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PROSPECTS

# Regulatory Functions of Cdk9 and of Cyclin T1 in HIV Tat Transactivation Pathway Gene Expression

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**Abstract** HIV-1 gene expression relies upon a complex machinery that is primarily controlled by two viral regulatory proteins, Tat and Rev. Rev is involved in regulating post-transcriptional events of HIV-1 gene expression. The Tat protein transactivates transcription from the HIV-1 5′ long terminal repeat (LTR) and acts in synergy with specific cellular factors. Recently, it has been shown that one set of these cellular factors is a protein kinase activity termed TAK (Tat-associated kinase), which activates transcription by hyperphosphorylation of the carboxyl-terminal domain (CTD) of the large subunit of RNA polymerase II. TAK also enhances transcription of HIV-2, together with the retroviral transactivator, Tat-2. The TAK activity appears to be related to the CTD kinase P-TEFb, which stabilizes transcription elongation of many genes and was originally isolated from *Drosophila* extracts. Both TAK and P-TEFb contain at least two subunits: the cyclin-dependent kinase, CDK9 (PITALRE), the catalytic subunit, and the regulatory subunit, cyclin T1. CDK9 and cyclin T1 are ubiquitous factors that affects many cellular processes, including cell differentiation and apoptosis. The involvement of TAK in HIV-1 and HIV-2 gene expression is an important aspect in the biology of these two retroviruses, and may lead to the development of novel antiretroviral drugs and/or gene therapy approaches for the treatment of patients with AIDS. J. Cell. Biochem. 75:357–368, 1999. 

**Key words:** PITALRE; Cdk9; cyclin T1; HIV; Tat; AIDS; antiretroviral therapy; gene therapy

There is a growing interest in developing novel therapeutic approaches to treat patients with the acquired immunodeficiency syndrome (AIDS). Indeed, the efficacy of antiretroviral therapies has remarkably improved in establishing a long-term suppression of human immunodeficiency virus type 1 (HIV-1) replication. This is clearly shown by the increased average survival period of patients with AIDS [Carpenter et al., 1997; Cavert et al., 1997; Gulick et al., 1997; Hammer et al., 1997; Palella et al., 1998; Wong et al., 1997], and by the substantial decline of the AIDS death rate during the last three years in industrialized countries [Global programme on AIDS, 1995]. To date, 11 antiretroviral compounds are currently used in AIDS therapy, however, they only target two viral enzymes: the reverse transcriptase and the protease [reviewed by Romano et al., 1998]. These drugs are more effective if administered in combination to patients, as they can delay the emergence of HIV-1 strains resistant to antiretroviral therapy [Gulick et al., 1997; Hammer et al., 1997; Foundraine et al., 1998; Weverling et al., 1998]. However, the onset of AIDS in patients is only a matter of time, as HIV-1 continues to mutate while replicating at high rates in the host over the entire course of infection [Embretson et al., 1993; Ho et al., 1995; Mansky et al., 1995; Nowak et al., 1991; Pantaleo et al., 1993; Wei et al., 1995], and may generate viral variants resistant to multidrug therapy. This event will result in acute syndrome and death of the patients. In

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addition, this represents a serious threat to the recent achievements in AIDS research and therapy programs. Worryingly, there has already been a number of reports of transmission of HIV-1 strains resistant to multidrug therapy [Angarano et al., 1994; Conlon et al., 1994; Erice et al., 1993; Hechts et al., 1998; Imrie et al., 1997; Ippolito et al., 1994; Veenstra et al., 1995]. The report by Hechts et al. [1998] raises additional concern, as the HIV-1 variant that was transmitted was resistant to both reverse transcriptase and to protease inhibitors. On these grounds it is essential to broaden the spectrum of therapeutics for the treatment of patients with AIDS. In this respect the HIV-1 accessory proteins (Vif, Vpr, Vpu, and Nef), the regulatory proteins (Tat and Rev), and the viral integrase are very attractive targets. This task now seems more feasible, if one considers the progress that has recently been achieved in understanding the biology and the pathogenesis of HIV-1 [Romano et al., 1998], which causes a wide variety of illnesses, such as: neurologic disorders, development of opportunistic diseases, onset of AIDS-related malignancies and higher incidence of other types of neoplasias, nephropathies, anemia, and cardiopathies.

The field of AIDS research is evaluating a variety of new therapeutic interventions. These include the production of chemokine-like molecules to inhibit viral infection; the generation of novel antiretroviral drugs to target other HIV-1 factors, in addition to the reverse transcriptase and protease; the development of gene therapy and of vaccines programs. The final aim of these new therapy programs is to eradicate HIV-1 from patients. In order to do so, it is also necessary to suppress HIV-1 in cells which are latently infected (quiescent T cells and promonocytes), and in parts of the body that are hard to reach, such as the central nervous system (CNS).

Among the various significant discoveries in the area of HIV-1 biology, the recent finding that the Tat associated kinase activity (TAK) is involved in the Tat transactivation pathway is of considerable interest [Cullen et al., 1998; Emerman and Malim, 1998; Jones, 1997]. Interestingly, TAK also contrives with Tat-2 in transactivating transcription from the HIV-2 5′-LTR [Herrmann and Rice, 1993; Yang et al., 1996]. The involvement of TAK in the Tat-mediated transactivation of HIV-1 and HIV-2 transcription will inevitably lead to greater understanding of viral gene expression, along with many other aspects of the biology of these two retroviruses, including the mechanism of gene regulation in latently infected cells. Two components of the TAK complex have been recently identified and are structurally related to critical cell cycle regulatory molecules. These cellular components comprise CDK9, a CDC2-related serine/threonine kinase, and its activating subunit, cyclin T1. While these molecules themselves are not involved in regulating the cell cycle, perhaps the information gained regarding these proteins may also result in therapeutic applications in the future. Indeed, some preliminary reports have shown an in vitro suppression of TAK transactivation by certain chemical inhibitors that target serine/threonine kinases [Mancebo et al., 1997]. Similar results were obtained in in vivo studies expressing a kinase inactive form of CDK9 [Mancebo et al., 1997; Gold et al., 1998], although the inhibitory effects of the mutant form on Tat protein function may be restricted to specific cell lines [Gold et al., 1998]. Taken together, these findings underline once more the relevance of TAK activity in HIV-1 and HIV-2 gene expression, and encourage the pursuit of research programs to develop novel antiretroviral inhibitors and/or gene-based therapy programs for the treatment of patients with AIDS.

## The Essential Role of Tat Protein in the Biology of HIV-1

The optimization of transcription levels is a key factor for the replication of HIV-1 and HIV-2, both of which encode for two closely related transactivator proteins named Tat-1 and Tat-2, respectively [reviewed by Jones and Peterlin, 1994]. For the sake of simplicity, and considering that HIV-1 and HIV-2 have a very similar biology, this review will focus on HIV-1 and Tat-1 will be referred to as Tat.

The genomic organization of HIV-1 is rather complex (Fig. 1), and has a size of 9.8 kb [Greene, 1991; Vaishnav and Wong-Staal, 1992]. In addition to the *gag, pol*, and envelope (*env*) genes, there are at least six other open reading frames (ORF) encoding for four accessory proteins (Vif, Vpr, Vpu, and Nef) and two regulatory proteins (Tat and Rev). The 5′- and the 3′-LTR contain the signals for integration into the cell's chromosomal DNA, for reverse transcription (the primer binding site for transfer RNA), the packaging signal ($\Psi$) in the untranslated 5′-mRNA

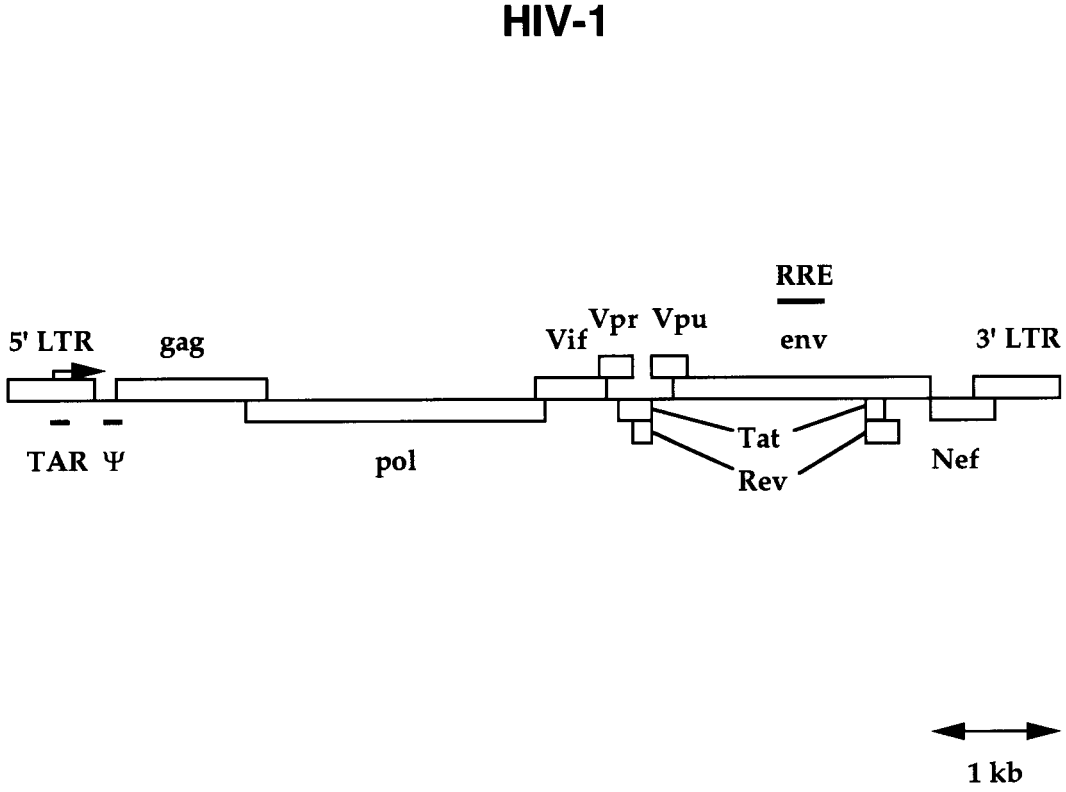
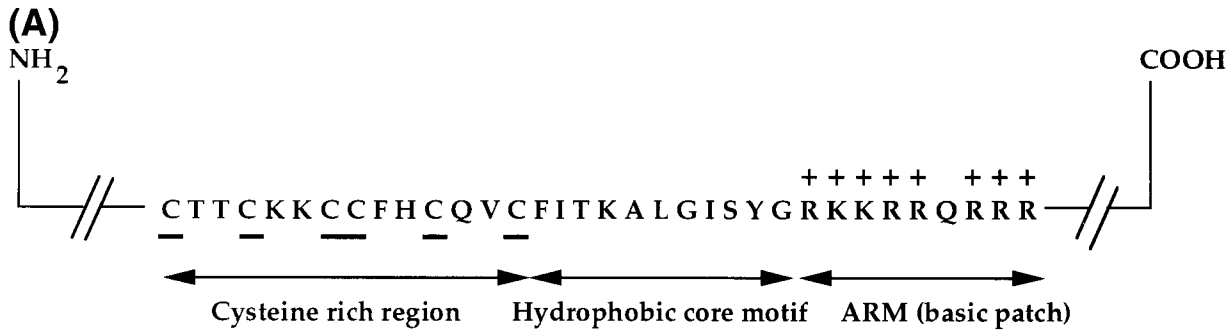



**Fig. 1.** The genome of HIV-1. LTR, long terminal repeat; TAR, transactivation response RNA element; Ψ, packaging signal; env, envelope; RRE, rev-response-element.

[Lever et al., 1989], and the polyadenylation signal in the 3′-LTR.

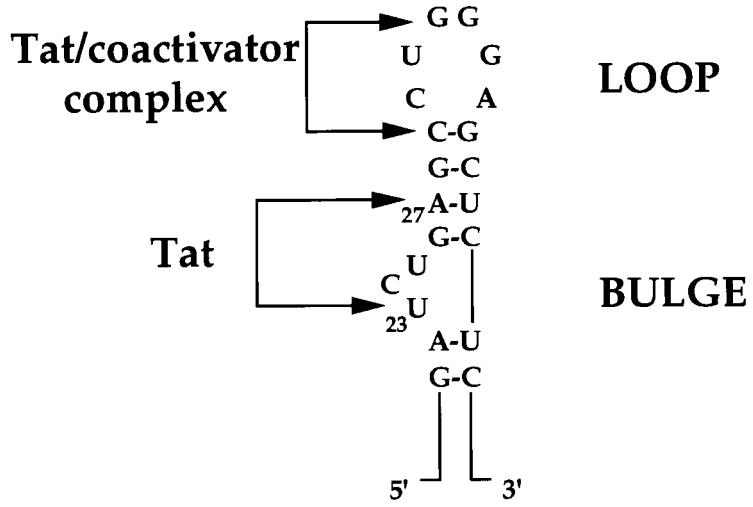
The primary transcript is the full-length viral mRNA, which is translated into the Gag and Pol proteins. Other HIV-1 proteins, such as Tat, Rev, Env, Vif, Vpr, Vpu, and Nef, originate from the translation of subgenomic mRNAs derived from different splicing events. The HIV-1 mRNA splicing pathway is orchestrated by the Rev protein, which is itself the product of spliced mRNA [Feinberg et al., 1986; Sodroski et al., 1986; Steffi et al., 1992]. The Rev and Tat proteins share a number of similarities, as they both bind to HIV-1 mRNA via a distinct small basic region [reviewed by Jones and Peterlin, 1994; Cullen, 1994]. The structure of the HIV-1 RNA-binding domain of Tat is shown in Figure 2A. The HIV-1 RNA motif recognized by the Rev protein is termed the rev-response-element (or RRE, see Fig. 1) [reviewed by Cullen, 1994]. The Tat protein activates transcription by binding to the transactivation response (TAR) RNA element (Fig. 2B), which is situated at the 5′-end of nascent retroviral transcripts [Rosen et al., 1985; Jakobovits et al., 1988; Garcia et al., 1988; Selby et al., 1989]. All HIV-1 mRNAs contain both RRE [reviewed by Cullen, 1994] and TAR sequences [Muesing et al., 1987].

As already mentioned, Tat is a potent transactivator of the HIV-1 5′-LTR, and is essential for efficient viral replication [reviewed by Jones and Peterlin, 1994]. An additional three *cis*-acting elements of the HIV-1 5′-LTR are also absolutely required for in vivo and in vitro Tat transactivation: the TATA box, a binding site for the Sp1 transcription factor and the TAR RNA element [Jones and Peterlin, 1994]. The TAR RNA sequence forms a stable stem-loop structure [Muesing et al., 1987] that is required for the Tat transactivation response [Berkhout et al., 1989]. Without the Tat protein, transcription from the HIV-1 5′-LTR would terminate within the first 60 ribonucleotides [Kao et al., 1987; Laspia et al., 1989; Feinberg, 1991; Marciniak and Sharp, 1991]. However, a number of studies also indicate that the Tat protein induces transcription activation from the HIV-1 5′-LTR in a TAR-independent fashion in some cell lines derived from the CNS [reviewed by




HIV-1 Tat protein

(B)




HIV-1 TAR

**Fig. 2.** **A**: Structure of the transcriptional activation domain of HIV-1 Tat protein. ARM, arginine-rich RNA-binding motif. **B**: The sequence of the RNA hairpin of the HIV-1 TAR element. Tat protein binds to the base triple of the bulge, whereas the loop sequence is thought to interact with a Tat-coactivator complex.

Taylor and Khalili, 1994; Yang et al., 1997; Buzy et al., 1995].

The transcriptional activation domain of Tat comprises a cysteine-rich region, followed by a hydrophobic core motif and by a basic patch. The basic patch consists of an arginine-rich RNA-binding motif (ARM, as shown in Fig. 2A), which allows binding of Tat to the base triplet in the bulge region of TAR (Fig. 2B) [Liu et al., 1996; Tao et al., 1997]. The base triplet struc-

ture in the bulge derives from Hoogstein interaction between U23 and AU27 in the upper stem (Fig. 2B) [Tao et al., 1997]. The HIV-1 TAR loop (Fig. 2B) appears to interact with a Tat-coactivator complex [reviewed by Jones, 1997; Zhou and Sharp, 1995]. Mutational analysis of the TAR loop sequence revealed a block necessary for Tat transactivation in vivo, without affecting the Tat-TAR interaction [reviewed by Cullen, 1990], suggesting the presence of a Tat-dependent cellular activity specific for the TAR loop sequence. A number of cellular proteins have been identified and shown to interact with the HIV-1 TAR loop sequence, however, none of them are essential for Tat transactivation of the HIV-1 5′-LTR [Gatignol et al., 1989; Marciniak et al., 1990b; Sheline et al., 1991; Wu et al., 1991]. TAK has also been proposed as the Tat-cofactor specific for the HIV-1 TAR stem loop sequence [Herrmann and Rice, 1995]. Additional cellular factors have been shown to bind in vitro to the bulge of HIV-1 TAR [Sheline et al., 1991], the upper stem [Gatignol et al., 1991], the lower stem [Gunnery et al., 1992], and to a combination of lower stem bulge and A27 of the upper stem [Rounseiville and Kumar, 1992]. However, the biochemical functions of these HIV-1 TAR bulge- and/or stem-binding factors have not been completely characterized, as are the various Tat-binding cellular proteins that have been identified [Desai et al., 1991; Nelbock et al., 1990; Ohana et al., 1993]. The HIV-1 5′-LTR can also be transactivated by a number of other viral factors including Epstein-Barr virus (EBV) [Lin, 1993; Quinlivan et al., 1990; Romano et al., 1997], herpes simplex virus (HSV) [Albrecht et al., 1989; Chapman et al., 1991], human herpesvirus 6 (HHV-6) [Geng et al., 1992; Di Luca et al., 1991], and cytomegalovirus (CMV) [Davis et al., 1987].

Another pathway for transcription activation of the HIV-1 5′-LTR is represented by the cellular factor NF-κB, which is induced by a variety of cytokines, and is inhibited by the HIV-1 accessory proteins Vpr and Nef [reviewed by Romano et al., 1998]. Interestingly, it has been found that NF-κB is involved in the TAR-independent Tat transactivation of the HIV-1 5′-LTR in certain cell lines derived from the CNS [Yang et al., 1997].

In vivo systems using transfected cell lines indicate that the Tat protein stabilizes the elongation of transcripts from the HIV-1 5′-LTR, and this stimulates a modest increase of the initiation rate [Kao et al., 1987; Laspia et al., 1989; Feinberg et al., 1991]. In in vitro cell extracts, however, Tat appears to only have an effect on the processivity of HIV-1 mRNA elongation [Marciniak and Sharp, 1991; Kato et al., 1992]. Conversely, in the CNS-derived U87-MG cell line, a cell line that supports TAR-independent Tat transactivation of the HIV-1 5′-LTR, the effects of the Tat protein are predominantly on the increase of transcription initiation in a TAR-deleted HIV-1 5′-LTR reporter construct. With TAR-containing LTR constructs, however, the Tat protein mediates its effects through stabilization of RNA elongation [Yang et al., 1997]. These findings suggest the existence of two alternative Tat transactivation pathways in CNS cell lines; one TAR dependent and the second TAR independent. In the in vitro system, the stimulation of transcription from the HIV-1 5′-LTR by Tat is in the range of a 10-fold increase [Marciniak et al., 1990a]. In the in vivo models, however, Tat stimulates a 100-fold activation observed in the in vivo models [Feinberg et al., 1986; Peterlin et al., 1986; Rosen et al., 1986; Wright et al., 1986; Hauber et al., 1987; Muesing et al., 1987; Rice and Mathews, 1988; Sadaie et al., 1988]. This finding strongly suggests that some cellular factors involved in the Tat transactivation pathway of HIV-1 5′-LTR are much more effective in intact cells in vivo than in cell-free systems. The Tat-induced increase of the initiation rate of transcription from the HIV-1 5′-LTR is too modest [reviewed by Jones, 1997] to justify the discrepancies between the in vivo and in vitro systems. Taken together, these findings indicate that the main function of the Tat protein is to stabilize the elongation of HIV-1 mRNA, and that certain cellular factors are required to optimize Tat transcription of the HIV-1 5′-LTR.

Interestingly, it has been observed that Tat transactivation of the HIV-1 5′-LTR is blocked by 5,6-dichloro-1-β-ribofurosylbenzimidazole (DRB) [Marciniak and Sharp, 1991], which is a purine nucleoside analog that inhibits early transcription elongation by inactivating protein kinases [Zandomeni and Weinmann, 1984; Zandomeni et al., 1986]. In fact, two kinase complexes have been found to be involved in the Tat transactivation pathway, TFIIH [Parada and Roeder, 1996; Garcia-Martinez et al., 1997; Cujec et al., 1997] and TAK [Herrmann and Rice, 1993, 1995; Yang et al., 1996; Zhu et al., 1997; Mancebo et al., 1997]. These findings can

be considered a breakthrough in understanding the transcriptional regulation mechanism of HIV-1, although many aspects still need further investigation. The Tat-mediated induction of the HIV-1 5′-LTR has been shown to be a useful model to study the mechanism of transcription in eukaryotic inducible genes. In such systems, the RNA polymerase II (RNA pol II) complexes pause shortly after the initiation of RNA transcription [Blair et al., 1996; Blau et al., 1996; Bentley, 1995]. Generally, the RNA elongation comes to a stop in the first 20 or 60 nucleotides downstream of the promoter. In the presence of either DNA- or RNA-binding factors, the elongation of RNA transcription is resumed. The function of these activators consists of either attracting and/or stimulating other cellular factors to rescue the stalled RNA elongation complexes, or to remove cellular inhibitors of transcription.

The HIV-1 TAR-dependent transactivation by the Tat protein is linked to the CTD of RNA polymerase II (RNA pol II) [Chun and Jeang, 1996; Okamoto et al., 1996; Parada and Roeder, 1996; Yang et al., 1996]. The tandem heptapeptide repeat (YSPTSPS) in the CTD of RNA pol II shows a differential phosphorylation pattern during the various phases of the transcription process [reviewed by Dahmus, 1996]. The CTD of RNA pol II is not phosphorylated in preinitiation complexes or in RNA elongation complexes paused at a very early stage of transcription initiation. Conversely, processive RNA pol II molecules have a hyperphosphorylated CTD. The enhanced RNA pol II processivity originates from conformational changes following the hyperphosphorylation of CTD. Hyperphosphorylation of the CTD is thought to release the elongation complex from the promoter and abolish interactions with other transcription factors that are not associated with elongating RNA pol II complexes [Svejstrup et al., 1997; Zawel et al., 1995], such as the TATA-binding protein.

According to in vitro and in vivo data [reviewed by Jones, 1997], the TFIIH complex appears to be essential to regulate promoter clearance and the early steps of transcription initiation [Dvir et al., 1996; Goodrich and Tjian, 1994]. The TAK complex, on the other hand, is required to sustain RNA pol II processivity during transcription elongation [Marshall and Price, 1992; Marshall et al., 1996; Zhu et al., 1997]. Several lines of evidence indicate there is an interaction between Tat and the TFIIH complex [Parada and Roeder, 1996; Cujec et al., 1997], and between Tat and the TAK complex [Herrmann and Rice, 1993, 1995; Yang et al., 1996; Zhu et al., 1997]. Interestingly, it has been recently demonstrated that PITALRE (or Cdk9) is the kinase required for TAK activity [Zhu et al., 1997].

A possible interpretation of the dynamics of Tat transactivation of the HIV-1 5′ LTR can be summarized as follows: 1) The Tat protein associates with various components of the TFIIH complex in the RNA pol II holoenzyme and in the preinitiation complex. 2) In parallel the Tat protein also associates with the TAK complex. 3) The TFIIH complex, possibly in concert with the Tat protein, triggers promoter clearance and transcription initiation, during which the CTD of RNA pol II is partially phosphorylated, causing the release of the mediator [Svejstrup et al., 1997], and the detachment of the preinitiation complex from other transcription factors of the promoter [Zawel et al., 1995]. 4) The TFIIH/Tat complex falls off the elongating RNA pol II after the first 20–60 nucleotides have been transcribed. 5) Simultaneous with the dissociation of the TFIIH/Tat complex, the nascent HIV-1 TAR RNA recruits the TAK/Tat complex, which hyperphosphorylates the CTD of RNA pol II, and, consequently, enhances the processivity of the transcription elongation complex.

The mechanism of Tat-2 transactivation of the HIV-2 5′-LTR mirrors that of the HIV-1 Tat protein, with a minor difference. The carboxyl-terminus of Tat-2 is phosphorylated in vitro and in vivo by the TAK complex [Dahmus, 1995; Finzi and Siciliano, 1998], while HIV-1 Tat protein is not.

### The TAK/Tat Complex

Recent reports indicate that the human TAK complex corresponds to the *Drosophila* positive acting transcription factor P-TEFb [Zhu et al., 1997; Mancebo et al., 1997]. TAK and P-TEFb complexes contain a serine/threonine kinase that phosphorylates the CTD of RNA pol II, a cyclin partner required for kinase activity, and a number of subunits that have yet to be identified [reviewed by Jones, 1997]. The kinase subunit of *Drosophila* P-TEFb shares 72% identity at the amino acid level with its human analog, CDK9 [Grana et al., 1994; Zhu et al., 1997; De Falco and Giordano, 1998], a CDC2-related kinase [Marshall and Price, 1992; Marshall et

al., 1996; Grana et al., 1994; De Falco and Giordano, 1998]. CDK9's cyclin partner in human P-TEFb complexes can be either cyclin T1, T2a, or T2b (collectively referred to as cyclin T) [Peng et al., 1998], whereas CDK9 in TAK complexes appears to solely associate with cyclin T1 [Wei et al., 1998; Yang et al., 1997; Zhu et al., 1997]. The exact dynamics of the interaction between the Tat protein and the TAK complex are currently under investigation. In vitro data show an electrophoretic profile of the TAK activity complex with an apparent molecular weight of 110 kd [Yang et al., 1996], which probably consists of CDK9 and cyclin T1. So far, there is no evidence for a direct association between CDK9 and the Tat protein. Instead, it appears that cyclin T1 specifically binds the Tat activation domain, which is required to enhance the binding affinity of Tat to the HIV-1 TAR RNA [Wei et al., 1998]. Thus, the recruitment of CDK9 to HIV-1 TAR RNA occurs through cyclin T1.

Cyclins and cyclin dependent kinases (CDKs) were originally identified as critical regulators of the cell cycle [for review see Morgan, 1997]. Cyclins are the activating subunit for CDKs and were originally named for their cyclical expression during the cell cycle; only during specific phases of the cell cycle would various cyclins be present and only then would their CDK partners be active. Therefore, the activity of CDK/cyclin complexes is typically dependent on the cell cycle stage. These CDK/cyclin complexes phosphorylate, and thereby regulate, proteins crucial to cell cycle progression. However, other CDK/cyclin complexes, such as CDK7/cyclin H and CDK8/cyclin C, are regulators of transcription. In turn, the activity of this subgroup of kinases is not modulated during the cell cycle. To date, all the CDK complexes involved in transcription are able to phosphorylate the CTD of RNA polymerase II in vitro. The CDK9/cyclin T complex falls into this transcription regulatory group of CDKs; its activity does not vary during the course of the cell cycle and it phosphorylates the CTD of RNA polymerase II. Figure 3 shows the functions of CDK9 in the biology of the cell. Phosphorylation of the CTD by CDK9/cyclin T complexes appears to enable

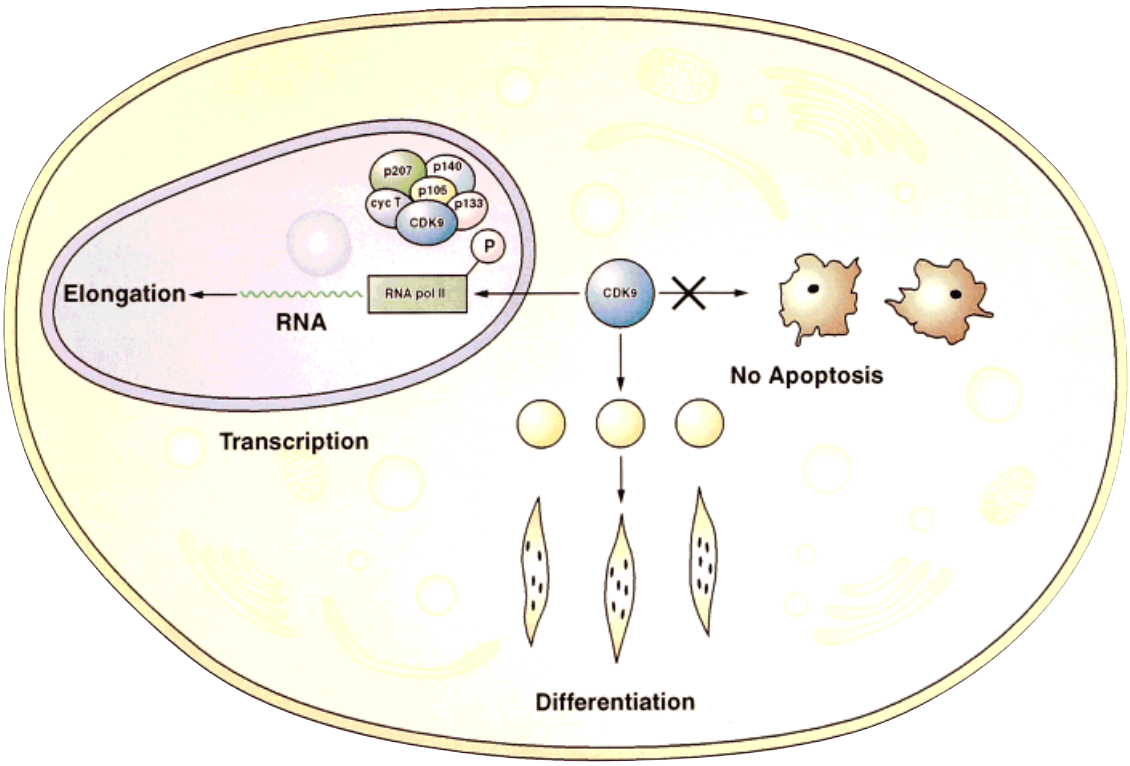



**Fig. 3.** Functions of CDK9 in the biology of the cell. Reprinted with permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc. [De Falco and Giordano, 1998].

RNA polymerase II to enter productive elongation by counteracting negative factors [Marshall et al., 1996]. It is proposed that this is the mechanism by which Tat and the TAK complex stimulate gene expression from the HIV LTR [Jones and Peterlin, 1994].

Many other lines of investigation underlie the crucial role of the TAK complex in the Tat transactivation pathway. For instance, it is of great interest that the expression of human cyclin T1 allows for Tat transactivation of the HIV-1 5′-LTR in murine cells, which normally are not permissive for Tat-mediated transactivation via TAR RNA [Wei et al., 1998]. This finding might have implications for the development of appropriate transgenic murine models susceptible to HIV-1 infection, which would greatly facilitate the study of in vivo viral transmission, and the in vivo analysis of novel inhibitors of viral replication. A recent study has identified the human cyclin T1 binding region to the Tat protein and to HIV-1 TAR RNA in the first 281 amino acid residues [Fujinaga et al., 1999]. Interestingly, the mouse cyclin T1 has a cysteine to tyrosine substitution at position 261 [Fujinaga et al., 1999; Garber et al., 1998], which seems to be the cause of the weak binding affinity to the Tat protein, and of the inability of mouse cyclin T1 to mediate the interaction of Tat with HIV-1 TAR RNA [Fujinaga et al., 1999; Wei et al., 1998]. In fact, if the tyrosine residue at position 261 is changed to a cysteine residue, mouse cyclin T1 becomes functional in the context of the HIV-1 TAR-dependent Tat transactivation pathway [Fujinaga et al., 1999]. Conversely, if the cysteine residue at position 261 of human cyclin T1 is changed to a tyrosine residue, the mutated human cyclin T1 behaves like the wild type mouse cyclin T1, and, therefore, does not permit Tat transactivation of the HIV-1 LTR [Fujinaga et al., 1999].

Other important findings are the high expression levels of CDK9 detected in primary human lymphoid tissues [De Luca et al., 1997], the ability of Tat mutant proteins to support Tat transactivation [Herrmann and Price, 1993; Herrmann et al., 1995; Yang et al., 1996], and the in vitro and in vivo inhibitory effects of a kinase inactive CDK9 over the Tat transactivation of the HIV-1 5'-LTR [Gold et al., 1998; Mancebo et al., 1997], which appear to be cell type dependent [Gold et al., 1998].

It should be noted that the regulation of TAK activity relies upon different mechanisms in various cell lines [Herrmann et al., 1998]. Up-regulation of TAK activity has been detected in activated peripheral blood lymphocytes (PBLs) and in promonocytic cell lines upon stimulation to differentiate into macrophage-like cells. Specifically, phorbol ester-activation of PBLs determines an up-regulation of TAK activity that correlates with enhanced expression levels of both CDK9 and cyclin T1. A different pattern of TAK activity up-regulation has been observed in phorbol ester-induced promonocytic cells differentiating into macrophages. In this case there is only an increase in cyclin T1 expression levels, whereas CDK9 expression remains constant.

## CONCLUSION

In conclusion, the study of TAK activity in HIV-1 infected PBLs and in cells of monocytic lineage will undoubtedly provide useful insights into the pathobiology of HIV-1. These insights may have particular relevance with regard to the establishment of viral transcription latency and in the mechanism of HIV-1 gene expression activation in chronically infected quiescent T cells and promonocytes. In addition, it will be interesting to determine whether the TAK/Tat complex is also involved in the TAR-independent Tat transactivation observed in some CNS-derived cell lines. A better understanding of TAK/Tat interaction may also lead to the design of novel antiretroviral inhibitors, and/or to the development of effective gene therapy approaches for the treatment of patients with AIDS.

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