Roles of HIV-1 auxiliary proteins in viral pathogenesis and host-pathogen interactions

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ABSTRACT

Active host-pathogen interactions take place during infection of human immunodeficiency virus type 1 (HIV-1). Outcomes of these interactions determine the efficiency of viral infection and subsequent disease progression. HIV-infected cells respond to viral invasion with various defensive strategies such as innate, cellular and humoral immune antiviral mechanisms. On the other hand, the virus has also developed various offensive tactics to suppress these host cellular responses. Among many of the viral offensive strategies, HIV-1 viral auxiliary proteins (Tat, Rev, Nef, Vif, Vpr and Vpu) play important roles in the host-pathogen interaction and thus have significant impacts on the outcome of HIV infection. One of the best examples is the interaction of Vif with a host cytidine deaminase APOBEC3G. Although specific roles of other auxiliary proteins are not as well described as Vif-APOBEC3G interaction, it is the goal of this brief review to summarize some of the preliminary findings with the hope to stimulate further discussion and investigation in this exhilarating area of research.

Keywords: HIV-1, auxiliary proteins, viral pathogenesis, host-pathogen interactions.

INTRODUCTION

In addition to the prototypical retroviral Gag, Pol, and Env proteins, HIV-1 produces six additional proteins, i.e., Tat, Rev, Nef, Vif, Vpr and Vpu (Fig. 1, adapted from [1]). While Tat and Rev are required for viral replication, Nef, Vif, Vpr and Vpu are usually dispensable for viral growth in many of the *in vitro* systems [2, 3], hence known as auxiliary proteins. However, these proteins are often necessary for viral replication and pathogenesis *in vivo* and they carry out many of the essential functions during the viral life cycle (Tab. 1). Consequently, presence or absence of these auxiliary proteins can significantly change the course and severity of the viral infection [4]. In the followings, main functions of these auxiliary proteins in the process of HIV-1 infection and their roles in HIV-host interactions are briefly described.

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Viral Protein U (Vpu)

Vpu is a small (9 kDa) membrane protein that enhances the release of progeny virions from infected cells and induces the degradation of the CD4 receptor. Vpu expressed in the ER interacts with a membrane-proximal domain of the cytoplasmic tail of CD4 and links it to h-βTrCP [5], a member of the F-box protein family first characterized as components of ubiquitin-ligase complexes [6]. The CD4–Vpu–h-βTrCP ternary complex then recruits SKP1, another member of the ubiquitination machinery [7]. As a result, CD4 is ubiquitinated and targeted to proteasomes for degradation. The ability of Vpu to increase progeny virus secretion from infected cells had been attributed initially to ion conductive membrane pore formation characteristic to cells over-expressing Vpu [8]. However, a later report [9] showed that the requirement for Vpu is host

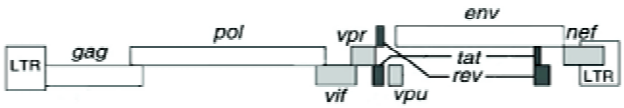

Fig. 1 Genome of HIV-1 (Adapted from [1]).

Tab. 1 HIV-1 auxiliary proteins and their major activities

Protein	Major Functions
Vpu	CD4 degradation
	Promoting virion release
Vif	Stimulation of reverse transcription
	Counteraction of host anti-virus factors, e.g., APOBEC3G
Vpr	Nuclear import of pre-integration complex
	Interference with host cell cycle progression (G2/M regulation)
	Induction of apoptosis
	Transactivation of HIV-LTR and host cellular genes
Nef	Modulation of cellular receptors including CD4, MHCI, MHCII and CD28
	Enhancement of viral infectivity
	Interference with host cell signal transduction
	Regulation of cholesterol trafficking
Rev	Nuclear export of un-spliced viral RNA
	Effects on the viral RNA stability and translation
Tat	Promoting viral RNA transcription
	Induction of apoptosis
	Inhibition of siRNA formation by Dicer

cell-dependent, suggesting that Vpu may counteract an inhibitory factor expressed in some, but not the other, cells. This factor was identified recently as TASK-1, a widely expressed acid-sensitive K^+hannel [10]. TASK-1 is structurally homologous to Vpu, suggesting oligomerization as a possible mechanism of inactivation of ion channel activity of these proteins. However, the mechanism by which TASK-1 inhibits virion release is still unclear.

Viral Protein R (Vpr)

The viral protein R (Vpr) is a 96 amino acids small basic protein, and is well conserved in HIV-1, HIV-2 and SIV [11]. Nuclear magnetic resonance (NMR) analysis suggests that Vpr protein of HIV-1 consists of an α-helix-turn-α-helix domain in the amino-terminal half from amino acids 17 to 46 and a long α-helix from 53 to 78 ended with an α turn in the carboxyl-terminal half [12, 13]. The Vpr protein can be found in virions [14], cells, sera and cerebrospinal fluid of AIDS patients, indicating that it may exert its biological functions on many different targets. Despite its small size, Vpr has been shown to have multiple activities during virus replication, including effects on the nuclear import of the proviral DNA as a component of the pre-integration complex (PIC), cell cycle G2/M progression, regulation of apoptosis, and transactivation of the HIV-1 LTR as well as host cell genes.

One of the Vpr functions in the viral infection process is to mediate the nuclear import of HIV-1 PIC [15]. In the cytoplasm, HIV viral RNA (in complex with several viral proteins) is reverse transcribed into DNA which then associates with the host cellular proteins to form PIC. Vpr is a component of this pre-integration complex [16-18]. Vpr moves with PIC along cytoskeletal filaments and accumulates at the perinuclear region close to centrosomes [19]. Though it is not yet known whether Vpr plays an active role during this movement of the PIC along microtubules, Vpr appears to participate in the subsequent steps, including the anchoring of the PIC to the nuclear envelope and the nuclear translocation of the viral DNA [15]. Experiments in macrophages strongly suggest an important role for Vpr in mediating the nuclear import of HIV-1 PICs into the nucleus of nondividing cells [20]. The mechanism of Vpr-mediated nuclear import is not clear, it is likely that Vpr interacts directly or indirectly with cellular machinery regulating the nucleo-cytoplasmic shuttling [21-25].

In addition to the effect in nuclear import, Vpr induces cell cycle G2 phase arrest in human and fission yeast cells suggesting a highly conserved effect of Vpr on cellular activities [26-32]. Progression of cells from G2 phase of the cell cycle to mitosis is a tightly regulated cellular process that requires activation of the Cdc2 kinase, which determines onset of mitosis in all eukaryotic cells. In human and fission yeast cells, the activity of Cdc2 is regulated in part by the phosphorylation status of Cdc2, which is phosphorylated by Wee1 kinase during late G2 and is rapidly dephosphorylated by the Cdc25 tyrosine phosphatase to trigger entry into mitosis. These Cdc2 regulators are the downstream targets of two well-characterized G2/M checkpoint pathways which prevent cells from entering mitosis when cellular DNA is damaged or when DNA replication is inhibited. Vpr also inhibits Cdc2 through hyperphosphorylation [29, 32]. However, the exact molecular mechanism leading to the hyper-phosphorylation of Cdc2 and G2 arrest is not yet clear. There are reports suggesting that Vpr induces G2 arrest by mimicking components of the DNA damage repair pathway involving ATR, Rad17 and Hus1 [33, 34]. However, other reports showed that Vpr modulates cell cycle G2/M transition through cellular mechanisms other than the classic mitotic checkpoints

[32, 35-37]. For example, Vpr-induced G2 arrest was shown to involve protein phosphatase 2A [32, 36] or a mitogen-activated protein kinase signal transduction pathway [37]. It is possible that there are multiple mechanisms leading to Vpr-induced G2 arrest. Alternatively, *vpr* gene expression may trigger a type of cellular surveillance responses other than the well-characterized DNA damage or replication checkpoints but results in G2 arrest by impinging upon the same cellular targets, i.e., Cdc2. This premise certainly needs to be further evaluated. Biological significance of Vpr-induced G2 arrest during viral infection is also not well understood. However, HIV-1 LTR seems to be more active in the G2 phase, implying that Vpr-induced G2 arrest may confer a favorable cellular environment for efficient transcription of HIV-1 [38].

Vpr also induces apoptosis in infected cells. Since a major mechanism for CD4+ T cell depletion in HIV-infected patients is apoptosis, which is induced by HIV through multiple pathways in both infected cells and non-infected "bystander" cells [39], it is expected that the apoptotic effect of Vpr may contribute to CD4+ T cell depletion. Although it is well accepted that Vpr induces apoptosis, there are studies suggesting that Vpr may also act as a negative regulator of T cell apoptosis [40, 41]. In addition, it is debated whether Vpr-induced apoptosis is a result of G2 arrest. The activity of the cell cycle regulatory Wee-1 kinase associates with a decrease in Vpr-induced apoptosis, indicating a direct correlation between G2 arrest and apoptotic properties of Vpr [42]. However, other reports suggested that these two Vpr activities can be separated [43-47]. Even though the molecular mechanism of Vpr-induced apoptosis is elusive, most researchers favor the idea that Vpr induces apoptosis through mitochondria-dependent pathway [48]. This intrinsic pathway for apoptosis is initiated by mitochondrial outer membrane permeabilization (MOMP) leading to release of the apoptotic factors from the space between the inner and outer mitochondrial membranes [49]. Vpr binds to ANT (adenine nucleotide transporter) protein of the inner mitochondrial membrane [48, 50, 51], and can move across the outer mitochondrial membrane leading to depolarization of the inner mitochondrial membrane, swelling of the inner mitochondria and ultimately MOMP with release of the apoptosis factors. There is considerable evidence supporting this hypothesis, including the finding that the cell killing induced by Vpr can be reduced by the down-regulation of ANT levels [48], and that Vpr activates caspase-9 which initiates caspases of the intrinsic apoptotic pathway [52]. However, other reports do not fit this hypothesis. Vpr was shown to locate predominantly in the nucleus or at the nuclear membrane [25, 53, 54], but not in the mitochondria. In addition, it has been reported that Vpr activates caspase-8 [55, 56] which should not be activated in the intrinsic MOMP pathway.

There are several reported host responses to Vpr. Vpr is targeted by the CD8+ T-lymphocytes during the acute phase of the viral infection [57, 58]. Production of some heat shock proteins (HSPs) is also responsive to *vpr* gene expression [59-61]. Furthermore, some of the heat shock proteins, such as yeast Hsp16 or human HSP70, exert effective protective effect against some or all of the Vpr activities [62-64]. Conversely, Vpr suppresses cellular [65] and humoral immune responses through adjusting the cell proliferation [40, 66, 67] or the production of the cytokines (TNF α and IL12) and chemokines (RANTES, MIP-1α and MIP-1β) [40, 67]. Thus there appears to be an active and antagonistic interaction between Vpr and host anti-Vpr responses. For detailed review on this subject see [68].

Virus Infectivity Factor (Vif)

Vif protein of HIV-1 is a 192 aa protein that expresses at high levels in the cytoplasm of infected cells. Vif was thought to be important because it is essential for the reproduction of HIV-1 in peripheral blood lymphocytes, macrophages, and certain cell lines known as 'nonpermissive' cells [69]. Vif-deficient virions produced from 'permissive' cells can infect 'nonpermissive' cells, but the virus subsequently produced is not infectious. The molecular nature of permissivity and the exact function of Vif in infection of nonpermissive cells was not known until recently when a series of reports showed that a host cellular protein known as APOBEC3G (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G) is a potent inhibitor of HIV infection in the nonpermissive cells [70, 71]. APOBEC3G is a member of the cytidine deaminase family, which prevents viral cDNA synthesis via deaminating deoxycytidines (dC) in the minus-strand retroviral cDNA replication intermediate [72-76]. As a result, it creates stop codons or G-A transitions in the newly synthesized viral cDNA which is then subjective to elimination by host DNA repair machinery [74, 76]. APOBEC3G confers its antiviral effect by encapsidating into the virus particles through interaction with viral Gag protein [77-81]. Thus, APOBEC3G represents an innate host defense mechanism against HIV infection. However, the virus has also developed an offensive strategy to suppress the antiviral effect of APOBEC3G through Vif. Vif binds directly to APOBEC3G and counteracts its anti-HIV activity by promoting its degradation. Vif-mediated APOBEC3G degradation involves the recruitment of a specific E3 ligase complex, which leads to the polyubiquitylation and proteasome-mediated degradation [82-86].

In addition to the counteracting effect of Vif on APOBEC3G, Vif protein is specifically packaged into vi-

rus particles, where it is processed by protease. Protease-processed Vif is believed to be an important step for production of infectious viruses [87]. Vif also stabilizes viral nucleoprotein complex through direct interaction with 5' region of HIV-1 genomic RNA [88-91]. Moreover, Vif modulates viral reverse transcriptase through its C-terminal domain either by stimulating the binding of RT and primer or increasing the polymerization rate of RT [92].

Negative Regulator Factor(Nef)

The HIV-1 Nef protein is a 27-kDa myristoylated protein that is abundantly produced during the early phase of viral replication cycle. It is highly conserved in all primate lentiviruses, suggesting that its function is essential for survival of these pathogens. Nevertheless, early publications reported a negative effect of Nef on viral replication, hence the name 'negative factor' or Nef [93, 94]. Subsequent studies, however, demonstrated that Nef plays an important role in several steps of HIV replication. In addition, it appears to be a critical pathogenic factor, as Nef-deficient SIV and HIV are significantly less pathogenic than the wild-type viruses [95-97], whereas Nef-transgenic mice show many features characteristic to HIV disease [98, 99].

The role of Nef in HIV-1 replication and disease pathogenesis is determined by at least four independent activities of this protein. First, Nef affects the cell surface expression of several cellular proteins. It down-regulates CD4 [100], CD8 [101], CD28 [102], major histocompatibility complex class I [103] and class II [104] proteins, but upregulates the invariant chain of MHC II (CD74) [104]. To modulate cell surface receptor expression, Nef utilizes several strategies, linked to distinct regions within the Nef protein (reviewed in [105]). For example, down-regulation of the CD4 and CD28 receptors involves a dileucine-based motif in the second disordered loop of Nef, which connects Nef to adaptor protein (AP) complex [106], which is a part of cellular endocytosis machinery. Nef also directly binds to CD4 and CD28 using overlapping sequences within its core structure [102], thus inducing accelerated endocytosis of these proteins via clathrin-coated pits followed by lysosomal degradation. Down-regulation of MHC class I involves Nef-mediated connection in the endosomes between MHC-I's cytoplasmic tail and the phosphofurin acidic cluster sorting protein-1 (PACS-1)-dependent protein-sorting pathway [107]. Since all these receptors are essential for proper functions of the immune system, modulation of their surface expression by Nef has profound effects on anti-HIV immune responses. Down-regulation of MHC I protects HIV-infected cells from host CTL response, whereas down-modulation of CD28 and CD4 probably limits the adhesion of a Nef-expressing T cell to the antigen-presenting cell, thus promoting the movement of HIV-infected cells into circulation and the spread of the virus. Another benefit for the virus from CD4 down-modulation is abolishment of interaction between the receptor and the Env protein of the budding virus, which likely increases HIV release from infected cell as well as infectivity of viral particles.

Second, Nef interferes with cellular signal transduction pathways. Nef is myristoylated on its amino-terminus and exhibits a proline-rich SH3-binding domain, both of which mediate Nef association with lipid rafts, cholesterol-rich membrane microdomains that concentrate potent signaling mediators [108]. Nef was found to complex with and activate serine/threonine protein kinase PAK-2 [109], which may contribute to activation of infected cell. *In vitro*, HIV-infected T cells produce enhanced levels of interleukin-2 during activation [108]. When expressed in macrophages, Nef intersects the CD40L signaling pathway inducing secretion of chemokines and other factors that attract resting T cells and promote their infection by HIV [110, 111].

Third, Nef enhances virion infectivity and viral replication [112]. This effect is mediated by Nef present in HIV virions and is due, at least in part, to the ability of Nef to induce actin remodeling and facilitate the movement of the viral core past a potentially obstructive cortical actin barrier [113]. In support of this model, the infectivity-enhancing properties of Nef are eliminated by disruption of actin cytoskeleton or pseudotyping of HIV virions with VSV-G glycoprotein, which targets viral entry to endocytosis-dependent pathway thus bypassing cortical actin.

Fourth, Nef regulates cholesterol trafficking in HIV-infected cells. Cholesterol plays an important role in the HIV life cycle, as HIV assembly and budding, as well as infection of target cells all depend on plasma membrane cholesterol. Depletion of cellular cholesterol markedly and specifically reduces HIV-1 particle production [114], and cholesterol-sequestering drugs, such as beta-cyclodextrin, render the virus incompetent for cell entry [115, 116]. Nef has been shown to bind cholesterol via a cholesterol-recognition motif at its carboxyl-terminus and to transport newly synthesized cholesterol to the site of viral budding [117]. In addition, Nef interferes with activity of cellular cholesterol efflux machinery (MB, unpublished result), thus effectively hijacking cholesterol transport in HIV-infected cell.

Regulator of Expression of the Virion (Rev)

Rev is a ~116 aa sequence-specific RNA binding phosphoprotein that is expressed during the early stages of HIV-1 replication [118, 119]. Rev transports to cytoplasm single-

spliced and un-spliced viral mRNAs that are required for expression of HIV structural proteins and production of genomic RNA. Eukaryotes have evolved a special mechanism to retain the incompletely spliced RNAs in the nucleus. This mechanism is undoubtedly beneficial to the host cell, but presents HIV-1 with a serious problem. Since HIV only has one LTR promoter, it encodes a single, genome-length primary transcript. In order to express the various incompletely spliced viral transcripts, some of HIV-1 transcripts must be transported out of the nucleus without splicing. Rev fulfills this function [120].

Rev contains at least three functional domains [119, 121]. An arginine-rich domain which mediates both specific RNA binding and nuclear/nucleolar localization [122, 123], a nuclear export signal (NES) [119, 124], and a homomultimerization domain [125, 126]. Homomultimerized Rev interacts directly with importin β and the nucleolar phosphopprotein B23 via its NLS domain [127, 128]. The Rev-importin β-B23 complex is recruited to the nuclear pore by the direct importin β-nucleoporin interaction. GTPase known as Ran plays a key role during the transporting process [129]. In the cytoplasm, Ran presents in a Ran-GDP form allowing Rev binding to importin β. Once the importin β-Rev complex reaches the nucleus, where Ran-GTP predominates due to high concentration of Ran-GEF (Ran-specific guaninenucleotide-exchange factor) and RCC1 (regulator of chromosomal condensation 1), the interaction of importin β with Ran-GTP results in the disassembly of the Rev-importin β-B23 complex and the release of Rev cargo. In the nucleus, Rev binds to a special 234-basepair region of complex HIV RNA secondary structure called the Rev Response Element (RRE), which is located within the second intron of HIV [120]. The high-affinity binding of first Rev monomer to its primary site in the RRE structure is followed by the binding of additional copies of Rev to form multimerized Rev [130-133]. The Rev protein, with its RNA cargo, will then bind to CRM1, also known as exportin-1 [134-136], through it's nuclear export signal (NES) domain. CRM-1 forms a complex with the GTP-bound Ran and the leucine-rich NES mediating the export of the NES-containing protein from the nucleus through the nuclear pore [135]. In the cytoplasm, a Ran-specific GTPase-activating protein (Ran-GAP) converts Ran-GTP to Ran-GDP, resulting in a Ran-GTP gradient across the nuclear membrane. Upon binding of RanBP1 (Ran binding protein 1) to Ran-GTP, the Crm1-Rev-Ran-GTP complex is disassembled and the Rev/RNA cargo is released. Asymmetric distribution of Ran-GEF and Ran-GAP between nucleus and cytoplasm ensures a constant Ran-GTP/GDP gradient to facilitate Crm1 recycling and continued Rev/RRE nuclear export [137-140]. This cycle of continuous protein shuttling between the nucleus and the cytoplasm generates a system where the small amounts of Rev present in an HIV-infected cell have the capacity to mediate the export of significant amounts of intron-containing HIV RNAs.

Besides Crm1, a number of other cellular proteins also participate in nuclear transport activity of Rev including eIF-5A (enkaryotic initiantion factor 5A) [141, 142], Sam68 (68 kDa Src-associated protein) [143], certain DEAD box protein RNA helicases (DDX3, DDX1) and hRIP (human Rev-interacting protein). The eIF-5A plays a crucial role in the nuclear export of Rev-RRE complexes and mutant eIF-5A inhibits HIV-1 replication in lymphocytes [141, 142]. The precise mechanism of eIF-5A activity in Rev function remains to be defined. However, it was proposed that eIF-5A acts as an adapter that targets the Rev-NES to the nucleoplasmic face of the NPC and mediates efficient binding to Crm1 [139, 144]. Sam68 promotes the nuclear export of Rev in astrocytes [145]. Sam68 is also required for Rev function and HIV-1 production in HeLa cells [146]. In 293T, Jurkat cells and peripheral blood mononuclear cells, down-modulation of endogenous Sam68 significantly lowers HIV expression by inhibiting the CRM1-mediated export of nuclear Rev, resulting in the nuclear retention of both Rev and Crm1[147]. Sam68 might function through enhancement of HIV-1 RNA 3' end processing [148]. Recent research showed that certain DEAD box (Asp-Glu-Ala-Asp) protein RNA helicases (DDX3 and DDX1) and hRIP (human Rev-interacting protein) play important roles in Rev functions and HIV-1 replication. DDX3 acts as a nucleo-cytoplasmic shuttling protein, which binds CRM1 and localizes to nuclear membrane pores [149]. DDX1 is a critical co-factor for Rev function, which helps maintain the proper subcellular distribution of Rev and functions through the Rev-RRE axis [150, 151]. The hRIP is an essential Rev cofactor required for virus replication. Ablation of hRIP activity by a dominant-negative mutant or RNA interference, inhibits virus production by mis-localizing Rev-directed RNAs to the nuclear periphery whereas reintroduction of hRIP protein restores virus production [152, 153].

In addition to facilitating nuclear export, Rev has several additional effects on HIV RNA. Rev increases stability and translation of HIV RNA [154, 155]. With the expression of Rev, the half-life of HIV RNAs in the nucleus of a T-cell line infected with HIV increases significantly [156]. If Rev function is inhibited by LMB, a nuclear export inhibitor, nuclear pool of RRE-containing RNA decreases even in the presence of Rev [157]. HIV-infected cells exert special mechanisms to counteract the function of Rev; the 16.4.1 protein is one of anti-Vif cellular proteins that also counteracts Rev activity. Overexpression of 16.4.1 inhibits Rev, whereas downregulation of 16.4.1

by siRNA stimulates Rev [158].

Transactivator of Transcription (Tat)

Tat is a small protein (101 amino acids in most clinical HIV-1 isolates, 86 amino acids in the laboratory HIV-1 HXB2 strain) which is essential for efficient transcription of viral genes and for viral replication. Tat potently transactivates LTR-driven transcription, resulting in a remarkable increase of viral gene expression [159-161].

Tat increases the transcriptional rate in three different ways. First, Tat modifies chromatin conformation at the proviral integration site and makes it more suitable to viral transcription. Tat binds to a structured RNA element (TAR, transactivation-responsive region) present at the 5'-end of viral leader mRNAs (nucleotide position +1 to +59 [163]) via cyclin T1 bridging between the activation domain of Tat and the TAR loop [164]. Through this interaction, Tat recruits a series of transcriptional complexes, including enzymes with histone and factor acetyl transferase (HAT and FAT) activities, which modify chromatin at the proviral integration site and make it more suitable to transcription. With Tat protein, long polyadenylated RNA and increased gene expression ensue [159-161, 165].

Second, Tat recruits P-TEFb to adjust the activity of polymerase II. In mammalian cells, RNA polymerase II activity is controlled by the phosphorylation status of its carboxyl-terminal domain (CTD). Hypophosphorylation of the CTD on Ser2 correlates with low processivity, whereas hyperphosphorylation increases the processivity of the enzyme complex [166]. In the absence of Tat, transcription from the HIV-1 LTR produces predominantly short RNA because hypophosphorylated RNAPII is arrested prematurely following the actions of negative elongation factors, including DSIF (5, 6-dichloro-1-beta-D-ribofuranosylbenzimidazolesensitivity-inducing factor) and NELF (negative elongation factor complex) [167]. P-TEFb, one of the kinase complexes that can phosphorylate the CTD of RNA Pol II in the mammalian cells, is composed of CKD9 kinase and its cyclin partner, cyclin T [168, 169]. Cdk9 kinase activity is naturally suppressed by interaction with 7SK RNA, hexamethylene bisacetamide-induced protein1 [170] and indirubin-3'-monoxime. Tat binds to the TAR structure on the viral RNA and recruits P-TEFb through binding to cyclin T1 [164, 169, 171]. Recruitment of P-TEFb to TAR stimulates RNAPII Ser2 phosphorylation by Cdk9 [170], and alters the substrate specificity of Cdk9 to include Ser5 phosphorylation of the CTD [172], resulting in the dissociation of DSIF and NELF. Recent studies demonstrated that human splicing factor SKIP (the splicing-associated c-Ski-interacting protein [173]) and PP1 (protein phosphatase-1) are also required in this step [174]. As a result, Tat facilitates the transcription initiation. On the other hand, Tat also facilitates transcription elongation. Acetylation of Tat at Lys50 caused by p300 or hGCN5 dissociates cyclinT1 and Tat from TAR RNA [175-177] and transfers Tat to the elongating RNAPII complex where it recruits PCAF (p300/CREB binding protein-associated factor) via the PCAF bromodomain and enhances the transcriptional elongation of HIV-1 [178-181]. It was proposed that arginine methylation within the arginine-rich motif of HIV-1 Tat by PRTM6 (protein arginine methyltransferases) triggers the dissociation of acetylated Tat from the polymerase complex and PCAF at the end of the transcription cycle [182, 183], and the ubiquitination and dimethylation of arginines mark Tat for degradation [184]. In addition, monomethylation can be reversed by the action of a Tat peptidyl arginine deaminase, and ubiquitinated Tat can be recycled after deacetylation by SIRT1 (the class III deacetylase sirtuin 1) into the transcription cycle [183, 185].

Third, Tat transactivates HIV-1 RNAs through the activation of NF-κB [186]. Protein members of the Rel/NF-κB family bind to the enhancer element of the viral LTR [187, 188]. In the un-stimulated normal mammalian cells, NF-κB is retained in the cytoplasm by its inhibitor protein IκB-α. Tat promotes NF-κB activation through a change in the redox state of the cell and IκB-α degradation.

In addition to its crucial role in activating viral transcription, Tat is associated with a number of additional activities [189]. Extracellular Tat induces production of cytokines such as transforming growth factor beta, IL-2, or IL-6 [190-193]. Tat causes neurotoxicity in the central nervous system [194-200] and apoptosis in cultured peripheral blood mononuclear cells and some CD4 T-cell lines [201-204]. Another report demonstrated that Tat contributes to cell survival through up-regulation of the anti-apoptotic gene Bcl-2 [205]. Recently, Benasser and coworkers [206] demonstrated that Tat plays important role in abrogating nucleic acid-based adaptive immunity, RNA silencing. It is suggested that Tat impairs the cell's RNA-silencing defense by inhibiting the ability of Dicer to process precursor double-stranded RNAs into siRNAs [206].

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