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# Transcriptional and post-transcriptional regulation of HIV-1 gene expression: role of cellular factors for Tat and Rev

Sergei Nekhai &  
Kuan-Teh Jeang<sup>†</sup>

<sup>†</sup>Author for correspondence  
NIH, Building 4, Room 306,  
9000 Rockville Pike,  
Bethesda, MD 20892, USA  
Tel.: +1 301 496 6680;  
Fax: +1 301 480 3686;  
kjeang@niaid.nih.gov

The emergence of drug-resistant HIV-1 strains presents a challenge for the design of new therapy. Targeting host cell factors that regulate HIV-1 replication might be one way to overcome the propensity for HIV-1 to mutate in order to develop resistance to antivirals. This article reviews the interplay between viral proteins Tat and Rev and their cellular cofactors in the transcriptional and post-transcriptional regulation of HIV-1 gene expression. HIV-1 Tat regulates viral transcription by recruiting cellular factors to the HIV promoter. Tat interacts with protein kinase complexes Cdk9/cyclin T1 and Cdk2/cyclin E; acetyltransferases p300/CBP, p300/CBP-associated factor and hGCN5; protein phosphatases and other factors. HIV-1 Rev regulates post-transcriptional processing of viral mRNAs. Rev primarily functions to export unspliced and partially spliced viral RNAs from the nucleus into the cytoplasm. For this activity, Rev cooperates with cellular transport protein CRM1 and RNA helicases DDX1 and DDX3, amongst others.

The emergence of drug-resistant HIV-1 presents a challenge for the design of new therapeutics. Targeting host cell factors might be one way to combat HIV-1 mutational changes that confer resistance to currently available antiviral agents. Transcriptional and post-transcriptional processes that regulate HIV-1 gene expression represent attractive targets for antiretroviral targeting, since they require interactions with host cell factors by unique viral proteins, Tat and Rev. This article surveys, in a nonexhaustive fashion, the understanding of cellular proteins that participate in transcription, splicing and export of HIV-1 mRNA from the nucleus into the cytoplasm.

## HIV-1 Tat activates transcription through TAR RNA & adapts coactivators to the viral LTR promoter

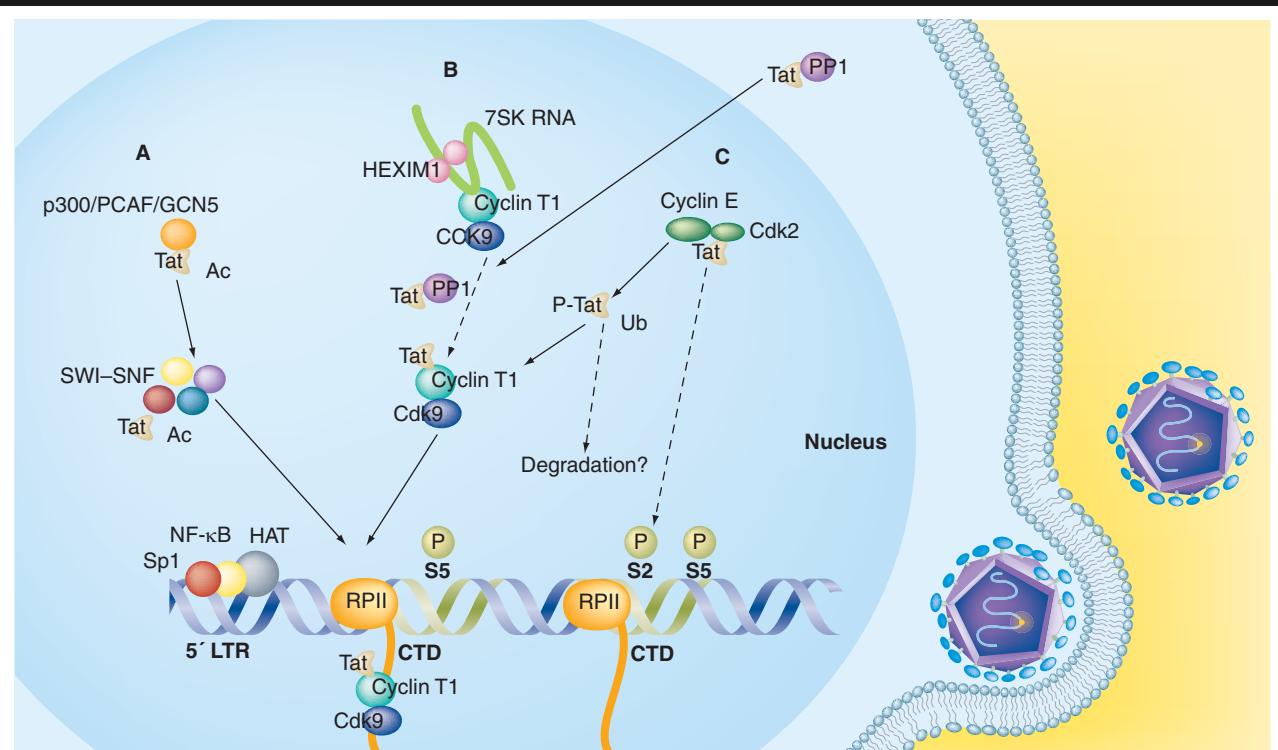
Expression of the HIV-1 provirus requires host cell transcription factors as well as the viral Tat protein (Figure 1) [1]. The HIV-1 promoter located in the 5' long-terminal repeat (LTR) is composed of U3 (nucleotides -454 to -1), R (nucleotides +1 to +60) and U5 (nucleotides +61 to +181) regions [2]. The U3 contains three Sp1 binding motifs and two nuclear factor (NF)- $\kappa$ B binding sites that serve to regulate basal HIV-1 transcription [2]. Additionally, the LTR contains DNA binding elements for positive transcriptional regulators, such as nuclear factor of activated T cells, AP-1 and others; and for negative regulatory factors, such as YY1 and LSF [2]. The importance of Sp1 for HIV-1 transcription is underscored by the finding that

induction of serine protease inhibitor SerpinB2 in activated monocytes significantly promoted HIV-1 transcription and viral replication [3]. SerpinB2 relieves HDM2-mediated inhibition of Sp1, supporting the importance of Sp1 to HIV-1 transcription [3]. Additionally, mutational analysis of Sp1 and NF- $\kappa$ B binding sites suggests functional cooperation between these two factors [4]. Recent findings indicate that inhibitors of NF- $\kappa$ B function can be anti-HIV-1 drug candidates. For example, HIV-1 replication was inhibited in OM10.1 cells when these cells were pretreated with 2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl-nicotinonitrile, a novel I $\kappa$ B kinase inhibitor [5]. Coumarins and structurally related compounds also inhibit HIV-1 replication by reducing the activity of the p65 subunit in of NF- $\kappa$ B cells [6].

Proinflammatory cytokines (such as tumor necrosis factor  $\alpha$ ) can induce NF- $\kappa$ B and activate HIV-1 transcription [7]. Currently, it is thought that activation of the HIV-1 provirus occurs with the remodeling of a single nucleosome, nuc-1, located immediately downstream of the HIV transcription start site. Nuc-1 is remodeled through post-translational acetylation of histones [7]. This acetylation of histones within the nucleosomally organized HIV-1 LTR is likely the initial step that initiates proviral transcription.

Once the LTR begins transcription, the first viral sequence transcribed is a short leader RNA, termed the transactivation responsive (TAR) RNA element (nucleotides +1 to +82), which is

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**Figure 1.** Schematic representation of Tat-mediated HIV-1 transcription.

Depicts a network of Tat-interacting host cell factors. Preinitiation, initiation and elongation of HIV-1 transcription are shown. Phosphorylation of RNA polymerase II (RPII) CTD on Ser5 and Ser2 residues is indicated. Solid arrows represent the data backed by cellular or *in vivo* studies and broken arrows represent more speculative data from *in vitro* studies. **(A)** Interaction of Tat with p300, PCAF and GCN5 leads to Tat acetylation and histone acetylation; the interaction with SWI-SNF complex leads to displacement of histone deacetylase 1 and recruitment of histone acetylases synergistically with Sp1 and NF-κB. **(B)** Tat translocates PP1 to the nucleus. Translocation of PP1 by Tat might result in dissociation of 7SK RNA from Cdk9/cyclin T1. Interaction of Tat with Cdk9/cyclin T1 recruits the latter to TAR RNA. **(C)** Tat interaction with Cdk2/cyclin E results in Tat phosphorylation, which may or may not precede Tat ubiquitination. Recruitment of Cdk2 by Tat to RPII CTD may induce phosphorylation of Ser2 residues in the RPII CTD. Ac: Acetylation; CTD: C-terminal domain; HAT: Histone acetyl transferase; LTR: Long-terminal repeat; NF-κB: Nuclear factor κB; PCAF: p300/CBP-associated factor; RPII: RNA polymerase; TAR: Transactivation responsive; Ub: Ubiquitination.

present at the 5'-end of all HIV-1 transcripts (Figure 1) [1]. TAR is important for further induction of HIV-1 transcription by Tat, a virally encoded transcriptional activator. To activate transcription, Tat directly binds a trinucleotide bulge with the hairpin-looped structure of TAR RNA. In cell-free assays, Tat exclusively induces elongation of transcription [8,9]. However, in contrast to this transcriptional elongation *in vitro*, intracellularly, Tat induces both initiation and elongation of transcription from the integrated HIV-1 provirus [10–12]. Inside the cell, there is evidence that contact between Tat and TAR RNA rather than RNA polymerase (RNAP) II-pausing is the rate-limiting step relieved by Tat transactivation [12]. Recent evidence shows that Tat stimulates formation of a promoter complex containing TATA-box-binding protein (TBP) but not TBP-associated factors, suggesting a role

for Tat in the initiation of transcription [10]. On the other hand, for transcripts that have already initiated from the promoter, Tat also recruits a positive transcription elongation factor (P-TEF)b composed of Cdk9/cyclin T1, which serves to phosphorylate the C-terminal domain (CTD) of RNAP II [9,13,14]. Phosphorylation of the CTD of RNAP II converts this polymerase to a more processive enzyme.

Tat has also been reported to recruit histone acetyl transferases (HATs) to the integrated provirus [15–17]. This activity may contribute to the remodeling of Nuc-1. Of interest, the ability of Tat to recruit P-TEFb may also assist the transcriptional function of NF-κB, since there are some indications that NF-κB may utilize Cdk9/cyclin T1 for the induction of transcription [17,18]. Finally, Tat can induce Sp1 phosphorylation by DNA-protein kinase, and

such phosphorylation has been demonstrated to increase the contribution of Sp1 to HIV-1 transcription [19].

#### Activation of transcription by Cdk9/cyclin T1

Tat associates with Cdk9 by direct binding of its cyclin partner, cyclin T1. In turn, cyclin T1 binds the loop of TAR RNA [20–22]. During elongation of transcription, a crucial event occurs when Cdk9 phosphorylates the CTD of RNAP II, which contains 52 consecutive Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 repeats [23]. This phosphorylation increases the processivity of RNAP II. Additional findings show that Cdk9 triggers the dissociation of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF) and negative elongation factor (NELF; Rd protein) from the elongation complex [24]. Mechanistically, Cdk9 phosphorylates human SPT5, a subunit of DSIF and the RD protein, a subunit of NELF. Phosphorylation of RD results in the loss of NELF-binding to the stem of TAR RNA, releasing NELF's inhibitory effect [25].

Studies from Andrew Rice's laboratory demonstrated that cyclin T1 expression is highly regulated during macrophage differentiation. In human primary monocytes, cyclin T1 protein expression is low but can be induced by treatment of macrophages with lipopolysaccharide through post-transcriptional mechanisms [26]. In late-differentiated macrophages, cyclin T1 undergoes proteasome-mediated proteolysis; however, HIV-1 infection in these cells leads to the reinduction of cyclin T1 [27]. DNA array analyses of the genes in differentiated macrophages show that more than 20% of genes induced by phorbol 12-myristate 13-acetate (PMA) require cyclin T1 for their normal level of induction, and approximately 15% of genes repressed by PMA require cyclin T1 for their normal level of repression [28]. This finding raises the concern that targeting cyclin T1 as an anti-HIV-1 approach might be toxic to cells [28]. Interestingly, the kinase activity of Cdk9/cyclin T1 in cells is physiologically regulated in a negative fashion by a small RNA, the 7SK small nuclear RNA [29,30] in association with a cellular protein termed HEXIM1 [31,32]. It remains to be determined whether there is a range of Cdk9/cyclin T1 down-regulation that can be therapeutic against the virus and still be compatible with normal cellular viability.

#### Contribution to HIV-1 transcription by Cdk2/cyclin E

Cdk2/cyclin E is active in the late G<sub>1</sub> phase and regulates the G<sub>1</sub>/S transition [33]. HIV-1 transcription is inducible by Tat primarily in G<sub>1</sub>, while in G<sub>2</sub> viral transcription appears to be Tat-independent [34,35]. In G<sub>2</sub>, transcription of the provirus is largely governed via recruitment of histone acetyltransferase cyclic-AMP binding protein (CBP) and transcription factors NF-κB and c-Jun, together with hyperacetylation of histones H3 and H4 in nucleosome nuc-1 [36]. It is known that HIV-1 Vpr can arrests cell in G<sub>2</sub> [37], allowing for the above mode of transcription.

Tat has been found to associate with a protein complex containing Cdk2 [38]. This finding is interesting because Cdk2/cyclin E can bind the CTD of RNAP II, can associate with transcriptional elongation complexes, and can phosphorylate Tat *in vitro* [39]. The significance of these findings was verified by experiments that employed roscovitine and its analog CYC202 to inhibit Cdk2/cyclin E kinase activity. Such inhibition of Cdk2/cyclin E activity halted HIV-1 replication in T cells, monocytes and peripheral blood mononuclear cells (PBMCs) [40]. Additionally, siRNA inhibition of Cdk2 expression also results in loss of Tat-induced transcription from the HIV-1 promoter and suppresses viral replication [41,42]. Finally, Tat is phosphorylated on Ser16 and Ser46 *in vivo*, presumptively by Cdk2, and mutations in these serines prevented HIV-1 transcription and viral replication [43]. Because Cdk2-specific siRNA does not disrupt binding of Tat to Cdk9/cyclin T1 [43] but does strongly inhibit HIV-1 replication [41,42], Tat apparently interacts with Cdk2/cyclin E prior to its binding to Cdk9/cyclin T1 and TAR RNA. Finally, findings that Cdk2 knock-out mice are viable [44] suggest that Cdk2 may be dispensable for cellular proliferation and survival. If true, inhibitors of Cdk2 could represent a novel class of anti-HIV-1 therapeutics.

#### Roles for cellular phosphatases: FCP1, PP1 & PP2A

Contrasted with the well-studied kinases, the contribution of protein phosphatases to HIV-1 transcription is poorly understood. The best-characterized RNAP II CTD phosphatase, FCP1, dephosphorylates *in vitro* both Ser2 and Ser5 in the CTD heptads [45,46]. Tat can bind amino acids 562–685 in FCP1. *In vitro* binding by Tat alleviates FCP-mediated inhibition of HIV-1 transcription [47,48].

Another phosphatase, protein phosphatase (PP)1 also dephosphorylates CTD and copurifies with RNAP II, and may be a physiological CTD phosphatase [49]. PP1 increases Tat-dependent HIV-1 transcription *in vitro* [50]. In cultured cells, inhibition of PP1 through expression of NIPP1 or catalytically inactive mutant of PP1 $\gamma$ , inhibits Tat-induced transcription and replication of HIV-1 [51,52]. The  $^{35}\text{QVCF}^{38}$  sequence of Tat, which resembles the RVxF motif present in cellular proteins that interact with PP1 [53], was found to bind directly the RVxF-binding pocket of PP1 [54]. That PP1 $\alpha$  is redistributed into a new location in the nucleus by Tat, but not by a  $^{35}\text{QACA}^{38}$  mutant of Tat, is evidence consistent with direct binding of PP1 by Tat [52].

In the scheme of HIV-1 transcription, Cdk9 is a logical substrate for dephosphorylation by PP1. Phosphorylation of Cdk9 on Thr 186 is required for its binding to 7SK RNA and HEXIM1 [55,56]. Dephosphorylation of the Thr 186 Cdk9 by PP1 *in vitro* dissociates 7SK RNA [55]. The relevance of the PP1–Cdk9 interaction is supported by experiments in cultured cells. Here, okadaic acid induces phosphorylation of Cdk9, and expression of NIPP1 prevents this phosphorylation [54].

A final phosphatase adds even more regulatory complexity. Autophosphorylation by Cdk9 of serines in its C terminus enhances association of P-TEFb with Tat and TAR RNA [57]. Protein phosphatase 2A (PP2A) was found to regulate HIV-1 transcription [58,59] by dephosphorylating autophosphorylated Cdk9 [54]. HIV-1 Tat binds to LIS1 protein, a product of the lissencephaly gene whose mutation causes a severe form of brain malformation [60]. LIS1 resembles the B-subunit of PP2A and interacts with the catalytic subunit of PP2A *in vitro* [61]. Interestingly, LIS1 over expression in cells increases HIV-1 transcription [61]. Thus, another scenario emerges in which HIV-1 Tat might interact with PP2A holoenzyme by binding to LIS1. Thus, HIV-1 transcription and phosphorylation of Cdk9 are apparently intricately regulated by several phosphatases, including both PP1 and PP2A.

#### Effects of post-translational modifications on HIV-1 transcription

Post-translational modifications of Tat influence its transcriptional function. Recently, nonproteolytic ubiquitination of Tat by Hdm2 was found to positively affect transcription [62]. Additionally, methylation of Tat on arginine residues by PRMT6 inhibited transcriptional

activity, suggesting that methylases might act to restrict HIV replication in cells [63]. Additional events that repress HIV-1 transcription arise from the recruitment of histone deacetylase (HDAC)1 to the HIV-1 LTR by YY1 and LSF [64], AP-4 [65], and the NF- $\kappa$ B p50 subunit [66]. Evidence supporting the repressive role of HDAC comes from findings that inhibition of HDAC1 by trichostatin A activates HIV-1 transcription [64,66]; and knockdown of NF- $\kappa$ B p50 with siRNA promoted processive transcription of RNAP II at the HIV-1 LTR [66].

Acetylation of Tat on Lys50 and Lys51 by p300/CBP has been shown to be important for transcription from an integrated HIV-1 promoter [17,67]. Another HAT, histone acetyltransferase hGCN5, also acetylates Tat lysines 50 and 51 [68]. Acetylation of Tat Lys50 by p300/CBP dissociates Tat from TAR RNA, whereas acetylation of Tat at Lys28 by p300/CBP-associated factor enhances Tat binding to Cdk9/cyclin T1 [15]. Acetylation of Tat at Lys50 regulates Tat binding to Brm, a DNA-dependent ATPase subunit of the SWI/SNF chromatin-remodeling complex, and activates HIV-1 transcription [69]. The INI-1 and BRG-1 components of SWI/SNF chromatin-remodeling complex synergize with p300 acetyltransferase and acetylated Tat to remodel the nucleosome at the HIV promoter in order to activate transcription [70]. Acetylated Tat has indeed been found in a complex with BRG-1, on chromatinized HIV-1 LTR [71]. Sirtuin 1, a deacetylase, can recycle acetylated Tat through deacetylation [72].

#### Post-transcriptional regulation of HIV-1

After synthesis, HIV transcripts are processed into unspliced, partially spliced and fully spliced RNAs. The unspliced 9-kb mRNAs encode Gag and Gag/Pol; the singly spliced 4 kb mRNAs encode Vif, Vpr, Vpu and Env; and the fully spliced 2-kb RNAs encode Tat, Rev and Nef. Just as Tat is the key transcription factor for HIV-1, the Rev protein is the critical viral protein for post-transcriptional regulation.

#### HIV-1 Rev protein

Like Tat, Rev is a small, positively charged RNA-binding protein that is approximately 116 amino acids in size. One can operationally divide the Rev protein into three physical/functional domains. This first is a basic arginine-rich domain that binds RNA and functions as a nuclear localization sequence (NLS); the second is a leucine-rich domain that contains the nuclear

export signal (NES), and the third is a protein self-multimerization domain [73]. The NLS and NES interdigitate Rev into cellular pathways normally used for nuclear import and export of macromolecules. Hence, in the setting of HIV-1 replication, the arginine-rich amino acids of Rev act as a NLS that serves in the import of Rev from the cytoplasm into the nucleus. For this nuclear entry, Rev is ferried through the nuclear pore by the nuclear import factor importin  $\beta$  in a complex with the nucleolar phosphoprotein B23 via importin  $\beta$ -nucleoporin interaction [74]; current evidence suggests that the NLS of Rev directly interacts with importin  $\beta$ . Once inside the nucleus, the arginine-rich RNA binding motif of Rev directs the protein to bind a stem-loop RNA structure, known as the Rev responsive element (RRE), which is resident within the HIV-1 Env RNA and other RNAs with overlapping reading frames. It is thought that binding between Rev and RRE is initiated first by high-affinity binding of a single Rev monomer, followed by the cooperative binding of up to 12 additional Rev molecules to the RRE. Rev bound to RRE-RNA then associates via its NES to a cellular CRM1 protein, also known as exportin-1. The Rev-RRE-CRM1 multimeric complex shuttles unspliced and partially spliced viral RNAs from the nucleus into the cytoplasm for their translation into protein. It should be noted that additional cellular factors such as hRIP [75], Sam68 [76] and eIF5a [77] also appear to contribute to the RNA export function of Rev.

#### *Splicing of HIV-1 RNA*

The HIV genome has five 5' splice donors and nine 3' splice acceptors. Through various permutations and combinations, these sites can generate 40 or more differently spliced mRNAs [78–80]. The abundance of alternatively spliced species is regulated by the efficiencies through which 5' and 3' splice sites are used. This, to a certain degree, is determined by the nature of the 5' splice donors and 3' splice acceptors [81,82], the cellular factors that interact at these elements [83–85], and *cis*-acting motifs that surround the splice sites. In general, HIV-1 splice sites are inherently suboptimal sequences and are used inefficiently. However, a further level of control over splicing of HIV-1 mRNAs is exerted by cellular factors that bind to exonic splicing enhancers (ESE) [86,87] and exonic/intronic splicing silencers (ESS/ISS) [88,89]. ESE, ESS, and ISS are short *cis*-acting sequences flanking HIV-1 splice sites [90].

How do ESS and ISS sequences regulate HIV-1 splicing? In general, ESS/ISS sequences in HIV-1 RNA contain a pyrimidine UAG motif. It has been found that these motifs are commonly bound by hnRNP A1 proteins, including A1, A1B, A2 and B1 [91]. Mechanistically, the binding of hnRNP A1 proteins to ESS/ISS occludes the formation of a spliceosome complex, leading to suppressed splicing [92].

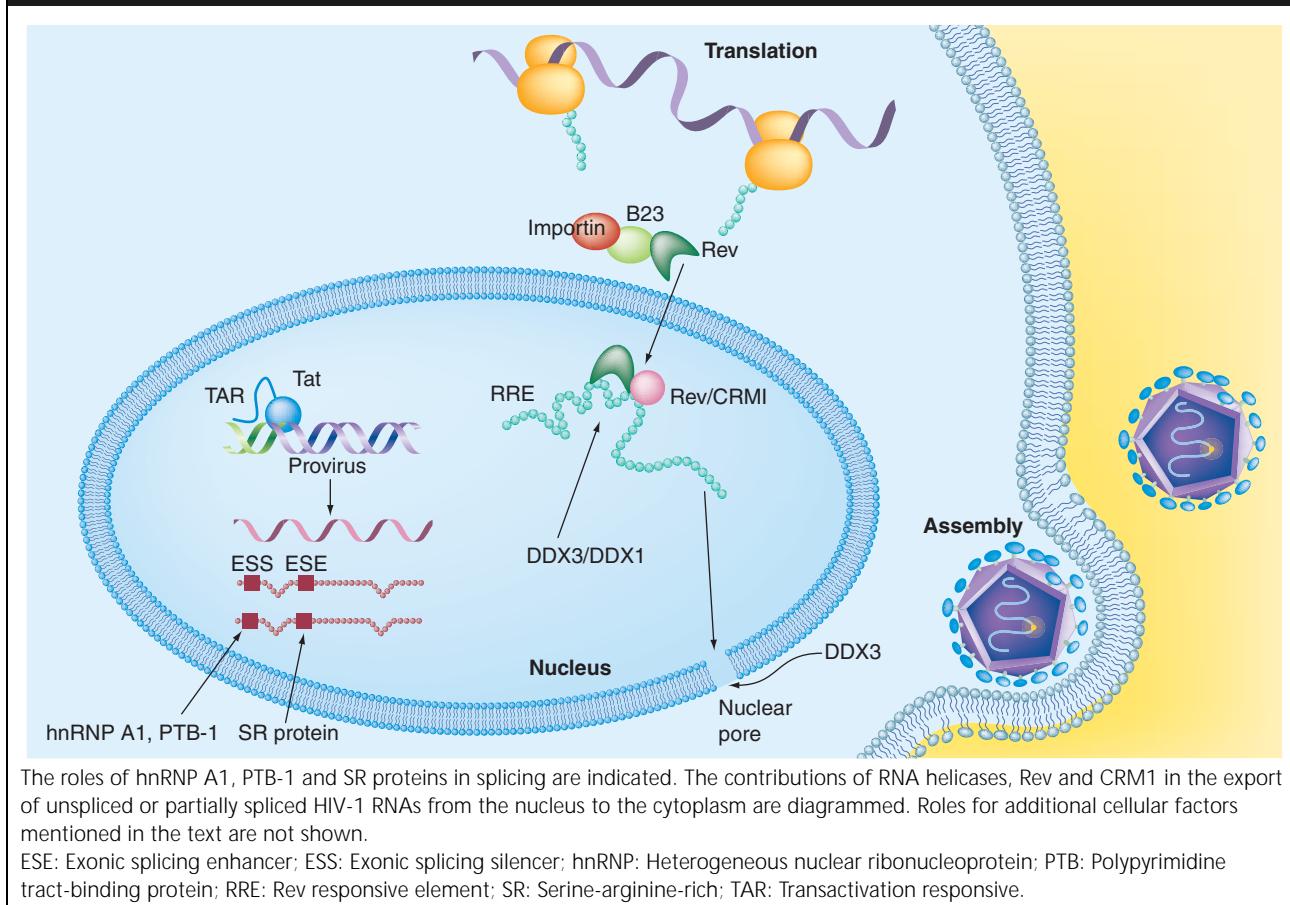
In a converse manner, utilization of some splicing sites in HIV-1 RNA is enhanced by the *cis*-ESE sequences [90]. Differing from exons with ESS, exons containing ESE are enhanced for splicing. While not all is yet understood, ESE motifs are recognized inside the nucleus by proteins of the serine-arginine-rich (SR) family. SR proteins appear to recruit splicing factors such as U2AF65, U2AF35 and the U1 snRNP component U1-70K to facilitate spliceosome assembly. In doing so, SR protein-ESE interaction promotes the utilization of flanking 5' and 3' splice sites. Recently, drugs that work to inhibit cellular kinases that phosphorylate SR proteins have been shown to inhibit HIV-1 replication [93]. This finding suggests that therapeutic molecules that modulate HIV-1 splicing may possibly be useful for intervening against viral replication.

#### *Export of HIV-1 RNA from the nucleus to the cytoplasm*

As alluded to above, the early HIV-1 regulatory proteins Tat, Rev and Nef are encoded by multiply spliced mRNAs. It is generally viewed that such fully spliced messages are constitutively exported from the nucleus into the cytoplasm where they are translated into proteins. However, there is a recent report that argues that in latently infected cells, spliced Tat, Rev and Nef transcripts remain retained in the nucleus; and that under such circumstance, their emergence into the cytoplasm requires the polypyrimidine tract binding protein [94].

On the other hand, multiply spliced viral RNAs aside, up to 50% of HIV-1 mRNAs in infected cells are unspliced or partially spliced. There is much evidence that unspliced mRNAs are retained in the nucleus. Yet unspliced HIV-1 RNAs need to exit the nucleus to locate into the cytoplasm in order that they can be translated into Env and Gag proteins and serve as genomic RNAs for progeny virions. How the unspliced and partially spliced HIV-1 transcripts reach the cytoplasm is now understood to depend on Rev function (Figure 2).

Figure 2. Rev function in the export of unspliced and partially spliced HIV-1 RNAs from the nucleus.



Early HIV-1 research found that when Rev was mutated, expression of Env and Gag proteins (translated from unspliced/partially spliced RNAs) was lost while expression of Tat, Rev and Nef proteins (translated from multiply spliced RNA) remained intact. Subsequently, the explanation for this finding was that Rev is needed to promote the export of unspliced/partially spliced viral RNAs from the nucleus into the cytoplasm. As mentioned above, Rev can bind through its basic domain the highly secondary structured RRE element present in all unspliced/partially spliced HIV transcripts [95–99]. The Rev–RRE complex then associates with CRM1 to shuttle from the nucleus into the cytoplasm [100–105]. The Rev–RRE–CRM1 route is independent of the path used by fully spliced HIV-1 mRNA, and cellular mRNAs to exit the nucleus [106,107] because inhibition of one pathway does not affect the other pathway [108,109].

Rev–RRE–CRM1 also engages the activity of cellular RNA helicases [110]. RNA helicases DDX1 and DDX3 are now known to associate with the Rev–CRM1–RRE complex [111,112].

DDX3 acts to enhance the Rev-dependent, but not other RNA export, pathway; and DDX3 can directly bind CRM1 and Rev and facilitate mRNA egress through the nuclear pore. DDX3 is postulated to remodel and ‘thread’ large unspliced HIV-1 RNAs through the nuclear pore, facilitating their final release to the cytoplasmic side of the nuclear pore complex. In this regard, DDX3 is functionally similar to its yeast analog, the Dbp5p helicase [113]. The function of DDX1 may be akin to DDX3; however, DDX1 seems to be restricted to assisting Rev activity in human astrocytes. Overall, the ability of Rev to cycle from the cytoplasm into the nucleus and back to the cytoplasm in concert with viral RNAs and cellular factors (CRM1 and RNA helicases) creates a continuous loop whereby a limiting amount of viral protein can regulate the export of a larger amount of unspliced/partially spliced viral mRNA.

#### Future perspective

The likelihood that cellular proteins can be drug targets for HIV-1 therapy hinges upon the ability to achieve efficacy with tolerable toxicity. Drugs

that work at lower concentrations to inhibit the contribution of cellular factors to viral processes without affecting the cell-endogenous function of these proteins need to be discovered. Careful empirical verification should be performed to investigate whether any of the cellular transcription and post-transcription factors mentioned in this survey may be candidates for HIV-1 therapy.

### Concluding remarks

This review has discussed in a nonexhaustive fashion some of the cellular factors that work together with Tat and Rev to regulate transcription and post-transcriptional expression of HIV-1 mRNAs.

What has not been discussed is the role of cellular proteins in the regulation of retroviral translation [114]. One of the interests in understanding the requisite cellular factors for viral replication is the potential to target these factors for HIV-1 therapy [115]. HIV-1 is much more mutable than cellular genes; accordingly, targeting the latter rather than the former is considerably less likely to elicit drug-resistant viruses.

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### Executive summary

#### HIV-1 Tat is requisite for viral transcription

- Tat interacts with histone acetyl transferases to loosen nucleosomes to allow for transcription of integrated long-terminal repeat (LTR).
- Tat assembles TATA-box-binding protein complexes at the LTR to initiate intracellular transcription.
- Tat engages cyclin T/Cdk9 (pTEFb) kinase complex for processive elongation of transcription.
- Tat also engages protein phosphatase (PP)1 and PP2A phosphatases to regulate HIV-1 transcription.

#### HIV-1 Rev is required for export from the nucleus of unspliced/partially spliced viral mRNAs

- Rev binds Rev responsive element (RRE)-containing viral RNA and associates with transport protein CRM1.
- Rev–RRE–CRM1 interacts with RNA helicases DDX1 and DDX3 to exit the nuclear pore.
- Splicing of HIV-1 RNAs is regulated by exon splicing silencer, intron splicing silencers and exon splicing enhancer.
- For mutable viruses such as HIV-1, drugs that target cellular factors needed for viral replication may elicit fewer drug-resistant viruses than drugs that target viral proteins.

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## Affiliations

- *Sergei Nekhai*  
Center for Sickle Cell Disease & Department of Biochemistry & Molecular Biology, Howard University, 520 W. Street, NW Washington, DC, 20059, USA  
Tel.: +1 202 865 4545;  
Fax: +1 202 865 7861;  
[snekhai@howard.edu](mailto:snekhai@howard.edu)
- *Kuan-Teh Jeang*  
Molecular Virology Section, Laboratory of Molecular Microbiology, NIAID, Building 4, Room 306, NIH, 9000 Rockville Pike, Bethesda, MD 20892, USA  
Tel.: +1 301 496 6680;  
Fax: +1 301 480 3686;  
[kjeang@niaid.nih.gov](mailto:kjeang@niaid.nih.gov)