Role of the HIV-1 Positive Elongation Factor P-TEFb and Inhibitors Thereof

Yan Wang[1], Xin-yong Liu[1,*] and Erik De Clercq[2]

[1]*Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, No.44 Wenhuaxi Road, Jinan 250012 , China;* [2]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

Abstract: Transcription is considered to be a crucial step in the replication cycle of HIV-1. Tat regulates an early step of transcription elongation. The positive elongation factor P-TEFb, a heterodimer containing a catalytic subunit (CDK9) and unique regulatory cyclins (CycT1), is required for HIV-1 Tat transcriptional activation. This is a potential target for new HIV-1 transcription inhibitors. Without P-TEFb, transactivation is restrained and only short transcripts are generated. All the P-TEFb inhibitors can suppress the HIV-1 transactivation process by inhibition of CycT1, CDK9 or their interaction. Several low-molecular-weight compounds such as flavopiridol, roscovitine and the human small nuclear RNA 7SK which have been showed to possess potent anti-HIV activity by interfering with P-TEFb functions are reviewed in this article.

Key Words: HIV-1, Tat, TAR, P-TEFb, transcription inhibitor.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is the communicable disease caused by the human immunodeficiency virus (HIV). HIV has two subtypes: HIV-1 and HIV-2, and HIV-1 is the main causative agent, with strong virulence. HIV-1 infects immune cells, and causes a depletion of CD4+ lymphocytes, eventually resulting in defective cellular immunity. Although the established combination therapies using fusion, reverse transcriptase and/or protease inhibitors has changed the destiny of HIV-1-infected individuals, leading to a dramatic reduction in AIDS-related morbidity and mortality [1], AIDS therapies still face many challenges. As of today exactly 25 approved drugs have appeared, and resistance has been shown to arise due to the high rate of genetic variation of the HIV-1 genome and mutation selection under drug suppression [2]. Therefore, identification of new targets and development of new drugs with a low resistance profile and high potency remains subject of intensive research.

In the HIV-1 life cycle, transcription from the proviral DNA is considered to be a crucial step for viral replication, and the transactivator of transcription (Tat) protein is absolutely required. Tat regulates an early step in transcription elongation that requires the positive transcription elongation factor b (P-TEFb) complex, consisting of cyclin-dependent kinase 9 (CDK9), cyclin T and other associated factors, to elongate the viral RNA genome [3, 4]. Tat complexes with P-TEFb to form the Tat/P-TEFb complex, which is tethered to the transactivation response element (TAR). The assembly of the Tat-TAR-P-TEFb complex autophosphorylates P-TEFb and hyperphosphorylates the C-terminal domain (CTD) of RNA polymerase II [5-7], thereby inducing the formation of processive elongation complexes that synthesize full-length HIV viral mRNA. Without P-TEFb, only short transcripts are generated. Thus, P-TEFb is required for HIV-1 Tat transcriptional activation [4, 8], and plays a key role in the formation of full-length transcript. The conserved domain of CycT1, as a part of P-TEFb, make it possible to detect new inhibitors with low resistance in the near future.

STRUCTURE OF P-TEFb

Information regarding the composition of P-TEFb was obtained by cloning of full-length cDNA encoding the small subunit of the *Drosophila* factor [9]. The deduced amino acid sequence revealed the small subunit of *Drosophila* P-TEFb as a member of the Cdc2-like cyclin-dependent kinase family. A search of the protein database revealed that a human protein of PITALRE exhibits 72% identity and 83% similarity to the *Drosophila* protein. The high level of sequence similarity indicated that PITALRE is a potential homolog of the small subunit of *Drosophila* P-TEFb and therefore may be a component of human P-TEFb. Depletion of PITALRE caused annihilation of human P-TEFb activity providing further proof that PITALRE is a component of human P-TEFb [9].

Full-length cDNA encoding the large subunit of *Drosophila* P-TEFb was also cloned using the protein sequence information [10]. The sequence of the cDNA indicated that the predicted protein contained a cyclin motif. The function of P-TEFb has been assigned to a kinase-cyclin pair, and the kinase subunit alone had no activity [10]. Recombinant proteins with simultaneous expression of both *Drosophila* subunits were produced in a baculovirus expression system and its ability to phosphorylate the CTD of RNA polymerase II was demonstrated [10]. Because of its similarity to other cyclin-dependent kinases, the kinase subunit was named cyclin-dependent kinase 9 (CDK9), and the cyclin subunit was named cyclin T because it is involved in transcription [10].

*Address correspondence to this author at the Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, No.44 Wenhuaxi Road, Jinan 250012 , China; Fax: +86 531 8838-2264;
E-mail: xinyongl@sdu.edu.cn

1389-5575/09 $55.00+.00

To further understand the function of P-TEFb, three human sequences were found to encode for two related human genes: cyclins T1 and T2 [11], and cyclins T2 splice variants termed T2a and T2b that share the first 642 amino acids but possess different carboxyl termini (Fig. (**1**)). All three proteins were coexpressed with CDK9 to produce active recombinant P-TEFb, the major form was cyclinT1. T2a and T2b were less abundant forms derived from a second gene. In the HeLa nuclear compartment, about 80% of the CDK9 was complexed with cyclin T1, 10% was complexed with T2a and 10% with T2b [11].

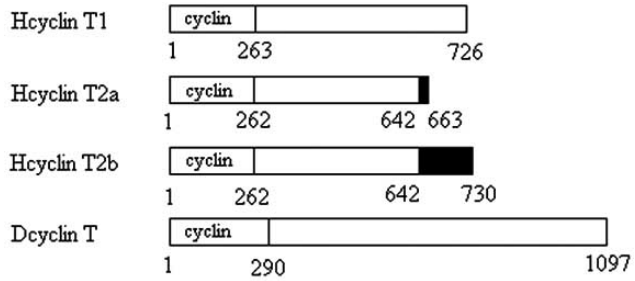

Fig. (1). Diagram of human cyclins T1, T2a, and T2b and *Drosophila* cyclin T [11]. Amino acids are numbered and the cyclin box is indicated in the diagram. Human cyclins T2a and T2b have 642 amino acids in common but different carboxyl termini (black boxes). In the cyclin box region, *Drosophila* cyclin T had 64% identity to either human cyclins T1 or T2, and human cyclins T1 and T2 shared 81% identity.

Human cyclin K was also identified as a component of P-TEFb. A CDK9-cyclin K heterodimer was extracted from insect cells infected with a baculovirus expressing both proteins [12]. A study showed that CycK, with the kinase activity of CDK9, could phosphorylate the CTD of RNA polymerase II when tethered heterologously to RNA, which provided another evidence that P-TEFb contains a common catalytic subunit (CDK9) and the unique regulatory cyclins CycT1, CycT2a, CycT2b, or CycK [13]. But which cyclin as the component of P-TEFb is still a problem.

In a study of a hamster ovary gene expression, the problem about the composition of P-TEFb was solved. Cyclin T1, not cyclins T2a or T2b, were encoded by human chromosome 12, and human cyclin T1 can rescue Tat transactivation in Chinese hamster ovary (CHO) cells [14]. *In vitro,* only human cyclin T1, but not rodent cyclin T1 or human cyclins T2a and T2b, could support interactions between Tat and TAR. Recruitment of the Tat-P-TEFb complex is more specific with only P-TEFb containing cyclin T1 allowing complex formation with TAR [15], and human cyclins T2a and T2b do not rescue Tat transactivation in rodent cells [12]. It is likely that cyclin K will not function because it lacks the cysteine residue at position 261 of cyclin T1 that is required for a zinc-dependent interaction between cyclin T1 and HIV-1 Tat [16]. Thus, human Cyclin T1 was identified as a Tat-associated protein, an important component of P-TEFb.

CRYSTALLOGRAPHIC ANALYSIS OF CYCLIN T1

Cyclin T1 has a conserved domain—the cyclin box domain, which is necessary and sufficient for its interaction with Tat protein [16, 17]. A recent study has shown the structure of the cyclin box domain (Fig. (**2a**)) of human cyclin T1 by crystallographic analysis of cyclin T1 in complex with the transactivator protein Tat from equine infectious anemia virus (EIAV), which is functionally and structurally related to HIV-1 Tat [18].

The cyclin box structure of Cyclin T1 consists of two characteristic α-helical repeats giving an elongated shape. Each repeat consists of five helices named as H1–H5 and H1′–H5′ respectively (Fig. (**2b**)). The N and C- termini of α-

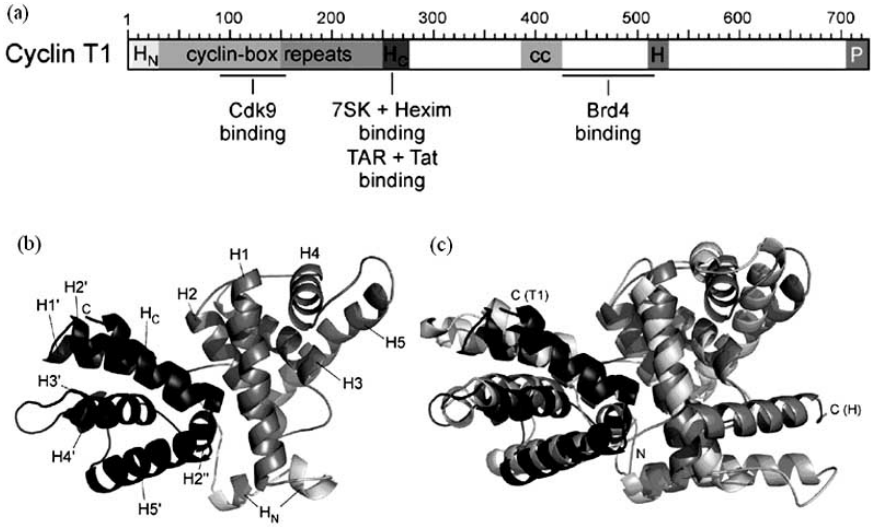

Fig. (2). Structure of the cyclin box domain of cyclin T1 [18]. (a) Schematic diagram of the modular domain organization of human CycT1. The N-terminal cyclin domain contains the two canonical cyclin-box repeats and accessory N and C-terminal helices. (b) Overall structure of human CycT1 (8–263). Helices of the two repeats are denoted H1-H5 and H1′-H5′. The N and C- termini (HN and HC) of α-helical repeats fold at the interface of the two repeats are shown respectively. (c) Superimposition of CycT1 and human cyclin H (RCSB accession number 1JKW). The two molecules are aligned on their cyclin box repeat structures (T1, 30–248; H, 49–262) resulting in an RMSD value of 2.2 Å.

helical repeats fold at the interface of the two repeats and are referred as HN and HC, respectively. Overall, the structure of Cyclin T1 (30–248), excluding both terminal helices, can superimpose cyclin H (RCSB accession number 1JKW). The higher structural and sequential similarity of the first repeat holds for cyclins in general indicates the conserved CDK-binding domain. On the opposite site of kinase-binding domain, there is a specific insert between helices H4 and H5 which may have potential to interact with effector molecules and is a potential target for research in the future. Also, the second repeat exhibits another characteristic. The specific insert between helices H3′and H4′ is unique to T-type cyclins and leads to an extended bulged loop with highly conserved aromatic residues [18].

STRUCTURE OF CDK9

CDK9 is a serine–threonine kinase involved in transcription and responsible for the activation of RNA polymerase II, which is a potential therapeutic target in human disease. Therefore, data on its 3D structure are essential for the development of new and specific inhibitors [19].

The human CDK9 protein was expressed in *E. coli* BL21 and purified from soluble fraction using ionic exchange and ATP-affinity chromatography [19]. Circular dichroism was used to determine the secondary structure of CDK9. The CDK9 CD spectrum revealed that it contains 30–34% helix, 33–36% beta, and 21–29% of coil.

The models for CDK9 show a typical bilobal structure (Fig. **3B**), in which the smaller N-terminal lobe consists of predominant β-sheet structure, which contains five antiparallel β-strands (β1–β5) and a single large helix (α1). The larger C-terminal lobe consists of α-helices primarily, with a pseudo-4-helical bundle (α2, 3, 4, 6), a small β- ribbon (β6–β8), and two additional helices (α5, 7) [19, 20]. The conserved core consists of a small ATP binding domain and a large lobe associated with peptide binding and catalysis [19]. The core (the β-sheet and the helical bundle) of the CDK9 structure is very similar to that of CDK2 [21, 22],as shown in Figs. (**3A-C**).

STRUCTURE ANALYSIS OF P-TEFB

The N-Terminus 188 Amino Acids of CycT1 is Necessary and Sufficient to Bind CDK9 *In Vivo*

To gain more insight in the critical region(s) of cyclinT1 responsible for interaction with both CDK9 and Tat, an extensive analysis of the *in vivo* interactions were performed using the yeast two- and three-hybrid systems [23]. CycT1 can interact efficiently with CDK9 in the two-hybrid interaction system. Through a series of cyclinT1 mutation experiment for the ability to associate with CDK9, it appeared that the region of cyclinT1 from amino acid 1 to 188 is necessary and sufficient to interact with CDK9 *in vivo* [23]. A similar conclusion has also been reached by others [24].

In Vivo Interaction Between CycT1 and Tat/TAR Requires the Integrity of the "Cyclin Box"

The ability of CycT1 to bind Tat protein has been previously reported and such interaction mediates Tat function by recruitment of P-TEFb complex to TAR. Experiments on the interaction of CycT1 or CycT1 mutants with Tat/TAR were carried out in a modified yeast three-hybrid system for finding the essential domain in CycT1. A series of CycT1 mutants were tested to identified a Tat/TAR recognition motif (TRM) at the carboxyl-terminal edge of "cyclin homology box" between aa 254 and 272. Moreover, no significant activation or LacZ gene was detected when CycT1(133△188) mutant bind to Tat/TAR. The CycT1(133△188) mutant encodes the first 487 amino acids of CycT1, with a deletion of 55 amino acids between 133 and 188 residues. This mutant encodes the TRM region (aa 254±272) and fails to bind Tat/TAR, which indicated that the integrity of the entire "cyclin box" is required for binding to Tat/TAR *in vivo* [23].

Cyclin T1 Mutants that Enhance Tat-Activation form a Complex with Tat on TAR RNA

Human cyclin T1 mutants experiments were performed to evaluate whether the ability of cyclin T1 mutants to enhance tat-activation in rodent cells correlated with the ability to form a complex with Tat bound to TAR RNA [25]. Tat formed a complex with the full-length human cyclin T1 protein, but did not form a complex with human cyclin T1 proteins containing either the amino-terminal 95 or 206 amino acid residues. In contrast, cyclin T1 mutants containing either the amino-terminal 298, 397, 405 or 595 amino acid residues formed gel-retarded complexes with Tat similar to those seen with full-length cyclin T1. Amino terminal deletions of cyclin T1 which removed either 66, 203, 301 or 400 amino acid residues, respectively, were unable to form a complex with Tat on TAR RNA. These results indicated that the ability of the amino terminus of cyclin T1 to form a complex with Tat on TAR RNA correlated with the ability of

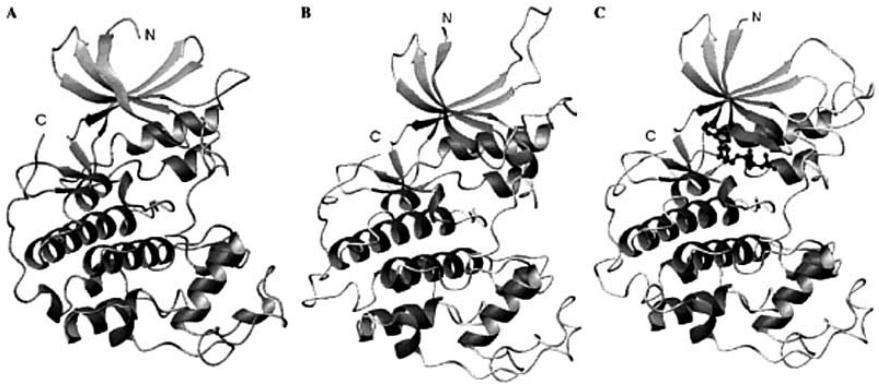

Fig. (3). Ribbon diagram of (A) CDK2 structure; (B) CDK9 model; and (C) CDK9:ATP model [19].

this portion of the cyclin T1 protein to markedly stimulate tat-activation of the HIV-1 long terminal repeat (LTR) [25].

THE FUNCTION OF P-TEFB IN THE RNA POLYMERASE II TRANSCRIPTION OF THE HIV-1 TAT TRANSACTIVATION

Transcription of the integrated provirus by RNA polymerase II is a key step in HIV-1 replication, which determines the amount of virus produced in the infected cell. The transcription of HIV-1 provirus can be divided into four steps: initiation, suspension, transactivation and transcription elongation, in which, P-TEFb plays an important role in the transcription stage.

(1) Initiation. CREB binding protein (P300/CBP), P300/CBP-associated factor (PCAF) and Histone acetyl transferase (HAT) are recruited to chromatin to acetylate the histone. The chromatin becomes loose in order to expose HIV-1 provirus genome. The 5′ LTR serves as the promoter of RNA polymerase II, and the transcription is initiated while RNA polymerase II binds to LTR [3, 26].

(2) Suspension. In the absence of viral trans-activator of transcription (Tat) protein, RNA polymerase II initiates transcription from the viral LTR, but is defective for elongation with the productive transcripts [20]. The suspension of transcription is achieved by the binding of 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) and negative elongation factor (NELF) bind to RNA polymerase II, which function together by blocking elongation [27].

(3) Transactivation. CyclinT1 binds to CDK9 to form P-TEFb. HAT p300 and the related p300/CBP and PCAF bind to Tat and acetylate the residue Lys28 thereby enhancing its transactivating function on HIV-1 LTR [28, 29]. Also, Ac28-Tat has high affinity for CycT1-CDK9 compared to PCAF. This results in increased binding of the Tat-P-TEFb complex to TAR RNA with the subsequently releases PCAF from Tat [30]. The full-length CycT1 protein is phosphorylated by CDK9, whereas ATP enhances binding to the TAR of both P-TEFb complexes [31]. The carboxyl terminal domain (CTD) of RNA polymerase II, the Spt5 subunit of DSIF and NELF are phosphorylated by Tat-P-TEFb-TAR complexes to overcome the action of negative factors that normally act to inhibit transcription elongation from the LTR [8, 32].

(4) Transcription elongation. A full-length RNA transcript is produced by RNA polymerase II [33]. P300/CBP is recruited to bind to Tat-P-TEFb-TAR complexes. The Ac50-Tat, acetylated by P300/CBP, has a lower affinity to Tarresulting in the release of P300/CBP-Tat-P-TEFb from Tar [34].

P-TEFB INHIBITORS

As mentioned above, human transcription elongation factor P-TEFb is a heterodimer consisting of CycT1 and CDK9. Whichever is inhibited, either CycT1 or CDK9 or the interaction between them, can repress P-TEFb function as Transactivation factor. Recently, a few P-TEFb inhibitors have been reported.

Flavopiridol

Flavopiridol is a semisynthetic small-molecular-weight derivative of rohitukine, an alkaloid isolated from *Dysoxylum binectariferum.* It is a CDK inhibitor and has been in clinical trials as an anticancer agent because of its antiproliferative properties [35].

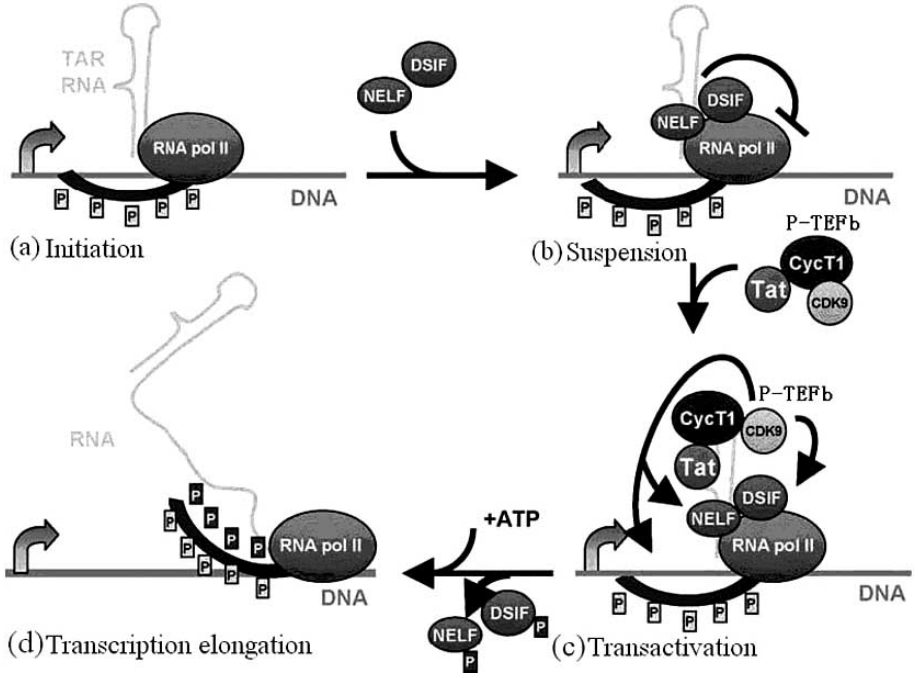

Fig. (4). The process of HIV-I Tat transactivation [5]. (a) RNA polymerase II binds to LTR to initiate transcription. (b) DSIF binds to RNA polymerase II, and they function together to block elongation. (c) With the aid of Tat-P-TEFb-TAR complexes, the CTD of RNA polymerase II, the Spt5 subunit of DSIF and NELF are phosphorylated, and, hence, transactivation is stimulated. (d) A full-length RNA transcription is produced by RNA polymerase II.

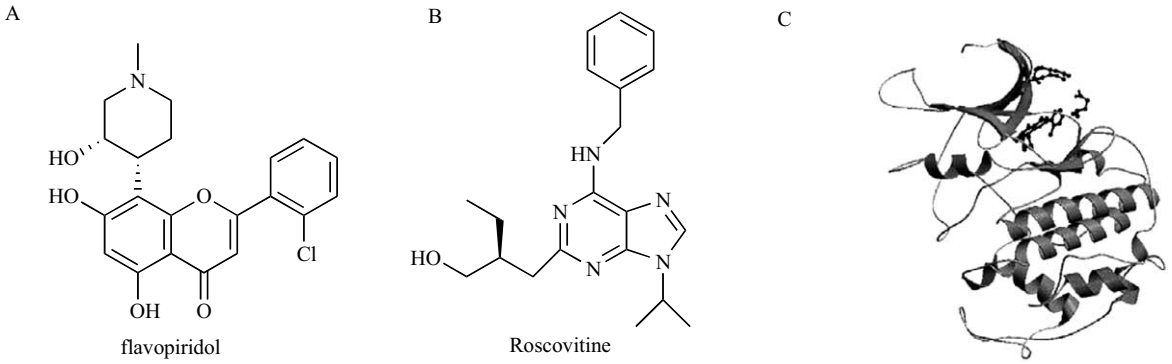

Fig. (5). (A, B) Structure of Flavopiridol and Roscovitine. (C) Ribbon diagram of the complex CDK9 with Flavopiridol.

Flavopiridol potently blocks transcriptional activity of RNA polymerase II *in vitro* by inhibiting P-TEFb, a process initially related to inhibition of HIV replication (IC_{50} < 10 nM) [36]. Flavopiridol inhibits the C-terminal domain kinase activity of P-TEFb. Furthermore, inhibition of CDK9 by flavopiridol was shown to be non-competitive with respect to ATP [37].

A number of studies elucidating the bonding model of the complex CDK9/Flavopiridol have been done in the past showing that the kinase is folded into the typical bilobal structure, with the smaller N-terminal lobe and the larger C-terminal lobe. The flavopiridol molecule is found to be in the cleft between the two lobes (Fig. **5**). Analysis of the interactions of flavopiridol with CDK9 indicates that flavopiridol binds to the ATP binding pocket of CDK9 with higher affinity than for CDK2 [20, 38]. *In vitro* and *in vivo*, flavopiridol inhibited transcription at concentrations much lower than those required for inhibition of other CDKs, even in the presence of ATP. DNA microarrays studies have demonstrated that flavopiridol inhibits gene expression by closely mimicking the actions of two specific transcription inhibitors (actinomycin D and DRB), and further indicating that flavopiridol might act as a global transcription inhibitor [39].

Roscovitine

Roscovitine has been reported to inhibit CDK1, -2, and -5 activity, but not of CDK3, -6, -7, -8, or -9. However, CDK9-cyclin T complex is critical in the control of the Tat protein function. New experiments were performed to examine the effect of Roscovitine on CDK9-cyclin T complex from both infected and uninfected cells. The IC_{50} value of Roscovitine on CDK9 was calculated to be~0.6 μM [40]. A recent study demonstrated that it effectively inhibited wild-type and resistant HIV-1 mutants in T cell lines, monocytes, and peripheral blood mononuclear cells (PBMCs) at low concentrations and sensitized these cells to apoptose resulting in a dramatic drop in viral titers [41].

7SK RNA

Two reports have shown a direct role of the most abundant human small nuclear RNA (snoRNA) 7SK in the inhibition of P-TEFb activity [42, 43]. Using different experimental approaches, Zhou and co-workers [42] and Bensaude and colleagues [43] identified the presence of two functionally different P-TEFb complexes *in vivo*. Both groups identified a P-TEFb-interacting RNA molecule capable of inhibiting its catalytic function. Sequence analysis of RNA molecule has confirmed that it is human snoRNA (7SK). Almost half of the P-TEFb complex in HeLa cells is associated to 7SK RNA, which impaired its catalytic and transcriptional activity. Disruption of the 7SK/P-TEFb complex using UV irradiation, actinomycin D treatments or RNA digestion led to increase of transcription elongation [42, 43]. Thus, there are two forms of P-TEFb complex which can be found *in vivo*, a free transcriptionally active complex and a 7SK-interacting form [44] (Fig. **6**). 7SK inhibits HIV-1 Tat specific transcriptional activities of P-TEFb *in vivo* and *in vitro* by inhibiting the kinase activity of CDK9 and preventing recruitment of P-TEFb to the HIV-1 promoter [42]. It has been proposed that

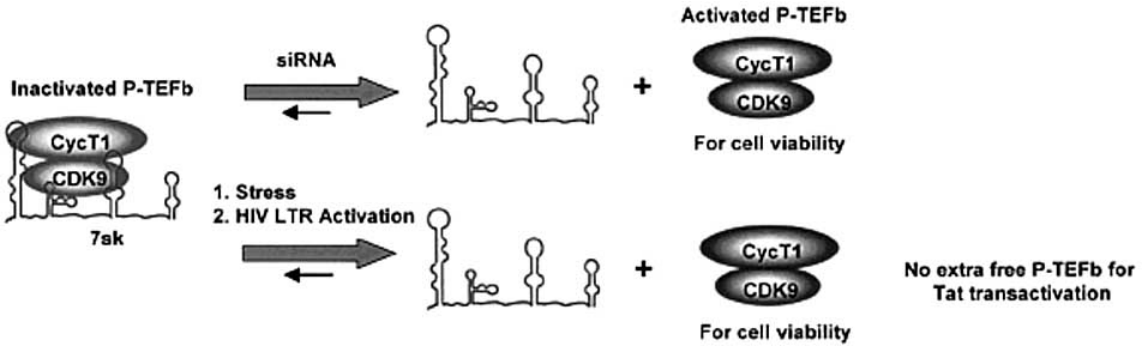

Fig. (6). Model of P-TEFb knockdown by siRNA treatment [44]. Under normal conditions, 7SK interacts with the majority of P-TEFb available in the cell, rendering it inactive, supported by biochemical data documenting strong interactions between 7SK and P-TEFb and its negative effect on P-TEFb kinase activity.

inhibition of CDK9 activity by 7SK might constitute a feedback loop modulating the efficiency of the elongation step of RNAPII-dependent gene transcription [43]. Therefore, the RNA-mediated regulation of transcription might be an evolutionarily conserved mechanism.

Also, the 7SK binding alone was not sufficient to inhibit P-TEFb [45]. All the 7SK-associated proteins from HeLa cells were screened for a factor(s) that could also interact with P-TEFb. One protein called HEXIM1 emerged from this screen. P-TEFb was inhibited by the HEXIM1 protein in a process that specifically required 7SK for mediating the HEXIM1: P-TEFb interaction [46], which allowed HEXIM1 to inhibit transcription both *in vivo* and *in vitro*. P-TEFb dissociated from HEXIM1 and 7SK in cells undergoing stress response leading to an increase in the level of active P-TEFb for stress-induced transcription. P-TEFb was the predominant HEXIM1-associated protein factor, and thus likely to be the principal target of inhibition coordinated by HEXIM1 and 7SK. Since HEXIM1 expression is induced in cells treated with hexamethylene bisacetamide, a potent inducer of cell differentiation, targeting the general transcription factor P-TEFb by HEXIM1/7SK may contribute to the global control of cell growth and differentiation [45].

PERSPECTIVES

To date, reverse transcriptase inhibitors, protease inhibitors and fusion inhibitors are widely used in clinic, but these drugs have all elicited resistance associated with the high rate of genetic variation of the HIV-1 genome. 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. P-TEFb influences a critical step, transactivation, which becomes a key target in the HIV-1 replication cycle. Using the conserved domain of CycT1 which is a part of P-TEFb, it is possible to make new inhibitors with low resistance in the near future therapies for HIV-1 infection.

ABBREVIATIONS

AIDS	=	Acquired immune deficiency syndrome
CDK9	=	Cyclin-dependent kinase 9
CTD	=	C-terminal domain
CHO	=	Chinese hamster ovary
DRB	=	5, 6-dichloro-1-beta-D-ribofuranosylbenzimidazole
DSIF	=	DRB-sensitivity inducing factor
EIAV	=	Equine infectious anemia virus
HIV	=	Human immunodeficiency virus
HAT	=	Histone acetyl transferase
LTR	=	Long terminal repeat
NELFs	=	Negative elongation factors
P-TEFb	=	Positive transcription elongation factor b
PBMCs	=	Peripheral blood mononuclear cells
PCAF	=	P300/CBP-associated factor
snoRNA	=	Small nucleolar RNA
Tat	=	Transactivator of transcription
TAR	=	Transactivation response element
TRM	=	Tat/TAR recognition motif

REFERENCES

[1] Butera, S.T. Therapeutic targeting of human immunodeficiency virus type-1 latency: current clinical realities and future scientific possibilities. *Antiviral Res*., **2000**, *48*, 143-76.

[2] Richman, D.D. Resistance of clinical isolates of human immunodeficiency virus to antiretroviral agents. *Antimicrob. Agents Chemother*., **1993**, *37*, 1207-13.

[3] Liou, L.Y.; Herrmann, C.H.; Rice, A.P. HIV-1 infection and regulation of Tat function in macrophages. *Int. J. Biochem. Cell. Biol*., **2004**, *36*, 1767-75.

[4] Ni, Z.; Saunders, A.; Fuda, N.J.; Yao, J.; Suarez, J.R.; Webb, W.W.; Lis, J.T. P-TEFb is critical for the maturation of RNA polymerase II into productive elongation *in vivo*. *Mol. Cell. Biol*., **2008**, *28*, 1161-70.

[5] Garriga, J.; Graña, X. Cellular control of gene expression by T-type cyclin/CDK9 complexes. *Gene*, **2004**, *337*, 15-23.

[6] Gargano, B.; Amente, S.; Majello, B.; Lania, L. P-TEFb is a crucial co-factor for Myc transactivation. *Cell Cycle*, **2007**, *16*, 2031-7.

[7] Brès, V.; Yoh, S.M.; Jones, K.A. The multi-tasking P-TEFb complex. *Curr. Opin. Cell. Biol.*, **2008**, *20*, 334-40.

[8] Mancebo, H.S.; Lee, G.; Flygare, J.; Tomassini, J.; Luu, P.; Zhu, Y.; Peng, J.; Blau, C.; Hazuda, D.; Price, D.; Flores, O. P-TEFb kinase is required for HIV Tat transcriptional activation *in vivo* and *in vitro*. *Genes Dev*., **1997**, *11*, 2633-44.

[9] Zhu, Y.; Pe'ery, T.; Peng, J.; Ramanathan, Y.; Marshall, N.; Marshall, T.; Amendt, B.; Mathews, M.B.; Price, D.H.; Transcription elongation factor P-TEFb is required for HIV-1 Tat transactivation *in vitro*. *Genes Dev*., **1997**, *11*, 2622-32

[10] Peng, J.; Marshall, N.F.; Price, D.H.; Identification of a cyclin subunit required for the function of *Drosophila* P-TEFb. *J. Biol. Chem*., **1998**, *273*, 13855-60.

[11] Peng, J.; Zhu, Y.; Milton, J.T.; Price, D.H.; Identification of multiple cyclin subunits of human P-TEFb. *Genes Dev*., **1998**, *12*, 755-62.

[12] Fu, T.J.; Peng, J.; Lee, G.; Price, D.H.; Flores, O. Cyclin K functions as a CDK9 regulatory subunit and participates in RNA polymerase II transcription. *J. Biol. Chem*., **1999**, *274*, 34527-30.

[13] Lin, X.; Taube, R.; Fujinaga, K.; Peterlin, B.M. P-TEFb containing cyclin K and CDK9 can activate transcription *via* RNA. *J. Biol. Chem*., **2002**, *277*, 16873-78.

[14] Wimmer, J.; Fujinaga, K.; Taube, R.; Cujec, T.P.; Zhu, Y.; Peng, J.; Price, D.H.; Peterlin, B.M. Interactions between Tat and TAR and human immunodeficiency virus replication are facilitated by human Cyclin T1 but not cyclins T2a or T2b. *Virology*, **1999**, *255*, 182-9.

[15] Taube, R.; Fujinaga, K.; Irwin, D.; Wimmer, J.; Geyer, M.; Peterlin, B.M. Interactions between equine cyclin T1, Tat, and TAR are disrupted by a leucine-to-valine substitution found in human cyclin T1. *J. Virol*., **2000**, *74*, 892-98.

[16] Garber, M.E.; Wei, P.; KewalRamani, V.N.; Mayall, T.P.; Herrmann, C.H.; Rice, A.P.; Littman, D.R.; Jones, K.A. The interaction between HIV-1 Tat and human cyclin T1 requires zinc and a critical cysteine residue that is not conserved in the murine CycT1 protein. *Genes Dev*., **1998**, *12*, 3512-27.

[17] Wei, P.; Garber, M.E.; Fang, S.M.; Fischer, W.H.; Jones, K.A. A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. *Cell*, **1998**, *92*, 451-62.

[18] Anand, K.; Schulte, A.; Fujinaga, K.; Scheffzek, K.; Geyer, M. Cyclin Box Structure of the P-TEFb subunit cyclin T1 derived from a fusion complex with EIAV tat. *J. Mol. Biol*., **2007**, *370*, 826–36.

[19] Leopoldino, A.M.; Canduri, F.; Cabral, H.; Junqueira, M.; de Marqui, A.B.; Apponi, L.H.; da Fonseca, I.O.; Domont, G.B.; Santos, D.S.; Valentini, S.; Bonilla-Rodriguez, G.O.; Fossey, M.A.; de Azevedo, W.F. Jr.; Tajara, E.H.; Expression, purification, and cir-

cular dichroism analysis of human CDK9. *Protein Exp. Purif*., **2006**, *47*, 614-20.

[20] de Azevedo, Jr. W.F.; Canduri, F.; Freitas da Silveira, N.J. Structural basis for inhibition of cyclin-dependent kinase 9 by flavopiridol. *Biochem. Biophys. Res. Commun*., **2002**, *293*, 566-71.

[21] Schulze-Gahmen, U.; De Bondt, H.L.; Kim, S.H. High-resolution crystal structures of human cyclin-dependent kinase 2 with and without ATP: bound waters and natural ligand as guides for inhibitor design. *J. Med. Chem*., **1996**, *39*, 4540-46.

[22] Canduri, F.; Uchoa, H.B.; de Azevedo, W.F. Jr. Molecular models of cyclin-dependent kinase 1 complexed with inhibitors. *Biochem. Biophys. Res. Commun*., **2004**, *324*, 661-66.

[23] Fraldi, A.; Licciardo, P.; Majello, B.; Giordano, A.; Lania, L. Distinct regions of cyclinT1 are required for binding to CDK9 and for recruitment to the HIV-1 Tat/TAR complex. *J. Cell. Biochem*., (Suppl) **2001**, *36*, 247-53

[24] Bieniasz, P.D.; Grdina, T.A.; Bogerd, H.P.; Cullen, B.R. Recruitment of cyclin T1/P-TEFb to an HIV type 1 long terminal repeat promoter proximal RNA target is both necessary and sufficient for full activation of transcription. Proc. *Natl. Acad. Sci. U. S. A*., **1999**, *96*, 7791-96.

[25] Ivanov, D.; Kwak, Y.T.; Nee, E.; Guo, J.; García-Martínez, L.F.; Gaynor, R.B. Cyclin T1 domains involved in complex formation with Tat and TAR RNA are critical for tat-activation. *J. Mol. Biol*., **1999**, *288*, 41-56.

[26] Bannwarth, S.; Gatignol, A. HIV-1 TAR RNA: The target of molecular interactions between the virus and its host. *Curr. HIV Res*., **2005**, *3*, 61-71.

[27] Nakatani, Y.; HIV-1 transcription: activation mediated by acetylation of Tat. *Structure*, **2002**, *10*, 443-44.

[28] Hottiger, M.O.; Nabel, G.J. Interaction of human immunodeficiency virus type 1 Tat with the transcriptional coactivators p300 and CREB binding protein. *J. Virol*., **1998**, *72*, 8252-56.

[29] Kiernan, R.E.; Vanhulle, C.; Schiltz, L.; Adam, E.; Xiao, H.; Maudoux, F.; Calomme, C.; Burny, A.; Nakatani, Y.; Jeang, K.T.; Benkirane, M.; Van Lint, C. HIV-1 tat transcriptional activity is regulated by acetylation. *EMBO J*., **1999**, *18*, 6106-18.

[30] Brès, V.; Tagami, H.; Péloponèse, J.M.; Loret, E.; Jeang, K.T.; Nakatani, Y.; Emiliani, S.; Benkirane, M.; Kiernan, R.E. Differential acetylation of Tat coordinates its interaction with the co-activators cyclin T1 and PCAF. *EMBO J*., **2002**, *21*, 6811-19.

[31] Garber, M.E.; Mayall, T.P.; Suess, E.M.; Meisenhelder, J.; Thompson, N.E.; Jones, K.A. CDK9 autophosphorylation regulates high-affinity binding of the human immunodeficiency virus type 1 Tat-P-TEFb complex to TAR RNA. *Mol. Cell. Biol*., **2000**, *20*, 6958-69.

[32] Kim, J.B.; Sharp, P.A. Positive transcription elongation factor B phosphorylates hSPT5 and RNA polymerase II carboxyl-terminal domain independently of cyclin-dependent kinase-activating kinase. *J. Biol. Chem*., **2001**, *276*, 12317-23.

[33] Brigati, C.; Giacca, M.; Noonan, D.M.; Albini, A. HIV Tat, its TARgets and the control of viral gene expression. *FEMS Microbiol. Lett*., **2003**, *220*, 57-65.

[34] Kaehlcke, K.; Dorr, A.; Hetzer-Egger, C.; Kiermer, V.; Henklein, P.; Schnoelzer, M.; Loret, E.; Cole, P.A.; Verdin, E.; Ott, M. Acetylation of Tat defines a cyclinT1-independent step in HIV transactivation. *Mol. Cell*., **2003**, *12*, 167-76.

[35] Zhai, S.; Senderowicz, A.M.; Sausville, E.A.; Figg, W.D. Flavopiridol, a novel cyclin-dependent kinase inhibitor, in clinical development. *Ann*. *Pharmacother*., **2002**, *36*, 905-11.

[36] Chao ,S.H.; Fujinaga, K.; Marion, J.E.; Taube, R.; Sausville, E.A.; Senderowicz, A.M.; Peterlin, B.M.; Price, D.H. Flavopiridol inhibits P-TEFb and blocks HIV-1 replication. *J. Biol. Chem*., **2000**, *275*, 28345-48.

[37] Chao, S.H.; Price, D.H. Flavopiridol inactivates P-TEFb and blocks most RNA polymerase II transcription *in vivo*. *J. Biol. Chem*., **2001**, *276*, 31793-99.

[38] Wang, S.; Fischer, P.M. Cyclin-dependent kinase 9: a key transcriptional regulator and potential drug target in oncology, virology and cardiology. *Trends Pharmacol. Sci*., **2008**, *29*, 302-13.

[39] Lam, L.T.; Pickeral, O.K.; Peng, A.C.; Rosenwald, A.; Hurt, E.M.; Giltnane, J.M.; Averett, L.M.; Zhao, H.; Davis, R.E.; Sathyamoorthy, M.; Wahl, L.M.; Harris, E.D.; Mikovits, J.A.; Monks, A.P.; Hollingshead, M.G.; Sausville, E.A.; Staudt, L.M. Genomic-scale measurement of mRNA turnover and the mechanisms of action of the anti-cancer drug flavopiridol. *Genome. Biol*., **2001**, *2*, research0041.1–0041.11.

[40] Wang, D.; de la Fuente, C.; Deng, L.; Wang, L.; Zilberman, I.; Eadie, C.; Healey, M.; Stein, D.; Denny, T.; Harrison, L.E.; Meijer, L.; Kashanchi, F. Inhibition of human immunodeficiency virus type 1 transcription by chemical cyclin-dependent kinase inhibitors. *J. Virol*., **2001**, *75*, 7266-79.

[41] Agbottah, E.; de La Fuente, C.; Nekhai, S.; Barnett, A.; Gianella-Borradori, A.; Pumfery, A.; Kashanchi, F. Antiviral activity of CYC202 in HIV-1-infected cells. *J. Biol. Chem*., **2005**, *280*, 3029-42.

[42] Yang, Z.; Zhu, Q.; Luo, K.; Zhou, Q. The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. *Nature*, **2001**, *414*, 317-22

[43] Nguyen, V.T.; Kiss, T.; Michels, A.A.; Bensaude, O. 7SK small nuclear RNA binds to and inhibits the activity of CDK9/cyclin T complexes. *Nature*, **2001**, *414*, 322-25.

[44] Chiu, Y.L.; Cao, H.; Jacque, J.M.; Stevenson, M.; Rana, T.M. Inhibition of human immunodeficiency virus type 1 replication by RNA interference directed against human transcription elongation factor P-TEFb (CDK9/CyclinT1). *J. Virol*., **2004**, *78*, 2517-29

[45] Yik, J.H.; Chen, R.; Nishimura, R.; Jennings, J.L.; Link, A.J.; Zhou, Q. Inhibition of P-TEFb (CDK9/cyclin T) kinase and RNA polymerase II transcription by the coordinated actions of HEXIM1 and 7SK snRNA. *Mol. Cell*., **2003**, *12*, 971-82.

[46] Yik, J.H.; Chen, R.; Pezda, A.C.; Samford, C.S.; Zhou, Q. A human immunodeficiency virus type 1 Tat-like arginine-rich RNA-binding domain is essential for HEXIM1 to inhibit RNA polymerase II transcription through 7SK snRNA-mediated inactivation of P-TEFb. *Mol. Cell. Biol*., **2004**, *24*, 5094-105.

Received: 22 August, 2008 **Revised: 06 November, 2008** **Accepted: 08 November, 2008**