

The Regulation of HIV-1 Transcription: Molecular Targets for Chemotherapeutic Intervention

Miguel Stevens, Erik De Clercq, Jan Balzarini

Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium

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Abstract: The regulation of transcription of the human immunodeficiency virus (HIV) is a complex event that requires the cooperative action of both viral and cellular components. In latently infected resting CD4⁺ T cells HIV-1 transcription seems to be repressed by deacetylation events mediated by histone deacetylases (HDACs). Upon reactivation of HIV-1 from latency, HDACs are displaced in response to the recruitment of histone acetyltransferases (HATs) by NF- κ B or the viral transcriptional activator Tat and result in multiple acetylation events. Following chromatin remodeling of the viral promoter region, transcription is initiated and leads to the formation of the TAR element. The complex of Tat with p-TEFb then binds the loop structures of TAR RNA thereby positioning CDK9 to phosphorylate the cellular RNA polymerase II. The Tat-TAR-dependent phosphorylation of RNA polymerase II plays an important role in transcriptional elongation as well as in other post-transcriptional events. As such, targeting of Tat protein (and/or cellular cofactors) provide an interesting perspective for therapeutic intervention in the HIV replicative cycle and may afford lifetime control of the HIV infection. © 2006 Wiley Periodicals, Inc. *Med Res Rev*, 26 No. 5, 595–625, 2006

Key words: HIV-1; LTR; transcription; latency; NF- κ B; Tat; TAR; RNA polymerase II

1. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), remains a global health threat. At present, treatment of HIV-infected individuals is based on combination therapy with HIV-1 reverse transcriptase (RT) and/or protease and/or gp41 inhibitors. Despite the notable success of highly active antiretroviral therapy (HAART) in reducing plasma viral loads to undetectable levels during HIV infection and slowing down clinical progression to AIDS, HAART fails to completely eradicate the virus in HIV-infected individuals. This curative failure is

Correspondence to: Jan Balzarini, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. E-mail: Jan.Balzarini@rega.kuleuven.be

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mainly the result of a small pool of chronically HIV-infected resting CD4⁺ T cells containing integrated but transcriptionally dormant HIV proviruses to persist. Additionally, emergence of multidrug-resistant viruses have increasingly been reported in patients receiving HAART, urging the need for new anti-HIV treatment strategies. Several targets in the HIV-1 replicative cycle other than RT, protease and virus entry have been identified as possible intervention sites for antiviral chemotherapy. Among these, HIV-1 transcriptional regulation seems to be very attractive, as it would open the possibility to control HIV-1 replication not only in acutely but also in chronically infected cells. In this way, inhibitors of HIV-1 transcriptional regulation may have great potential in anti-HIV drug combination therapy because they can force the virus to slow down its replication rate or even shut-off virus replication and may afford lifetime control of the HIV infection. Moreover, it may be argued that the use of antiretroviral drugs targeted at HIV-1 transcriptional regulation would result in a lower incidence of drug resistance, since the regulation of HIV-1 transcription requires the interplay of both viral and cellular components.

In this review, we will describe the regulation of HIV-1 transcription and identify the viral and cellular components that may serve as potential targets for antiviral chemotherapy.

2. THE HIV-1 PROMOTER

The long terminal repeat (LTR) region of the HIV-1 subtype B genome is divided into three regions which contain four functional domains: the transactivating region (TAR), the basal or core promoter, the enhancer region, and the regulatory elements. The last three are found within U3, whereas the TAR is found within the repeat (R) region (Fig. 1).

Several cellular proteins including Myb,¹ Ets-1,² USF,³ NFAT,⁴ C/EBP,⁵ AP-1,⁶ LEF-1,⁷ GR,⁸ RAR,⁹ and COUP-TF¹⁰ have been proposed to interact with the upstream regulatory region capable of up- or downregulating virus expression. For example, within the regulatory domain is a GGTC A palindrome situated to which COUP-TF and RAR, both members of the steroid/thyroid hormone receptor superfamily, bind. Retinoids have been shown to enhance HIV-1 expression through interaction with RAR. Since COUP-TF, a negative regulator of several retinoic acid responsive elements (RAREs), efficiently interact with the same binding site, the retinoid response of the HIV-1 RARE can be significantly repressed.⁹ Furthermore, synthetic retinoid antagonists are able to inhibit retinoic acid-induced transcriptional activation of the HIV-1 RARE.¹¹ In this way retinoid antagonists could provide an additional way of repressing HIV-1 activation by retinoids *in vivo*. Another approach for regulation of HIV gene expression, as well as a mechanism for inhibition of viral replication, is the ability of small DNA-binding molecules to target predetermined DNA sequences located within the LTR promoter. Sequence-specific pyrrole-imidazole polyamides have been designed to bind DNA sequences adjacent to recognition sites for the TBP subunit of TFIID, Ets-1, and LEF-1.¹² TBP is essential for initiation of HIV-1 transcription, and LEF-1, considered to be an architectural protein, plays a central role in coordinating activities of multiple transcription factors.¹³ LEF-1 was shown to bend DNA,¹⁴ which facilitates protein-protein interactions between transcription factors bound at distant sites, a property which has also been ascribed to the transcription factor USF.¹⁵ Both TBP and LEF-1 bind the minor groove of DNA and are likely to be inhibited by the minor groove-binding polyamides. The Ets-1 inhibitory polyamide, on the other hand, not only interferes with the binding of Ets-1 to its recognition helix, but also blocks the formation of the ternary Ets-1 NF- κ B DNA complex at the HIV-1 enhancer. This ternary complex formation can also be inhibited by polyamides which bind adjacent to the recognition sites for NF- κ B.¹⁶

The HIV-1 enhancer region consists of two tandem binding sites for the transcription factor NF- κ B. The AP-2 transcription factor binds between these two NF- κ B motifs and has positive

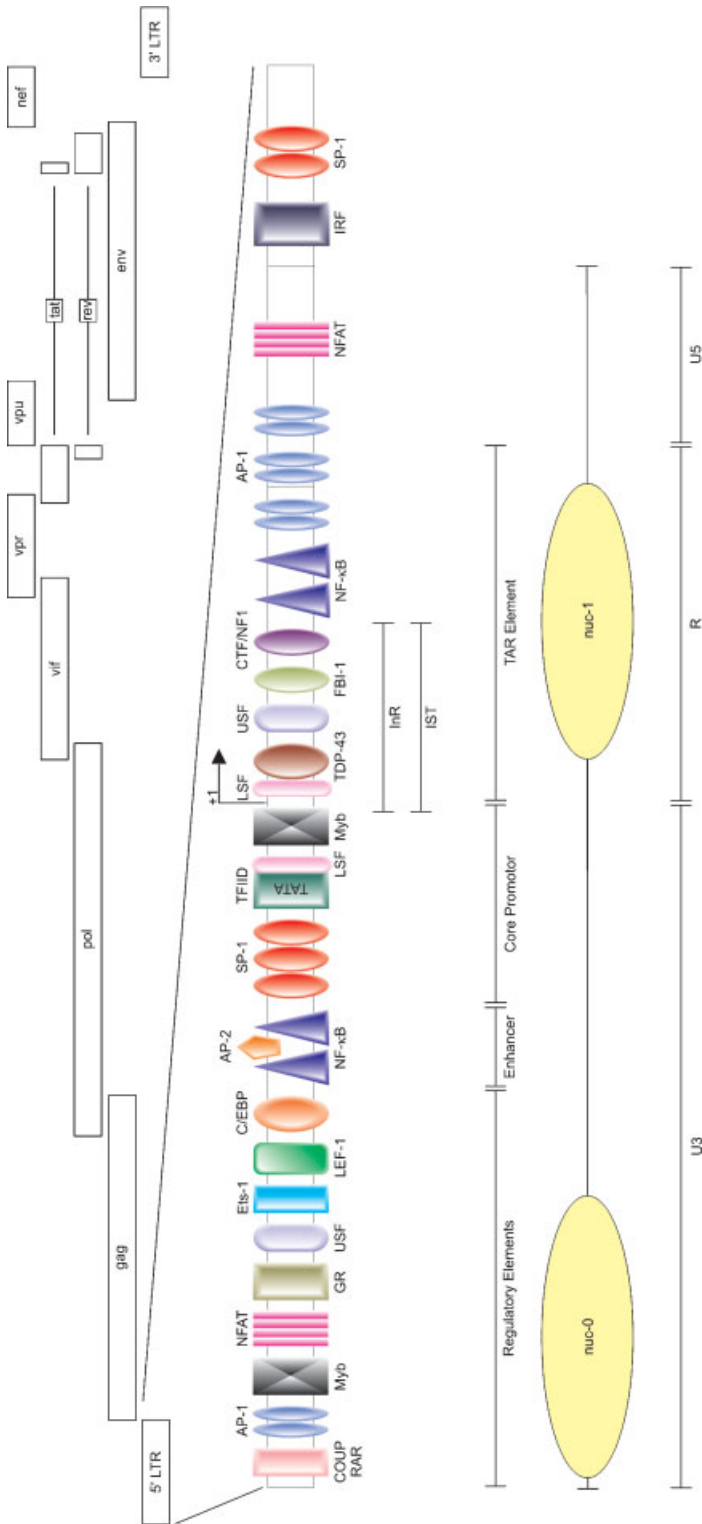


Figure 1. Structure of the HIV genome and the viral promoter elements. The 5' LTR promoter consists of three structural sequence elements, U3 (nt -453 to -1), R (nt +1 to +98), and U5 (nt +99 to +180) with +1 as the start site of transcription and four main functional domains: a regulatory region (nt -454 to -104), an enhancer element (nt -105 to -79), a core promoter (nt -78 to -1), and a TAR element (nt +1 to +60). Within the TAR element two initiator elements (InR) are located from -3 to +8 and +35 to +59, and an inducer of short transcripts (IST) spans the initiation region from positions -5 through +26 and +40 through +59. Regardless of the site of integration, the 5' LTR promoter of HIV is packaged by two nucleosomes, nuc-0, which is located between nucleotides -415 and -255, and nuc-1, which is located between nucleotides +10 and +155. The 5' LTR promoter incorporates several *Cis*-acting DNA sequences for binding cellular transcription factors including, COUP, chicken ovalbumin upstream promoter transcription factor; RAR, retinoic acid receptor; Myb, Myb binding protein; NFAT, nuclear factor of activated T cells; USF, upstream stimulating factor; Ets-1, Ets binding factor; LEF-1, lymphocyte enhancer factor; C/EBP, CCAAT/enhancer binding protein; NF-κB, nuclear factor kappa B; AP-2, activator protein 2; SP-1, SP1 binding protein; TFIID, transcription factor IID; LSF, late SV40 transcription factor; TDP-43, TAR DNA-binding protein; FBI-1, factor that binds to IST; CTF/NF1, CCAAT-box transcription factor/nuclear factor 1; AP-1, activator protein 1; IRF, interferon regulatory factors.

effects on the transcriptional level without interfering with the NF- κ B-mediated TNF- α induction.¹⁷

The core promoter region resembles that of many eukaryotic genes transcribed by the RNA polymerase II. In general, core promoters comprise either a TATA box or the initiator region (InR), a pyrimidine-rich site that spans the RNA start. The HIV-1 core promoter contains both type of elements though with a TATA motif that starts 2 nucleotides further upstream (CATATA box).¹⁸ General transcription factors are able to bind both type of elements, in addition with three tandem-repeat Sp1 binding sites.¹⁹ Numerous cellular factors interact extensively with this region and seem to play an important role in the assembly of a preinitiation complex (PIC) and in the transcriptional regulation of the viral promoter. A cellular DNA-binding protein, LSF (also known as LBP-1 or UBP), sequentially interacts in a concentration-dependent manner with two sites that surround the transcriptional initiation site of the HIV-1 promoter. Although sequences in the downstream site overlapping the InR were found to enhance transcription, purified LSF specifically repressed HIV transcription by binding to the upstream site which overlaps the TATA box element, preventing the TFIID complex to bind the promoter.²⁰ Furthermore, this cellular factor is able to restrict the elongation of HIV-1 transcripts after PIC formation suggesting that LSF could be part of a mechanism that blocks transcription processivity.²¹ The mechanism by which LSF represses HIV-1 transcription involves the transcriptional regulator YY1. A unique cooperation of both cellular factors seems to specifically synergize negative regulation of HIV-1 LTR expression.²² The transcriptional repression caused by YY1 leads to the recruitment of histone deacetylase (HDAC1), maintaining the nucleosome nuc-1 in a deacetylated state.^{23,24} This kind of potent repression of LTR transcription could allow an activated, HIV-infected cell to return to a dormant state and establish a stable non-productive infection. In addition, it was possible to counteract the repression from the LTR promoter by targeting DNA sequences adjacent to recognition sites for LSF with pyrrole-imidazole polyamides²⁵ or by inhibition of the mitogen-activated protein kinase (MAPK) p38 that regulates LSF binding to the LTR promoter.²⁶ Other cellular factors associated with the core promoter are TDP-43 and USF. TDP-43 has been shown to bind to the InR and is capable of modulating HIV-1 gene expression by either altering or blocking the assembly of transcription complexes either in the presence or absence of Tat.²⁷ On the other hand, USF showed a stimulatory effect on the transcriptional machinery.²⁸ Within the core promoter an unusual transcriptional element is located just downstream of the start site of transcription and is known as the inducer of short transcripts or IST. This bipartite DNA element promotes the synthesis of short transcripts *via* formation of transcription complexes that are unable to elongate efficiently.²⁹ Factor binding to IST (FBI-1) binds specifically to this IST element suggesting a role in the establishment of these abortive transcription complexes.³⁰

The RNA element TAR, which is encoded largely between the two IST half-elements, forms a stable secondary stem-loop structure and represents a key element in the Tat-mediated transcriptional activation (*vide infra*).

Finally, the 5'-untranslated leader region (5'-UTR) also contains important transcriptional elements for transcription factors such as AP-1, NF- κ B, NF-AT, IRF, and Sp1.^{31,32} IRF-1 is able to significantly increase the basal transcriptional activity of the HIV-1 LTR promoter by recruiting the acetyltransferase CBP.^{33,34} Consistent with this, only IRF-1 and IRF-2 bind the ISRE-like element on the HIV-1 LTR.³⁵

3. TRANSCRIPTIONAL STIMULATION

The transcription factor NF- κ B was found to be activated in many cell types in response to a broad range of stimuli and conditions.³⁶ Previously, it has been proposed that diverse stimuli all act through a common step involving an increase in oxidative stress within the cell. Evidence for

this oxidative stress response, defined as an increase in intracellular ROS, stemmed from in the induction of the expression and replication of HIV-1 by H_2O_2 ,³⁷ and the inhibition of the NF- κ B signaling pathway by antioxidants such as pyrrolidine dithiocarbamate (PDTC)³⁸ and *N*-acetyl-L-cysteine (NAC).³⁹ These effects seem to be cell or stimulus specific, suggesting that this oxidative stress model in NF- κ B activation is an exception rather than the rule.⁴⁰

Hence, stimulation with tumor necrosis factor alpha (TNF- α) or phorbol 12-myristate 13-acetate (PMA) decreased intracellular thiols levels, an observation that could be counteracted by NAC.⁴¹ Since then, more evidence appeared for a role of thiols within the cell, indicating that NF- κ B activity must be regulated in a redox modulatory way dependent on the intracellular thiol levels.

NF- κ B exists in the cytoplasm of unstimulated cells as a latent form of a transcriptionally active p50/p65 dimer bound to an inhibitory protein, I κ B α (Fig. 2). Activation of NF- κ B involves the phosphorylation of I κ B α at two serine residues (S32 and S36) by the IKK complex, which targets the inhibitor protein for ubiquitination and subsequent degradation by the 26S proteasome.⁴² In addition to the phosphorylation of I κ B α , the IKK complex also phosphorylates the p65 subunit of NF- κ B on serine 536 in the transactivation domain controlling the kinetics of the p65 nuclear import and cytosolic I κ B α localization.^{43,44} PDTC and NAC have been shown to prevent the phosphorylation of I κ B α which may account for their inhibition of NF- κ B activity.⁴⁵ As a result of this finding, various antioxidants have been examined for their inhibitory effects on HIV-1 activation. In this way, compounds such as α -lipoic acid,^{46,47} oltipraz,^{48,49} α -tocopherol (vitamin E), and butylated hydroxyanisole (BHA)⁵⁰ were found to inhibit HIV-1 LTR-directed gene expression and to suppress viral replication (Table I and Fig. 5). In addition to antioxidants, other compounds such as salicylates or aspirin-like drugs,⁵¹ pentoxifylline,^{52,53} coumarins,⁵⁴ acridone derivatives,⁵⁵ iron chelators,^{56,57} and the binding subunit of pertussis toxin^{58,59} were also found to be inhibitory to NF- κ B activation.

Upon release of I κ B α , the nuclear localization signal (NLS) on the p65 subunit becomes unmasked, which allows for a rapid translocation of NF- κ B to the nucleus.⁶⁰ Importantly, the p65 subunit becomes phosphorylated on serine 276 by protein kinase A (PKA) rendering NF- κ B transcriptional active through recruitment of the histone acetyltransferase p300/CBP to the viral promoter.^{61–63} It is known that p50 homodimers are also phosphorylated by PKA⁶⁴ and associate with HDAC-1 in unstimulated cells to bind to the viral promoter and cause a transcriptional repression. However, NF- κ B p50/p65 heterodimers bind with greater affinity to the promoter enabling signal-induced heterodimers to displace p50-HDAC-1 from the promoter and activate transcription.⁶⁵ As unphosphorylated p65 also interacts with HDAC-1,⁶⁶ the phosphorylation of nuclear NF- κ B determines whether it associates with p300/CBP or HDAC-1, ensuring that only signal-induced NF- κ B entering the nucleus can activate transcription. Once in the nucleus, a cellular reducing catalyst thioredoxin plays a major role in the stimulation of the DNA binding of NF- κ B by reduction of a disulphide bond involving cysteine 62 on the p50 subunit.^{67,68} This selective reduction of the oxidized p50 subunit is also mediated by the nuclear signaling protein redox factor-1 (Ref-1).⁶⁹ Both factors TRX and Ref-1 are not only required for NF- κ B binding but can also interact with each other to stimulate the DNA-binding activity of AP-1.⁷⁰ Although the activation of NF- κ B have been shown to occur under oxidative conditions through the release and degradation of I κ B α , the binding of NF- κ B to the LTR promoter seems to be dependent on reducing conditions. It is likely that various compounds may impair this NF- κ B DNA-binding activity and, as a result, inhibit LTR-driven gene expression. In this way, some cell systems showed that the effects of PDTC on NF- κ B were not primarily due to its antioxidant properties, but could be ascribed to a pro-oxidant effect of PDTC through a mechanism involving a shift towards oxidizing conditions.⁷¹ This observation supports the previous conclusion that the effects of antioxidants on NF- κ B activation are cell or stimulus dependent. The NF- κ B DNA binding impairment was also true not only for the oxidizing agent diamide, but also for the alkylating agent, *N*-ethylmaleimide⁷² and the pyridine *N*-oxide derivatives.⁷³ The oxidizing properties of these compounds can be reversed by treatment with β -mercaptoethanol, which provides reducing conditions for thiol components. The antiviral effects of nitric oxide (NO)

mediated through NF- κ B inhibition have been demonstrated with the use of NO-generating agents such as *S*-nitroso-*N*-acetylpenicillamine (SNAP) and sodium nitroprusside (SNP). Although NO production is increased in HIV-1-infected patients, this free radical seems to inhibit the NF- κ B signaling pathway at multiple levels, that is, at the activation level as well as the DNA-binding level.⁷⁴ Other compounds such as cepharanthine,⁷⁵ hydroxyurea,⁷⁶ and carboxyamidotriazole⁷⁷ also target the NF- κ B signaling pathway, but the molecular basis of their antiviral activity remains to be determined.

4. TRANSCRIPTIONAL ACTIVATION

The HIV-1 provirus is packaged into chromatin whereby, independently of the site of integration, nucleosomes are positioned precisely on the 5'-LTR promoter region with respect to *cis*-acting regulatory elements. This higher ordered chromatin structure negatively regulates gene expression by restricting access of the transcriptional machinery to the viral promoter. Multiprotein complexes bind the viral promoter to induce a nucleosomal rearrangement in such a way that DNA regulatory sequences become more accessible to other sequence-specific proteins. As a result, these changes frequently are identified as an increase in sensitivity at specific DNA sequences to digestion by the nuclease DNase I, and are, therefore, termed DNase I-hypersensitive sites (DHSs).

Two nucleosomes (called nuc-0 and nuc-1) are positioned within the viral promoter. Nuc-0 is positioned upstream of the modulatory region whereas nuc-1 is immediately downstream of the viral transcription start site (Fig. 1). These nucleosomes define two open regions, one encompassing the regulatory region plus the enhancer/core promoter region (nt 200–465), whereas the other region contains regulatory elements in the 5'-UTR downstream of the transcription start site (nt 610–720).⁷⁹ Importantly, upon stimulation with phorbol esters, TNF- α or histone deacetylase inhibitors, nuc-1 becomes rapidly and specifically disrupted by acetylation of specific lysine residues within histone H3 and H4 of nuc-1. This nucleosomal remodeling, which is insensitive to the transcription inhibitor α -amanitin, results in an open nucleosome-free region from nt 200 to nt 720 leading to a marked increase in sensitivity to DNase I.^{80,81} Nuc-1 disruption is necessary for transcriptional activation as it blocks the binding of transcription factors necessary for the assembly of a PIC and cause an arrest in the progression of RNA polymerase II (RNAPII), resulting in inefficient elongation and the accumulation of short attenuated transcripts. Since nuc-1 is the only disrupted nucleosome in the HIV-1 promoter after global hyperacetylation due to histone acetylase inhibitors, suggests that an additional level of specificity must exist in this system.^{81,82} This may be mediated through a mechanism which involves a displacement of corepressor complexes containing HDAC, which has been recruited to the viral promoter by host factors such as LSF with YY1 and the NF- κ B p50

Figure 2. NF- κ B transcriptional activation can be considered as two distinct steps with respect to the cellular compartment. One step involves the phosphorylation and subsequent degradation of I κ B α in the cytoplasm followed by the nuclear translocation of the active transcription factor. Another step is the DNA binding of the cellular transcription factor resulting in transcriptional activation of the viral genome in the nucleus. Upon stimulation I κ B α is phosphorylated by the I κ B kinase complex (IKK), containing IKK α /IKK1, IKK β /IKK2, and IKK γ /NEMO. Ubiquitination and degradation of I κ B α by the 26S proteasome complex activates PKA that phosphorylates the NF- κ B p65 subunit at Ser 276 rendering NF- κ B transcriptionally active through recruitment of p300/CBP. In addition to the release of I κ B α , the nuclear localization signal (NLS) on the p65 subunit becomes unmasked, which allows for a rapid translocation of NF- κ B to the nucleus and acetylation of p65 on Lys 221 by p300/CBP. Once in the nucleus, a disulphide bond involving cysteine 62 on the p50 subunit of the NF- κ B p50/p65 heterodimers becomes reduced by TRX and Ref-1, and allows for a rapid displacement of the Ser337-phosphorylated p50 homodimers associated with HDAC-1, which repress transcription. Transcriptional activation involves not only the viral genome but a wide variety of genes, including that of its own inhibitor I κ B α . The newly synthesized I κ B α enters the nucleus and removes NF- κ B from the viral promoter to transport it back to the cytoplasm leading to the termination of the NF- κ B-dependent transcription. In order to terminate the NF- κ B transcriptional response, the p65 subunit of the NF- κ B p50/p65 heterodimer must become deacetylated by HDAC-3 which allows the binding of deacetylated p65 to newly synthesized I κ B α proteins induced by NF- κ B.⁷⁸

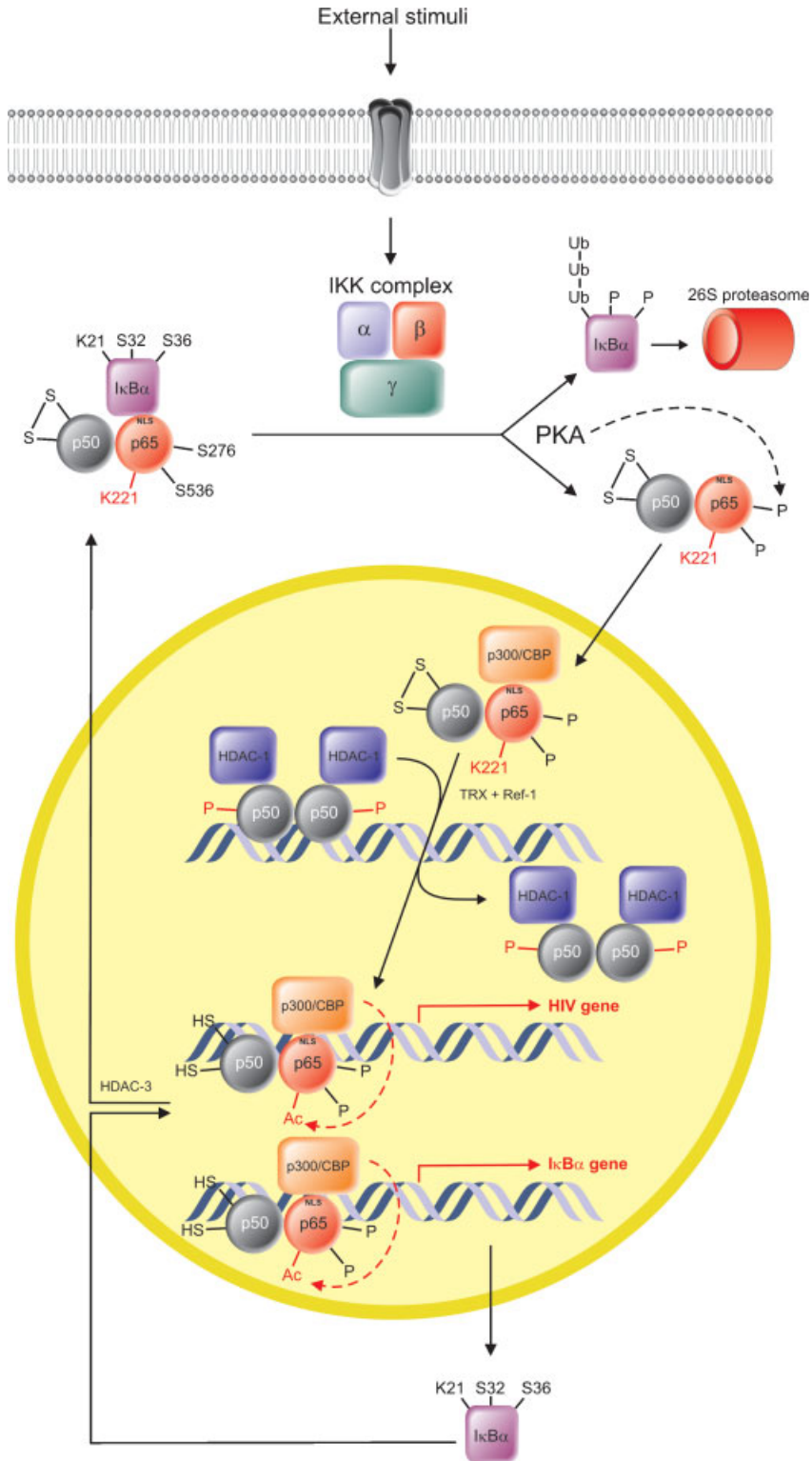


Figure 2.

homodimers, in response to the recruitment of chromatin remodeling and modifying complexes by NF- κ B p50/p65 heterodimers (or Tat) (Fig. 3A and B). The association of NF- κ B to a coactivator complex containing p300/CBP but possibly also p300/CBP-associated factor (P/CAF), steroid receptor coactivator-1 (SRC-1), and SRC-1-related proteins, not only results in the disruption of nuc-1, but may also lead to an increase in stability of the activator–DNA complex and to an increase in accessibility of promoter sequences for binding transcription factors and regulatory proteins indispensable for stimulation of the transcription initiation process.^{83–85}

An important consequence of histone acetylation appears to be the recruitment of the ATP-dependent chromatin remodeling complex SWI/SNF to the LTR promoter.⁸⁶ More specifically, the ATPase subunit of the SWI/SNF chromatin remodeling complex Brahma-related gene 1 (BRG-1) is recruited to the API-3 site located at the 3'-end of nuc-1. This event seems to be mediated by an inducible factor ATF-3 (with JunB as a possible dimerization partner) in the presence of HMGA1 proteins. Although ATF-3 and HMGA1 confer the specificity of recruiting SWI/SNF to nuc-1, histone acetylation stabilizes the interaction of the complex at the edge of nuc-1.^{87,88} In other words, during HIV-1 transcriptional activation histone acetylation acts as a signal for the recruitment of chromatin remodeling complexes. Upon interaction with nuc-1, SWI/SNF utilizes the energy from ATP hydrolysis to disrupt nuc-1 in a non-covalent manner. The exact mechanism by which SWI/SNF disrupts the histone–DNA interactions to facilitate transcription is yet not clear, but it is believed to either slide the DNA helix in a screw-like manner or to propagate a DNA bulge around the nucleosome interface.^{89,90} Retinoic acid inhibits HIV-1 transcriptional activation by interfering with nuc-1 remodeling without affecting histone acetylation.⁹¹

5. TRANSCRIPTIONAL MACHINERY

The synthesis of mature and functional HIV messenger RNA or transcription is a complex, multistage process that requires the cooperative action of viral and cellular proteins (Fig. 3C and D). At the initiation of transcription, the TATA box is recognized by the TBP. The binding of TBP usually

Figure 3. Transcriptional activation. **A:** In unstimulated cells the HIV-1 proviral DNA is assembled into chromatin with precisely positioned nucleosomes surrounding two NF- κ B binding sites, three Sp1 binding regions, and the transcription initiation site. Repression of HIV-1 transcription is mediated by HDAC-1 bound to the p50 homodimers that specifically interacts with the HIV-1 promoter thereby maintaining the nucleosome nuc-1 in a deacetylated state. Another form of transcriptional repression is mediated by the recruitment of HDAC-1 to the transcription initiation site by the cooperative interaction with host factors YY1 and LSF. **B:** In stimulated cells HIV-1 transcriptional repression is released resulting in TFIID binding to the TATA box. The NF- κ B p50/p65 heterodimers associates with the HAT CBP/p300 and displaces p50-HDAC-1 homodimers from the promoter. Recruitment of HAT complexes to the HIV-1 promoter by NF- κ B results in the acetylation of lysine residues on the N-termini of the histones H3 and H4 of nuc-1. Histone acetylation is thought to both decompact chromatin structure to allow greater access of the transcriptional machinery and acts as a signal for the recruitment of the ATPase-dependent chromatin remodeling complex SWI/SNF to the 3'-end of nuc-1 by cooperative interactions with HMGA1 and ATF-3. Upon interaction with nuc-1, SWI/SNF utilizes the energy from ATP hydrolysis to disrupt nuc-1 in a non-covalent manner. **C:** Transcriptional initiation begins with the assembly of a PIC by coordinated accretion of TFIIA, TFIIB, TFIIF in complex with the non-phosphorylated form of RNAPII, mediator, TFIIE, and finally TFIIH. TFIIH mediates the transition from transcription initiation to elongation by ATP-dependent DNA strand separation from positions -9 to +2 and phosphorylation of Ser5 of the heptapeptide repeat present in the CTD of RNAPII through its CDK7 kinase activity. Subsequently, the Ser-5-phosphorylated CTD of RNAPII recruits capping enzymes (CE) which mediate 5'-end RNA capping soon after promoter clearance. DSIF and NELF negatively regulate elongation through interactions with the hypophosphorylated form of RNAPII. This results in termination of the initiated polymerase and subsequent generation of short abortive transcripts. **D:** In order to release the repression caused by the DSIF/NELF complex and secure an efficient transcriptional elongation, the CTD of RNAPII must become hyperphosphorylated on Ser2 by the CDK9 subunit of p-TEFb. This hyperphosphorylation as well as the phosphorylation of DSIF and NELF are mediated by a direct interaction between the CTD of RNAPII and p-TEFb which can be recruited to the HIV-1 promoter by NF- κ B. Finally, CTD phosphorylation on Ser2 leads to recruitment of specific factors involved in RNA splicing, transcription termination, and 3'-end RNA-processing.

initiates a cascade of additional interactions with different proteins, also referred as the TBP-associated factors (TAF), to assemble as a multisubunit TFIID complex. Once TFIID is formed, additional multicomponent transcription factors are recruited including TFIIA, TFIIB, TFIIE, TFIIIF, and TFIIH into a PIC. Within the assembly of the PIC a multisubunit enzyme, RNA polymerase II (RNAPII), is found that catalyzes the synthesis of viral mRNA from the proviral DNA. The largest subunit of RNAPII is unique in that it contains a carboxyl-terminal domain (CTD) consisting of multiple-tandem repeats of the YSPTSPS heptapeptide, with both Ser2 and Ser5 the sites of reversible phosphorylation.^{92,93} Although the viral transcriptional activator Tat and its cellular cofactor p-TEFb are mainly ascribed to the transcription elongation step (*vide infra*), recent evidence points towards a role of both proteins in transcription initiation. Tat and p-TEFb facilitate the binding of TBP to the transcription complex enabling the assembly of the PIC.^{94,95}

Once the PIC assembly is complete, TFIIH performs at least three critical functions in RNAPII transcription: (1) it mediates ATP-dependent strand separation through its 3'-5' helicase activity at the transcription start site, resulting in an open DNA complex from positions -9 to +2,^{96,97} (2) it phosphorylates the fifth serine of the heptapeptide repeat present in the CTD of RNAPII through its CDK7 kinase subunit during the transition from transcription initiation complex to a stable elongation complex,^{98,99} and (3) it suppresses transcription arrest of early RNAPII elongation intermediates.^{100,101} Although CTD phosphorylation is temporally correlated with promoter clearance and thought to be a prerequisite for the formation of a stable elongation complex, the precise role of CTD phosphorylation remains obscure. The idea that phosphorylation of the CTD at multiple sites serves to disrupt interactions between the unmodified CTD and proteins necessary for the formation of a stable PIC remains an attractive possibility. Not only TFIIH, but also the p65 subunit of the transcriptional activator NF- κ B, was thought to influence RNAPII processivity. In this way, NF- κ B was able to stimulate transcriptional elongation probably due to the recruitment of the CTD kinase, p-TEFb, which catalyzes the phosphorylation of the second serine of the heptapeptide repeat present in the CTD of RNAPII.^{102,103} The phosphorylation pattern changes during the transcription cycle resulting in the recruitment of specific mRNA-processing factors. CTD phosphorylation on Ser5 must occur during the transition from transcription initiation to elongation, because phosphorylated CTD has a role in recruiting mRNA capping guanylyltransferase enzymes to the nascent transcript, and 5'-mRNA capping occurs soon after promoter clearance.^{104,105} Furthermore, CTD phosphorylation on Ser2 triggers binding of the splicing and the 3'-mRNA processing machinery, and recruits specific factors involved in transcription termination.^{106,107}

Following promoter clearance, and before entering productive elongation, the processivity of RNAPII is controlled by the action of both negative and positive elongation factors (N-TEFs and P-TEFs).^{108,109} Negative elongation factors (NELF), such as the DRB (5,6-dichloro-1- β -D-ribofuranosylbenzimidazole) sensitivity-inducing factor (DSIF, a human homolog of yeast Spt4-Spt5 complex) and the NELF, have been proposed to be responsible for premature stopping and termination of the initiated polymerase, resulting in the generation of short abortive transcripts. NELF acts cooperatively with DSIF through binding to the DSIF/RNAPII complex and to nascent transcripts.¹¹⁰ On the other hand, positive elongation factors, such as p-TEFb, are known to mediate the transition from abortive into a productive elongation complex by phosphorylating the RNAPII CTD.^{111,112} p-TEFb is composed of two subunits: the catalytic subunit cyclin-dependent kinase CDK9 (previously named PITALRE) and the regulatory subunit cyclin T1. The exact mechanism by which DSIF, NELF, and p-TEFb act together is still unclear, but evidence suggests that DSIF and NELF negatively regulate elongation through interactions with RNAPII containing a hypophosphorylated CTD. Phosphorylation of the RNAPII CTD, the hSpt5 subunit of DSIF, and the NELF by p-TEFb might overcome the repression and promote elongation.^{113,114}

Upon promoter clearance, the interaction between the hyperphosphorylated RNAPII and TFIIB, TFIIE, TFIIIF, and TFIIH must be broken to engender a fully competent transcriptional apparatus. During elongation, TFIID can remain bound to the core promoter supporting reinitiation of

transcription by RNAPII and the other transcription factors. However, since the dephosphorylated form of RNAPII preferentially enters the PIC, recycling of this enzyme is necessary and is likely to be mediated by a serine/threonine TFIIF-associated CTD phosphatase (FCP1).^{115,116} This RNAPII recycling process seems to be stimulated by TFIIF (RAP74), which is plausible as TFIIF is known to directly interact with RNAPII and to play a role in the recruitment of RNAPII to the PIC.^{117,118} Alternatively, small CTD phosphatase (SCP1) activity is also enhanced by TFIIF (RAP74) with preferential dephosphorylation of RNAPII on Ser5, whereas FCP1 dephosphorylates Ser2 and Ser5 with comparable efficiency.^{119,120} Efficient reinitiation of transcription can be achieved if recycled RNAPII re-enters the PIC before TFIID dissociates from the core promoter.

6. TAT-ACTIVATED TRANSCRIPTION

The HIV-1 promoter directs the synthesis of two classes of RNA molecules, short non-polyadenylated transcripts, and full-length polyadenylated transcripts. In the absence of the viral transcriptional activator Tat, efficient synthesis of the short transcripts is dependent on the IST and corresponds to RNAs with heterogeneous 3' ends located around positions +59 that contain the TAR element, the binding site for Tat.¹²¹ In the presence of Tat, the amount of short transcripts is decreased while the synthesis of full-length transcripts are dramatically increased. Thus, IST seems to stimulate the formation of abortive transcription elongation complexes so as to synthesize the binding site necessary for Tat function.¹²² A cellular POZ (for poxvirus and zinc fingers) domain containing factor FBI-1, binds specifically to this IST suggesting a role in the formation of the HIV-1 short transcripts. In addition, FBI-1 associates to, and stimulates Tat, implicating that repression of the short transcripts by Tat may be mediated through interactions between these two factors.^{123,124}

A. Tat–TAR Interaction

The TAR element, encoded largely between the two IST half-elements, forms a highly stable, nuclease-resistant, secondary stem-loop structure, and is directly bound by the Tat protein (Fig. 4). Tat recognition of TAR involves the presence of a pyrimidine-rich bulge near the apex of the TAR RNA stem and nucleotide pairs flanking the bulge (G₂₁:C₄₁ and A₂₂:U₄₀:G₂₆:C₃₉ and A₂₇:U₃₈). Analysis of compensatory mutations in the TAR RNA stem reveals that the primary nucleotide sequence throughout the TAR stem does not appear to be a prerequisite for Tat recognition. However, TAR RNA mutations that affect base-pairing and, hence, the secondary structure of the pyrimidine-rich bulge, abolish Tat activity.^{125–128} NMR studies have revealed that, in order to obtain an effective Tat–TAR interaction, Tat recognition requires conformational changes within the TAR RNA structure. This rearrangement process involves the displacement of the first residue in the bulge (U23) by one of the arginine side chains present in the basic binding domain of the Tat protein. The displaced U23 is brought into close proximity with G26 creating an arginine binding pocket and enabling TAR RNA to undergo a transition from a structure with an open and accessible major groove to a much more rigid structure.^{129–131} In addition, the conformational change also repositions critical phosphates on the TAR RNA backbone that can be easily contacted by other basic residues found in the TAR RNA-binding region. These contacts contribute not only to the affinity of the Tat–TAR interaction, but also to its specificity, by providing discrimination with respect to other bulged RNA structures.¹³² Different anti-HIV approaches were developed through interference with the Tat–TAR interaction, including the synthesis of peptoids (such as CGP64222 or TR87),^{133,134} Tat peptide mimetics,¹³⁵ quinolone derivatives,^{136,137} polyamide oligomers,¹³⁸ arginine-aminoglycoside conjugates,¹³⁹ intercalators,¹⁴⁰ chemically modified RNA aptamers,^{141,142} or TAR RNA decoys (Table I and Fig. 5).^{143–145} Other approaches that sequester Tat's function were based on the development of small interfering RNAs (siRNAs) directed against Tat expression,¹⁴⁶ the discovery of granulins that

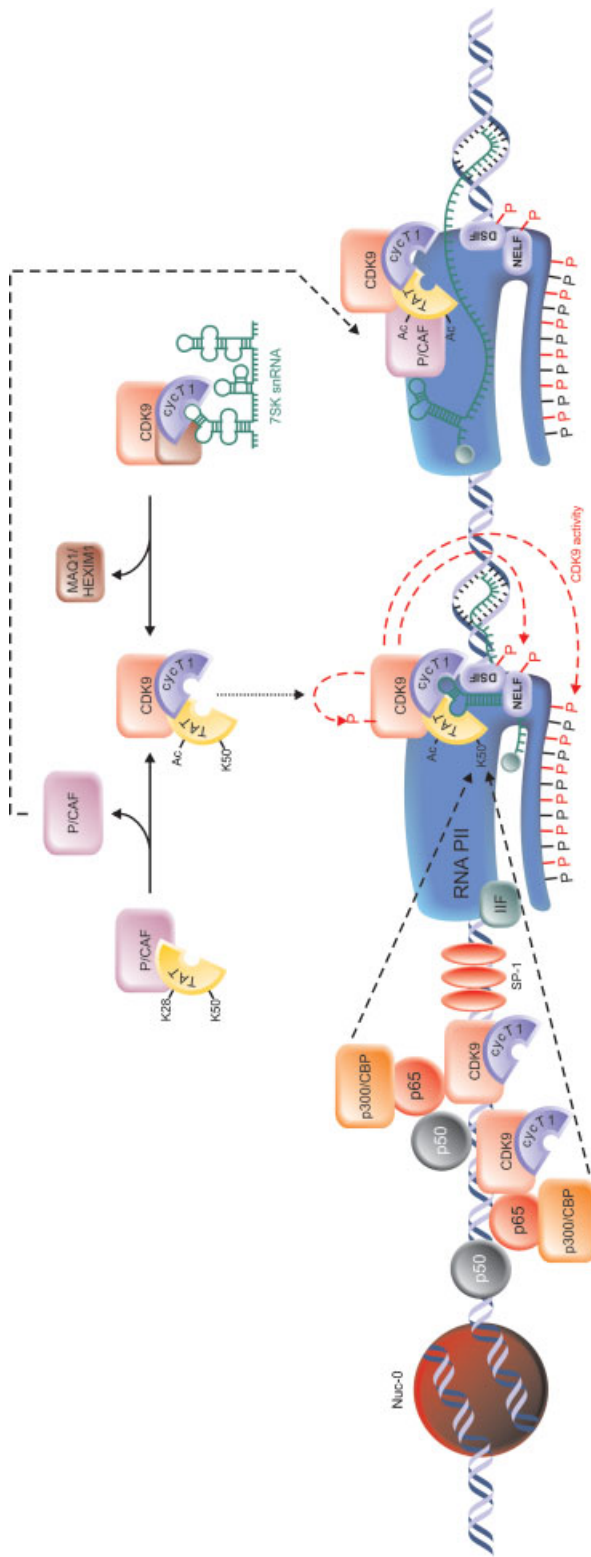


Figure 4. A model for the regulation of Tat-mediated transcription. Initially, non-acetylated Tat interacts with P/CAF, which was found to be associated with the HIV-1 promoter only in response to Tat. P/CAF acetylates Tat at position Lys28. This subsequently abrogates the interaction between P/CAF and Tat, but significantly enhances its interaction with p-TEFb which is released from its association with MAO1/HEXIM1 and 7SK snRNA upon different stimuli including stress, ultraviolet light, DRB, and hypertrophic signals. The complex of Tat with acetylated p-TEFb then binds the upper bulge and loop structures of TAR, thereby positioning CDK9 to phosphorylate also the negative elongation factors NELF and DSIF, which repress transcription by binding to the lower stem in TAR, as well as the CTD of RNAPII on both Ser2 and Ser5. These phosphorylation events result in the release of the transcriptional elongation block. In addition to the productive elongation mediated by the hyperphosphorylated RNAPII, Tat becomes acetylated by p300/CBP on Lys50 resulting in the dissociation of p-TEFb-Tat from TAR RNA and subsequent binding to the elongating RNAPII. Furthermore, the acetylation of Tat on Lys50 serves as a signal to recruit P/CAF generating a p-Tat-P/CAF ternary complex associated with RNAPII during transcriptional elongation.

Table 1. Overview of the HIV-1 Transcription Inhibitors with Their Target for Chemotherapeutic Intervention

Inhibitor	Molecular target	Reference
PDTC	NF- κ B activation	38, 45
NAC	NF- κ B activation	39, 45
α -lipoic acid	NF- κ B activation	46, 47
Oltipraz	NF- κ B activation	48, 49
α -tocopherol	NF- κ B activation	50
BHA	NF- κ B activation	50
Salicylates	NF- κ B activation	51
Pentoxifylline	NF- κ B activation	52, 53
Coumarins	NF- κ B activation	54
Acridone derivatives	NF- κ B activation	55
Iron chelators	NF- κ B activation	56, 57
B-oligomer of pertussis toxin	NF- κ B activation	58, 59
Diamide	NF- κ B binding	72
<i>N</i> -ethylmaleimide	NF- κ B binding	72
Pyridine <i>N</i> -oxide derivatives	NF- κ B binding	73
Cepharanthine	NF- κ B signalling pathway	75
Hydroxyurea	NF- κ B signalling pathway	76
Carboxyamidotriazole	NF- κ B signalling pathway	77
All-trans retinoic acid	Nuc-1 remodeling	91
CGP64222	Tat-TAR interaction	133
TR87	Tat-TAR interaction	134
Tat peptide mimetics	Tat-TAR interaction	135
Quinolone derivatives	Tat-TAR interaction	136, 137
Arginine-aminoglycoside conjugates	Tat-TAR interaction	139
Intercalators	Tat-TAR interaction	140
RNA aptamers	Tat-TAR interaction	141, 142
TAR RNA decoys	Tat-TAR interaction	143, 144, 145
Granulins	Tat or p-TEFb	147, 148
Flavopiridol	p-TEFb	187
R-roscovitine	p-TEFb	188, 189
Curcumin	p300/CBP	224, 225
HR73	SIRT1	226

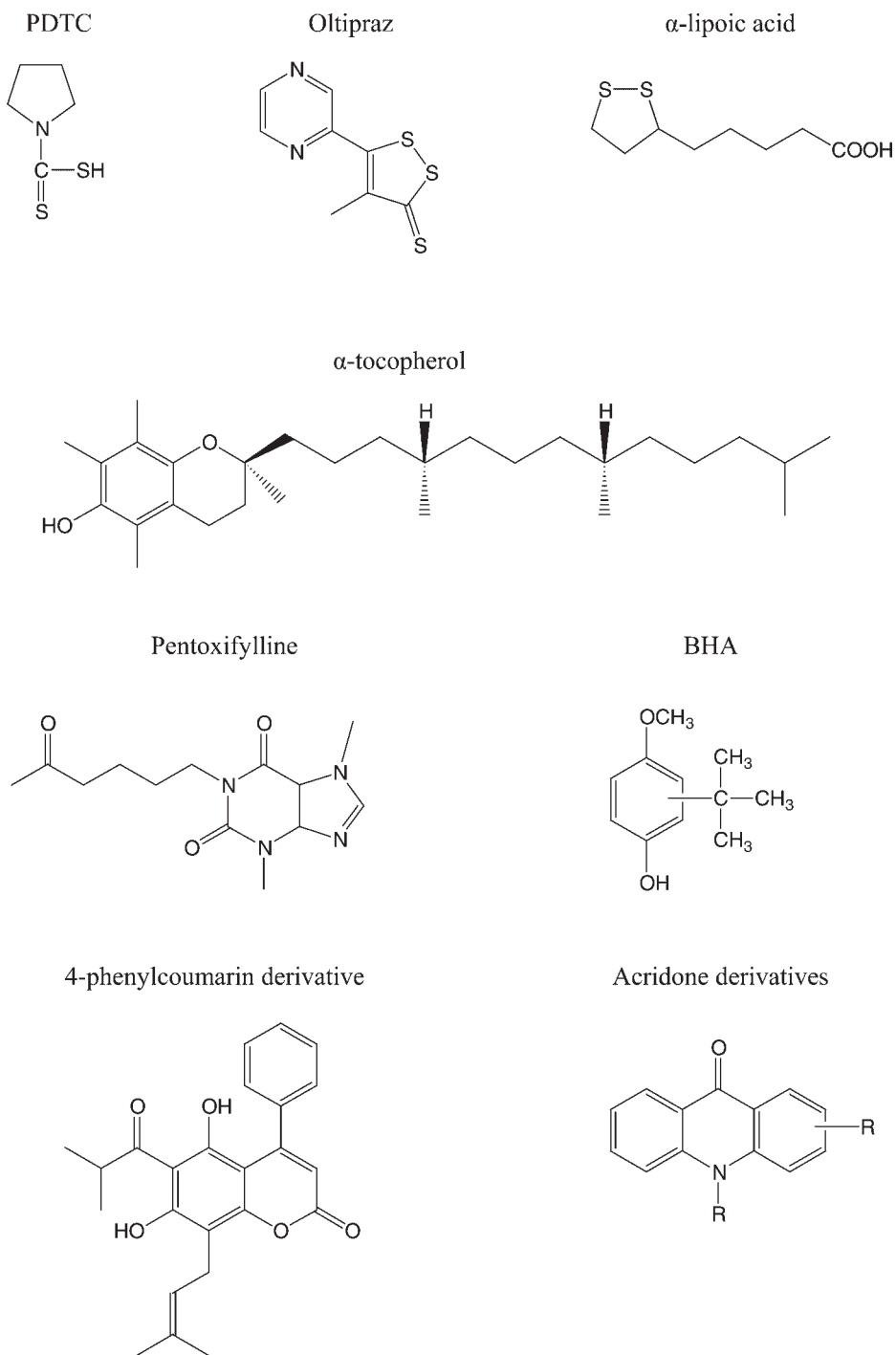
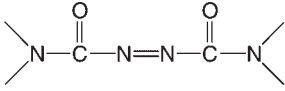
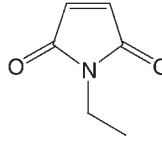


Figure 5. Chemical structures of the HIV-1 transcription inhibitors.

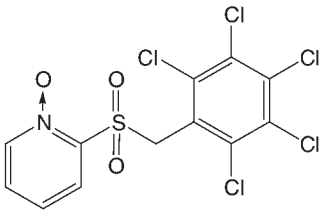
Diazinedicarboxylic acid
bis(*N,N*-dimethyl-amide)



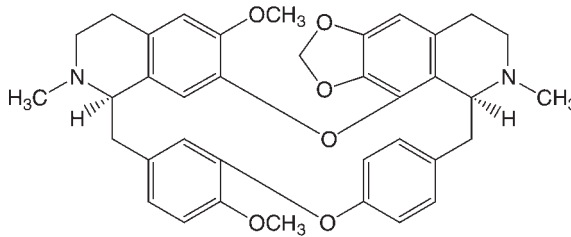
N-ethylmaleimide



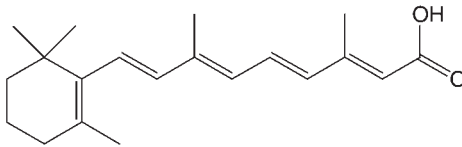
Pyridine *N*-oxide derivative
(JPL-32)



Cepharanthine



All-*trans* retinoic acid



TR87

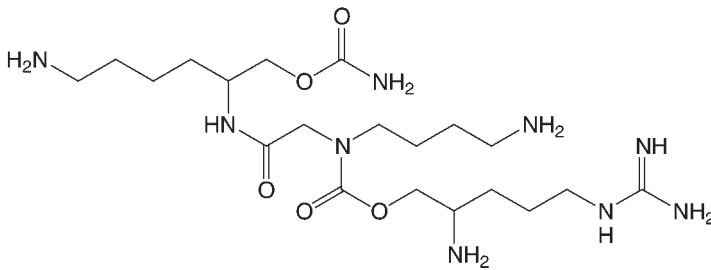
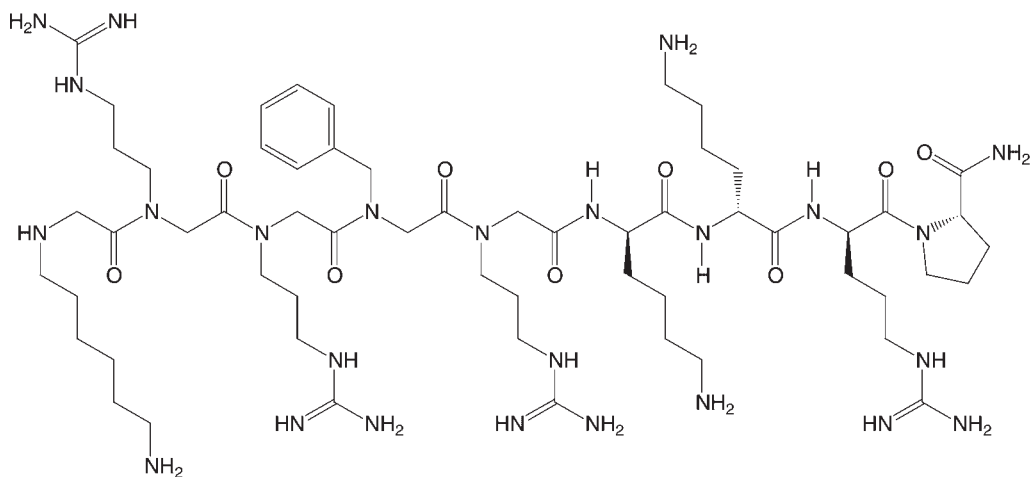


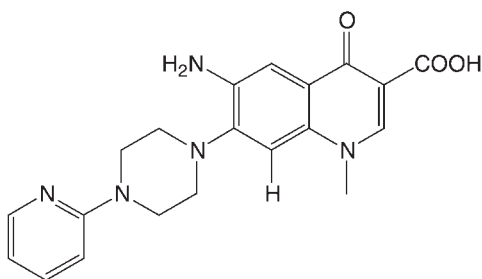
Figure 5. (Continued)

CGP64222

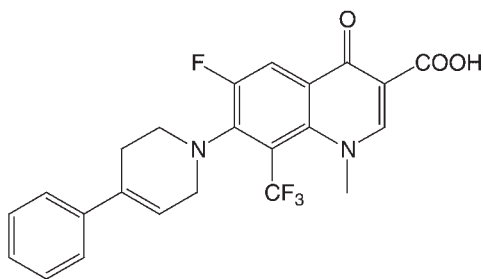


Quinolone derivatives

WM5



K-37



Arginine-aminoglycoside conjugate RMMP

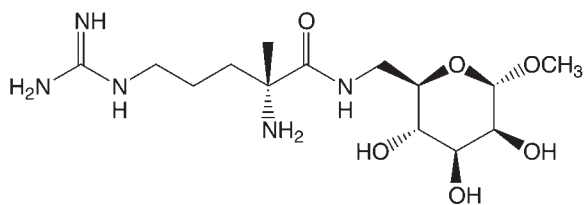
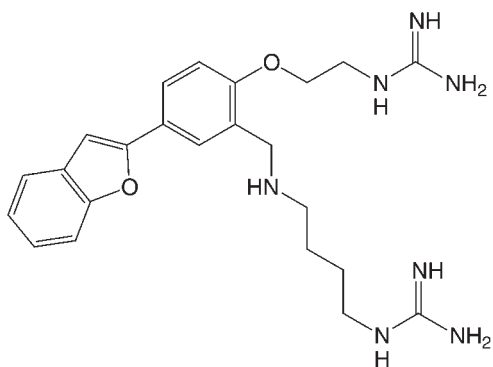


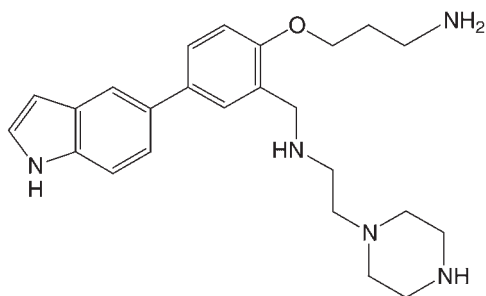
Figure 5. (Continued)

Intercalators

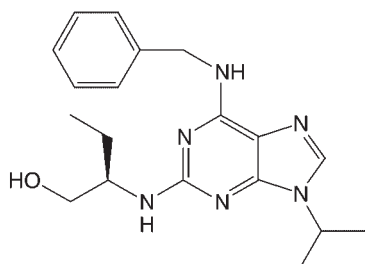
RBT418



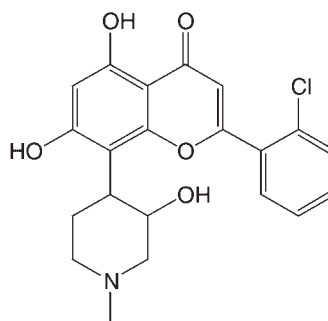
RBT550



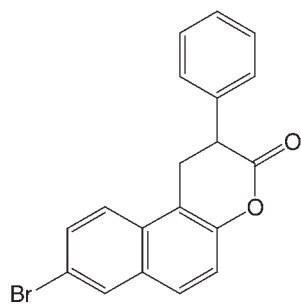
Roscovitine



Flavopiridol



HR73



Curcumin

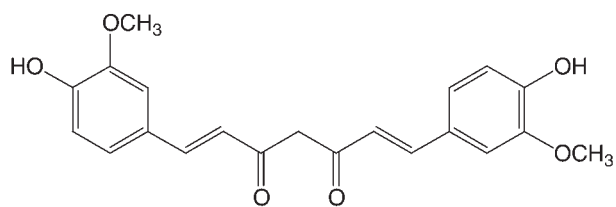


Figure 5. (Continued)

bind directly to Tat or hCycT1,^{147,148} and the introduction of antibodies directed to Tat^{149–151} or hCycT1.¹⁵²

The HIV-1 *trans*-activator protein Tat is a small polypeptide (101 amino acids in most clinical HIV-1 isolates; 86 amino acids in the laboratory strain HIV-1_{hxb2}) encoded from two separate exons and is essential for efficient transcription of the viral genome. In the first exon (amino acids 1–72) there is an *N*-terminal proline-rich/acidic region (amino acids 1–21), a region containing seven cysteines (amino acids 22–37), a core region (amino acids 38–48), and a basic region enriched with arginine and lysine amino acids that is highly conserved among different strains (amino acids 49–57). The second exon starts at amino acid position 73 and has a more variable sequence.¹⁵³ Based on mutational analysis, Tat can be divided into two functional domains. The first domain is the functionally autonomous transcription-activation domain (amino acids 1–48), which may be involved in metal ion binding.^{154,155} The second functional domain comprises the complete basic region (amino acids 49–57), which is essential for TAR RNA binding and functions as a NLS for Tat.¹⁵⁶ Furthermore, it appears that the basic domain of Tat is also important for translocation through the cell membrane.¹⁵⁷ It is quite unusual that Tat is able to leave the cell and enter adjacent cells without any loss of transcriptional activity. Although the precise mechanism by which this unique biological property occurs has not been clarified yet, it offers major possibilities for drug delivery as it would allow it to translocate less permeable molecules into the cell.¹⁵⁸ Other pleiotropic activities of extracellular Tat involves the production of cytokines and expression of cytokine receptors,^{159,160} modulation of survival, proliferation and migration of different cell types,^{161,162} and angiogenic properties.¹⁶³

B. Tat-Dependent Transcriptional Elongation

In the last decade, two classes of Tat-associated proteins have been attributed to the Tat-TAR-dependent transactivation: Tat-associated kinases (TAK) and Tat-associated acetyl-transferases. The association of Tat with cellular TAK resulted in a hyperphosphorylation of the RNAPII CTD and proved directly responsible for the enhanced transcriptional processivity of the elongation complex. In addition, it was observed that the transactivation domain of Tat interacted with TAK, which was shown to be identical to the kinase subunit of p-TEFb.^{164–168} In other words, not only NF- κ B but also Tat recruits p-TEFb to engender productive elongation (Fig. 4). Although we mentioned (*vide supra*) that the CDK9 kinase subunit of p-TEFb phosphorylates Ser2 of the CTD of RNAPII, the presence of Tat modifies the substrate specificity of CDK9 enabling the kinase to phosphorylate both Ser2 and Ser5 of the CTD.¹⁶⁹ Consistent with this role, p-TEFb is only able to phosphorylate the RNAPII after phosphorylation by TFIIF and travels with the elongating RNAPII, whereas TFIIF is released from the elongation complex between +14 and +36.^{111,170,171} Previously, we mentioned the role of DSIF and NELF in transcriptional repression at the early phase of elongation resulting in the generation of short abortive transcripts. Surprisingly, the presence of Tat converts DSIF from a negative to a positive elongation factor by phosphorylation of the hSpt5 subunit of DSIF by p-TEFb. As a result of this transition, DSIF cooperates with Tat in the productive elongation of transcription.^{172–174} Furthermore, not only DSIF is phosphorylated but also NELF becomes phosphorylated by p-TEFb. This causes NELF, that binds to TAR through its RNA recognition motif (RRM), to dissociate from the TAR RNA and relieve transcriptional pausing.^{175,176} Besides the DRB-sensitive CTD kinase activity engendered by the CDK9 subunit, the p-TEFb also consists of a cyclin T subunit, named after its involvement in transcription.¹¹² Human cyclin T1 (hCycT1) interacts directly with Tat and dramatically enhances its affinity for TAR RNA.¹⁷⁷ Interestingly, the interaction between Tat and hCycT1 requires zinc and a critical cysteine residue on position 261 within the TRM (Tat-TAR recognition motif) of hCycT1. Although murine Cyclin T1 (mCycT1) lacks this C261 residue, it forms also a weak zinc-independent complex with the transactivation domain of Tat but, unlike hCycT1, results in a dramatic reduction in binding efficiency to TAR RNA.¹⁷⁸ Consequently, Tat

function could be specifically inhibited upon overexpression of mCycT1 leading to the formation of an inactive Tat–mCycT1 complex.¹⁷⁹ Thus, the ability of Tat to recruit p-TEFb to TAR RNA not only stimulates transcriptional elongation, but also governs the species specificity of HIV-1. However, the recruitment of p-TEFb to the viral promoter was inhibited by 7SK small nuclear RNA (7SK snRNA) in order to control transcription (Fig. 4).^{180,181} This inhibitory mechanism required “ménage à quatre” 1 (MAQ1) and Hexam-ethylene bisacetamide-induced protein 1 (HEXIM1) to associate with p-TEFb and act as a CDK inhibitor. The HEXIM1 protein interacts directly with 7SK snRNA and the hCycT1 subunit of p-TEFb, by this means competing with Tat for hCycT1 binding. Activation of upstream signaling pathways leads to the abrogation of the interaction of p-TEFb with MAQ1/HEXIM1 and 7SK snRNA.^{182–186} Other CDK inhibitors were based on chemical synthesis with most prominent flavopiridol and R-roscovitine. Both compounds were not only able to inhibit CDK9, but proved also inhibitory to other CDKs involved in transcription such as CDK2 and CDK7.^{187–189} The exact role of CDK2 in Tat-dependent transcription is still unknown, but evidence suggests that CDK2 may function to maintain target-specific phosphorylation of the RNAPII CTD and possibly Tat itself.^{190,191}

Next to the CDK inhibitory mechanism involving MAQ1/HEXIM1 and 7SK snRNA, the formation of the p-TEFb–Tat–TAR ternary complex also requires the relief of two autoinhibitory mechanisms in p-TEFb. Autophosphorylation of CDK9 overcomes the first autoinhibition by creating a favorable p-TEFb conformation and exposing the TRM in hCycT1 for efficient interaction with TAR RNA. TFIIF inhibits CDK9 phosphorylation until it is released from the elongation complex.^{171,192} The second autoinhibition is caused by the intramolecular interaction between the *N*- and *C*-terminal regions of hCycT1, which blocks the access of TAR RNA to hCycT1 TRM, and is relieved by interaction of the hCycT1 *C*-terminal region with the Tat stimulatory factor, Tat-SF1.¹⁹³ In addition, the hCycT1–Tat–SF1 interaction may also recruit spliceosomal U small nuclear ribonucleoproteins (snRNPs), which interact with Tat-SF1, so as to elongating RNAPII, and subsequently function in both splicing and transcriptional elongation.^{194,195} Coupling of pre-mRNA splicing and transcription was also seen by two others splicing-associated proteins namely the coactivator of 150 kDa, CA150, and the *c*-Ski-interacting protein, SKIP. CA150 binds directly to the phosphorylated CTD of RNAPII through its FF repeat motif (so called because of flanking conserved phenylalanine residues) and represses RNAPII transcriptional elongation. Whether this CA150-mediated repression is caused by interaction with the pre-mRNA splicing factor SF1, which has been shown to repress transcription, remains to be clarified. Besides the coupling of transcription and splicing by the CA150–SF1 interaction, CA150 was also able to bind Tat-SF1 through its FF domain.^{196–198} In contrast to CA150, SKIP enhances transcriptional elongation by association with p-TEFb in the p-TEFb–Tat–TAR ternary complex. Furthermore, a direct interaction of SKIP with the spliceosomal protein tri-snRNP110K indicates that only a subset of slicing factors are recruited to the HIV-1 promoter.¹⁹⁹ Besides pre-mRNA splicing, mRNA capping was also found to be coupled with transcriptional elongation. The Tat–TAR-dependent Ser5 phosphorylation of RNAPII CTD is crucial not only in promoting transcription elongation but also in stimulating capping of nascent HIV-1 mRNA.^{200–202} The Tat protein not only regulates transcriptional elongation or post-transcriptional events through RNAPII CTD phosphorylation, but also interferes with the dephosphorylation of RNAPII so as to stimulate transcription. On the one hand, Tat inhibits the CTD phosphatase FCP1 and consequently may alleviate FCP1-mediated pausing of transcription.^{203–205} On the other hand, Tat interacts with the PPP-type protein phosphatase PP1, that also functions as a CTD phosphatase, and augments Tat-mediated transcription.^{206,207}

C. Tat Regulation by Post-Translational Modifications

A second class of Tat-associated proteins that contribute to the Tat–TAR dependent transactivation comprises the Tat-associated acetyltransferases. Tat is known to interact with different

acetyltransferases including the Tat-interactive proteins (Tip60 and Tip110),^{208–210} TAFII250,²¹¹ the human homolog of yeast GCN5 (hGCN5),²¹² p300/CBP, and P/CAF.^{213–215} P/CAF was able to bind to the cysteine-rich region of non-acetylated Tat and acetylated Lys28 within the transcription-activation domain of Tat. This post-translational modification abrogated the interaction between P/CAF and Tat, but significantly enhanced the recruitment of p-TEFb by Tat.^{216,217} The acetylation site of p300/CBP, as well as hGCN5, has been mapped to Lys50 (and, weakly, to Lys51), a highly conserved amino acid within the TAR RNA-binding domain of Tat. This second post-translational modification leads to the dissociation of pTEFb-Tat from TAR RNA and subsequent binding to the elongating RNAPII.^{212,216,218,219} Both Tat and p-TEFb were able to bind and travel with the elongating RNAPII.^{170,220,221} In addition, acetylation of Tat on Lys50 by p300/CBP also served as a signal for the bromodomain of P/CAF to recruit this transcriptional coactivator to the elongating RNAPII. This results in the formation of a p-TEFb-Tat-P/CAF ternary complex associated with the elongation complex during transcriptional elongation.^{216,217,222,223} The acetylation of Tat (and nuc-1) by p300/CBP could be inhibited by curcumin and resulted in a suppression of Tat-mediated transactivation and HIV replication in cell culture.^{224,225} Both suppressive effects were also found for HR73, an inhibitor of class III deacetylase sirtuin 1 (SIRT1) that is able to deacetylate Lys50 in Tat. This proved that both acetylation and deacetylation are important for the regulation of Tat.²²⁶

Other post-translational modifications of Tat that regulate Tat activity have been mapped to Lys 71, that becomes ubiquitinated by the proto-oncoprotein Hdm2, and the arginine-rich motif (amino acids 49–63) which undergoes methylation by protein arginine methyltransferase 6 (PRMT6). Both modifications regulate the transcriptional activity of Tat, positively regulated by modification with ubiquitin and negatively regulated by arginine methylation.^{227,228} As such, it has become increasingly clear that Tat activity needs to be fine-tuned by both positive and negative regulatory mechanisms within cells in order to achieve optimal viral gene expression.

7. CONCLUSIONS

The regulation of HIV-1 transcription is a complex, multistage process that requires the cooperative action of viral and cellular proteins. The position of nuc-1 at the transcription initiation site and its disruption upon transcriptional activation suggests that chromatin plays an essential role in the suppression of HIV-1 expression during latency. Reactivation of the HIV-1 provirus may be mediated by the displacement of HDACs in response to the recruitment of HATs by NF- κ B or the viral transactivator Tat. Additionally, productive transcription of the HIV-1 provirus requires the recruitment of CDK9 to the viral promoter by Tat. Although these crucial functions of Tat are indispensable for HIV-1 replication, the implications of Tat reach far beyond both inside and outside the cell. Tat has the unusual property to be released and to enter cells freely without losing activity, enabling the upregulation of numerous genes including HIV-1 gene expression. As such, targeting of Tat protein provides interesting perspectives for therapeutic intervention in HIV infection and offers major possibilities in drug delivery. An important and popular therapeutic approach is based on the interference of Tat interaction with the bulge of TAR RNA. Numerous Tat-TAR inhibitors have been designed and are presented in several classes including peptoids, Tat peptide mimetics, quinolone derivatives, polyamide oligomers, arginine-aminoglycoside conjugates, intercalators, and a large class of small RNA-binding molecules. Another approach to sequester Tat's function is based on targeting its crucial cofactor CDK9 by CDK inhibitors such as flavopiridol and R-roscovitine. Moreover, R-roscovitine is able to selectively induce apoptosis of HIV-infected cells without viral release opening major perspectives for this class of CDK inhibitors. The Tat-mediated transcription could also be inhibited by curcumin and HR73 which interfere with acetylation and deacetylation of Tat, respectively. This recent approach affecting Tat function emphasize the importance of post-translational modifications in the regulation of Tat. Thus, different anti-HIV strategies based on

directly or indirectly Tat targeting have recently been developed and may provide great promise in drug combination strategies. Once HIV replication is shut down and plasma viral loads have reached undetectable levels upon combination therapy, HIV transcription inhibitors may potentially be able to keep the virus in its dormant state and control the latent HIV-1 reservoir.

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Miguel Stevens obtained his degree in Pharmaceutical Sciences (1999) at the University of Ghent (UGent), Belgium, and his Master in Cell Biotechnology (2000) at the Katholieke Universiteit Leuven (K.U.Leuven), Belgium. In 2006, he obtained his Ph.D. as Doctor in Medical Sciences at the Rega Institute for Medical Research.

Erik De Clercq was born in Dendermonde, Belgium, on March 28, 1941. He received his M.D. degree in 1966 and his Ph.D. in 1972 both at the Katholieke Universiteit Leuven (K.U.Leuven) in Belgium. After having spent two years at Stanford University Medical School as a postdoctoral fellow, he returned to the K.U.Leuven Medical School, where he became docent (assistant professor) in 1973, professor in 1975, and full professor in 1977. As of today he is teaching the courses of Cell Biology, Biochemistry, Microbiology and Virology in the first, second and third undergraduate years of the Medical School at the K.U.Leuven and K.U.Leuven Campus Kortrijk. Prof. E. De Clercq served as chairman of the Department of Microbiology from 1986-1991. Since 1999 he is again serving as chairman of the Department of Microbiology and Immunology. In 1985, he became President of the Rega Foundation and, in 1986, Chairman of the Board of the Rega Institute for Medical Research, two positions which he still holds. He is a titular member of the Belgian Royal Academy of Medicine and the Academia Europaea, and has also held the Prof. P. De Somer Chair of Microbiology at the K.U.Leuven. Prof. De Clercq's scientific achievements are in the antiviral chemotherapy field, and, in particular, the development of new antiviral agents for the treatment of various viral infections, including AIDS. He has (co)-discovered a number of antiviral drugs, currently used in the treatment of various virus infections, such as herpes simplex (valaciclovir), herpes zoster (brivudin), CMV (cytomegalovirus) infections (cidofovir), HBV (hepatitis B virus) infections (adefovir), and HIV infections (AIDS) (tenofovir disoproxil fumarate, marketed as VireadTM, and, in combination with emtricitabine, as TruvadaTM).

Jan Balzarini obtained his degree in Master in Biological Sciences (1975) and his degree as Bio-Engineer in Biochemistry and Microbiology (1977) at the Katholieke Universiteit Leuven (K.U.Leuven), Belgium. In 1984, he obtained his Ph.D. as Doctor in the Bioengineering at the Rega Institute for Medical Research, and received a tenured research position at the K.U.Leuven. After a post-doctoral stay at the National Institutes of Health, Jan Balzarini became, respectively, assistant-, associate- and eventually full professor of Virology and Chemotherapy at the Faculty of Medicine of the K.U.Leuven. His research projects are focussed on combined gene/chemotherapy of cancer, nucleoside kinases, antiviral chemotherapy (with a focus on AIDS, influenza and coronaviruses), mechanism of action of antiviral/anticancer drugs and molecular mechanisms of drug resistance. Jan Balzarini has received several awards, including the International René-Descartes Prize-2001 of the European Commission. He is on the Board of Directors of the International Society of Antiviral Research (ISAR) and the Rega Foundation of the K.U.Leuven.