Control of RNA initiation and elongation at the HIV promoter

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Abstract

Regulation of HIV-1 transcription involves a complex interplay between cis-acting DNA and RNA elements present within the chromatin-associated, proviral long terminal repeats (LTRs), cellular transcription factors (DNA targeted), and the viral trans-regulatory protein Tat (RNA targeted). Recently, it has been observed that Tat associates with a cellular kinase that phosphorylates the carboxyl-terminal domain of RNA polymerase II and hence stimulates transcriptional elongation. Here we review the control of HIV-1 transcription from the LTR promoter. We describe the HIV-1 promoter elements, how the cellular RNA polymerase II transcription complex initiates, and how Tat stimulates this transcription. Furthermore, in brief, we give a picture of the activation of the chromatin-associated integrated provirus and the importance of post-translational modification of Tat.

Key words

HIV. Tat. Elongation. Transcription. Activation

The HIV-1 promoter

Transcription of the integrated proviral DNA is initiated at the HIV-1 promoter, which is located in the U3 region of the 5' long terminal repeat (LTR). It contains a number of regulatory elements important for RNA polymerase II transcription. An important feature of the HIV-1 LTR is the presence of sites for several cellular transcription factors located both upstream and downstream of the start site[1] (Fig. 1). These cellular factors help control the rate of transcription initiation from the integrated provirus, and their abundance in different cell types, or at different times, likely determines whether a provirus is quiescent or actively replicating. The LTR can be divided into several discrete functional domains: the upstream regulatory region containing elements for cell type-specific expression, the enhancer, the basal promoter, TAR, and the downstream regulatory region.

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The upstream regulatory region contains numerous protein-binding sites and many are occupied, as observed by foot-printing[2]. These include binding sites for LEF, Ets-1, USF, C/EBP, AP-1, COUP, and NF-AT. This region appears to be a modulator region capable of up- or down-regulating virus expression, depending on the cell type and activation state of the cell. For example, the C/EBP binding sites are critical for HIV-1 expression in macrophages but not in T-cells[3]. In contrast, C/EBP inhibits expression in brain-derived cells[4]. The C/EBP binding site can also cooperate with the adjacent NF-κB binding site to enhance HIV-1 expression[5]. The upstream stimulating factor (USF) footprints over nucleotides from position -173 to -157 upstream of the transcription start site, and forms symmetric contacts with the guanines of the palindromic CACGTG core of the recognized sequence. Upon binding, USF is supposed to bend the LTR DNA template[6,7]. Ets proteins activate transcription via binding to a purine-rich GGAA core sequence located at position -141 to -149[8,9]. USF-1 and Ets-1 synergise in specific DNA binding as well as in the trans-activation of transcription. An interaction between Ets-1 and

USF-1 is required for full transcriptional activity of the HIV-1 LTR in T cells[10]. Ets-1 has also been shown to interact with NF-κB/NF-AT proteins and form a ternary complex on the HIV-1 enhancer[11]. The distal region of the HIV-1 LTR also contains binding sites for lymphoid enhancer-binding factor 1 LEF-1 and mutations in these sites inhibit the activity of the HIV-1 enhancer in Jurkat T cells. (LEF-1) is considered to be an architectural protein and plays a central role in coordinating activities of multiple transcription factors. It was shown to bend DNA approximately 130 degrees, which facilitates communication between widely separated protein-binding sites[13,14]. AP-1 mediates activation signals elicited by tumour-promoting phorbol esters (TPA and PMA)[15]. It binds TPA responsive element (TRE) sites (TGAGTCAG) in response to phorbol ester stimulation via protein kinase C (PKC) activation. NF-AT is an inducible transcription factor related to the NF-κB family. It is present in most immune cells and can physically interact with AP-1[16].

The enhancer region possesses two 10-bp conserved binding-sites for the nuclear factor kappa B (NF-κB) family of transcription factors. The NF-κB proteins are major regulators of gene activation in a developing immune system[17], including many genes in activated T cells. Stimulation of the HIV enhancer in response to mitogens has been attributed to the binding of NF-κB to the viral enhancer. The binding of NF-κB is, by itself, sufficient to induce HIV gene expression[18]. A protein-protein interaction can occur between NF-κB and Sp1 bound to an adjacent site[19,20] but is not essential, as proviruses deleted in Sp1 sites generally replicate in several types of T-cells[21]. NF-κB is generally considered to be inactive and cytoplasmic until stimulated by an inducer. The basis for the latent nature of NF-κB and for its inducibility is the association of NF-κB with a cytoplasmic inhibitory protein called IkB[22]. IkB contacts the nuclear localization signal (NLS) of NF-κB and this appears to play critical functional roles in inhibiting nuclear localization of NF-κB[23,24]. The release from IkB allows the extraordinarily rapid appearance of NF-κB in the nucleus. Transcriptional activation by NF-κB can occur within minutes subsequent to exposure to the inducer and is correlated with hyper-phosphorylation of IkB. The current model is that targeted phosphorylation of IkB leads to the ubiquity of this protein, which leads to degradation by the proteasome. There is a mutual regulation of NF-κB and IkB, as the IkB gene promoter contains multiple NF-κB binding sites that are functional in the up-regulation of gene expression in response to inducers that activate NF-κB[23,25]. Within an hour, IkB levels recover and this appears to repress NF-κB activity following induction, because re-synthesised IkB enters the nucleus, interacts with NF-κB, and inhibits DNA binding[26].

The basal promoter region of HIV contains three tandem-repeat Sp1-binding sites and a TATA box. At the initiation of transcription, the TATA box is recognised by transcription factor (TF) II B (TFIIB), also called the TATA-binding protein (TBP). Sp1 binds directly to the CG-rich region extending from –46 to -78 and can also interact with other DNA-associated factors. These Sp1 mediated protein interactions may stabilise the formation of the pre-initiation complex.

The TATA-box, Sp1, and NF-κB binding sites are considered to be the most influential, positive-acting elements in primary monocytes and macrophages, whereas NF-κB and NF-AT have strong effects in activated T-cells[27].

In addition to the upstream promoter, new studies indicate that the sequences downstream of transcription in the transcribed 5'-untranslated leader region are important for viral expression[28-31]. Footprinting studies have demonstrated that host-cell transcription factors interact extensively with this region[32]. Immediately downstream of the transcription initiation site are binding sites for the transcription factor LBP (also called UBP or LSF)[1]. The recognition sequence and nucleotide position of other transcription factor binding sites that have been identified in this 5'-untranslated leader region are also shown in figure 1. These include several well-characterised transcription factors such as AP-1, CREB/ATF, Sp1, NF-κB, IRF, NF-AT, and CTF/NF-1.

Mechanism of HIV transcriptional activation

Despite the importance of cellular factors for HIV transcription, transcription complexes initiated at the HIV promoter are rather inefficient at elongation and require the viral protein Tat to enhance the process of transcribing polymerases. Under some conditions, Tat may also enhance the rate of transcription initiation[33]. Tat increases production of viral mRNAs ~100-fold and consequently is essential for viral replication. In the absence of Tat, polymerases generally do not transcribe beyond a few hundred nucleotides, though they do not appear to terminate at specific sites.

In addition to Tat, another viral protein Vpr was shown to activate HIV-1 transcription[34]. Vpr is a viron-associated regulatory protein that has been shown to interact with TFIIB and Sp1[35,36], and cooperates with the co-activator CBP to activate HIV-1 transcription[37].

Tat protein

The first coding exon of tat is located in the central region of the viral genome between vpr and env; the second exon overlaps the translation frame for both rev and the gp41 subunit of env (see Fig. 1). Tat is translated from multiply spliced mono-cistronic transcripts that are synthesised early in infection and are not dependent on Rev function. It is a 14- to 15-kDa protein comprised of 101 amino acids[40,41]. Tat enhances transcriptional elongation by binding to a secondary RNA stem loop structure TAR (transactivation responsive element). The transcriptional activation domain of Tat comprises a cysteine-rich region (AA 22-37), followed by a hydrophobic core motif and by a basic patch (AA 49-57) (Fig. 2). The Tat activation domain has been shown to specifically bind human cyclin T1, which is required to en-

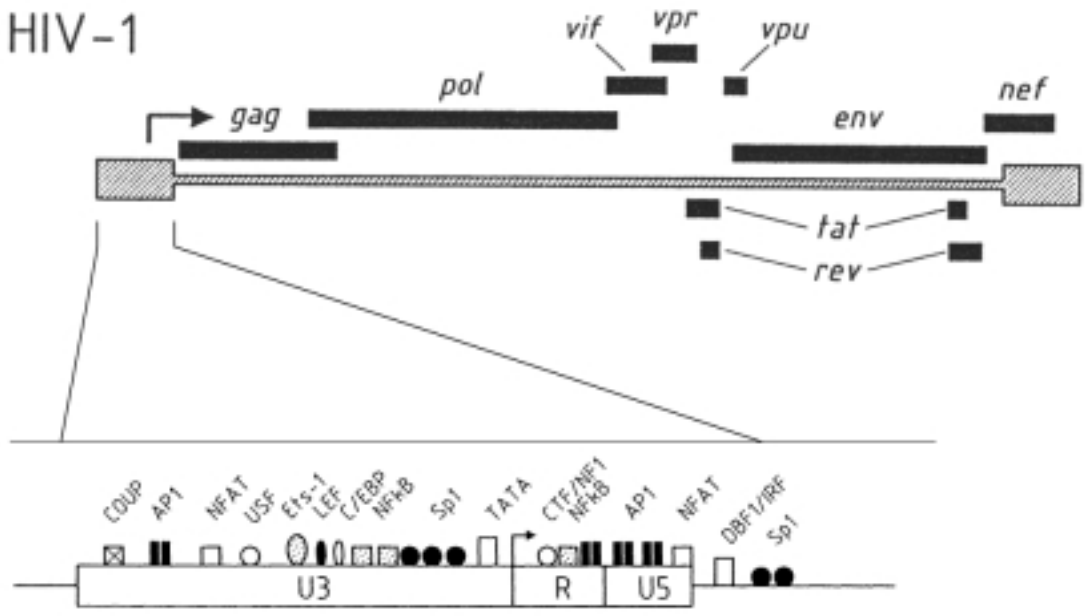

Fig. 1. *Schematic organization of the HIV-1 genetic structure. The positions of the various genes. (Gray boxes) The 5'- and 3'-long terminal repeats (LTRs). (Horizontal arrow) The site of transcription initiation. The primary transcripts are shown below the provirus together with the positions of known 5'-splice sites (downward-pointing arrows), known 3'-splice acceptor (upward-pointing arrows), and the Rev responsive element (RRE). The numbering of the splice sites is as previously described[38] (adapted from Frankel and Young, 1998)[39]. The HIV-1 LTR is represented with regard to the structural organization of the cis-acting elements. The location of the transcription initiation site is indicated by the arrow. COUP: AP-1; NFAT: nuclear factor from activated T-cells; USF: upstream stimulating factor; NF-kB: Nuclear factor kappa B; Sp1: TATA: TATA-box; NF1: nuclear factor 1; DBF: adapted from Van Lint* et al., *1997[31].*

hance the binding affinity and specificity of Tat to the HIV-1 TAR RNA[42]. More recent observations indicate that a critical cysteine residue (C261) is important for the formation of the tripartite complex between Tat, cyclin T1, and TAR[43]. The core domain is highly conserved in the Tat proteins of HIV and SIV. It influences the selectivity of the basic region to interact with wild-type TAR[44,45]. The basic patch consists of an Arg/Lys-rich RNA-binding motif (ARM), which is responsible for both direct interaction of Tat to the base triplet in the bulge region of TAR[46,47] and for nuclear/nucleolar localisation[48]. The arginine at position 52 is critical for the specific association with TAR. Mutations that truncate or substitute neutral amino acids for lysine and arginine residues in the basic domain have a dominant negative phenotype[49]. It is hypothesised that these dominant mutants may sequester cellular factors required for trans-activation. The carboxy-terminal region encoded by exon 2, is dispensable for transactivation function.

Tat/TAR interaction

The most interesting and unique function of Tat is that it acts through an RNA target, termed TAR, located immediately 3' to the LTR transcription start site[50]. The minimal TAR for HIV-1 trans-activation has been mapped to a stem-loop structure at the 5' end of all viral transcripts (+19 to +43), whereas TAR for HIV-2 and SIV is encompassed within the first 130 nucleotides of viral transcripts and appears to fold into a structure with two stem-loops. The HIV-1 TAR consists of a stem of 24 nucleotide pairs with a six-nucleotide loop (+30 to +35) and a small pyrimidine-rich bulge (+23 to +25) (Fig. 3). Tat forms a one-to-one complex with TAR and recognises the pyrimidine-rich bulge[45]. It interacts with the U_{23} in the bulge and with the major groove of two nucleotide pairs on either side of the bulge (G_{26}-C_{39} and A_{27}-U_{38}, and A_{22}-U_{40} and G_{21}-C_{41})[44]. Analysis of compensatory mutations in the TAR stem reveals that the primary nucleotide sequence can be altered without loss of activity, as long as base-pairing is maintained[51]. Mutational analysis of TAR loop sequences revealed a block necessary for Tat transactivation *in vivo* without affecting the Tat-TAR interaction[52], suggesting the presence of a Tat-dependent cellular activity specific for the TAR loop.

HIV-1 transcription

The synthesis of mature and functional HIV messenger RNA occurs by eukaryotic RNA polymerase II (RNAPol II) and is a complex, multistage process requiring the cooperative action of viral and many cellular proteins[53-56]. This process, transcription, proceeds via five stages: pre-initiation, initiation, promoter clearance, elongation and termination. The RNAPol II is first pre-initiated at the promoter,

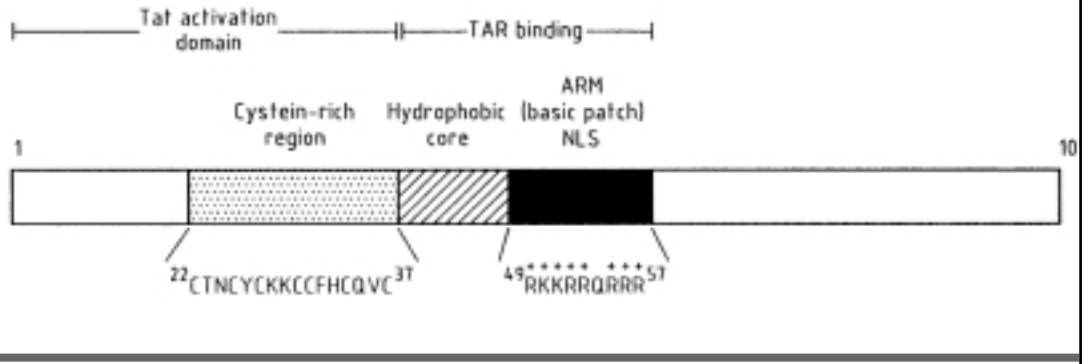

Fig. 2. *Amino acid sequence of the HIV-1 Tat protein. ARM: arginine-rich RNA-binding motif.*

where it interacts with DNA and general transcription factors. Initiation results in a separation of the DNA strands around the transcription start-site. Then phosphorylation causes the RNAPol II complex to be cleared from the promoter and elongated along the DNA. At the end of the HIV provirus, the complex is terminated and released from the DNA template, ready to start a new round of transcription.

Transcription initiation of all protein encoding genes requires the formation of a pre-initiation complex (PIC), which consists of RNA polymerase II and the basal transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (Fig. 4). TFIID is a multi-protein complex consisting of the TATA-binding protein (TBP) at its core, and TBP-associated factors (TAFs), which are essential for regulated transcription *in vitro*[57], but are not required for basal transcription[58,59]. It is generally accepted that binding of TBP to the TATA-box nucleates the formation of the PIC either through a stepwise assembly of basal factors, or through recruitment of a holoenzyme[60-63]. TBP interacts with the carboxyl-terminal domain (CTD) of RNAPol II, consisting of multiple-tandem repeats of the YSPTSPS heptapeptide. Due to phos-

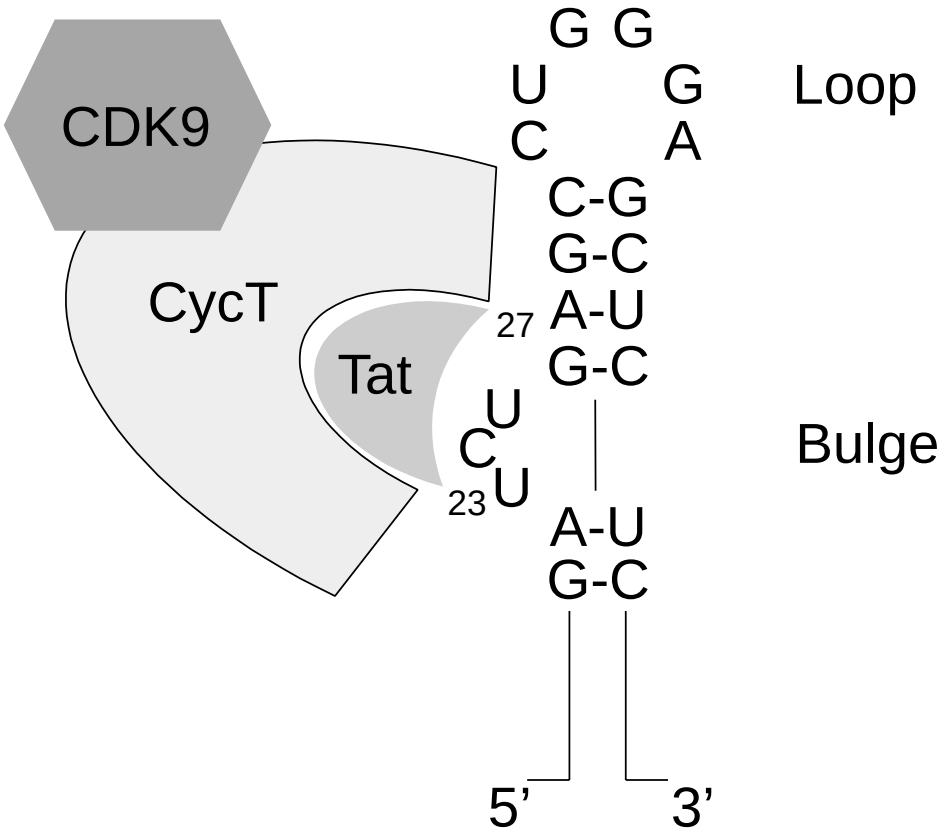

Fig. 3. *The sequence of the RNA hairpin of the HIV-1 TAR element. Tat protein binds to the base triplet of the bulge, whereas the loop sequence interacts with a Tat-coactivator complex. Cyc T: cyclin T; CDK9: cyclin-dependent kinase 9.*

phorylation of the repeats, two forms of the polymerase are observed, a highly phosphorylated and an un-phosphorylated form. The highly phosphorylated form is the RNAPol II species involved in transcription elongation, while the un-phosphorylated form is the species that preferentially associates with the assembling pre-initiation complex.

After assembly of the complete pre-initiation complex, addition of ATP results in separation of the DNA strands from positions –9 to +2. This constitutes the first transition in the RNAPol II initiation complex, and DNA helicase activity of TFIIH is responsible for the formation of this open complex[64] (Fig. 4). It is in dynamic equilibrium between open and closed conformation, and ATP hydrolysis by TFIIH-associated DNA helicase is required to sustain promoter opening. The open –9/+2 complex can fall back to the closed complex or initiate synthesis of an RNA chain by formation of the first phosphodiester bond, which stabilizes the open complex. After formation of a 4 nt product, a stable transcription complex is obtained[65]. Although the RNAPol II complex is capable of abortive transcription until 10 nt, the efficiency of abortive synthesis decreases with RNA length (Fig. 4).

In a next step, the promoter is cleared; this is the RNAPol II, which leaves the promoter in a way that allows a new polymerase to enter and form a re-initiation complex. At promoter clearance, the interaction between TFIIB, TFIIE, TFIIF, and TFIIH and RNAPol II must be broken to allow translocation of the polymerase II enzyme and re-closure of the –9/+2 region, whereas TFIID remains bound to the promoter[66] (Fig. 4). Probably, TFIIB dissociates at an early stage prior to re-closure and TFIIE releases between nt 1 and 10 synthesised, while TFIIF releases between 10 and 50 and TFIIH between 30 and 50 nt synthesised.

Following promoter clearance, and before entering productive elongation, the processivity of RNAPol II is controlled by the action of both negative and positive elongation factors (N-TEFs and P-TEFs). Negative transcription-elongation factors have been proposed to be responsible for abortive elongation. Handa and co-workers have recently identified two multisubunit complexes, termed DSIF and NELF[67-71]. The RNAPol II complex is blocked after initiation, due to the combined action of DSIF and NELF. These factors are bound tightly to hypophosphorylated RNAPol II but not to its hyperphosphorylated form, suggesting that phosphorylation may cause the dissociation of DSIF and NELF from RNAPol II. Phosphorylation of the RNAPol II CTD is a key-step in regulating the transition from initiation to elongation[72]. The presence of an RNA-recognition motif in NELF raises the interesting possibility that NELF could bind directly to the emerging RNA chain to enhance pausing proximal to the promoter.

The transition from abortive elongation to productive elongation is mediated by the action of positive elongation factors. One such factor is the positive transcription-elongation factor P-TEFb. It possesses a protein kinase activity, phosphorylating the CTD of Pol II[73,74].

Mechanism of action of Tat

In the absence of Tat, most of the viral transcripts terminate prematurely, producing short RNA molecules ranging in size from 60 to 80 nucleotides[75]. Upon binding to the TAR RNA sequence, Tat causes a substantial increase in transcript levels. The increased efficiency in transcription may result from preventing premature termination of the transcriptional elongation complex, and by augmenting the processivity of transcription complexes. It is not yet clear what the cause is of this abortive elongation in the absence of Tat.

The HIV-1 TAR-dependent trans-activation by Tat is linked to the CTD of RNA polymerase II[76-78]. Hyperphosphorylation of the tandem heptapeptide repeat (YSPTSPS) in the CTD of RNAPol II is thought to release the transcription complex from the promoter and process elongation.

Tat has been reported to interact with a number of cellular proteins associated with transcription, including TFIID[79], Sp1[80], TAFII-55[81], and RNAPol II[82]. Furthermore, two cyclin-dependent kinase (CDK)-cyclin pairs, present in two distinct transcription factor complexes, have been implicated as Tat cofactors which could phosphorylate CTD[73,83,84]. TFIIH, a general transcription factor, possesses CTD kinase activity, which resides in the CDK7/cyclin H subunit[83]. Several lines of evidence indicate there is an interaction between Tat and the TFIIH complex[78,85]. The second CTD kinase, a human transcription elongation factor complex known as p-TEFb, was also found to interact with the trans-activation domain of Tat[86,87]. It was first identified in and purified from Drosophila extracts, and sequence analysis of the small unit revealed its extensive sequence identity to a previously identified, cdc2-related kinase termed PITALRE (now referred to as CDK9)[74,87,88]. The CDK9 subunit of pTEFb is a serine/threonine kinase, which is capable of hyper-phosphorylating the CTD of RNAPII[73]. The importance of p-TEFb and its kinase activity in Tat trans-activation was suggested by a selective inhibition of Tat activity by low molecular weight kinase inhibitors. Tat-dependent transcription from the HIV LTR is strongly inhibited by low concentrations of 5,6-dichloro-1-β-D-ribofuranoslybenzimidazole (DRB)[89] and a set of related protein kinase inhibitors[90], due to the selective inhibition of CDK9 by these compounds. Direct evidence of the role of pTEFb in transcriptional regulation of the HIV LTR comes from experiments using inactive mutants of CDK9 kinase expressed in trans to inhibit transcription[90-92]. CDK9 immuno-depletion of HeLa nuclear extracts was unable to produce long transcripts from the HIV-1 LTR[90,92]. Furthermore, it has been known for several years that mutations in the apical loop region of TAR abolish Tat activity[93,94], yet this region of TAR is not required for binding by recombinant Tat protein *in vitro,* suggesting that the loop region acts as a binding site for essential cellular cofactors[95,96].

Recently, cyclin T1 (CycT1) was identified as a major partner of CDK9 in human cells[97], and it has been shown to interact with Tat and participate in TAR recognition[42]. This function of CycT1 fits the cri-

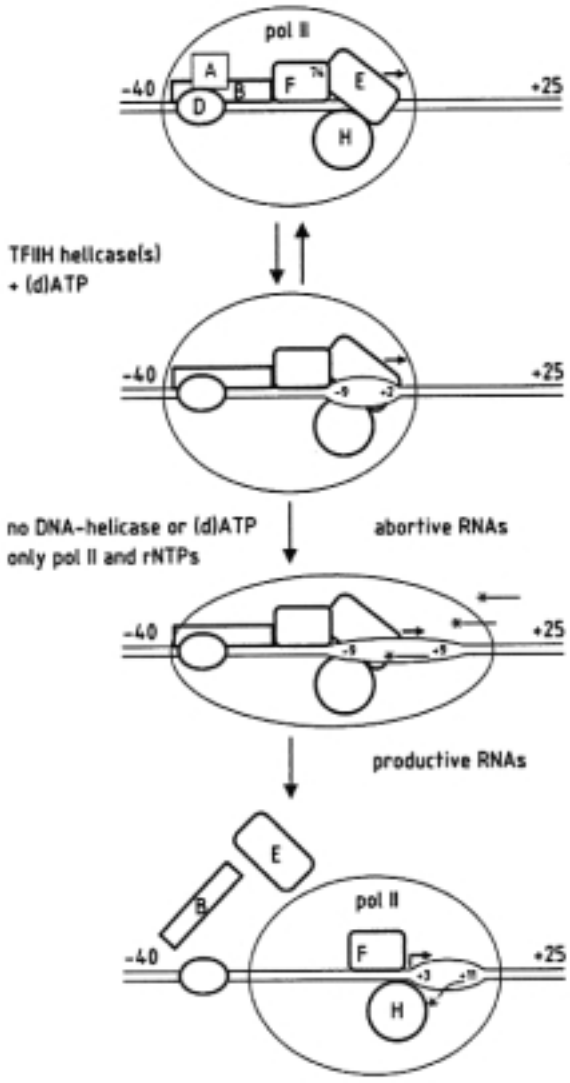

Fig. 4. *A model for the transcription initiation of RNAPolII. The assembled initiation complex is depicted on the top of the figure, with the basal transcription factors (TFIIA, TFIIB, TFIIF, TFIIE, and TFIIH) and RNAPolII as balloons. The TBP is the unlabeled open balloon over the TATA-box, whereas the other basal factors are indicated by a letter. The numbers indicate positions of the DNA template and are relative to the transcription start-site indicated by the arrow. The asterisks indicate the first residue of the RNA product. Adapted from Holstege* et al., *1997*[102].

teria established previously for the species-specific Tat cofactor[56,98]. Additional evidence in support of this hypothesis came from the observations that over-expression of the human CycT1 enhanced Tat trans-activation in rodent cells[42]. More recent observations indicate that a critical cysteine residue (C261), which is not conserved in the murine cyclin T1 protein (Y261), is important for the formation of the Tat/cyclin T1/TAR complex[99-101]. Interestingly, a reciprocal exchange of a cysteine to a tyrosine at position 261 (C261 ↔ Y261) between human cylin T1 (hT1) and the murine T1 (mT1) renders hT1 inactive and mT1 active for human Tat trans-activation. Thus, the ability of Tat to recruit cyclin T1/CDK9 (which together form pTEFb) to TAR, not only stimulates HIV-1 transcriptional elongation, but also governs the species specificity of HIV-1. In addition to CycT1, two additional CDK9-associated cyclins, cyclin T2a and cyclin T2b, have now been identified in human p-TEFb[97]. However, only cyclin T1 is able to associate with Tat and modulate recognition of TAR RNA[104]. The exact dynamics of the interaction between the Tat protein and p-TEFb are currently under investigation. So far, there is no evidence of 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. Unlike other cyclins and cyclin dependent kinases (CDKs), the activity of the CDK/cyclin complexes involved in transcription regulation (CDK7/cyclin H, CDK8/ cylcin C, CDK9/cyclin T) does not vary during the course of the cell cycle.

It was originally proposed that both TFIIH and pTEFb might act sequentially and in a concerted manner, promoting hyper-phosphorylation of RNAP II

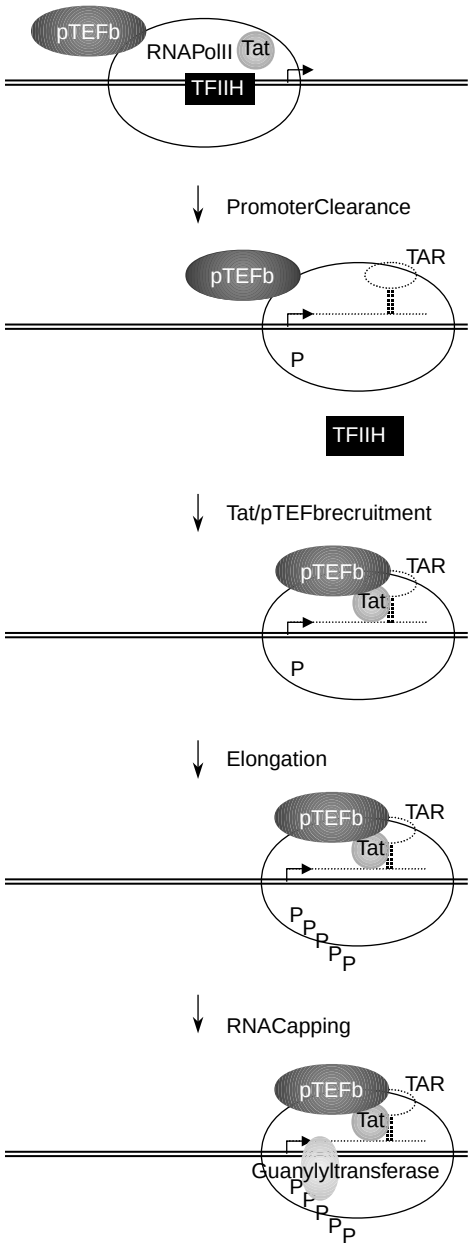

Fig. 5. *A model for the formation of processive RNAPol II elongation complexes by Tat protein. See text for details. pTEFb: cyclin T/CDK9 complex; Tat: HIV viral trans-activator of transcription; TAR: Tat responsive element; RNAPol II: RNA polymerase II; TFIIH: transcription factor IIH (cyclin /CDK7 complex).*

CTD and increasing polymerase processivity[105,106]. At present, however, the role of TFIIH in HIV-1 transcription is controversial[107].

During transcription, the mammalian CTD is phosphorylated mostly at the serine residues. However, the exact mechanism of CTD phosphorylation remains elusive[72]. It has been shown that CDK7 (TFIIH) and CDK9 (pTEFb) both associate with the HIV-1 pre-initiation complex (PIC) and function to hyper-phosphorylate the CTD of RNAP II. In basal transcription, CDK7 and CDK9 facilitate transcription activity: CDK7 phosphorylates serine 5[108] and CDK9 serine 2 of the CTD[109,110]. In the presence of Tat, CDK7 is not required for HIV-1 transcription[107]. Remarkably, Tat modifies the substrate specificity of CDK9, allowing CDK9 to phosphorylate serine 2 and serine 5[110]. In the absence of Tat, CDK9 phosphorylates the CTD at serine 2.

From these data, a model for the HIV-1 Tat transactivation can be drawn (Fig. 6). Tat binds the pre-initiation complex and interacts with TFIIH; however its interaction with other proteins present in the initiation complex cannot be ruled out[79-82]. It has been reported that Tat associates with purified transcription pre-initiation complexes[111]. pTEFb is also a component of the HIV-1 pre-initiation complexes but

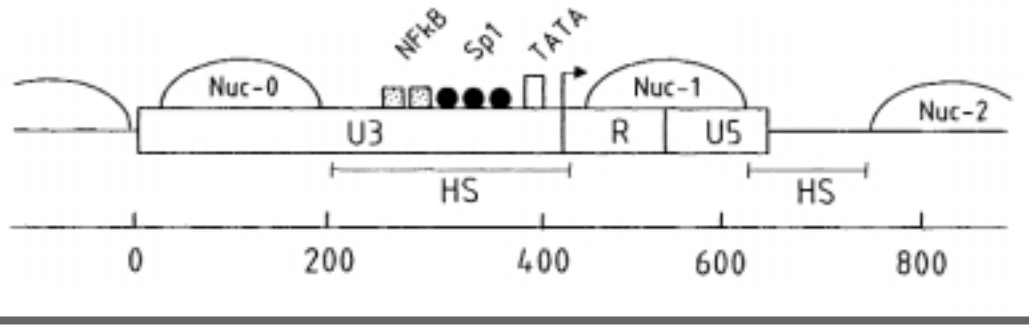

Fig. 6. *Model for the chromatin organization of the 5' LTR of HIV-1. A tentative assignment of nucleosome positions (Nuc-0, Nuc-1, and Nuc-2) based on nuclease digestion is shown. Two nucleosome-free regions, hypersensitive (HS) nuclease sites in purified nuclei, are indicated. Adapted from Verdin* et al., *1993.*[103]

it may not be involved in Tat binding at this point[110,112]. As described above, TFIIH plays a critical role in transcription initiation and promoter clearance[113,114], and is bound to non-phosphorylated RNAPol II holoenzyme. TFIIH, alone or with Tat, phosphorylates the CTD and assists in promoter clearance. The TFIIH complex dissociates from the RNAPol II 30 to 50 nucleotides after the initiation and is not part of the elongation complex[115]. After the transcription of a functional TAR RNA structure, Tat binds to TAR and repositions pTEFb in the vicinity of the CTD of RNAPol II. In the presence of Tat, the substrate specificity of pTEFb is altered, such that it phosphorylates serine 2 and serine 5 of the CTD and processive elongation complexes are formed.

Finally it is of interest to consider the possibility that phosphorylation of the RNAPol II CTD may have a direct effect on capping of the pre-mRNAs. Capping is targeted to nascent RNAs through binding of the guanyltransferase to the phosphorylated CTD[116]. Guanylterasferase binds CTD peptides containing phosphate groups at either serine 2 or serine 5. Interestingly, it has recently been reported that binding of guanylyltransferase to CTDs containing a phosphorylated serine 5 specifically stimulates enzymatic activity by enhancing the affinity for GTP and increasing the yield of enzyme-GMP intermediate[117]. Since Tat stimulates the serine 5 phosphorylation of the CTD it has also an indirect stimulatory effect on HIV-1 mRNA capping and hence translation. These observations are in agreement with several studies that have previously shown that Tat enhances the translation of mRNAs synthesised from the HIV-1 LTR[52,118].

Activation of integrated provirus requires histone acetyltransferase

Like all retroviruses, an essential step in the life cycle of HIV-1 is the integration of proviral DNA into the host-cell chromosomes. When integrated into cellular genomic DNA, the HIV-1 provirus becomes packaged into chromatin[103,119]. In the nucleosome, the basic subunit of chromatin, DNA is tightly wound around an octamer of histon proteins, thereby restricting access by DNA-binding factors. Physiological mechanisms for proviral transcription must thus account for the activation of the chromatin-associated viral promoter. In addition, there is evidence that the activity of the virally encoded Tat protein describes a rate-limiting step for activation of integrated HIV-1 genomes[120].

Precisely positioned nucleosomes and intervening nucleosome-free regions are found in the 5' LTR region where viral cis-acting elements are located (Fig. 6). A large nucleosome-free region is found associated with the enhancer/promoter region (nt 200 to nt 452-465) and with a region downstream of the site of transcription initiation (nt 610-720). Two nucleosomes are precisely positioned in the 5' LTR encompassing nt 40-200 (nuc-0) and nt 465-610 (nuc-1). Nuc-0 encompasses a portion of the upstream regulatory region, and several binding sites for factors have been described in this region. However, packaging of DNA in a nucleosome distorts the helical path of DNA[121] and consequently the recognition of a DNA-binding site by its cognate factor can be impaired[122]. For this reason, the biological significance of the binding sites described in this region should be re-evaluated in the context of chromatin structure. A second nucleosome in the 5' LTR (nuc-1) is positioned between nt 465 and nt 610 in basal conditions. This nucleosome is rapidly (within 1 hr) and specifically disrupted following treatment with PMA or TNF-α, two agents known to increase HIV-1 transcription in these cells. The specificity of this disruption is established by the observation that other nucleosomes in the region are not affected by PMA treatment. The disruption occurs precedent to, and independent from, transcriptional activation.

Chromatin remodelling associated with activation of transcription generally is accomplished by reversible acetylation of lysine residues in the amino-terminal domains of the core histones. This modification, induced by proteins having histone acetyltransferase (HAT) activity, weakens histone-DNA interactions, thereby relieving the repressive effects of the chromatin scaffold[123-124]. Consistently, the silent, integrated LTR also can be strongly activated by drugs inducing sustained high levels of histone acetylation in latently infected cells. It has been shown that trapoxin (TPX) and trichostatin A (TSA), which are

specific inhibitors of histone deacetylase and hence cause histone hyperacetylation, activate the transcription of the integrated, chromatin-packaged HIV-1 promoter from its normally silent state[125-129]. The fact that nuc-1 is the only disrupted nucleosome in the HIV-1 promoter in the presence of a global hyperacetylation of all nucleosomes due to TPX or TSA treatment, suggests that an additional level of specificity must exist in this system. The HIV transcriptional activator, Tat, has been shown to specifically associate with p300 and p300/CBP-associating factor (P/CAF), two histone acetyltransferases (HATs), *in vitro* and in within cells[130,131]. Moreover, the interactions of Tat with p300 and P/CAF actually occur at the LTR[131]. It has been suggested that an additional function of Tat is to relieve chromatin inhibition on transcription by recruiting p300 and P/CAF to the LTR promoter.

Furthermore, it has been shown that p300 and PCAF also directly acetylate Tat[132]. There are two sites of acetylation located in different functional domains of Tat. p300 acetylates Lys50 in the RNA binding domain, while P/CAF targets Lys28 in the activation domain of Tat. In support of a functional role for acetylation *in vivo*, trichostatin A, a histone deacetylase inhibitor, synergises with Tat in transcriptional activation of the HIV-1 LTR. Synergism was TAR-dependent and required the intact presence of both Lys28 and Lys50. Acetylation of Tat regulates two discrete and functionally critical steps in transcription. Acetylation at Lys28 by P/CAF enhances Tat binding to pTEFb, while acetylation by p300 at Lys50 of Tat promoted the dissociation of Tat from TAR RNA that occurs during early transcription elongation. Several observations suggest that a mechanism that regulates dissociation of Tat from TAR might be physiologically important. Firstly, Tat protein is expressed at exceedingly low to virtually undetectable levels in virus-infected cells[133,134]. Hence, in order for a limiting amount of Tat to be functional in a setting of large TAR RNA excess, a selective mechanism that effects Tat dissociation from TAR should exist. Secondly, the associative half-life of Tat for TAR is 41 seconds *in vitro*[135]; but this duration is incompatible with the rapid kinetics required for Tat-directed transcription from the LTR[136]. Hence, it seems likely that a post-translationally modified form of Tat that has a shorter half-life for TAR would exist intracellularly.

Conclusions

The mechanism of action of the HIV-1 Tat transactivation has been studied in detail. However, the mechanism by which HIV-1 is able to abort its transcription in the absence of Tat is not yet clear. Additional research is needed to complete our knowledge on the fine-tuned regulation of HIV trans-activation. Current knowledge already shows that Tat is an essential factor for the replication of HIV. Therefore, it is a good target for antiviral therapy. Mapping of the Tat interaction sites would be helpful for the design of specific inhibitors. For example, the Tat-CycT interaction could be a possible target for antiviral therapy. Therefore, specific assays for these interactions could be developed. However, the involvement of the different essential steps in HIV-1 Tat trans-activation, such as the Tat-CycT interaction, for importance cellular functions has not yet been extensively investigated. These data are needed to appreciate the possible toxicity of potential new antiviral drugs against these targets. Further detailed studies of the mechanism of action of Tat could expose additional possible targets.

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