) and recycling endosomes [35-38]. Since current models of the Vpu–BST-2 interaction draw heavily on the cellular localization and trafficking pathways of both proteins, we first describe the physiologic pathways of intracellular BST-2 trafficking. As shown in Fig. (**1**, i and ii), membrane-bound BST-2 is synthesized in the endoplasmic reticulum (ER) and traverses the Golgi cisternae and TGN. The latter acts as an exocytic hub, where outgoing material is sorted and packed into distinct secretory vesicles. Once it reaches the plasma membrane (PM), BST-2 localizes specifically to cholesterol-enriched lipid rafts, binding through its GPI anchor [7, 39-41]. This localization allows BST-2 to effectively tether virus particles, since several enveloped viruses preferentially bud from these microdomains [42-44].

From the PM, BST-2 and other surface markers undergo constitutive endocytosis, followed by their transport to early/sorting and recycling endosomes [45-48], where incoming material is sorted and then sent to different cellular destinations (Fig. **1**, IV). Thus, from this sorting hub, internalized proteins can be recycled directly back to the PM [49-51]. Alternatively, they may be sent to late endosomes and multi-vesicular bodies, where they are subjected to lysosomal degradation (Fig. **1**, V) [52-54], or transported to the biosynthetic/secretory compartments in a process known as retrograde transport [55-57].

These trafficking pathways are regulated by a complex molecular machinery (see Bonifacino *et al.* [58]) that includes small membrane-bound proteins called soluble N-ethylmaleimide-sensitive fusion factor attachment receptors (SNAREs) [59, 60], clathrin adaptor protein (AP) complex members, and small GTPases of the Rab family [61-65]. Evidence suggests that a clathrin-dependent mechanism is required for BST-2 internalization [39, 41] in a process facilitated by the non-canonical dual tyrosine motif at residues 6 and 8 of the BST-2 CT domain. This YxY_{6-8} motif, conserved through all mammalian BST-2 orthologs, is important in determining the protein's trafficking pathways, as it has been shown to interact with both AP-2 and AP-1 complexes, which play a role in directing cargo membrane proteins to clathrin-coated vesicles [65-67]. AP-2 mediates BST-2 internalization at the PM by endocytosis, whereas AP-1 is associated with the transport of BST-2 from recycling endosomes to the TGN [39, 41].

OVERVIEW OF VPU FUNCTION

Vpu has been known to mediate the ubiquitin-induced degradation of CD4 [24], through a conserved phospho-serine motif (DS52GxxS56) in the cytoplasmic domain (CT) of Vpu which recruits β-transducin repeat-containing protein (β-TrCP) and the E3 ligase complex. This results in proteasomal degradation of newly synthesized CD4 receptors [68, 69] in a process coupled to an ER-associated protein degradation (ERAD)-like pathway [70-72]. CD4 receptor degradation minimizes the superinfection of target cells and prevents the retention of Env precursors in the ER, in addition to guaranteeing the release of progeny virions from the PM [73-75]. Vpu was also found to be required for the efficient release of HIV-1 virions from susceptible cell lines. This activity was later shown to be derived from its antagonism of the restriction factor BST-2 [29, 76, 77], which is discussed extensively in the remainder of this review. Two recent reports have described the novel activity of Vpu that interferes with innate cellular immune responses of natural killer and natural killer T cells [78, 79].

Vpu has been shown to localize primarily in endosomes and the TGN [80, 81], where it is thought to interact with BST-2. Expression of Vpu results in reduced levels of BST-2 at the host cell membrane [3, 4, 40, 82, 83] and either degradation [84-88] or sequestration of the host factor in intracellular compartments [36, 89, 90] leading to increased virus release. In previous work, we and others showed that interaction of Vpu with BST-2 depends on their mutual TM domains, leading to downregulation of the latter from the cell surface and subsequent degradation. Evidence for these sequential events comes from data showing that the antiviral activity of BST-2 is restored in the presence of Vpu harboring mutations that disrupt the mutual association of the two proteins through their TM domains [85, 91-94]. However, in certain cell lines (CEMx174, H9) the interaction of Vpu and BST-2 does not effectively reduce the surface levels of the latter in spite of enhanced virion production [90]. This in turn suggests that downregulation at the PM is not the only anti-BST-2 function of Vpu. The implications of this observation remain to be determined in further studies.

SITES AND MODELS OF VPU'S ACTION IN BST-2 DOWNREGULATION

It is widely recognized that Vpu antagonizes BST-2 function as a virion tether by directly mediating the removal of the restriction factor from its site of action at the cell membrane [4, 35, 85, 95, 96]. Although the exact mechanisms of this antagonism are not well understood, current data suggest that APs are required for downregulation of BST-2 from the cell surface [35, 36]. Likewise, we and others have shown that one of the cellular co-factors required for BST-2 downregulation is β-TrCP [36, 84, 85, 88, 97] that regulates either proteasomal, or ubiquitin-dependent lysosomal degradation [98]. However, the requirement appears to be partial because mutations in the β-TrCP-binding motif of Vpu do not entirely abrogate its antagonism of BST-2 [4, 85, 99]. Overall, based on three putative cellular locations (the biosynthetic/secretory pathway, the PM, and the TGN/recycling endosomes) and three probable effector mechanisms (intracellular sequestration and either proteasomal or lysosomal degradation, as described later), several distinct models of the Vpu–BST-2 interaction leading to cell-surface downregulation of BST-2 have been postulated. These are discussed below.

Interference with the membrane transport of newly synthesized BST-2. Current evidence suggests that Vpu antagonizes BST-2 by altering its anterograde transport *via* sequestration in the biosynthetic/secretory pathway, which in

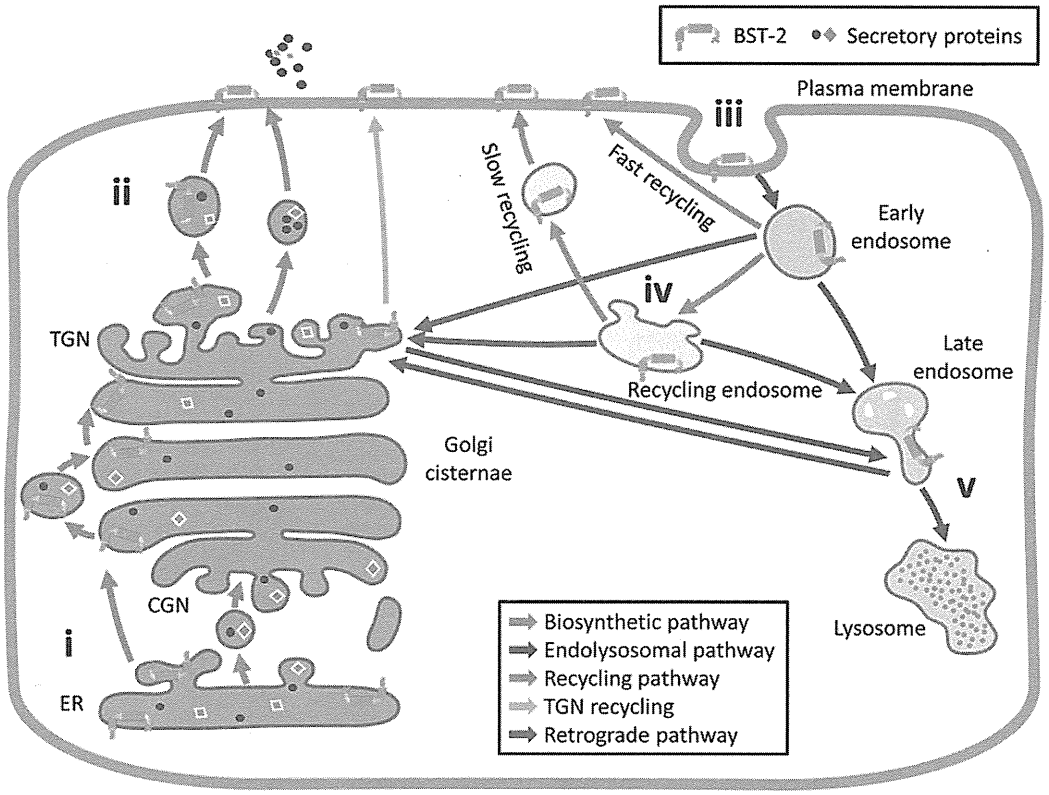

Fig. (1). Physiologic intracellular trafficking pathways of BST-2. Schematic representation of a cell, with the physiologic protein trafficking pathways depicted. The nucleus and other organelles are not shown. The biosynthetic/secretory pathway on the left is represented by red arrows. BST-2 is synthesized in the endoplasmic reticulum (ER) and travels through the *cis*-Golgi network (CGN), Golgi cisternae, and the *trans*-Golgi network (TGN) to finally reach the plasma membrane (PM) *via* secretory vesicles. From the cell surface, BST-2 is internalized, as seen on the right, and then travels either through the endolysosomal pathway, leading to its degradation (purple arrows) or the recycling pathway (blue arrows). The endosomal compartments act as a sorting hub from where BST-2 either cycles directly back to the PM (fast and slow recycling, represented by blue arrows) or relocates to the TGN after which it is sent back to the PM (green arrow). Alternatively, BST-2 can travel through the retrograde pathway (brown arrows), resulting in its transport to the TGN, Golgi membranes, or in some cases even to the ER. Another possibility is that, from recycling endosomes, BST-2 is re-routed to the late endocytic pathway, leading to its degradation in lysosomes.

turn reduces the supply of newly synthesized BST-2 to the cell membrane (Fig. **2A**). The model based on these observations draws support from studies showing that Vpu co-localizes with BST-2 in post-ER membranes [37, 84, 100, 101]. One of these reports has shown that Vpu harboring mutations that altered its intracellular localization and prevented its co-localization with BST-2 in the TGN, is unable to exert its antagonistic effects, suggesting that Vpu targets BST-2 in this compartment [37]. More importantly, Vpu-mediated downregulation of cell-surface BST-2 was strongly attenuated by treatment with Brefeldin A, which blocks ER-to-Golgi transport [37], or with B18R, a vaccinia-encoded antagonist of the type I IFN receptor (since BST-2 synthesis is type I IFN-inducible) [102]. These results suggest that Vpu-induced BST-2 downregulation is dependent on the *de novo* synthesis of the latter protein. It is therefore likely that Vpu blocks the pool of newly synthesized BST-2 which is trafficking to the PM (Fig. **2A**, i), as suggested by subcellular localization studies. BST-2 downregulation might therefore be accomplished simply by its sequestration in the TGN, or alternatively by sorting to the endolysosomal pathway, resulting in its degradation (Fig. **2A**, ii) [86, 102, 103]. Additionally, proteasomal degradation in an ERAD-like process has been suggested as a putative Vpu-mediated degradation mechanism [88] (Fig. **2A**, iii), but the intracellular pathways involved remain to be identified.

Inhibition of BST-2 recycling. Other lines of evidence suggest that Vpu downregulates BST-2 from the plasma membrane, followed by re-localization of the host factor to endosomal (Fig. **2B**, i) and TGN compartments (Fig. **2B**, ii), where BST-2 is sequestered such that its recycling to the cell surface is blocked. Constitutive clathrin-dependent endocytosis mediated by the AP-2 adapter complex [41] or α-adaptin [39] delivers BST-2 to endosomes, where Vpu is located. This results in the sequestration of BST-2 in a post-endocytic compartment and inhibition of its recycling

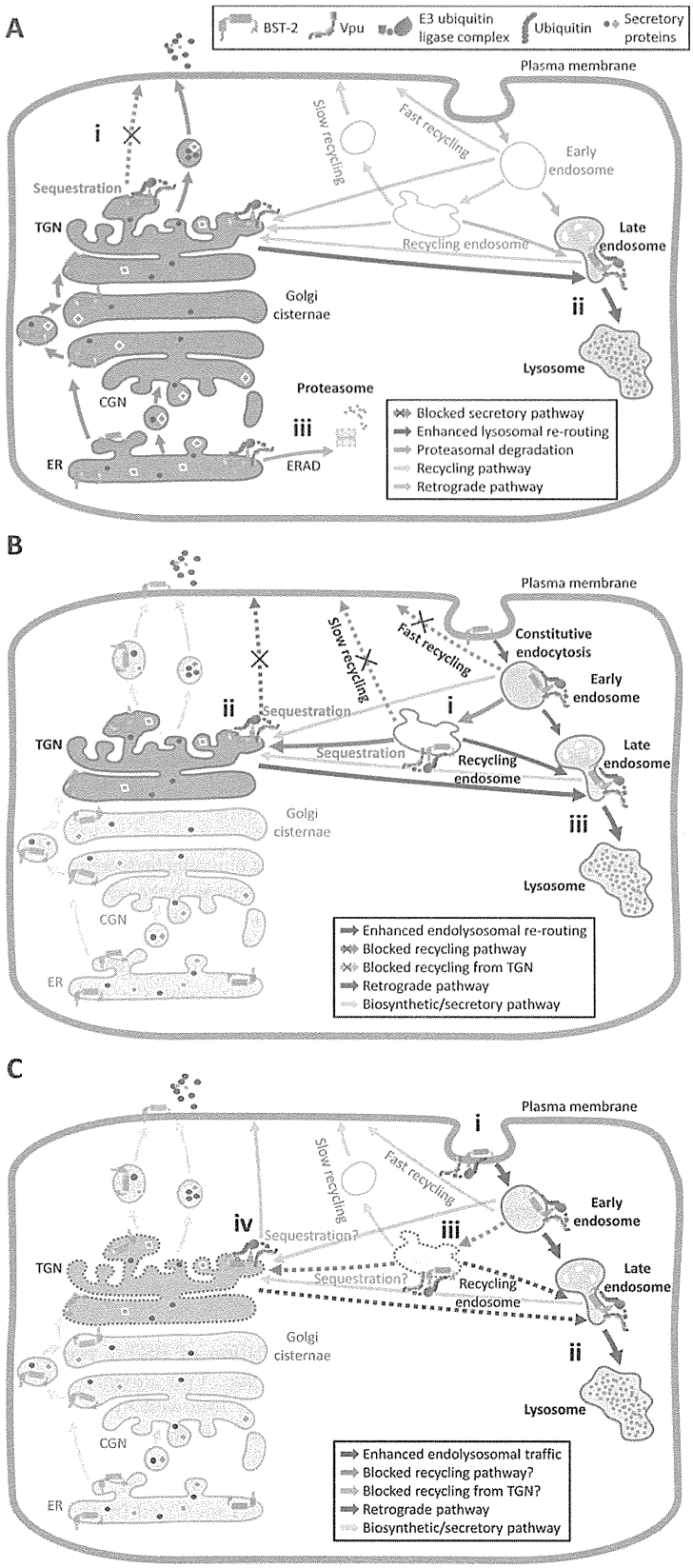
A
BST-2
Vpu
E3 ubiquitin ligase complex
Ubiquitin
Secretory proteins
Plasma membrane
Slow recycling
Fast recycling
Early endosome
i
Sequestration
TGN
Recycling endosome
Late endosome
Golgi cisternae
ii
Lysosome
Proteasome
CGN
iii
ER
ERAD
Blocked secretory pathway
Enhanced lysosomal re-routing
Proteasomal degradation
Recycling pathway
Retrograde pathway
B
Plasma membrane
Slow recycling
Fast recycling
Constitutive endocytosis
Early endosome
ii
Sequestration
i
TGN
Sequestration
Recycling endosome
Late endosome
Golgi cisternae
iii
Lysosome
CGN
ER
Enhanced endolysosomal re-routing
Blocked recycling pathway
Blocked recycling from TGN
Retrograde pathway
Biosynthetic/secretory pathway
C
Plasma membrane
i
Slow recycling
Fast recycling
Early endosome
iv
Sequestration?
iii
TGN
Sequestration?
Recycling endosome
Late endosome
Golgi cisternae
ii
Lysosome
CGN
ER
Enhanced endolysosomal traffic
Blocked recycling pathway?
Blocked recycling from TGN?
Retrograde pathway
Biosynthetic/secretory pathway

Fig. (2). Putative sites of interaction and potential alterations of trafficking involved in the downregulation of cell-surface BST-2 by HIV-1 Vpu. Schematic representation of BST-2 trafficking in the presence of Vpu, with the relevant trafficking pathways depicted. The nucleus and other organelles are not shown. (**A**) Vpu-mediated sequestration of BST-2 in biosynthetic/secretory compartments and either subsequent re-routing to the endolysosomal pathway or ERAD-like proteasomal degradation. (**B**) Vpu-mediated blockade of BST-2 recycling, resulting from the sequestration of BST-2 in the TGN and/or recycling endosomes, with subsequent relocation to the endolysosomal pathway for degradation. (**C**) Vpu-mediated direct internalization of BST-2 from the cell surface followed by the consequent enhancement of endolysosomal trafficking or the temporary sequestration of BST-2 in intracellular compartments with its subsequent relocation to the endolysosomal pathway for degradation.

pathway (Fig. **2B**, i) [35, 36, 104]. As discussed above, co-localization studies show that Vpu sequesters BST-2 in the TGN [37, 102, 105]. This trafficking blockade was shown to involve not only the pool of newly synthesized BST-2 but also endocytosed BST-2 recycling back to the cell surface *via* the TGN (Fig. **2B**, ii) [37, 105]. A Vpu-induced defect in BST-2 recycling is supported by the results of flow cytometric assays measuring newly deposited BST-2 at the cell surface. In one study, inhibition of the recycling pathway by the antimalarial drug primaquine induced BST-2 downregulation recapitulating the effect of HIV-1 Vpu expression [105], and in another study, Vpu also inhibited the membrane transport of BST-2 from a brefeldin A-insensitive compartment that presumably corresponds to recycling endosomes [35]. Taken together, these data suggest that inhibition of the BST-2 recycling pathway contributes significantly to Vpu-mediated downregulation of the restriction factor. Indeed, Vpu causes the relocation of BST-2 to an intracellular compartment containing transferrin, a marker of recycling endosomes (Fig. **2B**, i) [35]. This finding is complemented by recent studies showing the subcellular localization of Vpu and BST-2 in the TGN and recycling endosomes [36, 81, 102]. Taken together, these studies support a model in which Vpu interacts with BST-2 in intracellular compartments, most likely recycling endosomes (Fig. **2B**, i) and the TGN (Fig. **2B**, ii), effectively preventing the recycling transport of BST-2. From these compartments, BST-2 is subsequently re-routed to the endolysosomal pathway, ultimately resulting in its lysosomal degradation (Fig. **2B**, iii) [86, 102, 103].

Direct internalization of BST-2 from the cell surface. Although it is clear that Vpu-mediated antagonism requires the downregulation of BST-2 cell-surface expression, the precise role of the cellular internalization machinery in this process is unclear. We previously found that Vpu internalizes BST-2 from the plasma membrane. Overexpression of a dominant-negative form of dynamin-2, which inhibits both clathrin-dependent and -independent endocytosis, abrogated the Vpu-mediated downregulation of cell-surface BST-2 [85]. Our data is supported by reports showing that the Vpu-mediated BST-2 downregulation was inhibited by either siRNA suppression of AP-2 [36], or by the C-terminal fragment of the clathrin assembly protein AP180 [35]. Additionally, we have shown that a constitutive endocytosis-deficient BST-2 mutant harboring mutations in the non-canonical YxY motif of the CT domain [41] is still sensitive to Vpu-induced downregulation [85]. However, it has been recently reported that the rate of constitutive endocytosis in this mutant is not completely impaired [35].

Furthermore, although some reports suggest that Vpu does not upregulate the rate of BST-2 internalization [36, 37, 90], those studies were performed at steady-state and therefore potentially overlooked the early effects of Vpu on the downregulation of cell-surface BST-2. To overcome this limitation, we detected the continuous *de novo* cell-surface expression of BST-2 to enable its visualization at very low levels. The 37°C preincubation during the antibody internalization assay allowed us to detect the direct internalization of cell-surface BST-2 in the presence of Vpu [96]. Dube *et al.* validated this approach in a recent report employing similar conditions [102]. They showed that Vpu enhances the cell-surface clearance of BST-2, moderately in HeLa cells but, more importantly, significantly in $CD4^+$ Jurkat T-cells, the natural HIV-1 target. This suggests that Vpu-induced internalization is cell-type specific, which might depend upon unknown cellular cofactor(s). Taken together, these data suggest that Vpu directly and actively internalizes BST-2 from the PM (Fig. **2C**, i). We also observed that, upon Vpu-mediated internalization, BST-2 trafficking towards the endolysosomal compartment is enhanced, resulting in degradation of the host factor (Fig. **2C**, ii) [85]. It is nonetheless possible that other intracellular sorting pathways (i.e., retrograde and recycling transport) become involved after BST-2 internalization from the PM, resulting in temporary sequestration of the restriction factor in recycling endosomes (Fig. **2C**, iii) or the TGN (Fig. **2C**, iv), with subsequent re-routing to the endolysosomal pathway (Fig. **2C**, ii) for final degradation [86, 102, 103].

INTRACELLULAR FATE OF BST-2 IN THE PRESENCE OF VPU

In addition to the three potential sites of Vpu-BST-2 interaction, three different effector mechanisms have been proposed to account for the Vpu-induced downregulation of cell-surface BST-2, as follows; 1) intracellular sequestration; 2) proteasomal degradation; 3) lysosomal degradation. First, the inhibitory effects of Vpu on BST-2 do not necessarily involve degradation. It has been shown that Vpu decreases total cellular BST-2 levels to a lesser extent than cell-surface BST-2 [36, 89, 90]. As described above, co-localization studies suggest that the downregulation of surface BST-2 depends on Vpu-induced re-localization and accumulation in intracellular compartments overlapping with markers of the TGN [38, 103] or recycling endosomes [35, 36, 104]. Such accumulation could involve the pool of internalized BST-2 recycling back to the cell surface, or the newly synthesized protein in the biosynthetic pathway. Thus, Vpu might sequester BST-2 intracellularly, effectively preventing its constitutive trafficking towards its site of action at the PM.

On the other hand, increasing evidence supports the importance of a degradation pathway in BST-2 antagonism by a mechanism that is dependent upon β-TrCP [36, 84, 85, 88, 97]. It has been shown that the use of proteasomal

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

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

CONCLUSIONS

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

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

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CONFLICT OF INTEREST

Declared none.

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Retroelements versus APOBEC3 family members: No great escape from the magnificent seven

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Retroelements comprise a large and successful family of transposable genetic elements that, through intensive infiltration, have shaped the genomes of humans and other mammals over millions of years. In fact, retrotransposons now account for approximately 45% of the human genome. Because of their genomic mobility called retrotransposition, some retroelements can cause genetic diseases; such retrotransposition events occur not only in germ cells but also in somatic cells, posing a threat to genomic stability throughout all cellular populations. In response, mammals have developed intrinsic immunity mechanisms that provide resistance against the deleterious effects of retrotransposition. Among these, seven members of the APOBEC3 (A3) family of cytidine deaminases serve as highly active, intrinsic, antiretroviral host factors. Certain A3 proteins effectively counteract infections of retroviruses such as HIV-1, as well as those of other virus families, while also blocking the transposition of retroelements. Based on their preferential expression in the germ cells, in which retrotransposons may be active, it is likely that A3 proteins were acquired through mammalian evolution primarily to inhibit retrotransposition and thereby maintain genomic stability in these cells. This review summarizes the recent advances in our understanding of the interplay between the retroelements currently active in the human genome and the anti-retroelement A3 proteins.

Keywords: retroelements, retrotransposition, LINE-1, Alu, APOBEC3, HIV-1, Vif, restriction factors

INTRODUCTION

The evolution of vertebrate genomes has been driven in part by the long history of their interaction with genetic transposable elements. These so-called retrotransposons, which replicate via RNA intermediates, can be divided into two groups depending on the presence or absence of long terminal repeats (LTRs). LTR retrotransposons are endogenous retroviruses that constitute nearly 10% of murine and human genomes, but they have been rendered mostly inactive due to the accumulation of mutations, although some murine intracisternal A-particles (IAP) and MusD sequences remain viable (Dewannieux et al., 2004; Ribet et al., 2004). Non-LTR retrotransposons comprise the majority of transposable elements; in fact, collectively, they account for more than one third of the human genome. They can be further subdivided into three types; long interspersed elements (LINEs), short interspersed elements (SINEs), and the composite hominid-specific retrotransposons, each of which contain the only transposable elements currently active in the human genome, i.e., LINE-1, Alu, and *SINE-VNTR-Alu* (SVA), respectively (Deininger and Batzer, 2002; Ostertag et al., 2003).

Retrotransposition, discussed in greater detail below, involves the reverse transcription of an RNA intermediate with subsequent genomic integration in a process driven by retrotransposon-encoded RNA-dependent DNA polymerase and endonuclease. The integration of these elements may have harmful consequences for the host, compromising genomic stability via insertions, deletions, and DNA rearrangements and thereby posing a threat to human health, as described in several reports of retrotransposition-induced genetic disorders (Kazazian et al., 1988; Wallace et al., 1991; Kobayashi et al., 1998). In response, eukaryotic organisms have evolved mechanisms to restrict uncontrolled retrotransposition. Anti-retroelement strategies include transcriptional silencing through DNA methylation (Walsh et al., 1998; Bourc'his and Bestor, 2004; Burden et al., 2005), post-transcriptional silencing via RNA interference (Soifer et al., 2005; Yang and Kazazian, 2006), and some cellular factors inhibiting retrotransposition at the post-translational level. Of these cellular factors, seven members of the apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3; referred to hereafter as the A3) family of cytidine deaminases have been shown to act as potent inhibitors of a wide range of both exogenous retroviruses and endogenous retroelements (Sheehy et al., 2002; Esnault et al., 2005; Chen et al., 2006; Kinomoto et al., 2007). In this review, we focus on active endogenous retroelements, their deleterious effects on the human genome, and the anti-retroelement activity of A3 proteins.

RETROTRANSPOSONS: AN OVERVIEW

Unlike the murine LTR retrotransposons IAP and MusD, human versions, such as human endogenous retroviruses (HERV), have been mostly fossilized, and even those that are not are non-transposable. In contrast, many copies of human non-LTR retrotransposons can replicate through an RNA/protein complex intermediate and integrate into the host genome at a new site.

The LINE retrotransposons, typified by LINE-1 (L1), account for approximately 17% of the human genome, corresponding to >500,000 copies (of which 100 copies are retrotransposition-competent). L1 retrotransposons are 6 kb in length and contain a 5′ untranslated region (UTR) that harbors a Pol II promoter; two ORFs necessary for their own replication; and a 3′ UTR containing a polyadenylation signal, followed by a poly(A) tail (**Figure 1A**, top). Briefly, L1 elements are first transcribed by RNA-polymerase II using a promoter located at the L1 5′ region (Ostertag and Kazazian, 2001). ORF1, encoding an RNA-binding protein, and ORF2, encoding a protein with reverse transcriptase and endonuclease activity, are then translated in the cytoplasm. The resulting proteins associate with L1 RNA to form a ribonucleoprotein (RNP) complex (Martin, 1991; Hohjoh and Singer, 1996; **Figure 1B**) that is transported back into the nucleus, where L1 is integrated into the host genome through a target-primed reverse transcription (Cost et al., 2002).

The human genome also contains more than 1 million copies of Alu elements; these are the most common SINE retrotransposons, representing 11% of our genome. The typical Alu element is approximately 300 bp in length and is formed by the fusion of two 7SL-RNA gene-derived monomers separated by an A-rich linker, followed by a poly(A) tail (Kriegs et al., 2007; **Figure 1A**, middle). Likewise, there are ~2700 copies of the composite SVA elements in the human genome. SVAs, which are approximately 2 kb long, are composed of CCCTCT hexameric repeats that are followed by an inverted Alu-like region, a region containing a variable number of tandem repeats (VNTRs), and a partial HERV-K *env*–LTR sequence termed SINE-R that ends with a polyadenylation signal, followed by a poly(A) tail (Ostertag et al., 2003; **Figure 1A**, bottom). Unlike L1, Alu and SVA elements are non-autonomous since they do not encode functional reverse transcriptase or endonuclease; instead, they use the enzymatic machinery of L1 for retrotransposition. Once Alu and SVA elements have been transcribed and exported to the cytoplasm, they hijack the L1-encoded enzymes in the vicinity of the ribosomes through mechanisms that are as-yet unclear (**Figure 1C**; Dewannieux et al., 2003; Ostertag et al., 2003).

RETROTRANSPOSONS IN HUMAN DISEASES

Approximately 100 examples of disease-causing retrotransposon insertions are currently reported in the literature. It is estimated that *de novo* insertions of L1, Alu, and SVA elements are responsible for approximately 0.3% of all disease-causing human mutations, corresponding to event rates of 1:100, 1:20, and 1:900 births, respectively (Cordaux and Batzer, 2009). L1-induced genetic diseases include the following: Duchenne muscular dystrophy and X-linked dilated cardiomyopathy, resulting from insertions in the dystrophin gene (Narita et al., 1993; Yoshida et al., 1998); progressive chorioretinal degeneration, caused by the CHM gene disruption (van den Hurk et al., 2003); hemophilia A and B, due to insertions in the factor VIII and IX genes, respectively (Kazazian et al., 1988; Li et al., 2001; Mukherjee et al., 2004); and chronic granulomatous disease, the result of a mutation arising from an insertion in the CYBB gene (Meischl et al., 2000). Genetic diseases linked to Alu integration events include neurofibromatosis via an insertion in the NF1 gene (Wallace et al., 1991; Wimmer et al., 2011); Apert syndrome, a severe autosomal dominant disorder, due to integration of the element into the fibroblast growth-factor receptor 2 (FGFR2) gene (Oldridge et al., 1999); and progressive renal failure (Dent's disease) due to disruption of the renal chloride channel (CLCN5) gene (Claverie-Martin et al., 2005). The involvement of SVA retrotransposition in human diseases has also been documented; namely, an insertion in the ARH gene leads to autosomal recessive hypercholesterolemia (Wilund et al., 2002); disruption of the BTK gene causes X-linked agammaglobulinemia (XLA; Rohrer et al., 1999); and disruption of the fukutin gene results in Fukuyama-type congenital muscular dystrophy (Kobayashi et al., 1998). Importantly, ongoing retrotransposon insertions seem to occur not only in germ cells and early embryos but also in brain tissues (Coufal et al., 2009; Baillie et al., 2011), somatic cells *in vitro* (Kubo et al., 2006; Rangwala et al., 2009), and somatic malignant tissues (Economou-Pachnis and Tsichlis, 1985; Morse et al., 1988; Miki et al., 1992). Several reports have also shown retrotransposon-induced recombination in certain types of cancer (Schichman et al., 1994; Jeffs et al., 1998).

CELLULAR MECHANISMS LIMITING THE ACTIVITY OF RETROELEMENTS AND RETROVIRUSES

As noted above, since unrestricted retrotransposition would result in genome instability, eukaryotic organisms have developed several strategies to restrict these mobile elements. Firstly, retrotransposition can be regulated at the transcriptional level through several transcription factors. For example, L1 transcription is positively regulated by SOX11 (Tchenio et al., 2000), RUNX3 (Yang et al., 2003) and YY1 (Athanikar et al., 2004), and negatively regulated by SRY (Tchenio et al., 2000) and SOX2 (Muotri et al., 2005). DNA methylation by the methyl-CpG-binding protein MeCP2 results in the repression of L1 transcription in neurons (Walsh et al., 1998; Burden et al., 2005; Muotri et al., 2010). Secondly, retrotransposable elements are also susceptible to post-transcriptional regulation. For instance, endogenously encoded small interfering RNAs have been shown to reduce L1 retrotransposition *in vitro* (Soifer et al., 2005; Yang and Kazazian, 2006). Additionally, L1 transcripts that contain multiple polyadenylation signals lead to premature polyadenylation, resulting in the attenuation of L1 activity via truncation of its full-length transcripts (Perepelitsa-Belancio and Deininger, 2003). Thirdly, some cellular factors regulate retrotransposition at the post-translational level. In mice, the 3′–5′ exonuclease Trex1 digests retroelement-derived DNA to suppress the autoimmune response (Stetson et al., 2008), Consistent with this, mutations in human Trex1 cause autoimmune diseases like familial chilblain lupus and Aicardi-Goutieres syndrome (Crow et al., 2006). Likewise, HIV-1 restriction factors such as the cytidine deaminases, the focus of this review, can inhibit L1 and Alu retrotransposition through a mechanism that is still unknown.

In humans, the cellular cytidine deaminase family comprises several members, including activation-induced cytidine

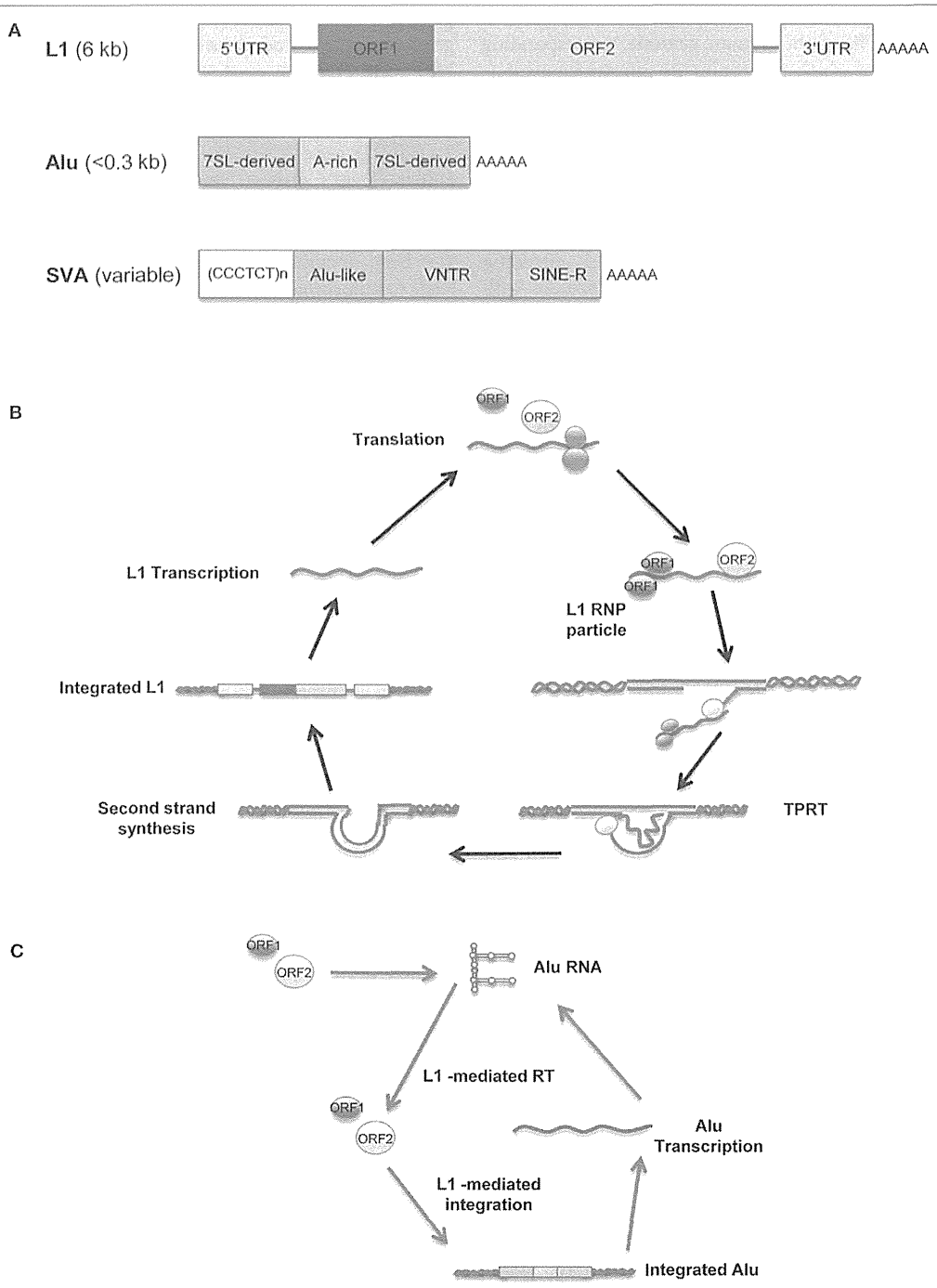

FIGURE 1 | Retrotransposition cycle. Schematic representation of active human retrotransposons. **(A)** *Top*: L1 genomic organization, from the left: 5′ UTR, untranslated region; ORF-1, encoding an RNA-binding protein; linker region; ORF-2, encoding reverse transcriptase and endonuclease; 3′ UTR; AAA, poly(A) tail. *Middle*: Alu organization, from the left: 7SL-derived monomer; A-rich linker, A_5TACA_6; 7SL-derived monomer; AAA, poly(A) tail. *Bottom*: SVA organization, from the left: (CCCTCT)n, hexamer repeat; inverted Alu-like sequence; VNTR, variable number of tandem repeats; SINE-R, HERV-K-derived sequence; AAA, poly(A) tail. **(B)** Retrotransposition cycle: L1 elements are transcribed by RNA-polymerase II from an L1 promoter sequence. The L1 mRNA template is exported to the cytoplasm and translated. Retrotransposon-encoded proteins actively bind the L1 RNA transcript, forming a ribonucleoprotein particle (RNP) that is imported back into the nucleus. There, the L1-encoded endonuclease nicks an L1 target sequence (5′-TTTT/AA-3′) and the 3′-OH generated is used as a primer for target-primed reverse transcription (TPRT) by the L1-encoded reverse transcriptase, resulting in *de novo* integration into the host genome. **(C)** Alu as well as SVA elements are transcribed and hijack the L1-encoded enzymatic machinery to complete their respective retrotransposition cycles.

deaminase (AID), APOBEC1, APOBEC2, the A3 family, and APOBEC4 (Harris and Liddament, 2004; Conticello, 2008; Smith et al., 2012). APOBEC1 is the catalytic subunit of an RNA-editing complex that deaminates $C^{6666} \rightarrow U$ in the mRNA of the lipid-transport protein apolipoprotein B, thereby creating a premature stop codon that leads to a truncated protein in gastrointestinal tissues (Teng et al., 1993). APOBEC1 proteins from multiple small-animal species exhibit inhibitory activity against not only exogenous retroviruses (Ikeda et al., 2008) but also endogenous retroviruses, such as murine IAP and MusD sequences, as well as L1 elements (Ikeda et al., 2011). AID plays a role in the adaptive humoral immune system by inducing somatic hypermutations and class switch recombination, which allows affinity maturation and memory development; however, its precise mechanism of action remains to be determined (Honjo et al., 2005). As described in detail in a subsequent section, members of the A3 family are potent inhibitors of both exogenous retroviruses and endogenous retroelements. A3G, the most extensively studied member of the A3 family, was the first cytidine deaminase shown to restrict infection by Vif-deficient HIV-1 viruses. Briefly, as depicted in **Figure 2**, A3G is incorporated into budding virions and thus exerts its antiviral effect at the post-entry step in target cells, either by mediating extensive deamination of the minus-strand of viral DNA during reverse transcription, which results in G → A hypermutations in the proviral DNA plus strand (deaminase-dependent mechanism) (Harris et al., 2003; Mangeat et al., 2003; Zhang et al., 2003), or by binding to HIV-1 RNA, leading to physical impairment of reverse transcription (deaminase-independent mechanism; Newman et al., 2005; Bishop et al., 2006; Iwatani et al., 2007). Consequently, primate lentiviruses have evolved to counteract the antiretroviral activity of A3G by acquiring Vif. This accessory protein prevents A3G incorporation into virions through its proteasomal degradation (Marin et al., 2003; Sheehy et al., 2003; Stopak et al., 2003). We and others have shown that Vif proteins derived from different HIV-1 subtypes differ in their potency of A3G inhibition, suggesting differential levels of viral fitness among clades (Iwabu et al., 2010; Binka et al., 2012). APOBEC2, a cardiac- and skeletal muscle-specific cytidine deaminase, is required for muscle development and early embryogenesis (Etard et al., 2010; Sato et al., 2010; Vonica et al., 2011). The physiological role of APOBEC4 remains to be determined.

DIFFERENTIAL ANTIVIRAL AND ANTI-RETROELEMENT ACTIVITIES OF A3 CYTIDINE DEAMINASES

Members of the A3 family contain either single (A3A, A3C, A3H) or double (A3B, A3DE, A3F, and A3G) cytidine deaminase domains (CDA). In A3G and A3F, the N-terminal CDA is responsible for RNA-dependent oligomerization, while the C-terminal CDA mainly mediates the deamination of single-stranded DNA (Hache et al., 2005; Newman et al., 2005). Some A3 family members strongly inhibit a wide range of exogenous retroviruses, as well as other viral pathogens, including herpesviruses, parvoviruses, papillomaviruses, and hepadnaviruses (Baumert et al., 2007; Vartanian et al., 2008; Narvaiza et al., 2009; Suspène et al., 2011b). The importance of A3 proteins *in vivo* has been demonstrated in murine studies in which mice lacking the A3 gene were shown to be more susceptible to viral infection than their wild-type counterparts (Okeoma et al., 2007, 2009; Takeda et al., 2008). A3 proteins also inhibit the mobilization of endogenous retroviruses, such as MusD, IAP, and the yeast LTR-retrotransposon Ty1 (Esnault et al., 2005; Schumacher et al., 2008), in addition to their inhibitory activity on L1 and Alu retrotransposition. The gene copy number of A3 family members is species-specific in mammals, in which except for primates, one, two, or three A3 proteins are encoded, whereas in humans and in non-human primates, seven A3 proteins have been recognized (A3A, A3B, A3C, A3DE, A3F, A3G, and A3H; Sawyer et al., 2004; OhAinle et al., 2006). Of note, expansion of the A3 gene cluster in primate genomes correlates with a

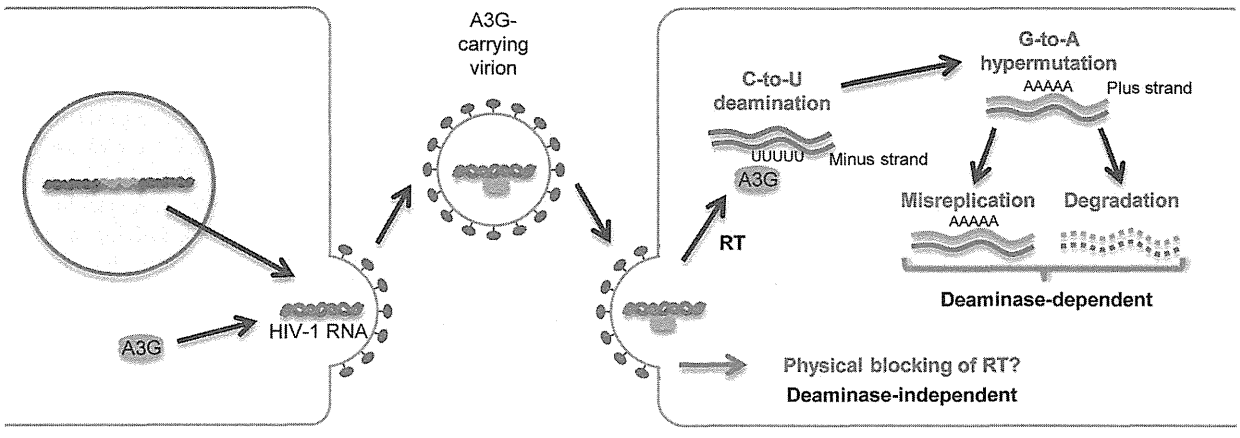

FIGURE 2 | Mechanisms of antiretroviral and anti-retroelement activity of A3G protein. A3G potently restricts Vif-deficient HIV-1 viruses: A3G is incorporated into virus particles in virus-producing cells. Following the infection of target cells, A3G inhibits viral replication either by binding to HIV-1 RNA, leading to physical blocking of reverse transcription (deaminase-independent mechanism), or by deaminating the viral minus-strand DNA during reverse transcription, thus generating G → A hypermutations in the proviral DNA plus strand.

sharp reduction in retrotransposition activity, suggesting that these restriction factors have evolved to protect mammalian hosts from retroelements (Sawyer et al., 2004; Schumann, 2007). Antiretroviral and anti-retroelement potencies were shown to differ in the seven members of A3 family, independently of their subcellular localization (Kinomoto et al., 2007). However, the exact mechanism by which A3 proteins inhibit retrotransposition is unclear. The current findings on antiviral and anti-retroelement activities of A3 members are summarized below and in **Table 1**.

A3A

Human A3A (hA3A) lacks inhibitory activity against HIV-1 produced from 293T cells overexpressing this protein (Bishop et al., 2004; Bogerd et al., 2006a; Kinomoto et al., 2007; Hultquist et al., 2011), since it is not incorporated into virions (Goila-Gaur et al., 2007; Aguiar et al., 2008). In human monocytic cells as targets, however, hA3A blocks the early phase of HIV-1 infection (Peng et al., 2007; Koning et al., 2011) but is counteracted by the HIV-2/SIV (simian immunodeficiency virus) accessory protein Vpx (Berger et al., 2010, 2011). Also, hA3A can inhibit infections by adeno-associated virus (Chen et al., 2006; Narvaiza et al., 2009), human papillomavirus (HPV; Vartanian et al., 2008), porcine endogenous retrovirus (PERV; Dörrschuck et al., 2011), and human T-cell leukemia virus type 1 (HTLV-1; Ooms et al., 2012). Importantly, *in vitro* overexpression experiments have demonstrated that hA3A effectively inhibits the retrotranspositions of L1, Alu (Bogerd et al., 2006b; Chen et al., 2006; Muckenfuss et al., 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Tan et al., 2009; Khatua et al., 2010; Ikeda et al., 2011), IAP, and MusD (Bogerd et al., 2006a; Chen et al., 2006; Ikeda et al., 2011) through a deaminase-independent mechanism. hA3A is intrinsically able to restrict infection of the genetically reconstituted HERV-K in a deaminase-dependent manner (Lee et al., 2008). In a recent report, hA3A was shown to induce somatic hypermutation in human mitochondrial and nuclear DNA; in the latter, this included genes associated with the development of cancer (Suspène et al., 2011a).

A3B

Human A3B (hA3B) is the sole member of the A3 family with an exclusive nuclear localization (Bogerd et al., 2006a; Muckenfuss et al., 2006; Stenglein and Harris, 2006; Kinomoto et al., 2007; Pak et al., 2011), sharing a common nuclear import mechanism with AID (Lackey et al., 2012). hA3B inhibits infections of HIV-1 and SIV, independently of the presence of Vif (Bishop et al., 2004; Yu et al., 2004; Doehle et al., 2005a; Hultquist et al., 2011). Since hA3B expression is extremely low in target $CD4^+$ T-cells (Bishop et al., 2004; Doehle et al., 2005a; Koning et al., 2009; Refsland et al., 2010), Vif might have failed to evolve the mechanism to antagonize this antiretroviral factor. Also, hA3B restricts infections by murine leukemia virus (MLV; Doehle et al., 2005b; Kinomoto et al., 2007), PERV (Dörrschuck et al., 2011), HTLV-1 (Ooms et al., 2012), and Rous sarcoma virus (RSV; Wiegand and Cullen, 2007). Like hA3A, *in vitro* overexpression of hA3B inhibits the retrotranspositions of L1, Alu (Bogerd et al., 2006b; Muckenfuss et al., 2006; Stenglein and Harris, 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Khatua et al., 2010; Ikeda et al., 2011), IAP, and MusD (Bogerd et al., 2006a; Chen et al., 2006; Ikeda et al., 2011) in a deaminase-independent manner, while inhibiting the reconstituted HERV-K infection through a deaminase-dependent mechanism (Lee et al., 2008). In a recent study, endogenously expressed hA3B effectively restricted L1 retrotransposition in both transformed cells and human embryonic stem cells (Wissing et al., 2011).

A3C

Human A3C (hA3C) is abundantly expressed in numerous tissues and cell types (Jarmuz et al., 2002), and its expression is unresponsive to interferon-α (Koning et al., 2009). Although hA3C is efficiently incorporated into retroviral particles, it exhibits only partial antiviral activity against HIV-1, with or without Vif (Bishop et al., 2004; Yu et al., 2004; Bogerd et al., 2006a; Hultquist et al., 2011). By contrast, hA3C is able to efficiently block the replication of SIV, which also encapsidates this protein but is readily antagonized by SIV Vif (Yu et al., 2004). hA3C can inhibit infection of primate foamy virus (PFV), which carries an hA3 antagonistic Bet protein (Russell et al., 2005; Perković et al., 2009). The overexpression of hA3C results in a moderate inhibition of L1 and Alu retrotranspositions (Bogerd et al., 2006b; Chen et al., 2006; Muckenfuss et al., 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Khatua et al., 2010) but effectively inhibits those of IAP, MusD, and Ty1 (Dutko et al., 2005; Chen et al., 2006). In a recent study, hA3C was shown to restrict infections by MLV (Langlois et al., 2005; Kinomoto et al., 2007), hepatitis B virus (HBV; Baumert et al., 2007; Köck and Blum, 2008), HPV (Vartanian et al., 2008), herpes simplex virus 1 and Epstein-Barr virus (Suspène et al., 2011b).

A3DE

Human A3DE (hA3DE) overexpression has moderate effects on L1 and Alu retrotransposition (Stenglein and Harris, 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Tan et al., 2009; Duggal et al., 2011). Similarly, hA3DE exhibits low levels of anti-HIV-1 and anti-SIV activities, both of which are antagonized by the respective Vif proteins (Dang et al., 2006; Hultquist et al., 2011). The reduced activity is determined by a cysteine residue located at amino acid position 320 of hA3DE. Substitution with the corresponding tyrosine present in A3F resulted in a 20-fold increase of A3DE activity (Dang et al., 2011). Indeed, the chimpanzee version of A3DE, carrying a tyrosine residue at this position, shows much higher antiretroviral activity, while both human and chimpanzee A3DEs exhibit similar levels of inhibition against retroelements, suggesting that the host defense activity of A3DE against retroelements has been evolutionarily conserved (Duggal et al., 2011).

A3F/A3G

With regard to the antiretroviral potencies of human A3G (hA3G) and A3F (hA3F) proteins, overwhelming amount of information is well-summarized elsewhere (Harris and Liddament, 2004; Huthoff and Towers, 2008; Malim, 2009). Similar to hA3G, as introduced in the previous section, hA3F has been shown to

Table 1 | Antiviral and anti-retroelement spectrum of A3 family members.

A3 family	Exogenous		Endogenous	
	Non-retroviruses	**Retroviruses**	**Non-human retroelements**	**Human retroelements**
A3A (C/N)	AAV (Chen et al., 2006; Narvaiza et al., 2009)	HIV-1 (Peng et al., 2007; Koning et al., 2011; Schmitt et al., 2011)	IAP (Chen et al., 2006; Ikeda et al., 2011) MusD (Bogerd et al., 2006a; Chen et al., 2006)	L1 (Bogerd et al., 2006b; Chen et al., 2006; Muckenfuss et al., 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Tan et al., 2009; Khatua et al., 2010; Ikeda et al., 2011)
	HPV (Vartanian et al., 2008)	SIV (Schmitt et al., 2011)	PERV (Dörrschuck et al., 2011)	Alu (Bogerd et al., 2006b; Muckenfuss et al., 2006; Niewiadomska et al., 2007; Tan et al., 2009; Khatua et al., 2010)
		HTLV-1 (Ooms et al., 2012)		Reconstituted HERV-K (Lee et al., 2008)
A3B (N)		HIV-1 (Bishop et al., 2004; Doehle et al., 2005a; Bogerd et al., 2006a; Kinomoto et al., 2007; Hultquist et al., 2011) SIV (Yu et al., 2004)	IAP (Bogerd et al., 2006a; Chen et al., 2006; Ikeda et al., 2011) MusD (Chen et al., 2006; Ikeda et al., 2011)	L1 (Bogerd et al., 2006b; Muckenfuss et al., 2006; Stenglein and Harris, 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Khatua et al., 2010; Ikeda et al., 2011; Wissing et al., 2011)
		HTLV-1 (Ooms et al., 2012)	PERV (Dörrschuck et al., 2011)	Alu (Bogerd et al., 2006b; Muckenfuss et al., 2006; Stenglein and Harris, 2006; Niewiadomska et al., 2007)
		MLV (Doehle et al., 2005b; Kinomoto et al., 2007) RSV (Wiegand and Cullen, 2007)		Reconstituted HERV-K (Lee et al., 2008)
A3C (C/N)	HBV (Baumert et al., 2007; Köck and Blum, 2008) HPV (Vartanian et al., 2008)	HIV-1 (Bishop et al., 2004; Yu et al., 2004; Bogerd et al., 2006a; Hultquist et al., 2011)	IAP (Chen et al., 2006) MusD (Chen et al., 2006)	L1 (Bogerd et al., 2006b; Chen et al., 2006; Muckenfuss et al., 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Khatua et al., 2010)
	HSV-1 (Suspène et al., 2011b)	SIV (Yu et al., 2004) PFV (Russell et al., 2005; Perković et al., 2009)	Ty1 (Dutko et al., 2005)	Alu (Bogerd et al., 2006b; Muckenfuss et al., 2006; Niewiadomska et al., 2007; Khatua et al., 2010)
	EBV (Suspène et al., 2011b)	MLV (Langlois et al., 2005; Kinomoto et al., 2007)		
A3DE (C)		HIV-1 (Dang et al., 2006; Hultquist et al., 2011)		L1 (Stenglein and Harris, 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Duggal et al., 2011)
		SIV (Dang et al., 2006)		Alu (Tan et al., 2009)
A3F (C)		HIV-1 (Wiegand et al., 2004; Zheng et al., 2004; Bishop et al., 2006; Holmes et al., 2007; Yang et al., 2007)	IAP (Chen et al., 2006) MusD (Chen et al., 2006)	L1 (Turelli et al., 2004a; Muckenfuss et al., 2006; Stenglein and Harris, 2006; Kinomoto et al., 2007; Niewiadomska et al., 2007; Khatua et al., 2010)
		SIV (Bogerd et al., 2004; Mangeat et al., 2004; Schröfelbauer et al., 2004) XMRV (Paprotka et al., 2010)	Ty1 (Dutko et al., 2005; Schumacher et al., 2005) PERV (Dörrschuck et al., 2011)	Reconstituted HERV-K (Lee and Bieniasz, 2007; Lee et al., 2008)
		PFV (Russell et al., 2005; Delebecque et al., 2006) MLV (Langlois et al., 2005) RSV (Wiegand and Cullen, 2007) MPMV (Doehle et al., 2006)		

(Continued)