

HIV-1 TAR RNA: The Target of Molecular Interactions Between the Virus and its Host

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Abstract: HIV-1 TAR RNA is the binding site of the viral protein Tat, the trans-activator of the HIV-1 LTR. It is present at the 5' end of all HIV-1 spliced and unspliced mRNAs in the nucleus as well as in the cytoplasm. It has a highly folded stem-bulge-loop structure, which also binds cellular proteins to form ribonucleoprotein complexes. The Tat-Cyclin T1-CDK9 complex is the main component in the trans-activation of HIV-1 and its affinity for TAR is regulated through Tat acetylation by histone acetyl transferases. Recent studies show that this complex is able to recruit other cellular partners to mediate efficient transcriptional elongation. TRBP, PKR and La bind directly to the TAR RNA structure and influence translation of HIV-1 in either positive or negative manners. Some mutations in TAR RNA severely impair HIV-1 trans-activation, translation and viral production, showing its functional importance. The overexpression or suppression of several TAR RNA-binding proteins has a strong impact on viral replication pointing out their major role in the viral life cycle. TAR RNA has been the target of drug development to inhibit viral replication. Recent data using small molecules or RNA-based technologies show that acting on the TAR RNA or on its viral and cellular binding factors effectively decreases virion production.

Keywords: TAR RNA, Tat, acetylation, trans-activation, translation, TAR RNA binding proteins.

INTRODUCTION

When integrated into the host genome, the HIV-1 5' long terminal repeat (LTR) becomes an eukaryotic transcriptional unit that contains downstream and upstream promoter elements [143, 146]. The 5' end of all HIV mRNAs, whether they are spliced or not, start with the formation of an identical stem-bulge-loop structure called the Trans-Activation Responsive (TAR) element located from nucleotide position +1 to +59. TAR was originally identified as the target for the trans-activator of HIV, the Tat protein, which is essential for efficient transcription of viral genes and for viral replication [22, 73, 113]. The studies that have examined the structure and the sequence of TAR have shown that the stem structure, the sequence in the bulge and in the loop, as well as the distance between the bulge and the loop are all required for Tat-mediated trans-activation. The TAR RNA needs to be in its nascent form and located closely from the 5' end to mediate efficient Tat function [144]. The functional importance of the TAR RNA has been emphasized by the influence of mutations in the context of HIV replication. Several mutations that affect the TAR sequence or structure decrease the viral kinetics [102]. Recent developments on the mechanism of Tat trans-activation and the regulation of Tat-TAR interaction by acetylating enzymes bring new light to some of the early results. Although TAR function was mainly studied in the context of Tat trans-activation, data on its role in translation show

that it is the target for a number of cellular RNA binding proteins that influence the rate of HIV translation (Table 1). Because of its importance in the virus life cycle, the HIV TAR RNA has been the target of drug development that lead to small molecules and RNA-based strategies for gene therapy.

I - TAT-TAR RNA INTERACTIONS FOR TRANS-ACTIVATION

A) Tat-Mediated Trans-Activation

As the basal transcription of HIV is very low, the viral Tat protein and host factors increase the mRNA production from the viral genome. In contrast to other known trans-activators that act on DNA, Tat acts through the TAR RNA located in the R region of the LTR. In addition to TAR, Tat requires the functional carboxy terminal domain (CTD) of the RNA polymerase II (RNAP II) for function. Tat binds to the TAR RNA and to the positive transcription-elongation factor b (P-TEFb), a complex composed of Cyclin T1 (CycT1) and the cyclin-dependent kinase 9 (CDK9) [134, 170]. Whether Tat binds alone to TAR RNA *in vivo* or only when complexed to P-TEFb remains to be determined. Several data favor the second hypothesis but do not exclude that the first one also occurs: i) the preexistence of CycT1 in the cell prior to Tat synthesis, ii) the higher affinity of the Tat-P-TEFb complex for TAR compared with Tat alone in gel mobility shift experiments [61, 64, 66, 170] and iii) the high affinity between the Tat and CycT1 proteins [10, 14, 170]. P-TEFb phosphorylates the RNAP II CTD and marks the transition from initiation to elongation of eukaryotic transcription [32, 82, 175, 187]. Thus Tat promotes the polymerase departure, promoter clearance, and efficient elongation through CTD phosphorylation by initiating the

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Table 1. Viral and Cellular Proteins Involved in HIV-1 TAR RNA Functions

	Viral Function	Cellular Function	References
Tat	HIV trans-activator, binds TAR RNA		[22, 65, 73, 91, 113, 144]
hCycT1	Binds Tat and increases its affinity for TAR RNA	Cyclin	[134, 170]
CDK9	Binds CycT1 and phosphorylates RNAP II CTD	Kinase, phosphorylates RNAP II CTD	[75, 140]
p300/CBP	Acetylates Tat at K50, K51	Histone Acetyl Transferase	[13, 86, 115]
PCAF	Acetylates Tat at K28	Histone Acetyl Transferase	[99]
hGCN5	Acetylates Tat at K50, K51	Histone Acetyl Transferase	[35]
PKR	Binds TAR RNA, inhibits translation	Kinase, inhibits translation, controls cell growth	[12, 38, 63]
TRBP	Binds TAR RNA, enhances translation	Inhibits PKR, increases cell growth, controls spermatogenesis	[12, 38, 69, 106, 183]
La autoantigen	Binds TAR RNA, enhances translation	RNAP III transcription	[26, 157]

formation of an elongation competent transcription complex [22, 65, 73, 91, 113, 144].

B) Components of the TAR/Tat/P-TEFb Complex

Many cellular factors bind the TAR RNA and act on transcription or trans-activation [4, 29, 59, 72]. CDK9 (part of TAK, P-TEFb, PITALRE) is an HIV-1 Tat-associated kinase [66, 75, 111, 176, 187]. Within the P-TEFb complex, CDK9 phosphorylates the CTD of the RNAP II and thus induces efficient promoter clearance and transcriptional elongation. CDK7 (part of TFIIF) was also reported to bind directly or indirectly to Tat [37, 67], but further investigations showed no involvement of CDK7 in transcriptional elongation *in vitro* and no direct or indirect interaction between CDK7 and Tat [10, 28]. Therefore, CDK9 is likely the sole component that mediates CTD phosphorylation induced by Tat but a coordinated activity between the two kinases is required for optimum transcription [185, 186]. CDK9 autophosphorylation is required for high-affinity binding of Tat-P-TEFb to TAR RNA that regulates Tat trans-activation *in vivo* and Tat modifies the phosphorylating activity of CDK9 on the RNAP II CTD during HIV-1 transcription [66, 185].

Human (h) CycT1 is a member of a family of proteins involved in cell cycle regulation [140]. In the HIV context, hCycT1 was isolated based on its affinity with CDK9 and Tat [134, 170]. It binds Tat in a Zn²⁺-dependent manner via the cysteine at position 261 and facilitates the binding of Tat to TAR [64]. When the Tat-CycT1 complex is formed, Tat promotes CycT1 nt 252-260 binding to the U31, whereas CycT1 promotes Tat K50 interaction with G34 in the TAR loop [145]. hCycT1 increases trans-activation in rodent cells and is one missing permissivity factor in these cells [170]. Murine (mu) CycT1 binds to Tat like its human homologue but does not restore trans-activation in rodent cells [14, 61, 64]. Both CycT1 and CDK9 are essential for Tat trans-activation. Genetic studies have shown that chimeric CycT1 or CDK9 proteins can activate transcription if tethered

directly to a nascent heterologous RNA [15, 60, 75]. Indeed, P-TEFb can also activate the HIV promoter in the absence of Tat and TAR if it is targeted to an upstream DNA sequence, indicating that the primary role of Tat and TAR is to recruit CycT1-CDK9 to the initiation complex. In this context, muCycT1 is also functional and Sp1 binding sites are required for function [178].

C) Cellular Interactions of the TAR/Tat/P-TEFb Complex

In uninfected cells, part of P-TEFb is localized in nuclear speckles, which may be the site of its function [83] and part is inactive due to its binding to the 7SK small nuclear RNA that inhibits CDK9 kinase activity [128, 177]. The 7SK RNA/P-TEFb complex associates with the CDK inhibitor MAQ1 and constitutes the inactive stock of P-TEFb. As the 7SK RNA/MAQ1/P-TEFb complex and the Tat-CycT1 interaction are mutually exclusive, the TAR RNA/Tat system likely diverts P-TEFb from its inactive form or co-opts the free form of P-TEFb that will no longer be available to form the 7SK RNA/MAQ1/P-TEFb complex [118]. In addition, P-TEFb modulates gene expression by interacting, either directly or indirectly, with a number of cellular transcription factors. It binds to the major histocompatibility complex class II transactivator CIITA [93] and NF- κ B [8] and the addition of Tat diverts these proteins from their cellular function. The p160 coactivator GRIP1 [101], Pur α [39] and the human I-mfa domain-containing protein (HIC) [180] also interact with P-TEFb and Tat to potentiate Tat activity on HIV and heterologous promoters. On the opposite, granulin binds CycT1 and Tat but reduces the RNAP II CTD phosphorylation and Tat activity [85].

P-TEFb also binds to negative transcription-elongation factors (N-TEFs), which include the DRB-sensitivity inducing factor (DSIF composed of SPT5 and SPT4) [105, 164] and negative-elongation factors (NELFs) [125, 174]. During elongation, CDK9 phosphorylates SPT5 and the RNAP II CTD, which alleviates the repression of

transcriptional elongation mediated by N-TEFs [105, 174]. This activity initiated by the recruitment of P-TEFb by Tat and TAR RNA to the pre-initiation complex will go on in the elongation complex after the complex has been released from TAR [135, 136, 184].

D) Cellular Models for Defective Tat-Mediated Trans-Activation

Specific steps in the HIV life cycle can be elucidated using defective cellular models. An example of a chronically infected cell defective for TAR function is the ACH2 cell line in which a single point mutation in TAR (C³⁷ to T) inactivates Tat-mediated trans-activation and viral replication [54]. Murine cells also provide a good example of a block of the trans-activation process. In these cells, the muCycT1 has a single change that mutates C²⁶¹ to a Y and renders the Tat-muCycT1-CDK9 complex unable to bind the TAR RNA and therefore to initiate trans-activation of the LTR [14, 61, 64]. The muCycT1 function in trans-activation is restored when the protein is targeted to DNA emphasizing the importance of transferring the Tat-CycT1-CDK9 complex from TAR to the transcription complex in the natural context [178]. This model has been very useful to elucidate the trans-activation mechanism and to pursue the search for additional factors required for full restoration of viral replication in murine cells [10, 16, 114, 182].

II - REGULATION OF TAR/TAT/P-TEFB INTER-ACTIONS BY HISTONE ACETYL TRANSFERASES

A) Histone Acetyl Transferases (HATs) and Chromatin Structure

Chromatin structure and the formation of nucleosomes with histones are modulated by the activity of multiprotein complexes including histone acetyl transferases (HATs) and deacetylases (HDACs) [11, 126]. Indeed, HATs are proteins that acetylate histones and facilitate chromatin unfolding to allow transcription [80, 165]. Some viral proteins interact directly with HATs (also called factor acetyl transferases, FATs) and HDACs to disrupt cellular gene expression and increase their activity, thereby contributing to viral life cycle and cellular dysfunction [24, 62]. Following integration into the host cell genome, the HIV-1 provirus is organized into the chromatin although a non-integrated form can remain. The formation of nucleosomes in the 5'LTR is precisely positioned with respect to *cis*-acting regulatory elements [141, 160, 172], but can be disrupted by histone acetylation or by Tat [51, 109].

B) Tat acetylation by HATs Regulates its Binding to TAR RNA

HATs p300 and the related CREB binding protein (called p300/CBP) and p300/CBP-associated factor (PCAF) bind Tat, mediate its acetylation and increase its trans-activating function on HIV-1 LTR [13, 86, 115]. The analyses of the interaction between various HATs and Tat have elucidated the sites of Tat acetylation. As a first step, PCAF acetylates Tat at K28 [99]. Ac28-Tat has an increased affinity for CycT1-CDK9, but a decreased affinity for PCAF, which enhances the binding of the Tat-P-TEFb

complex to TAR RNA and subsequently releases PCAF from Tat [20, 99]. p300/CBP and hGCN5 acetylate Tat at K50 and K51 [35, 44, 99, 129]. In some assays, Ac50Tat has no affinity for TAR [20, 44, 99], whereas in another one, only the Ac50Tat-CycT1 complex and not Ac50Tat shows a decreased TAR binding [92]. These data lead to two different models in which Tat acetylation at K50 by p300/CBP promotes the dissociation of either Tat-P-TEFb complex from TAR [20] or P-TEFb alone [92]. In addition, the bromodomain of PCAF binds specifically to Ac50Tat and requires Y47 and R53 in Tat [48, 124]. Unacetylated Tat interacts with PCAF through another domain, which suggests two different steps of PCAF-Tat binding [20]. Mutations in either K28 or K50 decrease Tat trans-activation and HIV replication, whereas treatment with deacetylase inhibitors synergize with Tat function [19, 44, 99, 129, 147].

C) Sequential Steps of the *In Vivo* Regulation of Tat-TAR Binding for Trans-Activation

A mechanism that reconciles all data about the steps leading to activated transcription elongation by Tat has not yet reached a consensus. One main discrepancy in the current models is whether the Tat-CycT1-CDK9 complex is transferred from TAR to the elongating complex paused after TAR or to the next pre-initiation complex (PIC) on the promoter. Models based on *in vitro* studies have favored the first mechanism [94]. However, a direct comparison of the transcriptional activity of Tat and P-TEFb shows a much stronger increase when their recruitment occurs during the PIC formation rather than after the transcription elongation complex (TEC) has been formed [185]. These data explain the limited Tat activity in *in vitro* assays measuring only elongation [100]. Furthermore, the phosphorylating activity of CDK9 on RNAPII CTD starts between 14 and 36 nt after the release of TFIIH, which corresponds to the transition between initiation and elongation prior to the TAR RNA synthesis [186]. In addition, *in vitro* models do not evaluate HIV trans-activation by Tat in the context of the chromatin structure. In contrast, *in vivo* studies lead to strong Tat trans-activation and favor a dual model in which Tat promotes the formation of a processive transcription elongation complex at the initiation site. This model is fully compatible with the most recent *in vivo* data and complements previous models [73, 113, 141]. This new information is brought by chromatin-immunoprecipitation (ChIP) experiments. These data show that p300, CBP, NF- κ B p65, and PCAF are recruited early to the HIV-1 promoter upon Tat activation *in vivo* [109, 115] and that Ac50Tat, but not unacetylated Tat is associated with the HIV-1 promoter *in vivo* [92]. Several data have shown that the Tat and the P-TEFb complex are present in both the PIC and in the transcription elongation complex (TEC) [68, 97, 135, 185] and that the formation of short TAR transcripts *in vivo* is linked to the inducer of short transcripts, which is a DNA element uncoupled to the trans-activation mechanism [133, 151]. Tat also has direct or indirect affinity for Sp1, TBP, TAF55, TAF250, TFIIB and RNAPII, which are all within or in the proximity of the PIC [30, 33, 45, 95, 163, 171]. Overall, these and the recent data showing the requirement of Sp1 for the activity of DNA-bound Tat and CycT1 [178] strongly suggest an *in vivo* step

of Tat-P-TEFb activity at the PIC level and a limited activity by direct recruitment to the TEC.

In the model presented in (Fig. 1), the basal level of transcription generates a few TAR and Tat molecules, but it can be boosted by cellular events. The newly formed unacetylated Tat binds PCAF, which generates Ac28Tat that has an increased affinity for CycT1 already bound to CDK9. PCAF is then released from Ac28Tat. The Tat-CycT1-CDK9 complex binds to the low amount of nascent TAR RNA

present in the cell. P300/CBP and possibly hGCN5 are recruited at this site and acetylate Tat at K50 and maybe at K51. The p300/CBP-Ac50Tat-P-TEFb is then released from TAR and transferred to the next PIC either on the promoter or paused at around 15 nt following hypophosphorylation of the RNAP II CTD on Ser 5 by CDK7/TFIIH. CDK9 will be phosphorylated and active after TFIIH release from the initiation complex, which occurs after a 14-36 nucleotides-RNA has been synthesized [186]. The role of NF-κB p65,

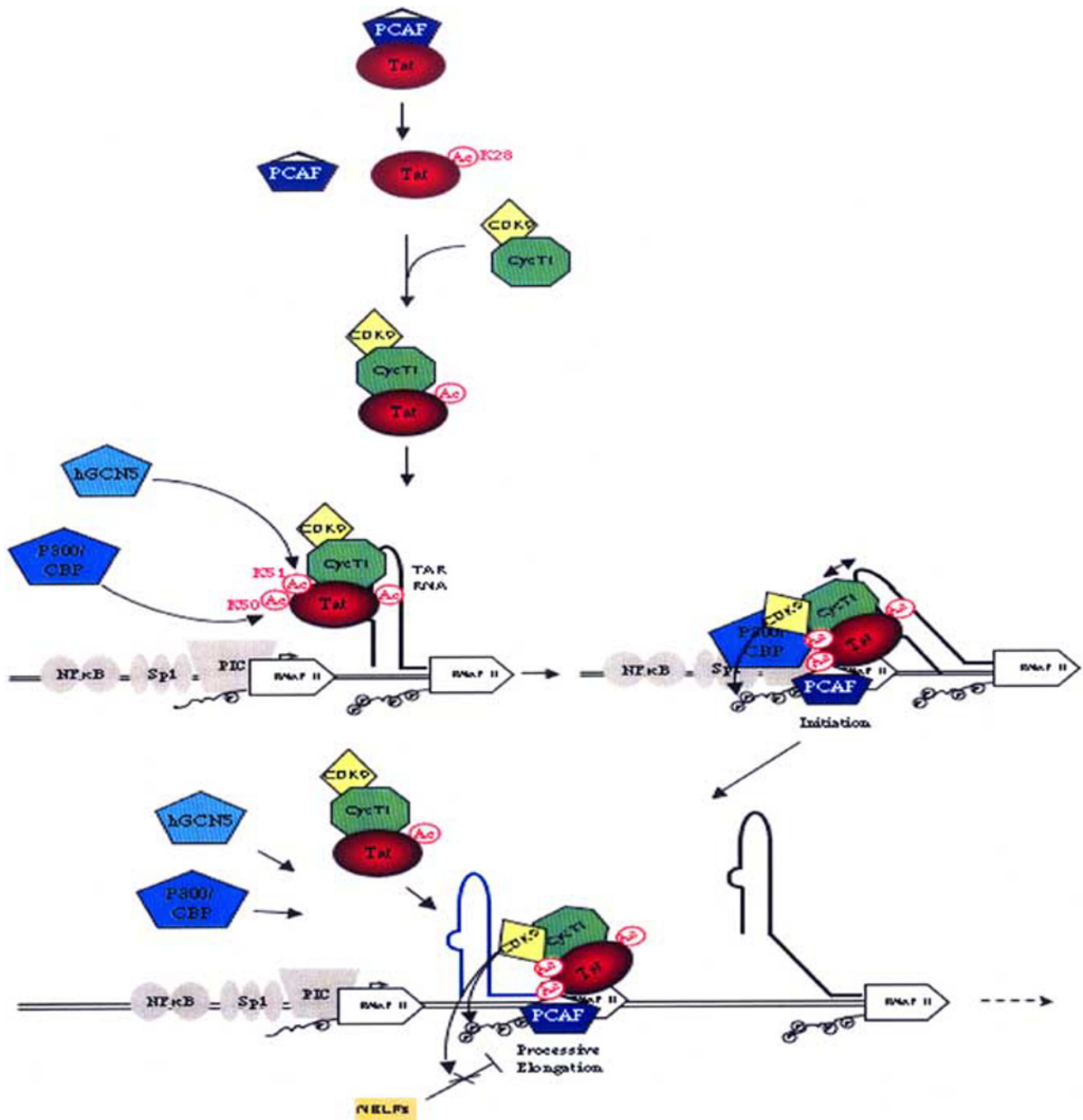


Fig. (1). Model for the regulation of Tat-TAR binding by acetylation and its implications for the *in vivo* trans-activation mechanism. PIC is the transcription preinitiation complex before or after hypophosphorylation of the RNAPII CTD by CDK7/TFIIH. The double-sided arrow shows the release the Tat-CycT1-CDK9 complex from TAR RNA. Newly synthesized TAR RNA is in blue.

PCAF, and later hGCN5 recruitment by Tat to the promoter is not yet elucidated but probably contributes to the formation of an open chromatin that increases transcription efficiency. The association of p300/CBP-Ac50Tat-P-TEFb with the initiation complex then forms an efficient transcription elongation complex, in which CDK9 hyperphosphorylates the RNAP II CTD. This new complex recruits PCAF via its bromodomain and the basic region of Ac50Tat. Whether K28 in Tat remains acetylated or not at this step and the role of PCAF in the elongation complex remain unknown. The role of CDK9 in the elongating complex is crucial in maintaining the RNAP II CTD in an hyperphosphorylated form, both by a direct phosphorylation activity and by inhibiting NELFs that would otherwise slow down elongation. Because of the continued function of CDK9 from the initiation step after TFIIH activity to the end of the elongation process, a release of P-TEFb from TAR independently from Tat [92] seems unlikely at this point, but we cannot exclude a partial release of P-TEFb or a partial retention of Tat alone on TAR. This model points out the importance of acetylation in Tat-TAR RNA binding and release that mediate Tat trans-activation *in vivo*.

III - TAR RNA AND THE TRANSLATIONAL CONTROL OF HIV-1

A) Post-Transcriptional Control of HIV-1 Expression

After its synthesis in the nucleus, the 9 kb HIV-1 RNA is differentially spliced to produce mRNAs for the regulatory/accessory and structural proteins [142]. In the early phase, HIV-1 mRNA is multiply spliced to produce a 2kb mRNA that is constitutively exported to the cytoplasm and translated to produce Rev, Tat and Nef. In the late phase, in the presence of Rev, unspliced and incompletely spliced RNAs are exported to the cytoplasm. These RNAs are translated to produce gag, pol, env, vif, vpr and vpu. The mechanism of Rev-mediated RNA export occurs through its RNA binding domain and its nuclear export signal [139]. Once in the cytoplasm, HIV mRNAs are translated via the cellular translational machinery [63]. HIV RNA translation is modulated by the TAR RNA structure and by cellular factors that bind to it in the cytoplasm. Following translation, the HIV genomic RNA is transported for viral packaging that also involves additional cellular RNA binding proteins [27, 123].

B) TAR RNA Downregulates Translation

In vitro translation assays, and transient transfections have shown that the presence of the TAR RNA at the 5' end of RNA transcripts inhibits translation. The mechanism of this inhibition occurs either through a direct block by the secondary structure of TAR, by the activation of the interferon (IFN)-induced double stranded (ds) RNA-activated protein kinase PKR or both mechanisms. *In vitro* translation assays in which PKR is activated regardless of the presence of TAR show that only constructs that possess TAR have a decreased translation [47]. Point mutations that do not affect the TAR stem structure maintain the translation inhibition, whereas mutations that affect the stem stability restore a normal translation indicating a main role of the RNA structure in the translation block. Activated PKR mediates

an additional translational repression of TAR containing RNAs, whereas two cellular proteins, the TAR RNA Binding Protein (TRBP) and the La autoantigen bind to TAR and alleviate this inhibition very efficiently [47, 157] (Fig. 2).

C) PKR binds TAR RNA and Inhibits Translation

PKR is a cytoplasmic protein that binds to the stem of TAR [25, 127, 149]. PKR belongs to the family of double-stranded (ds) RNA binding proteins [98, 150, 154]. It binds TAR RNA with a specific binding of the dsRNA binding domain (RBD)1 around the bulge, whereas dsRBD2 binds to the lower stem [153]. This binding at a 2:1 protein:RNA ratio mediates PKR activation and translational inhibition [25]. TAR RNA ds structure activates PKR by mediating its autophosphorylation. Activated PKR phosphorylates the α subunit of the eukaryotic initiation factor 2 (eIF2 α), which blocks the translation initiation step and leads to an antiviral and antigrowth activity. Although PKR and IFNs can effectively inhibit HIV-1 LTR expression and viral replication in tissue culture experiments [12, 38, 137], treatment of HIV-1 infection with IFN has consistently failed [103] suggesting high defense mechanisms from either the cell or the virus.

D) TRBP Increases Translation from TAR RNA by Two Mechanisms

TRBP1 and TRBP2 were the first cDNAs to be cloned based on the protein's properties to bind HIV-1 TAR [69-72] and RRE [130] RNAs. They stimulate the expression of the HIV-1 LTR in human and murine cells [38, 49, 69]. TRBPs are dsRNA binding proteins with two dsRBDs [70, 150]. A KR-helix motif in which lysines and arginines mediate TAR binding is located in the dsRBD2 [43, 55]. TRBP also binds to PKR and blocks the inhibitory effects of PKR on translation [130], on HIV-1 LTR expression [38, 56], and on HIV-1 replication [12]. In an *in vitro* translation assay, TRBP releases the translation block due to TAR. This increased translation is also observed *in vivo* in PKR $-/-$ cells and is dependent on the presence of an intact TAR RNA structure [47]. Current knowledge indicates that TRBP facilitates HIV-1 replication via translational control that consists in two mechanisms: PKR inhibition and increased translation of TAR-containing RNAs (Fig. 2). Other mechanisms may also be involved such as the interaction with RRE RNA or with the closely related PKR activator (PACT) protein ([132]; Laraki G. and Gatignol A., unpublished) but await further confirmation.

E) La Autoantigen Alleviates Translation Inhibition Mediated by TAR

The La autoantigen forms ribonucleoprotein particles in the cell, influences RNA polymerase III transcriptional termination and reinitiation and is involved in the translation of internal ribosome entry sites [112]. Like TRBP, La cDNA was also cloned from an expression library as the protein has the capacity to bind the TAR RNA [26, 72]. Its role in HIV expression is to alleviate the translation inhibition mediated by the TAR structure located at the 5'

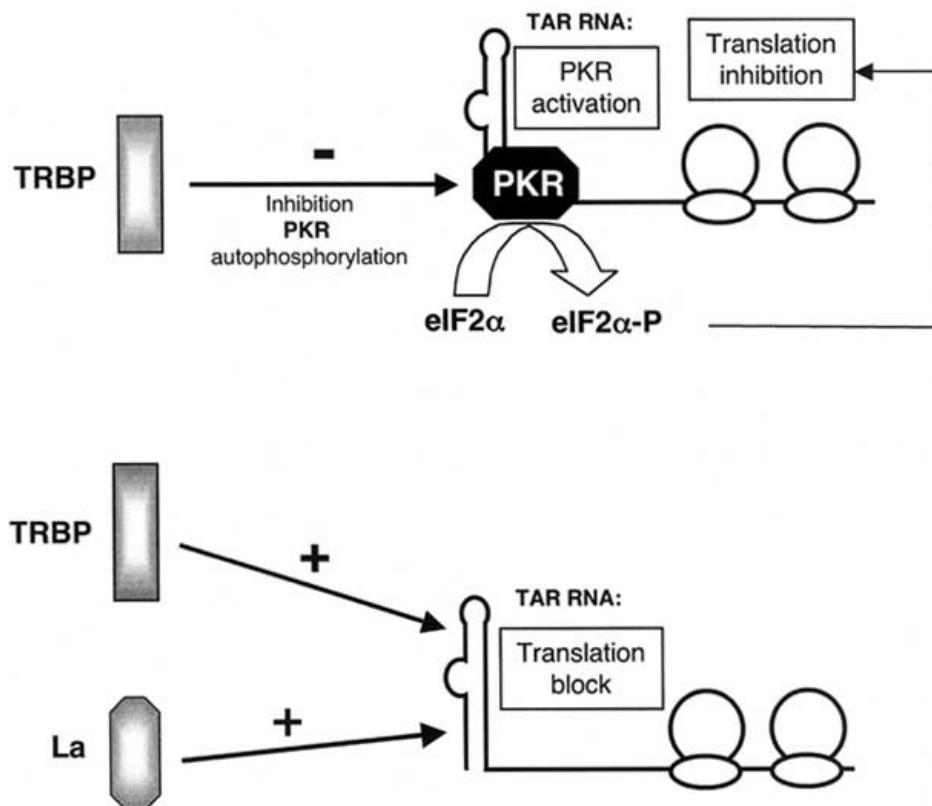


Fig. (2). Role of TAR RNA and its interacting cellular proteins in the regulation of HIV-1 translation.

end of mRNA transcripts [36, 157, 168], but its activity in the context of the entire virus has not been tested (Fig. 2).

F) Cellular Model Defective for the Translation of HIV Proteins

Astrocytes provide a cellular model that is not permissive to HIV-1 translation and should help in the elucidation of the different steps necessary to produce viral proteins. Astrocytes are localized in the brain and are infected by HIV. While they produce very small amounts of virus, they participate to the AIDS-associated dementia [77]. Although some productive infection has been described with pseudotyped HIV that bypasses the envelope requirement, several intracellular blocks in viral replication have been characterized [77]. One of them is a lack of translation of structural proteins [76]. Recent data indicate that TRBP can greatly increase HIV translation and replication in these cells¹ [77]. The restriction is due to a weak TRBP promoter expression in astrocytes compared with HIV-permissive cells [6, 7]. In this context, a combined functional association to the TAR and RRE RNAs as well as to PKR will likely mediate TRBP activity.

IV - TARGETING TAR RNA TO INACTIVATE HIV-1 REPLICATION.

In the virological context, the 5' leader RNA can have alternative structures, but the TAR hairpin does not exhibit variability [88]. Mutations in the TAR sequence generally

affect Tat function and/or HIV replication indicating a strong requirement for this structure during the expression of the complete genome [41, 81, 87, 102, 162]. Therefore, the TAR RNA is a potential therapeutic target and anti-TAR drugs would add to the action of other inhibitors [58, 73]. New therapeutic strategies against HIV in the recent years include hybrid small molecules, modified oligonucleotides and gene therapy based on ribozymes, RNA decoys, and siRNAs [2, 58].

A) Small Molecules

Large-scale screenings based on Tat-TAR inhibition gave rise to the selection of several compounds but none of them is currently used in therapeutics, emphasizing the need for new compounds [58, 73]. Recent screenings have identified small molecules such as aminoquinolones [131], trehalose derivatives [166], polyamidoamine dendrimers [181], phenothiazines [116], arginamides [138] and peptides [74, 173] as TAR binders and/or inhibitors of Tat-TAR interaction. Hybrid molecules have also been identified for their TAR affinity and their inhibitory activities of Tat-mediated trans-activation [89, 122]. One of these small molecules TAR-binders, TR87 composed of three unnatural modified peptides, was tested for inhibition of HIV-1 replication. It showed a potent and sustained suppression of HIV-1 production in cultured cells over 24 days without cellular toxicity [89]. In other experiments, a PBD-oligopyrroles hybrid compound showed low toxicity combined to an interruption of protein/TAR RNA interaction and Tat-induced trans-activation [122].

¹ Ong CL, Thorne JC, Gorry PR, Bannwarth S, Jawarowski A, Howard JL, Chung S, Moulard A, Gatignol A, Purcell DF. (in preparation).

B) Modified Oligonucleotides

Derivatives of antisense oligonucleotides that induce a steric blocking of the translation machinery by targeting 5' RNAs are more potent inhibitors than those inducing RNase H cleavage. They combine a high nuclease resistance with tight binding to single stranded RNA that efficiently inhibits protein synthesis [159]. The 5' position of the TAR RNA was therefore a target of choice for these new steric ribonucleoside analogues. Anti-TAR oligonucleotides have been designed or isolated as aptamers in large-scale screenings. They successfully block the TAR RNA structure and Tat trans-activation *in vitro* and in cell culture [5, 40, 79, 84]. Their activity against viral replication has not yet been tested. Polyamide nucleotide analogs (PNA) are nucleotides in which the bases are linked with peptide bonds instead of a sugar phosphate backbone and are resistant to nucleases and proteases. PNAs against TAR have been designed and tested for their activities on trans-activation or HIV-1 replication [117, 158]. To facilitate the PNA internalization into the cells, a 15-mer PNA targeted to the loop and the bulge region of TAR was conjugated with a membrane-permeating peptide vector, transportan. This conjugate blocks Tat trans-activation *in vitro* and in cell culture and inhibits HIV-1 replication [96].

C) Ribozymes

Ribozymes are catalytic RNA molecules that combine an antisense RNA to an enzymatic activity that specifically cleaves the targeted RNA. Several ribozymes that target HIV-1 RNA inhibit HIV replication [110, 148]. Ribozymes that directly target the TAR structure show limited activity due to the inaccessibility of the highly folded structure [161, 169]. The addition of an RNA structure that recruits RNA helicases improves the activity of ribozymes that target highly folded RNAs because the helicase moiety unfolds the RNA. This additional enzyme lead to the synthesis of an anti-TAR "maxizyme" that cleaves TAR *in vivo* [104, 167]. This new approach may bring anti-TAR ribozymes in gene therapy assays in complement to other ribozymes [3, 108, 120].

D) TAR decoys

TAR decoys, which are short RNA nucleotides mimicking the TAR structure, inhibit Tat trans-activation, HIV-1 and HIV-2 replication in lymphocytes [23, 107]. To improve their efficacy, TAR was co-transcribed from a U6 promoter with U16snoRNA to make U16TAR targeted to the nucleolus. In this context, the TAR decoy colocalized with Tat-EGFP in the nucleolus and sequestered Tat and CycT1 in this compartment. As a consequence, Tat-mediated trans-activation was unable to proceed and the viral replication in CEM cells was blocked very efficiently [119]. These TAR decoys have been included on retroviral vectors in combination with other molecules against HIV for gene therapy [46, 108].

E) siRNAs

Post-transcriptional regulation of gene expression is regulated in part by small non coding dsRNAs such as short

interfering RNA (siRNA), short hairpin RNA (shRNA) or microRNA (miRNA) through a mechanism known as RNA interference (RNAi) [1, 50, 121]. 21-23-bp siRNAs can be used in mammalian cells to induce the degradation of homologous mRNA by RNAi. These short dsRNAs do not generally induce the IFN response pathway [9, 52], although some PKR activation may occur [21, 152]. As HIV uses RNA intermediates for its replication, it was an obvious target for RNAi and various siRNAs have shown to be effective in reducing viral replication [78, 155]. Although the 5' ends of mRNAs [53] and the tight TAR structure [179] are less favorable to RNAi, the TAR RNA has been targeted successfully. siRNAs against TAR showed a maximum of 50% inhibition measured in a luciferase reporter gene assay due to the tight stem-loop structure of TAR [179] but one siRNA that can anneal from the stem to the loop part of TAR inhibited HIV-1 replication very efficiently [90]. Indirect targeting of TAR with si- sh- or miRNAs include the viral Tat protein [2, 18, 34, 108, 156] and cellular factors CycT1 and CDK9 [31] that all affect the trans-activation process and viral replication. Recent results also show that decreasing TRBP expression also decreases viral expression and replication indicating that affecting the TAR function in translation is also a useful antiviral strategy [57]. Because HIV can escape from siRNA inactivation [17, 42], multiple targets and combination therapies are necessary for AIDS treatment. As advances are being made in the design of vectors for the transduction of haematopoietic progenitor cells [2], future strategies may include anti-TAR siRNAs in combination with drugs or other RNA-based strategies that affect other HIV mRNAs or HIV-associated proteins.

CONCLUSIONS

The TAR RNA, present in all HIV mRNAs, is an integral component of the HIV life cycle that has roles from the reverse transcriptase step to genomic RNA encapsidation. Recent studies have shown that its *in vivo* interactions with various cellular proteins tightly regulate the Tat-mediated trans-activation process and the translational mechanism. The TAR RNA and its interacting proteins are therefore obvious targets in the design of anti-HIV therapies. Recently developed small molecules and RNA-based strategies show promising therapeutic approaches. As with antiviral drug therapy regimens, multiple molecules and RNAs must be used to target several different highly conserved regions essential for the viral life cycle. A combination of antisense, RNA decoys, ribozyme and siRNAs approaches in which TAR RNA will be a target holds promise in future therapies for HIV-1 infection.

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ABBREVIATIONS

AIDS	=	Acquired immunodeficiency syndrome
CIITA	=	Class II transactivator
CBP	=	cAMP-responsive element binding (CREB) binding protein
CDK9	=	Cyclin-dependent kinase 9
ChIP	=	Chromatin immunoprecipitation
CTD	=	Carboxy terminal domain
CycT1	=	Cyclin T1
DSIF	=	DRB-sensitivity inducing factor
dsRNA	=	Double stranded RNA
eIF2 α	=	Eukaryotic initiation factor 2 alpha
FAT	=	Factor acetyl transferase
GRIP1	=	Glucocorticoid receptor-interacting polypeptide-1
HAT	=	Histone acetyl transferase
HDAC	=	Histone deacetylase
HIC	=	Human I-mfa domain-containing protein
HIV-1	=	Human immunodeficiency virus type 1
IFN	=	Interferon
LTR	=	Long terminal repeat
MAQ1	=	Ménage à quatre 1
miRNA	=	Micro RNA
NELFs	=	Negative elongation factors
NF- κ B	=	Nuclear factor kappa b
N-TEFs	=	Negative transcription elongation factors
PACT	=	PKR Activator
PCAF	=	P300/CBP-associated factor
PIC	=	Pre-initiation complex
PKR	=	dsRNA activated protein kinase R
P-TEFb	=	Positive transcription elongation factor b
RNAi	=	RNA interference
RNAP II	=	RNA polymerase II
shRNA	=	Short hairpin RNA
siRNA	=	Small interfering RNA
snoRNA	=	Small nucleolar RNA
TAR	=	Trans-activating response
Tat	=	Trans-activator of transcription
TEC	=	Transcription elongation complex
TRBP	=	TAR RNA binding protein

REFERENCES

- [1] Agrawal N, Dasaradhi PV, Mohammed A, Malhotra P, Bhatnagar RK, Mukherjee SK. (2003). *Microbiology and Molecular Biology Reviews*. 67: 657-685.
- [2] Akkina R, Banerjee A, Bai J, Anderson J, Li MJ, Rossi J. (2003). *Anticancer Research*. 23: 1997-2005.
- [3] Amado RG, Mitsuyasu RT, Rosenblatt JD, Ngok FK, Bakker A, Cole S, Chorn N, Lin LS, Bristol G, Boyd MP, MacPherson JL, Fanning GC, Todd AV, Ely JA, Zack JA, Symonds GP. (2004). *Human Gene Therapy*. 15: 251-262.
- [4] Ansari SA, Safak M, Gallia GL, Sawaya BE, Amini S, Khalili K. (1999). *Journal of General Virology*. 80: 2629-2638.
- [5] Arzumanov A, Stetsenko DA, Malakhov AD, Reichelt S, Sorensen MD, Babu BR, Wengel J, Gait MJ. (2003). *Oligonucleotides*. 13: 435-453.
- [6] Bannwarth S, Talakoub L, Letourneur F, Duarte M, Purcell DF, Hiscott J, Gatignol A. (2001). *The Journal of Biological Chemistry*. 276: 48803-48813.
- [7] Bannwarth S, Daher A, Grandvaux N, Hiscott J, Gatignol A. (submitted).
- [8] Barboric M, Nissen RM, Kanasawa S, Jabrane-Ferret N, Peterlin BM. (2001). *Molecular Cell*. 8: 327-337.
- [9] Bass BL. (2001). *Nature*. 411: 428-429.
- [10] Battisti P-L, Daher A, Bannwarth S, Voortman J, Peden KWC, Hiscott J, Mouland AJ, Benarous R, Gatignol A. (2003). *AIDS Research and Human Retroviruses*. 19: 767-778.
- [11] Becker PB, Horz W. (2002). *Annual Review of Biochemistry*. 71: 247-273.
- [12] Benkirane M, Neuveut C, Chun RF, Smith SM, Samuel CE, Gatignol A, Jeang K-T. (1997). *The EMBO Journal*. 16: 611-624.
- [13] Benkirane M, Chun RF, Xiao H, Ogryzko VV, Howard BH, Nakatani Y, Jeang KT. (1998). *The Journal of Biological Chemistry*. 273: 24898-24905.
- [14] Bieniasz PD, Grdina TA, Bogerd HP, Cullen BR. (1998). *The EMBO Journal*. 17: 7056-7065.
- [15] Bieniasz PD, Grdina TA, Bogerd HP, Cullen BR. (1999). *Proceedings of the National Academy of Science USA*. 96: 7791-7796.
- [16] Bieniasz PD, Cullen BR. (2000). *Journal of Virology*. 74: 9868-9877.
- [17] Boden D, Pusch O, Lee F, Tucker L, Ramratnam B. (2003). *Journal of Virology*. 77: 11531-11535.
- [18] Boden D, Pusch O, Silbermann R, Lee F, Tucker L, Ramratnam B. (2004). *Nucleic Acids Research*. 32: 1154-1158.
- [19] Bres V, Kiernan R, Emiliani S, Benkirane M. (2002). *The Journal of Biological Chemistry*. 277: 22215-22221.
- [20] Bres V, Tagami H, Peloponese JM, Loret E, Jeang KT, Nakatani Y, Emiliani S, Benkirane M, Kiernan RE. (2002). *The EMBO Journal*. 21: 6811-6819.
- [21] Bridge AJ, Pebernard S, Ducraux A, Nicoulaz AL, Iggo R. (2003). *Nature Genetics*. 34: 263-264.
- [22] Brigati C, Giacca M, Noonan DM, Albin A. (2003). *FEMS Microbiol Lett*. 220: 57-65.
- [23] Browning CM, Cagnon L, Good PD, Rossi J, Engelke DR, Markovitz DM. (1999). *Journal of Virology*. 73: 5191-5195.
- [24] Caron C, Col E, Khochbin S. (2003). *BioEssays*. 25: 58-65.
- [25] Carpick BW, Graziano V, Schneider D, Maitra RK, Lee X, Williams BR. (1997). *The Journal of Biological Chemistry*. 272: 9510-9516.
- [26] Chang Y-N, Kenan DJ, Keene JD, Gatignol A, Jeang K-T. (1994). *Journal of Virology*. 68: 7008-7020.
- [27] Chatel-Chaix L, Clément JF, Martel C, Bériault V, Gatignol A, DesGroseillers L, Mouland AJ. (2004). *Molecular and Cellular Biology*. 24: 2637-2648.
- [28] Chen D, Zhou Q. (1999). *Molecular and Cellular Biology*. 19: 2863-2871.
- [29] Chepenik LG, Tretiakova AP, Krachmarov CP, Johnson EM, Khalili K. (1998). *Gene*. 210: 37-44.
- [30] Chiang CM, Roeder RG. (1995). *Science*. 267: 531-536.
- [31] Chiu YL, Cao H, Jacque JM, Stevenson M, Rana TM. (2004). *Journal of Virology*. 78: 2517-2529.
- [32] Chun RF, Jeang K-T. (1996). *The Journal of Biological Chemistry*. 271: 27888-27894.
- [33] Chun RF, Semmes OJ, Neuveut C, Jeang K-T. (1998). *Journal of Virology*. 72: 2615-2629.
- [34] Coburn GA, Cullen BR. (2002). *Journal of Virology*. 76: 9225-9231.
- [35] Col E, Caron C, Seigneurin-Berny D, Gracia J, Favier A, Khochbin S. (2001). *The Journal of Biological Chemistry*. 276: 28179-28184.
- [36] Craig AW, Svitkin YV, Lee HS, Belsham GJ, Sonenberg N. (1997). *Molecular and Cellular Biology*. 17: 163-169.
- [37] Cujec TP, Okamoto H, Fujinaga K, Meyer J, Chamberlin H, Morgan DO, Peterlin BM. (1997). *Genes and Development*. 11: 2645-2657.

- [38] Daher A, Longuet M, Dorin D, Bois F, Segeral E, Bannwarth S, Battisti P-L, Purcell D, Benarous R, Vaquero C, Meurs EF, Gatignol A. (2001). *The Journal of Biological Chemistry*. 276: 33899-33905.
- [39] Darbinian N, Sawaya BE, Khalili K, Jaffe N, Wortman B, Giordano A, Amini S. (2001). *Journal of Neuroimmunology*. 121: 3-11.
- [40] Darfeuille F, Arzumanov A, Gryaznov S, Gait MJ, Di Primo C, Toulme JJ. (2002). *Proceedings of the National Academy of Science USA*. 99: 9709-9714.
- [41] Das AT, Klaver B, Berkhout B. (1998). *Journal of Virology*. 72: 9217-9223.
- [42] Das AT, Brummelkamp TR, Westerhout EM, Vink M, Madiredjo M, Bernards R, Berkhout B. (2004). *Journal of Virology*. 78: 2601-2605.
- [43] Daviet L, Erard M, Dorin D, Duarte M, Vaquero C, Gatignol A. (2000). *European Journal of Biochemistry*. 267: 2419-2431.
- [44] Deng L, de la Fuente C, Fu P, Wang L, Donnelly R, Wade JD, Lambert P, Li H, Lee CG, Kashanchi F. (2000). *Virology*. 277: 278-295.
- [45] Deng L, Ammosova T, Pumfery A, Kashanchi F, Nekhai S. (2002). *The Journal of Biological Chemistry*. 277: 33922-33929.
- [46] Ding SF, Lombardi R, Nazari R, Joshi S. (2002). *Frontiers in Bioscience*. 7: a15-28.
- [47] Dorin D, Bonnet MC, Bannwarth S, Gatignol A, Meurs EF, Vaquero C. (2003). *The Journal of Biological Chemistry*. 278: 4440-4448.
- [48] Dorr A, Kiermer V, Pedal A, Rackwitz HR, Henklein P, Schubert U, Zhou MM, Verdin E, Ott M. (2002). *The EMBO Journal*. 21: 2715-2723.
- [49] Duarte M, Graham K, Daher A, Battisti P-L, Bannwarth S, Segeral E, Jeang K-T, Gatignol A. (2000). *Journal of Biomedical Science*. 7: 494-506.
- [50] Dykxhoorn DM, Novina CD, Sharp PA. (2003). *Nature Reviews Molecular Cell Biology*. 4: 457-467.
- [51] El Kharroubi A, Piras G, Zensen R, Martin MA. (1998). *Molecular and Cellular Biology*. 18: 2535-2544.
- [52] Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. (2001). *Nature*. 411: 494-498.
- [53] Elbashir SM, Harborth J, Weber K, Tuschl T. (2002). *Methods*. 26: 199-213.
- [54] Emiliani S, Van Lint C, Fischle W, Paras PJ, Ott M, Brady J, Verdin E. (1996). *Proceedings of the National Academy of Science USA*. 93: 6377-6381.
- [55] Erard M, Barker DG, Amalric F, Jeang K-T, Gatignol A. (1998). *Journal of Molecular Biology*. 279: 1085-1099.
- [56] François C, Duverlie G, Rebouillat D, Khorsi H, Castelain S, Blum HE, Gatignol A, Wychowksi C, Moradpour D, Meurs EF. (2000). *Journal of Virology*. 74: 5587-5596.
- [57] Frankel L, Bannwarth S, Christensen H, Daher A, Chung S, Ong C, Purcell D, Gatignol A. (2004). *Canadian Journal of Infectious Diseases*. 15, supplement A: 13A (abstract).
- [58] Froeyen M, Herdewijn P. (2002). *Current Topics in Medicinal Chemistry*. 2: 1123-1145.
- [59] Fujii R, Okamoto M, Aratani S, Oishi T, Ohshima T, Taira K, Baba M, Fukamizu A, Nakajima T. (2001). *The Journal of Biological Chemistry*. 276: 5445-5451.
- [60] Fujinaga K, Cujec TP, Peng J, Garriga J, Price DH, Grana X, Peterlin BM. (1998). *Journal of Virology*. 72: 7154-7159.
- [61] Fujinaga K, Taube R, Wimmer J, Cujec TP, Peterlin BM. (1999). *Proceedings of the National Academy of Science USA*. 96: 1285-1290.
- [62] Furia B, Deng L, Wu K, Baylor S, Kehn K, Li H, Donnelly R, Coleman T, Kashanchi F. (2002). *The Journal of Biological Chemistry*. 277: 4973-4980.
- [63] Gale M, Tan SL, Katze MG. (2000). *Microbiology and Molecular Biology Reviews*. 64: 239-280.
- [64] Garber ME, Wei P, KewalRamani VN, Mayall TP, Herrmann CH, Rice AP, Littman DR, Jones KA. (1998). *Genes and Development*. 12: 3512-3527.
- [65] Garber ME, Jones KA. (1999). *Current Opinion in Immunology*. 11: 460-465.
- [66] Garber ME, Mayall TP, Suess EM, Meisenhelder J, Thompson NE, Jones KA. (2000). *Molecular and Cellular Biology*. 20: 6958-6969.
- [67] Garcia-Martinez LF, Mavankal G, Neveu JM, Lane WS, Ivanov D, Gaynor RB. (1997). *The EMBO Journal*. 16: 2836-2850.
- [68] Garcia-Martinez LF, Ivanov D, Gaynor RB. (1997). *The Journal of Biological Chemistry*. 272: 6951-6958.
- [69] Gatignol A, Buckler-White A, Berkhout B, Jeang K-T. (1991). *Science*. 251: 1597-1600.
- [70] Gatignol A, Buckler C, Jeang K-T. (1993). *Molecular and Cellular Biology*. 13: 2193-2202.
- [71] Gatignol A, Jeang K-T (1994). In *Methods in Molecular Genetics: Molecular Virology Techniques, part A, Volume 4*, Adolph KW, ed. (San Diego, CA: Academic press), pp. 18-28.
- [72] Gatignol A, Duarte M, Daviet L, Chang Y-N, Jeang K-T. (1996). *Gene Expression*. 5: 217-228.
- [73] Gatignol A, Jeang K-T (2000). In *Advances in Pharmacology, Volume 48*, Jeang K-T, ed. (Academic Press), pp. 209-227.
- [74] Gelman MA, Richter S, Cao H, Umezawa N, Gellman SH, Rana TM. (2003). *Organic Letters*. 5: 3563-3565.
- [75] Gold MO, Yang X, Herrmann CH, Rice AP. (1998). *Journal of Virology*. 72: 4448-4453.
- [76] Gorry PR, Howard JL, Churchill MJ, Anderson JL, Cunningham A, Adrian D, McPhee DA, Purcell DF. (1999). *Journal of Virology*. 73: 352-361.
- [77] Gorry PR, Ong C, Thorpe J, Bannwarth S, Thompson KA, Gatignol A, Wesselingh SL, Purcell DFJ. (2003). *Current HIV Research*. 1: 463-473.
- [78] Haasnoot J, Cupac D, Berkhout B. (2003). *Journal of Biomedical Science*. 10: 607-616.
- [79] Hama T, Saleh A, Huq I, Rana TM, Miller PS. (2003). *Bioorganic and Medicinal Chemistry Letters*. 13: 1845-1848.
- [80] Hardy S, Brand M, Mittler G, Yanagisawa J, Kato S, Meisterernst M, Tora L. (2002). *The Journal of Biological Chemistry*. 277: 32875-32882.
- [81] Harrich D, Hooker CW, Parry E. (2000). *Journal of Virology*. 74: 5639-5646.
- [82] Herrmann CH, Rice AP. (1995). *Journal of Virology*. 69: 1612-1620.
- [83] Herrmann CH, Mancini MA. (2001). *Journal of Cell Science*. 114: 1491-1503.
- [84] Holmes SC, Arzumanov AA, Gait MJ. (2003). *Nucleic Acids Research*. 31: 2759-2768.
- [85] Hoque M, Young TM, Lee CG, Serrero G, Mathews MB, Pe'ery T. (2003). *Molecular and Cellular Biology*. 23: 1688-1702.
- [86] Hottiger MO, Nabel GJ. (1998). *Journal of Virology*. 72: 8252-8256.
- [87] Huthoff H, Berkhout B. (2001). *Nucleic Acids Research*. 29: 2594-2600.
- [88] Huthoff H, Berkhout B. (2001). *RNA*. 7: 143-157.
- [89] Hwang S, Tamilarasu N, Kibler K, Cao H, Ali A, Ping YH, Jeang KT, Rana TM. (2003). *The Journal of Biological Chemistry*. 278: 39092-39103.
- [90] Jacque JM, Triques K, Stevenson M. (2002). *Nature*. 418: 435-438.
- [91] Jeang K-T, Xiao H, Rich EA. (1999). *The Journal of Biological Chemistry*. 274: 28837-28840.
- [92] Kaehlecke K, Dorr A, Hetzer-Egger C, Kiermer V, Henklein P, Schnoelzer M, Loret E, Cole PA, Verdin E, Ott M. (2003). *Molecular Cell*. 12: 167-176.
- [93] Kanazawa S, Okamoto T, Peterlin BM. (2000). *Immunity*. 12: 61-70.
- [94] Karn J. (1999). *Journal of Molecular Biology*. 293: 235-254.
- [95] Kashanchi F, Piras G, Radonovich MF, Duvall JF, Fattaey A, Chiang C-M, Roeder RG, Brady JN. (1994). *Nature*. 367: 295-299.
- [96] Kaushik N, Basu A, Palumbo P, Myers RL, Pandey VN. (2002). *Journal of Virology*. 76: 3881-3891.
- [97] Keen NJ, Gait MJ, Karn J. (1996). *Proceedings of the National Academy of Science USA*. 93: 2505-2510.
- [98] Kharrat A, Macias MJ, Gibson TJ, Nilges M, Pastore A. (1995). *The EMBO Journal*. 14: 3572-3584.
- [99] Kiernan RE, Vanhulle C, Schiltz L, Adam E, Xiao H, Maudoux F, Calomme C, Burny A, Nakatani Y, Jeang KT, Benkirane M, Van Lint C. (1999). *The EMBO Journal*. 18: 6106-6118.
- [100] Kim YK, Bourgeois CF, Isel C, Churcher MJ, Karn J. (2002). *Molecular and Cellular Biology*. 22: 4622-4637.
- [101] Kino T, Slobodskaya O, Pavlakis GN, Chrousos GP. (2002). *The Journal of Biological Chemistry*. 277: 2396-2405.
- [102] Klaver B, Berkhout B. (1994). *The EMBO Journal*. 13: 2650-2659.

- [103] Krown SE, Aeppli D, Balfour HH. (1999). *Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology*. 20: 245-254.
- [104] Kuwabara T, Warashina M, Taira K. (2002). *Journal of Biochemistry (Tokyo)*. 132: 149-155.
- [105] Kwak YT, Guo J, Prajapati S, Park KJ, Surabhi RM, Miller B, Gehrig P, Gaynor RB. (2003). *Molecular Cell*. 11: 1055-1066.
- [106] Lee JY, Kim H, Ryu CH, Kim JY, Choi BH, Lim Y, Huh PW, Kim YH, Lee KH, Jun TY, Rha HK, Kang JK, Choi CR. (2004). *The Journal of Biological Chemistry*. 279: 30265-30273.
- [107] Lee SW, Gallardo HF, Gaspar O, Smith C, Gilboa E. (1995). *Gene Therapy*. 2: 377-384.
- [108] Li MJ, Bauer G, Michienzi A, Yee JK, Lee NS, Kim J, Li S, Castanotto D, Zaia J, Rossi JJ. (2003). *Molecular Therapy*. 8: 196-206.
- [109] Lusic M, Marcello A, Cereseto A, Giacca M. (2003). *The EMBO Journal*. 22: 6550-6561.
- [110] Lustig B, Jeang KT. (2001). *Current Medicinal Chemistry*. 8: 1181-1187.
- [111] Mancebo HS, Lee G, Flygare J, Tomassini J, Luu P, Zhu Y, Peng J, Blau C, Hazuda D, Price D, Flores O. (1997). *Genes and Development*. 11: 2633-2644.
- [112] Maraia RJ, Intine RV. (2002). *Gene Expression*. 10: 41-57.
- [113] Marcello A, Zoppe M, Giacca M. (2001). *IUBMB Life*. 51: 175-181.
- [114] Mariani R, Rutter G, Harris ME, Hope TJ, Krausslich HG, Landau NR. (2000). *Journal of Virology*. 74: 3859-3870.
- [115] Marzio G, Tyagi M, Gutierrez MI, Giacca M. (1998). *Proceedings of the National Academy of Science USA*. 95: 13519-13524.
- [116] Mayer M, James TL. (2004). *Journal of the American Chemical Society*. 126: 4453-4460.
- [117] Mayhood T, Kaushik N, Pandey PK, Kashanchi F, Deng L, Pandey VN. (2000). *Biochemistry*. 39: 11532-11539.
- [118] Michels AA, Nguyen VT, Fraldi A, Labas V, Edwards M, Bonnet F, Lania L, Bensaude O. (2003). *Molecular and Cellular Biology*. 23: 4859-4869.
- [119] Michienzi A, Li S, Zaia JA, Rossi JJ. (2002). *Proceedings of the National Academy of Science USA*. 99: 14047-14052.
- [120] Michienzi A, Castanotto D, Lee N, Li S, Zaia JA, Rossi JJ. (2003). *Annals New York Academy of Sciences*. 1002: 63-71.
- [121] Milhavet O, Gary DS, Mattson MP. (2003). *Pharmacological Reviews*. 55: 629-648.
- [122] Mischiati C, Finotti A, Sereni A, Boschetti S, Baraldi PG, Romagnoli R, Feriotto G, Jeang KT, Bianchi N, Borgatti M, Gambari R. (2004). *Biochemical Pharmacology*. 67: 401-410.
- [123] Moulard AJ, Xu H, Cui H, Krueger W, Munro TP, Prasol M, Mercier J, Rekosh D, Smith R, Barbarese E, Cohen EA, Carson JH. (2001). *Molecular and Cellular Biology*. 21: 2133-2143.
- [124] Mujtaba S, He Y, Zeng L, Farooq A, Carlson JE, Ott M, Verdin E, Zhou MM. (2002). *Molecular Cell*. 9: 575-586.
- [125] Narita T, Yamaguchi Y, Yano K, Sugimoto S, Chanarat S, Wada T, Kim DK, Hasegawa J, Omori M, Inukai N, Endoh M, Yamada T, Handa H. (2003). *Molecular and Cellular Biology*. 23: 1863-1873.
- [126] Narlikar GJ, Fan HY, Kingston RE. (2002). *Cell*. 108: 475-487.
- [127] Nekhai S, Kumar A, Bottaro DP, Petryshyn R. (1996). *Virology*. 222: 193-200.
- [128] Nguyen VT, Kiss T, Michels AA, Bensaude O. (2001). *Nature*. 414: 322-325.
- [129] Ott M, Schnolzer M, Garnica J, Fischle W, Emiliani S, Rackwitz HR, Verdin E. (1999). *Current Biology*. 9: 1489-1492.
- [130] Park H, Davies MV, Langland JO, Chang H-W, Nam YS, Tartaglia J, Paoletti E, Jacobs BL, Kaufman RJ, Venkatesan S. (1994). *Proceedings of the National Academy of Science USA*. 91: 4713-4717.
- [131] Parolin C, Gatto B, Del Vecchio C, Pecere T, Tramontano E, Cecchetti V, Fravolini A, Masiero S, Palumbo M, Palu G. (2003). *Antimicrobial Agents and Chemotherapy*. 47: 889-896.
- [132] Patel RC, Sen GC. (1998). *The EMBO Journal*. 17: 4379-4390.
- [133] Pendergrast PS, Hernandez N. (1997). *Journal of Virology*. 71: 910-917.
- [134] Peng J, Zhu Y, Milton JT, Price DH. (1998). *Genes and Development*. 12: 755-762.
- [135] Ping YH, Rana TM. (1999). *The Journal of Biological Chemistry*. 274: 7399-7404.
- [136] Ping YH, Rana TM. (2001). *The Journal of Biological Chemistry*. 276: 12951-12958.
- [137] Pitha PM. (1994). *Antiviral Research*. 24: 205-219.
- [138] Pitt SW, Majumdar A, Serganov A, Patel DJ, Al-Hashimi HM. (2004). *Journal of Molecular Biology*. 338: 7-16.
- [139] Pollard VW, Malim MH. (1998). *Annual Review of Microbiology*. 52: 491-532.
- [140] Price DH. (2000). *Molecular and Cellular Biology*. 20: 2629-2634.
- [141] Pumfery A, Deng L, Maddukuri A, de la Fuente C, Li H, Wade JD, Lambert P, Kumar A, Kashanchi F. (2003). *Current HIV Research*. 1: 343-362.
- [142] Purcell DF, Martin MA. (1993). *Journal of Virology*. 67: 6365-6378.
- [143] Quivy V, Van Lint C. (2002). *Biochemical Pharmacology*. 64: 925-934.
- [144] Rana TM, Jeang K-T. (1999). *Archives of Biochemistry and Biophysics*. 365: 175-185.
- [145] Richter S, Ping YH, Rana TM. (2002). *Proceedings of the National Academy of Science USA*. 99: 7928-7933.
- [146] Rohr O, Marban C, Aunis D, Schaeffer E. (2003). *Journal of Leukocyte Biology*. 74: 736-749.
- [147] Roof P, Ricci M, Genin P, Montano MA, Essex M, Wainberg MA, Gatignol A, Hiscott J. (2002). *Virology*. 296: 77-83.
- [148] Rossi JJ. (2000). *Advanced Drug Delivery Reviews*. 44: 71-78.
- [149] Roy S, Agy M, Hovanessian AG, Sonenberg N, Katze MG. (1991). *Journal of Virology*. 65: 632-640.
- [150] Saunders LR, Barber GN. (2003). *The FASEB Journal*. 17: 961-983.
- [151] Sheldon M, Ratnasabapathy R, Hernandez N. (1993). *Molecular and Cellular Biology*. 13: 1251-1263.
- [152] Sledz CA, Holko M, de Veer MJ, Silverman RH, Williams BR. (2003). *Nature Cell Biology*. 5: 834-839.
- [153] Spangord RJ, Vuyisich M, Beal PA. (2002). *Biochemistry*. 41: 4511-4520.
- [154] St Johnston D, Brown NH, Gall JG, Jantsch M. (1992). *Proceedings of the National Academy of Science USA*. 89: 10979-10983.
- [155] Stevenson M. (2003). *Nature Reviews Immunology*. 3: 851-858.
- [156] Surabhi RM, Gaynor RB. (2002). *Journal of Virology*. 76: 12963-12973.
- [157] Svitkin YV, Pause A, Sonenberg N. (1994). *Journal of Virology*. 68: 7001-7007.
- [158] Terreux R, Pairot S, Cabrol-Bass D, Patino N, Condom R. (2001). *Journal of Molecular Graphics and Modelling*. 19: 579-585.
- [159] Toulme JJ. (2001). *Nature Biotechnology*. 19: 17-18.
- [160] Van Lint C (2000). In *Advances in Pharmacology*, Volume 48, Jeang K-T, ed. (Academic Press), pp. 121-160.
- [161] Ventura M, Wang P, Franck N, Saragosti S. (1994). *Biochemical and Biophysical Research Communications*. 203: 889-898.
- [162] Verhoef K, Tijms M, Berkhout B. (1997). *Nucleic Acids Research*. 25: 496-502.
- [163] Veschambre P, Roisin A, Jalinot P. (1997). *Journal of General Virology*. 78: 2235-2245.
- [164] Wada T, Takagi T, Yamaguchi Y, Watanabe D, Handa H. (1998). *The EMBO Journal*. 17: 7395-7403.
- [165] Wallberg AE, Pedersen K, Lendahl U, Roeder RG. (2002). *Molecular and Cellular Biology*. 22: 7812-7819.
- [166] Wang M, Xu Z, Tu P, Yu X, Xiao S, Yang M. (2004). *Bioorganic and Medicinal Chemistry Letters*. 14: 2585-2588.
- [167] Warashina M, Kuwabara T, Kato Y, Sano M, Taira K. (2001). *Proceedings of the National Academy of Science USA*. 98: 5572-5577.
- [168] Waysbort A, Bonnal S, Audigier S, Esteve JP, Prats AC. (2001). *FEBS Letters*. 490: 54-58.
- [169] Weerasinghe M, Liem SE, Asad S, Read SE, Joshi S. (1991). *Journal of Virology*. 65: 5531-5534.
- [170] Wei P, Garber ME, Fang SM, Fischer WH, Jones KA. (1998). *Cell*. 92: 451-462.
- [171] Weissman JD, Brown JA, Howcroft TK, Hwang J, Chawla A, Roche PA, Schiltz L, Nakatani Y, Singer DS. (1998). *Proceedings of the National Academy of Science USA*. 95: 11601-11606.
- [172] Wu Y. (2004). *Retrovirology*. 1: 13.
- [173] Xie B, Calabro V, Wainberg MA, Frankel AD. (2004). *Journal of Virology*. 78: 1456-1463.
- [174] Yamaguchi Y, Takagi T, Wada T, Yano K, Furuya A, Sugimoto S, Hasegawa J, Handa H. (1999). *Cell*. 97: 41-51.

- [175] Yang X, Herrmann CH, Rice AP. (1996). *Journal of Virology*. 70: 4576-4584.
- [176] Yang X, Gold MO, Tang DN, Lewis DE, Aguilar-Cordova E, Rice AP, Herrmann CH. (1997). *Proceedings of the National Academy of Science USA*. 94: 12331-12336.
- [177] Yang Z, Zhu Q, Luo K, Zhou Q. (2001). *Nature*. 414: 317-322.
- [178] Yedavalli VS, Benkirane M, Jeang KT. (2003). *The Journal of Biological Chemistry*. 278: 6404-6410.
- [179] Yoshinari K, Miyagishi M, Taira K. (2004). *Nucleic Acids Research*. 32: 691-699.
- [180] Young TM, Wang Q, Pe'ery T, Mathews MB. (2003). *Molecular and Cellular Biology*. 23: 6373-6384.
- [181] Zhao H, Li J, Xi F, Jiang L. (2004). *FEBS Letters*. 563: 241-245.
- [182] Zheng YH, Yu HF, Peterlin BM. (2003). *Nature Cell Biology*. 5: 611-618. published erratum *Nat Cell Biol* (2003) 2005:2839.
- [183] Zhong J, Peters AH, Lee K, Braun RE. (1999). *Nature Genetics*. 22: 171-174.
- [184] Zhou C, Rana TM. (2002). *Journal of Molecular Biology*. 320: 925-942.
- [185] Zhou M, Halanski MA, Radonovich MF, Kashanchi F, Peng J, Price DH, Brady JN. (2000). *Molecular and Cellular Biology*. 20: 5077-5086.
- [186] Zhou M, Nekhai S, Bharucha DC, Kumar A, Ge H, Price DH, Egly JM, Brady JN. (2001). *The Journal of Biological Chemistry*. 276: 44633-44640.
- [187] Zhu Y, Pe'ery T, Peng J, Ramanathan Y, Marshall N, Marshall T, Amendt B, Mathews MB, Price DH. (1997). *Genes and Development*. 11: 2622-2632.

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