Novel host restriction factors implicated in HIV-1 replication

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Abstract

Human immunodeficiency virus-1 (HIV-1) is known to interact with multiple host cellular proteins during its replication in the target cell. While many of these host cellular proteins facilitate viral replication, a number of them are reported to inhibit HIV-1 replication at various stages of its life cycle. These host cellular proteins, which are known as restriction factors, constitute an integral part of the host’s first line of defence against the viral pathogen. Since the discovery of apolipoprotein B mRNA-editing enzyme 3G (APOBEC3G) as an HIV-1 restriction factor, several human proteins have been identified that exhibit anti-HIV-1 restriction. While each restriction factor employs a distinct mechanism of inhibition, the HIV-1 virus has equally evolved complex counter strategies to neutralize their inhibitory effect. APOBEC3G, tetherin, sterile alpha motif and histidine-aspartate domain 1 (SAMHD1), and trim-5α are some of the best known HIV-1 restriction factors that have been studied in great detail. Recently, six novel restriction factors were discovered that exhibit significant antiviral activity: endoplasmic reticulum α,2-mannosidase I (ERManI), translocator protein (TSP0), guanylate-binding protein 5 (GBP5), serine incorporator (SERINC3/5) and zinc-finger antiviral protein (ZAP). The focus of this review is to discuss the antiviral mechanism of action of these six restriction factors and provide insights into the probable counter-evasion strategies employed by the HIV-1 virus. The recent discovery of new restriction factors substantiates the complex host–pathogen interactions occurring during HIV-1 pathogenesis and makes it imperative that further investigations are conducted to elucidate the molecular basis of HIV-1 replication.

BACKGROUND

HIV-1 AND AIDS

Acquired immunodeficiency syndrome (AIDS) was discovered in 1981 when a group of homosexual men in the United States were diagnosed with opportunistic infections leading to their death [1]. In 1983, the infectious causative agent of this fatal disease was isolated [2] and later termed the human immunodeficiency virus (HIV-1) by the International Taxonomy of Virologists [3]. Since the start of the epidemic, around 78 million people across the world have been infected with the HIV-1 virus, resulting in 39 million global deaths. According to WHO statistics, 35 million people were living with HIV-1 infection by the end of 2013 [4].

HIV-1 is an obligate parasite and, like other viruses, it is dependent on host proteins to complete its lifecycle. The HIV-1 replication cycle initiates with the attachment of the viral envelope glycoprotein to the CD4 receptor and a co-receptor present on the surface of immune cells that could be either CC chemokine receptor 5 (CCR5) or CX chemokine receptor 4 (CXCR4). This is followed by the fusion of the viral and the host cell membranes. Post-fusion, the HIV-1 capsid, along with the viral RNA genome, is released into the cytoplasm. The viral RNA is reverse-transcribed into double-stranded DNA that enters the nucleus and integrates into the host genome. The viral genome is transcribed and translated by the host cell machinery. The viral proteins assemble with the viral RNA genome at the plasma membrane, leading to the release of the fully assembled virus, which initiates a new round of infection. Since the discovery of HIV-1, extensive research has been conducted in order to understand the molecular mechanisms underlying viral pathogenesis, host–virus interactions and the development of antiviral intervention strategies. The current management of HIV/AIDS is based on highly active antiretroviral therapy (HAART). HAART therapy aims to reduce the viral load in patients, maintain T cell counts and prevent opportunistic infections that lead to death [5]. HAART does not clear the virus completely and despite three decades of intensive research efforts, we are still far from a successful cure for HIV-1/AIDS. Several factors prevent a complete cure of HIV/AIDS, such as drug toxicity over a long treatment period, high mutation rates leading to the evolution of
drug-resistant variants and a tendency to remain latent in the host genome [6]. A successful HIV-1 vaccine also remains elusive and efforts are underway to develop HIV-1 vaccines, with several candidates being in clinical trials [7].

In order to counter HIV/AIDS, it is crucial to gain a better understanding of HIV-1 biology, and in particular how the virus exploits the host machinery to its advantage. Host proteins are known to interact with viral proteins, and elucidating the mechanism of these interactions would be a significant advance for the development of novel intervention strategies for antiretroviral therapy.

RESTRICTION FACTORS

Mammalian cells express a number of host proteins that inhibit HIV-1 replication at various stages of its life cycle. These host cellular proteins are termed restriction factors. They are induced by interferon and provide the initial line of defence against HIV-1 infection as components of the innate immune response.

For example, one of the most extensively studied HIV-1 restriction factors, APOBEC3G (A3G), a protein member of the family of cytidine deaminases, was discovered in 2002 [8–10]. Other members of the APOBEC3 family (APOBEC3B, APOBEC3D, APOBEC3F, APOBEC3G and APOBEC3H) have also been reported to inhibit HIV-1 replication [11]. However, among the APOBEC3 family, A3G has been demonstrated to exhibit the most potent anti-HIV-1 activity [12].

It was observed that A3G/3F is packaged into viral particles in the absence of the HIV-1 viral infectivity factor (Vif) protein and causes deamination of the nascent DNA minus strand (deoxycytidine to deoxyuridine) during reverse transcription of the HIV-1 RNA in the second round of infection. The resulting plus strand DNA contains multiple guanine to adenine hypermutations, leading to the formation of premature stop codons and the production of aberrant viral transcripts, which eventually undergo degradation. The antiviral activity of A3G/3F is counteracted by aberrant viral transcripts, which eventually undergo degradation 

The HIV-1 envelope (Env) protein plays a crucial role during the first step of the viral life cycle, i.e. attachment to its host cell receptor. HIV-1 Env is glycosylated with the carbohydrate moieties, playing an essential role in the proper folding of the native protein, which is crucial for both viral infection and immune evasion [17–20]. Like other N-linked glycoproteins, Env is expressed through the classical secretory pathway [21]. Initially, nascent polypeptide chains are co-translationally transferred to the endoplasmic reticulum (ER) lumen, where they are covalently attached to pre-assembled oligosaccharides (Glc3Man9GlcNAc2) at Asn residues present in the consensus Asn-X-(Ser/Thr) motifs [22, 23]. Sequential trimming of three glucose and six mannose residues followed by an extension mediated by Golgi glycosyltransferases leads to the production of diversified glycoproteins [24]. The Env glycan intermediates are correctly folded through the actions of the ER chaperones, calnexin and calreticulin. The misfolded proteins are recognized in the ER by the key quality-control player, UDP-glucose glycoprotein glucosyltransferase 1 (UGT1), which provides another opportunity for the correct folding of the proteins. Thereafter, the remaining misfolded proteins are eventually degraded by the ER-associated degradation (ERAD) pathway [25–28]. The trimming of α1,2-mannose residues from the Man9GlcNAc2 precursor is performed by a set of Carbohydrate Active Enzymes Database (CAzy) glycoside hydrolase family 47 (GH47) α-mannosidases [29, 30], which includes seven GH47 members [27], including ER α1,2-mannosidase I (ERManI), three Golgi α1,2-mannosidases (GMIA, GMIB, and GMIC) and three ER degradation-enhancing α-mannosidase-like (EDEM) proteins (EDEM1, EDEM2 and EDEM3) [27, 28]. In classical models of mammalian N-glycan maturation, ERManI is the first enzyme to act upon Man9GlcNAc2 to cleave a single α1,2-mannose residue (M10) from the glycan B branch and generate a Man8GlcNAc2 B isomer (Man8B) [30, 31]. However, under conditions of overexpression it may also continue to cleave α1,2-linked mannose residues on other branches [32]. Excessive processing acts as a signal targeting the glycoprotein for degradation via the ubiquitin–proteasome pathway [33, 34].

ENDOPLASMIC RETICULUM

α1,2-MANNOSIDASE I (ERMANI)

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TRANSLOCATOR PROTEIN (TSPO)

The degradation of the misfolded Env glycoprotein is triggered by a mitochondrial translocator protein (TSPO) [35]. This protein was identified as a high-affinity diazepam-
binding protein in peripheral tissues [36]. It plays a role in steroid biosynthesis [37] and is widely expressed in steroid-producing tissues. TSPO is localized on the outer mitochondrial membrane [38] and contains a C-terminal cholesterol interaction sequence, which mediates the import of cholesterol from the outer to the inner mitochondrial membrane [39]. TSPO interacts with an outer mitochondrial membrane protein, the voltage-dependent anion channel (VDAC) protein [40], which is a component of the mitochondrial permeability transition pore (mPTP) complex [41]. This complex controls mitochondrial membrane integrity by maintaining a proton gradient across the inner mitochondrial membrane, with a negative charge towards the inner matrix [42]. This function of TSPO has been reported to play an important role in antiviral activity, as explained in the next section.

The antiretroviral activity of A3G and its counteraction by HIV-1 Vif was discovered by studies based on a non-permissive CEM and permissive CEM-SS cells [12]. It was later observed that a variant of CEM cells called CEM.NKR cells also restricted the replication of wild type HIV-1 [43]. CEM.NKR cells are derivatives of CEM cells that are resistant to natural killer (NK) cell-mediated lysis [44] and lack the expression of two ER chaperone proteins, calnexin and calreticulin, which help in the correct refolding of proteins in the ER [45–47]. It was speculated that the absence of calnexin or calreticulin might play a role in restricting productive replication of HIV-1, which formed the basis of two hypotheses: either CEM.NKR cells lack a cofactor(s) that is essential for HIV-1 replication or these cells express a dominant inhibitor(s) that blocks HIV-1 replication. Heterokaryons formed between non-permissive CEM.NKR cells and permissive 293T failed to produce infectious viral particles, indicating that the restriction of HIV-1 replication in CEM.NKR cells was not due to a lack of cofactors (such as calnexin and calreticulin), but rather due to the expression of a dominant inhibitor(s) that blocks HIV-1 replication [43]. Overexpression of calnexin did not rescue HIV-1 replication in CEM.NKR cells, confirming the heterokaryon analysis [48].

These results suggested the possibility of a cellular inhibitor(s) in CEM.NKR cells that potently blocked the replication of HIV-1 at a post-entry step like A3G/A3F [43], except that it also inhibited the wild-type virus [49]. Replacing the vif gene of HIV-1 with SIVmac vif did not rescue the inhibition of HIV-1 replication [48]. It was further observed that expression of the mitochondrial translocator protein, TSPO, was significantly upregulated in CEM.NKR cells in comparison to CEM.SS and CEM.T4 cells, which are both permissive for HIV-1 replication [35]. Knocking down the expression of TSPO restored HIV-1 replication in CEM.NKR cells. Further detailed investigations identified that the overexpression of the ER-associated glycosidase hydrolase family 47 (GH47) alpha mannosidase, ERManI, also inhibited HIV-1 Env expression. Inhibiting the CAZy GH47 alpha-mannosidase activity of ERManI with kifunesine, or by small interfering RNA (siRNA)-mediated gene knockdown, inhibited the ERAD pathway and rescued HIV-1 Env expression [33, 34, 50].

HIV-1 Env is expressed through the classical secretory pathway, which is dependent on the ER for its proper folding [21]. The ER protein folding process is error-prone, so eukaryotes have evolved the ERAD quality-control pathway to specifically degrade misfolded glycoproteins. HIV-1 Env glycoproteins have a large number of cysteine residues that are cross-linked to form 10 disulfide bonds. The Env folding efficiency is extremely low, with over 80% of Env proteins being misfolded and retained in the ER for degradation. As has been reported for other glycoproteins, the most oxidized redox status in the ER is necessary for the proper folding of Env [51], which is regulated by the mitochondria, most likely through the release of reactive oxygen species [52]. These reactive oxygen species are connected to the ER via a mitochondrial-associated ER membrane that is supported by a protein complex consisting of a voltage-dependent anion channel and several other proteins [53]. It has been reported that TSPO interacts with the voltage-dependent anion channel [40]. An increase in ERManI expression during HIV-1 infection may increase the expression of TSPO, which could further reduce the oxidative redox status in the ER, most likely by blocking the mitochondria–ER communication. Misfolded Env are recognized by ERManI that non-specifically cleave α1,2-linked mannose residues on the first and third branches of the gp160 precursor protein (Fig. 1). The accumulation of misfolded Env results in their recognition and degradation via the ERAD pathway [16], which results in the production of HIV-1 viruses lacking Env and with reduced infectivity.

The HIV-1 Env degradation activity of ERManI resides in its catalytic domain. Structurally, the catalytic domain of ERManI contains an αααα7-barrel composed of 14 consecutive helices, of which Glu-330, Asp-463 and Glu-599 act as the catalytic residues [54, 55]. The ERManI mutants, E330A, R334C, E397K, D463A, C527A, C556A and E599A were defective in their Env degradation activity.

Interestingly, TSPO overexpression in the absence of ERManI reduced Env inhibition [16], suggesting that TSPO induces HIV-1 Env degradation via the ERAD pathway. However, ERManI acts as an initiator of this degradation, which resulted in the inhibition of HIV-1 replication [16]. The regulation of TSPO activity by ERManI is yet to be fully understood. Whether ERManI enhances the expression of TSPO directly or indirectly via other factors remains unknown. Furthermore, the HIV-1 viral protein that counteracts the activity of ERManI has not yet been identified. Recently, it was reported that the HIV-1 Vpr protein promotes Env folding in the ER, which is otherwise misfolded in its absence and eventually degraded in CEM.NKR cells [56]. This activity of Vpr resides in its N-terminal domain, in which the mutation of residue 30 (A30L) led to the abrogation of its activity in Env refolding [56]. Vpr has also been reported to increase the expression of Env in dendritic cells...
and macrophages [57]. Thus, it is highly plausible that Vpr may increase HIV-1 replication by directly or indirectly targeting ERManI and preventing it from initiating the misfolding and degradation of the Env protein. ERManI-mediated regulation of Env degradation has only been reported in CEM.NKR cells. It is thus important to study this phenomenon in other cells, such as CD4+ T cells, macrophages and monocytes, which would help to improve our understanding of ERManI’s mechanism of action. Degradation of the viral Env protein has the potential to be targeted to suppress HIV-1 infection and this warrants further investigation of the in vivo effect of ERManI on HIV-1 infection.

GUANYLATE-BINDING PROTEIN-5 (GBP5)

Guanylate-binding protein 5 is a member of the interferon-inducible guanosine triphosphatase (GTPases) superfamily that plays a role in intrinsic immunity against bacteria, protozoa and viruses. Guanylate-binding proteins hydrolyze guanosine triphosphate (GTP) to GDP and GMP [58]. Humans possess seven guanylate-binding proteins (GBP1–GBP7), whose genes are located in a single cluster on chromosome 1 [58, 59]. Human GBP1, 2 and 3 are induced by IFN-γ, TNF-α and IL-1β, whereas human GBP4 and GBP5 are robustly induced by IFN-γ alone. GBP1 is involved in host defence against dengue, hepatitis C, VSV and encephalomyocarditis [60, 61]. GBP5 is primarily expressed in the cytosol and endosomal membranes. However, upon infection it co-localizes with the pathogen [62] and reduces the production of infectious HIV-1 particles.

GBP5 is expressed in both macrophages and CD4+ T cells. Analysis of the Genomic Utility for Association and Viral Analyses in HIV (GuavaH) database indicated an enhanced expression of GBP5 in HIV-1-infected patients [63, 64]. The mechanism underlying the increase of GBP5 expression remains unknown. One report suggested that the HIV-1 Tat protein increases GBP5 expression in human primary T cells [65], while another report suggested that HIV-1 Vif decreases GBP5 expression in Vif-expressing primary T cells [66]. Interestingly, GBP5 has been identified as an interferon-inducible inhibitor of HIV-1 infectivity [67].

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**Fig. 1.** Degradation of Env by ERManI and interference by GBP5 in various steps of Env processing. During HIV-1 infection in CEM.NKR cells, high expression of ERManI in ER increases the expression of TSPO in mitochondria through an unknown mechanism, which in turn blocks the ion transport from mitochondria to the ER. This creates an imbalance in the optimal redox state of the ER and triggers increased processing of the Env glycoprotein, resulting in the misfolding of Env and eventually its degradation. GBP5 interferes with N-linked glycosylation modification of the Env glycoprotein in Golgi, which increases the number of unprocessed immature gp160. This subsequently increases the incorporation of immature Env trimers into progeny virions and reduces the infectivity of the virions.
that works by impairing the function of the viral envelope glycoprotein gp120 [68].

GBP5 has been reported to affect the processing of the HIV-1 envelope glycoprotein by interfering with the N-linked oligosaccharide glycosylation modification of Env glycoproteins in the Golgi (Fig. 1). This subsequently increases the incorporation of unprocessed immature gp160 into progeny virions, thus impairing its trafficking to the cell surface and the infectivity of the virions [68].

Recent reports suggest that there is an indirect mechanism of evasion that works through a naturally occurring mutation in the start codon of the HIV-1 Vpu gene in both macrophage-tropic and brain-derived HIV-1 strains. Vpu is a HIV-1 protein that counteracts the antiviral activity of another host restriction factor, tetherin. The HIV-1 Env glycoprotein is translated along with Vpu from a single bicistronic mRNA transcript. Env expression depends on the leaky scanning of the Vpu AUG initiation codon or ribosomal shunting [69]. Therefore, a mutation in the Vpu start codon leads to increased expression of the Env glycoprotein [70], countering GBP5 inhibition but making the virus susceptible to restriction by tetherin. These events block the release of progeny virions and increase their accumulation at the host cell surface [68]. Since HIV-1 is a highly adaptable virus, some M-tropic and brain-derived strains have evolved, containing disruptive mutations in Vpu that confer resistance to GBP5 in addition to a selective survival advantage [68].

The HIV-1 protein, negative regulatory factor (Nef), has multiple well-characterized activities, including the downregulation of cell surface molecules (CD4, MHC-I and T cell receptors) and alterations in the activation of T cells, macrophages and the actin cytoskeleton [72, 73]. In addition to these activities, the role of HIV-1 Nef in enhancing the infectivity of the virion is still not well understood. Nef-deficient viruses have been reported to have highly reduced infectivity, which suggests that an inhibitory factor is present in these virions [74]. Furthermore, heterokaryons formed by the fusion of lymphoid cells (high Nef responsive) and fibrosarcoma cells (low Nef responsive) produced HIV-1 viruses with a high dependence on Nef for their infectivity, which suggests that Nef rescued HIV-1 infectivity by overcoming the presence of a transdominant cellular inhibitor. Transcriptome analysis of high and low Nef-responsive cells identified SERINC5 as a putative protein responsible for regulating HIV-1 infectivity [75]. This was confirmed by another independent study [76] involving proteome analysis of virions produced by T lymphoid cells infected with wild-type HIV-1 or Nef-deleted HIV NL4-3, or murine leukaemia virus (MLV) with a glycosylated Gag instead of Nef. This study also identified two SERINC proteins, i.e. SERINC3 and SERINC5, as cellular proteins whose overexpression reduced the infectivity of Nef-defective virions, thus confirming their antiviral activity. Furthermore, the depletion or knockdown of SERINC3 and SERINC5 in T cells increased the infectivity of HIV-1 [76].

SERINC3 and SERINC5 are constitutively expressed intrinsic restriction factors whose expression is independent of interferon-α (IFN-α) [75, 76]. A recent report suggested that the SERINC-mediated restriction or inhibition of HIV-1 occurs post-entry at an early stage in the HIV-1 life cycle, with SERINC5 affecting the ability of the viral particle to translocate its content to the cytoplasm [75]. A reduced accumulation of reverse transcription products is observed in these target cells in the absence of Nef inhibiting HIV-1 replication [75, 76]. The precise mechanism by which SERINC3/5 proteins prevent the cytoplasmic transport of viral contents in the absence of Nef remains unclear. SERINC5-mediated restriction of HIV-1 does not depend on alterations in the lipid composition and organization of HIV-1 particles, but might involve different cellular functions of SERINC5 [77]. A recent report suggested that SERINC proteins inhibit the formation of small fusion pores between viruses and cells. These proteins further alter the conformation of Env proteins, inactivating them and thereby restricting HIV-1 fusion [78]. It has been demonstrated that among the five alternatively spliced isoforms of SERINC5, one isoform, Ser5-001, predominantly restricts HIV-1 replication. The presence of the tenth transmembrane domain in Ser5-001 plays a role in its expression and plasma membrane targeting that is essential for its antiviral activity [79].

The antiviral activity of SERINC3/5 is counteracted by three distinct viral proteins, Nef (HIV-1), glycosylated Gag (MLV) and S2 (EIAV). The mechanisms through which SERINC3/5 renders HIV-1 particles non-infectious are depicted in Fig. 2. In the absence of Nef or overexpression of SERINC5, almost all SERINC5 proteins are found on the plasma membrane and incorporate into the budding virions, resulting in their reduced infectivity [75, 76]. HIV-1
Nef reduces SERINC5 expression in the plasma membrane by localizing it to late endosomes, resulting in the enhancement of HIV-1 infectivity [75]. The ability of Nef to counteract SERINC5 is conserved across different primate lentiviruses and is dependent upon the nature of the viral envelope glycoprotein. Pseudotyping the HIV-1 virus with either vesicular stomatitis virus G protein (VSV-G) or Ebola virus glycoprotein (EBOV GP) has been shown to confer resistance against SERINC5. On the other hand, MLV-A or MLV-X Env pseudotyped HIV-1 remains sensitive to SERINC5 restriction (Fig. 2a). The Nef protein from two divergent SIV lineages (SIVmac239 and SIVagm) has been reported to counteract SERINC5 with a 10-fold higher efficacy than the HIV-1 LAI strain [75].

In addition to Nef, glycosylated Gag (glycoGag), an accessory protein of MLV, is also capable of rescuing the infectivity of Nef-defective HIV-1 [80]. MLV glycoGag is a type II transmembrane protein with an amino-terminal cytosolic non-Gag portion and an extracellular Gag domain [81]. In the absence of glycoGag, SERINC5 expressed in the producer cells potently inhibited the infectivity of MLV [75]. The equine infectious anaemia virus (EIAV) accessory protein S2 is the third protein reported to counteract the restriction by SERINC5. S2 plays an important role in viral

**Fig. 2.** The effect of SERINC on HIV-1. (a) In the presence of Nef, SERINC5 is removed from the plasma membrane and relocalized to late endosomes together with Nef. This prevents its incorporation into the nascent virions. These virions are able to deliver viral contents to the cytoplasm efficiently and continue HIV-1 infection. In the absence of Nef or overexpression of SERINC5 protein, its antiviral activity depends on the nature of the viral envelope protein. In the case of viruses with resistant envelope proteins, the mature virions lack SERINC5 protein and can continue with the infection of target cells. In the case of viruses with sensitive envelopes, SERINC5 being incorporated into the mature virus prevents proper binding and fusion of the virus to new target cells and hence terminates viral replication. (b) SERINC proteins may interact with sensitive Env protein and induce conformational changes in the Env protein, which slows down the fusion process and exposes the epitopes responsible to bind with various neutralizing antibodies.
replication and disease progression in horses. Although the three retroviral factors, Nef, GlycoGag and S2 protein, share no sequence homology and have evolved independently to counteract SERINC5, they do share three common features. Firstly, the three proteins bear putative motifs to bind clathrin adaptors such as AP2; secondly, they are expressed on the membrane; lastly, all three proteins require functional host cell endocytosis machinery to degrade SERINC3/5 and enhance virion infectivity [75, 82]. Hence, impairing clathrin-mediated endocytosis through inhibitors that block the function of endosomes or Nef/Glycogag/S2-mediated relocation of SERINC3/5 proteins to late endosomes may inhibit the production of infectious HIV-1/MLV/EIAV particles.

Many restriction factors, such as APOBEC3G, tetherin and TRIM5α, have co-evolved with the host over years of continuous host–pathogen interactions [83–85]. In contrast, no sign of positive evolutionary selection was observed in SERINC genes, despite the biological interaction of SERINC genes with the viral protein Nef. Thus, it is highly plausible that the antiretroviral function of the SERINC proteins may be a relatively new evolutionary advancement, which might have resulted from the arms race between the host and viral proteins [86].

In addition to the antagonism by Nef, HIV-1 Env is also able to restrict the activity of SERINC5 without excluding its incorporation into the viral particles, thus suggesting that Nef and Env counteract SERINC5 through distinct mechanisms [87]. The determinants in Env have been mapped to the V1/V2 loop [76] and the V3 loop [87]. Folding of the V1, V2 and V3 loops is an interdependent process that determines Env stability, conformation, co-receptor usage and the SERINC5 counteracting activity. However, HIV-1 Env is unable to prevent the incorporation of SERINC5 into the virion, which could result in the increased vulnerability of Env to inhibition by neutralizing antibodies (Fig. 2b). This could probably explain why the role of Nef in excluding SERINC5 from virion incorporation has been conserved among all HIV-1 isolates [87].

ZINC-FINGER ANTIVIRAL PROTEIN (ZAP)
Zinc-finger antiviral protein [ZAP; also termed zinc-finger CCCH-type, antiviral 1 (ZC3HAV1), or poly (ADP-ribose) polymerase 13 (PARP13)] is an inhibitor of multiple retroviruses. ZAP is encoded by an IFN-stimulated gene (ISG), ZC3HAV1 [88].

ZAP was initially discovered as a host factor that inhibited the replication of MLV. The screening of mammalian complementary DNA (cDNA) libraries from MLV-resistant cells led to the identification of a gene that encoded a CCCH-type zinc-finger protein, ZAP. ZAP expression produces significant loss of viral messenger RNAs (mRNAs) from the cytoplasm but not from the nucleus. There was no effect on the integration of proviral DNA within the genome, which indicated that ZAP expression does not affect the nuclear entry of viral DNA [89]. Subsequently, it was observed that ZAP could target several RNA viruses, including Retroviridae (HIV-1, MoLV and XMRV), Filoviridae (Ebola and Marburg), Togaviridae (alphavirus, Sindbis, Semliki Forest and Ross River viruses) and Hepadnaviridae (hepatitis B) [90–96]. In addition, ZAP inhibits the double-stranded DNA murine gamma herpesvirus [97], but has no effect on the vesicular stomatitis, poliovirus, yellow fever and herpes simplex I viruses [90]. ZAP has also been reported to restrict human retrotransposons [98]. Hence, ZAP has a wide range of antiretroviral activity. Being obligatory parasites, viruses utilize host cellular machinery to replicate their genome. As ZAP targets viral mRNA directly, it might have gained antiviral activity against broad range of viruses. It has been observed that ZAP overexpression inhibits HIV-1 replication by translational repression followed by viral mRNA degradation through recruiting the cellular mRNA degradation machinery [96, 99]. The proposed mechanisms of action of ZAP are depicted in Fig. 3. ZAP sequesters elf4G, impeding its interaction with elf4A and leading to the translation repression of viral mRNAs (Fig. 3a), which is a prerequisite for viral mRNA degradation [96]. Following the translational repression of HIV-1 mRNA, ZAP recruits either poly(A)-specific ribonuclease (PARN) to remove the poly (A) tail (Fig. 3b) or decapping enzyme with the help of its cofactor p72/DDX17 (Fig. 3c). The viral RNA is targeted to exosomes and degraded by XrnI from the exposed 5′end. Similarly, ZAP also recruits the exosome complex leading to 3′ to 5′ mRNA decay (Fig. 3d). Both human and rat ZAP inhibit the propagation of replication-competent HIV-1, suggesting that the antiretroviral activity of ZAP is not species-specific [96]. In the case of HIV-1, it has been reported that ZAP specifically targets newly synthesized HIV-1 mRNA that has been spliced at multiple sites and not at a single splice site through an unknown mechanism. It has been suggested that the target of ZAP is the 5′ UTR of HIV-1 Nef mRNA, which contains the second splicing junction [96]. Recently, TRIM25, an E3 ubiquitin and ISG15 ligase that is responsible for the poly-ubiquitination and activation of RIG-I [100–102], was found to be a cofactor of ZAP [88]. TRIM25 helps ZAP to block the replication of the Sindbis virus (SINV) [88].

The viral protein that counteracts the restriction activity of ZAP to establish productive infection remains unknown. Overexpression of human and rat ZAP inhibits the proliferation of replication-competent HIV-1, suggesting the absence of a viral protein that antagonizes the ZAP activity [96]. It was later reported that the nuclear matrix protein, Matrin 3, acts as a negative regulator of the anti-HIV-1 activity of ZAP. In addition to Matrin 3, the other nuclear matrix proteins, heterogeneous nuclear ribonucleoprotein U (hnRNP U) and splicing factor proline/glutamine-rich (SFPQ), also inhibit the anti-HIV-1 activity of ZAP [103]. Matrin 3 is a highly conserved 125 kDa nuclear inner matrix protein. It contains two RNA recognition motifs (RRMs) that bind RNA [104, 105] and two zinc fingers that are
reported to bind DNA [105–107]. It has been demonstrated that Matrin 3 binds HIV-1 RNAs through its RNA recognition motifs (RRMs) and acts as a Rev cofactor, promoting RNA stabilization and expression of Gag protein [104, 108]. The role of Matrin 3 against ZAP is distinct from its function as a Rev cofactor [103].

Matrin 3-associated proteins interact with those of the ZAP degradation complex, DEAD (Asp-Glu-Ala-Asp) box Helicase 17 (DDX17 or p72) and exosome component 3 (EXOSC3) in an RNA-dependent manner [103]. p72/DDX17 is a cofactor of ZAP [109] and EXOSC3 is a core component of the human exosome complex responsible for 3′–5′ exonuclease activity [110]. It has been observed that viral RNA mediates the interactions between these proteins. The mechanism through which Matrin 3 regulates ZAP’s antiviral activity is shown in Fig. 4. ZAP molecules interact [111] and form dimers on viral RNAs [112]. Matrin 3 may inhibit ZAP from dimerizing on HIV-1 RNAs (Fig. 4a). Both Matrin 3 and ZAP may compete for similar sites on viral RNAs (Fig. 4b). Matrin 3 may also interfere with ZAP’s interaction with DDX17 and EXOSC3 proteins, which is required for proper RNA degradation activity (Fig. 4c, d) [103].

Most of the findings were observed while ZAP was overexpressed. Further studies are now required to investigate the detailed regulatory mechanism of ZAP-mediated restriction of retroviruses by Matrin 3. The functional and mechanistic activity of ZAP needs to be elucidated in greater detail using CD4+T cells, macrophages/monocytes and in vivo model systems. Such studies would help in designing novel antiretroviral strategies that target the interaction between Matrin 3 and ZAP. Another mechanistic insight into the activity of ZAP was recently reported by Takata et al. [113], which suggested that ZAP binds directly to HIV-1 RNA sequences containing the CG dinucleotide and inhibits HIV-1 replication [113].

CONCLUSIONS

The discovery of host restriction factors that could serve as a natural defence against HIV-1 has been the focus of past research efforts, and these continue with great vigour. We have thus focused on reviewing six such newly discovered HIV-1 restriction factors, ERManI, TSPO, GBP5, SERINC3/5 and ZAP. The current review assimilates our present day knowledge of their mechanisms of action and the viral strategies to counteract these restriction factors. These
factors have the hallmarks of other well-known retroviral restriction factors, but also have certain unique characteristics. Unlike most other restriction factors, ERManI and SERINC3/5 are not interferon-inducible and hence can be categorized as constitutively expressed intrinsic restriction factors. On the other hand, GBP5 is highly induced by interferon, and targets the HIV-1 envelope protein. Env plays a crucial role in the first step of HIV-1 viral entry and is an attractive target for cellular restriction factors. While many target the viral proteins, ZAP is unique as it targets the host cell proteins utilized for viral replication and thus exhibits broader activity against several retroviruses. Hence, it is apparent that these newly discovered restriction factors demonstrate some distinctive properties that have not been observed with previously known restriction factors.

Since these are recent discoveries, there are a number of unanswered questions regarding the functional characteristics of these new restriction factors and their potential as antiretrovirals, and these need to be addressed. The antiviral activity of several of these restriction factors is cell-type-specific and needs to be demonstrated in all HIV-1-permissive cells. The viral countermeasure for the ERManI and ZAP restriction factors has not yet been determined. The identification and characterization of the direct viral evasion mechanism against these restriction factors may help to develop their potential as therapeutic targets against HIV-1 and associated disorders. The activity of these restriction factors is directly dependent on their levels of expression. Ectopic expression of SERINC5 potently inhibits HIV-1, even in the presence of Nef. The question of whether the viral protein Vpu counteracts the activity of GBP5 directly, or if changes in the expression levels of Vpu and Env affect HIV-1 inhibition by GBP5 remains to be clarified. It is postulated that increasing the cellular expression levels of ERManI, GBP5, SERINC5 and ZAP through exogenous factors may enhance the natural defence of the host and potentially reduce HIV-1 replication. Both SERINC3/5 and ZAP can inhibit diverse retroviruses. The question of whether the antiviral activity of ERManI and GBP5 is specific to HIV-1 or extends to other retroviruses needs to be examined.
Various approaches are currently being used to identify novel restriction factors. In a recent report, the expression profiling of host restriction factors in HIV-1 elite controllers identified schlafen 11 as a potential restriction factor that has yet to be functionally characterized [114]. As more restriction factors are being discovered, their exploitation as therapeutic targets has attracted widespread attention. Enhancing the expression of restriction factors or inhibiting the interaction of their viral antagonist using small molecule inhibitors or other pharmacological approaches could indeed be an effective antiviral therapy against HIV-1.

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Conflicts of interest
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