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Diversity of acetylation targets and roles in transcriptional regulation: the human immunodeficiency virus type 1 promoter as a model system

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Abstract

Persuasive evidence has accumulated that reversible acetylation of proteins is key post-translational modification regulating transcription in eukaryotes. Deacetylase inhibitors (such as trichostatin A) modulate the expression of ~2% of all cellular genes. We and others have demonstrated a marked transcriptional activation of the human immunodeficiency virus type 1 (HIV-1) promoter in response to deacetylase inhibitors. Deacetylation events seem to be an important mechanism of HIV-1 transcriptional repression during latency, whereas acetylation events play critical functional roles in HIV-1 reactivation from latency. These deacetylation/acetylation events are implicated in chromatin remodeling of the viral promoter region, as well as in modulating the functional properties of cellular and viral transcription factors binding to this promoter region. Thereby, the HIV-1 promoter constitutes a unique regulatory model system to study the complex relationship between acetylation processes and transcriptional activity.

Keywords: HIV-1; Transcription; Chromatin; Acetylation; Trichostatin A; NF-κB

1. Introduction

There is now strong evidence that both transcriptional activation and silencing are mediated at least in part through the recruitment of enzymes that control protein acetylation. Acetylation of specific lysine residues within nucleosomal histones is closely linked to chromatin disruption and transcriptional activation in many genes. Consistent with their role in altering chromatin structure, many transcriptional co-activators (including hGCN5, CBP/p300, P/CAF, SRC-1) possess intrinsic acetyltransferase activity that is critical for their function [1–3]. Similarly, co-repressor complexes include proteins that have deacetylase activity (reviewed in [4–8]). Importantly, reversible acetylation is also a critical post-translational modification of non-histone proteins, including general and specific transcription factors, transcriptional co-activators, non-histone structural chromosomal proteins, and nuclear import factors. Protein acetylation regulates many diverse functions, including DNA binding, protein/protein interaction, protein stability and cellular localization (reviewed in [2,9–12]). Hence, acetylation may rival phosphorylation as a mechanism for the transduction of cellular regulatory signals.

Previously, we and others have demonstrated the transcriptional activation of the HIV-1 promoter in response to deacetylase inhibitors [such as trichostatin A (TSA), trapoxin (TPX), valproic acid (VPA), sodium butyrate (NaBut)]. This occurred in *ex vivo* transiently or stably transfected HIV-1 long terminal repeat (LTR) promoter reporter constructs [13–15], as well as in latently HIV-1-infected cell lines [16–18], on *in vitro* chromatin-reconstituted HIV-1 templates [19,20], as well as in the context of a *de novo* HIV-1 infection.[1]

Deacetylase inhibitors, such as TSA, strongly induce HIV-1 transcription, although only a small subset (<2%) of cellular genes have their expression upregulated by TSA,

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as determined by RNA differential display analysis [21]. The ability of the HIV-1 promoter to respond specifically to TSA might reflect a complex regulatory link between deacetylation and transcriptional repression of the LTR. In this review, we will describe recent developments establishing the HIV-1 promoter as a regulatory model system to study the complex relationship between acetylation processes and transcriptional activity.

2. Chromatin environment

The eukaryotic genome is compacted with histones and other proteins to form chromatin [22], which allows for efficient storage of genetic information. However, this packaging also prevents the transcription machinery from gaining access to the DNA template [23]. Chromatin is heterogeneous in the nucleus: transcriptionally active genes are characterized by a more diffuse chromatin structure (active chromatin or euchromatin), whereas inactive genes are packaged in a highly condensed chromatin configuration (inactive chromatin or heterochromatin) [24]. Active chromatin has different characteristics, among those, a more relaxed structure when compared to the condensed nature of inactive chromatin and a high rate of acetylated histones.

For HIV-1, chromatin represents a very heterogeneous environment. The activity of a *de novo* integrated HIV-1 promoter can be dramatically affected by the site of integration and thus by the histone acetylation status at this site. However, although the site of integration appeared non-random [14,25], many studies have failed to define the molecular mechanism of integration site selection [26,27].

3. Local nucleosomal organization

Chromatin organization is not static and disruption of specific nucleosomes has been observed during transcriptional activation of a number of promoters [28,29]. Core histones are not only the primary proteins that fold DNA into chromatin, thereby limiting passively DNA accessibility, but they also play an active key role in transcriptional regulation [30]. Therefore, determination of the mechanisms responsible for both the positioning and the disruption of specific nucleosomes in a given gene is crucial for the understanding of its transcriptional regulation. Multiprotein complexes exist whose primary function is to help activate gene expression by altering chromatin so that DNA regulatory sequences become more accessible to sequence-specific proteins and to the general transcription machinery [31]. At least two different, yet highly conserved, mechanisms are at work to alter chromatin structure: chromatin remodeling activities/factors and post-translational modifications of chromatin components, in particular, histone acetylation.

Our laboratory has studied the chromatin organization of HIV-1 proviruses integrated in five different latently infected cell lines [32]. Independently of the site of integration, two nucleosomes (called nuc-0 and nuc-1) are positioned at the viral promoter DNA at precise locations with respect to regulatory elements [32]. Nuc-0 is positioned immediately upstream of the modulatory region and nuc-1 immediately downstream of the viral transcription start site (Fig. 1A). These nucleosomes define two open regions corresponding, respectively, to the modulatory region plus the enhancer/core promoter region (nt 200–465) and to a regulatory domain in the leader region downstream of the transcription start site (nt 610–720), where transcription factors have been found to bind *in vitro* and *in vivo* (Fig. 1A) [33–38]. Importantly, activation of the integrated HIV-1 promoter by cytokine tumor necrosis factor α (TNF-α) or phorbol ester TPA is accompanied by the loss or rearrangement of the nucleosome nuc-1 near the transcription start site [32]. Therefore, chromatin modifications might result in HIV-1 promoter activation. Consistently, the silent, integrated viral LTR can be strongly activated by drugs such as NaBut, TSA or TPX, which inhibit deacetylases and thereby cause a global hyperacetylation of all cellular histones [16,21,39–43]. Remarkably, despite this global histone hyperacetylation observed following treatment with deacetylase inhibitors, the only detectable modification at the level of the HIV-1 chromatin is the disruption of nuc-1 in the 5′ LTR [16], whereas other nucleosomes remain unaffected. This disruption is accompanied by an activation of virus production, which occurs at the transcriptional level [16].

In vitro studies examining chromatin-reconstituted HIV-1 templates also support a role for acetylation in LTR-directed transcription [19,20]. Experiments from the Jones laboratory have shown that TSA strongly induces HIV-1 transcription on nucleosomal DNA *in vitro*, concomitant with an enhancer-dependent increase in the level of acetylated histones [19]. They conclude that HIV-1 enhancer complexes greatly facilitate transcription reinitiation on chromatin *in vitro* and act at a limiting step to promote acetylation of histones and/or other, as yet undefined, regulatory transcription factors required for HIV-1 enhancer activity [19] (see Section 4). Another study by Steger *et al.* [20] has demonstrated that, *in vitro*, histone acetyltransferase (HAT) activities acetylating either histone H3 or H4 stimulate HIV-1 transcription in a chromatin-specific fashion. Acetylation of only histone proteins mediates enhanced transcription, suggesting that these HAT activities facilitate transcription at least in part by modifying histones. In addition, HATs increase accessibility of HIV-1 chromatin in the absence of transcription, suggesting that histone acetylation leads to nucleosome remodeling [20].

The observations already described suggest that under latency conditions, the viral promoter is poised for transcription but repressed by the presence of a single nucleosome, nuc-1. The fact that inhibition of deacetylase activity

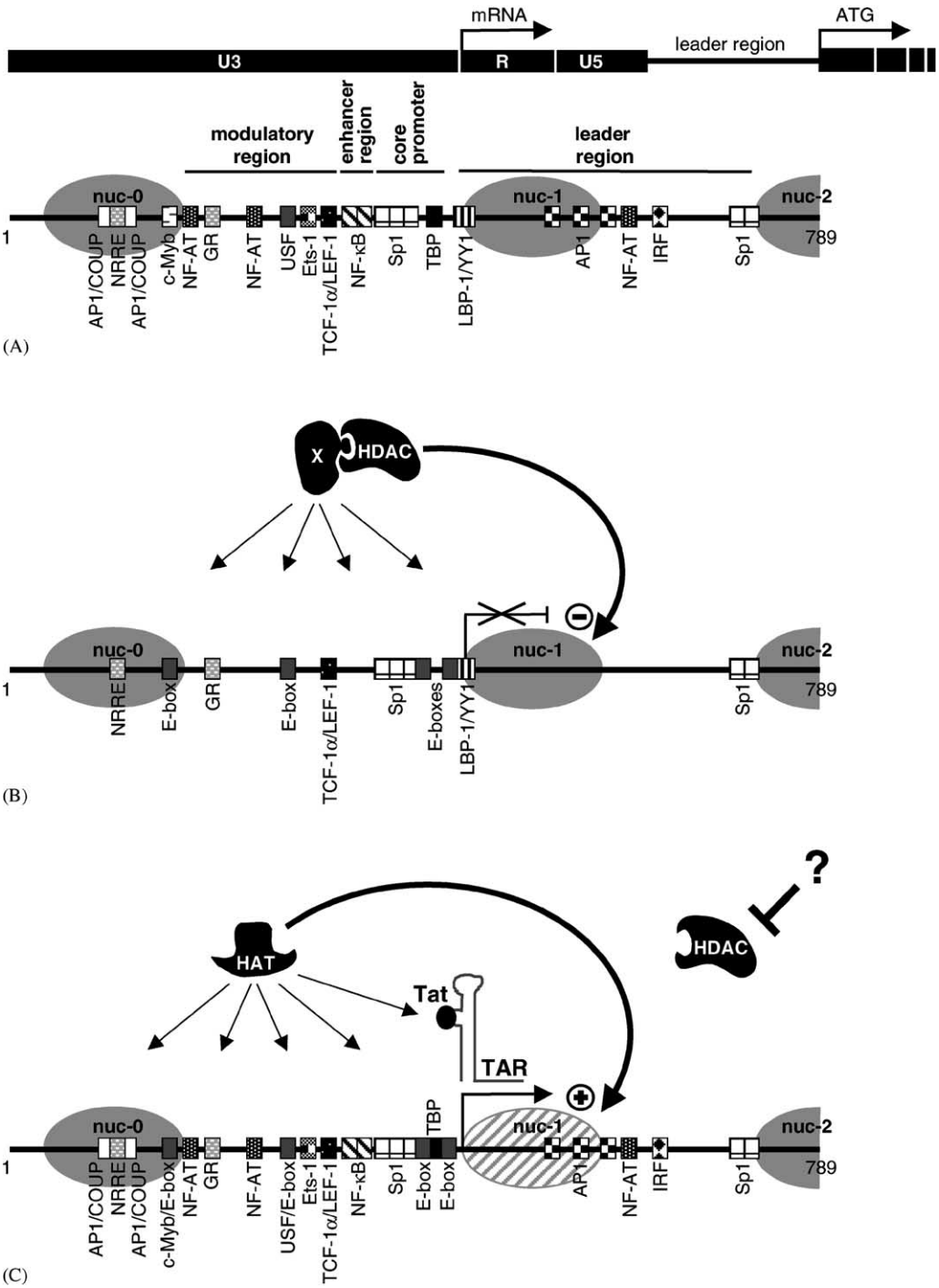

Fig. 1. (A) Organization of the 5′ LTR region of the HIV-1 retrovirus. The LTR is composed of the U3, R and U5 regions. The complete LTR and the leader region (nt 1–789) (where nt +1 is the start of U3 in the 5′ LTR) are shown. The location of the transcription initiation site at the U3–R junction is indicated by an arrow. The transcription factor binding sites present in the different regulatory regions are aligned with nucleosome positioning (grey spheres). From 5′ to 3′, these transcription factor binding sites are as follows (reviewed in [38,52]): two AP1/COUP binding sites (nt 94–112 and nt 118–131), the nuclear receptor-responsive element (NRRE) (nt 98–134), one c-Myb binding site (nt 151–156), two NF-AT binding sites (nt 162–199 and nt 238–251), one glucocorticoid receptor (GR) binding site (nt 192–207), one USF binding site (nt 288–293), one Ets-1 binding site (nt 305–313) one TCF-1α/LEF-1 binding site (nt 315–330), two κB sites (nt 350–373), three Sp1 binding sites (nt 377–408), the TATA box (nt 427–431), one LBP-1/YY1 binding site (nt 445–481), three AP-1 binding sites (nt 540–616), one NF-AT binding site (nt 618–631), one IRF binding site (nt 652–677) and two Sp1 binding sites (nt 724–743). (B) Model for transcriptional repression of the HIV-1 provirus during latency. Under latency conditions, nuc-1 blocks transcriptional initiation and/or elongation because this nucleosome is maintained hypoacetylated by nearby deacetylases (HDAC). The targeting of these HDACs to nuc-1 is mediated by interactions with transcription factors binding to DNA in the HIV-1 promoter region. These interactions take place directly or indirectly through another protein X. (C) Model for transcriptional activation of the HIV-1 provirus. Nuc-1 is a major obstacle to transcription. To activate transciption, nuc-1 must be remodeled. Nuc-1 remodeling could happen by inhibiting the deacetylases. How is this accomplished *in vivo* is unknown. The disruption could also happen by local recruitment of acetyltransferases (HAT) by DNA-binding factors and/or by the viral protein Tat, which binds to the neosynthesized RNA hairpin TAR. This would result in nuc-1 hyperacetylation and remodeling, thereby eliminating the block to transcription.

is sufficient for transcriptional activation suggests that nuc-1 is constitutively deacetylated by targeted deacetylase(s). This model is supported by data showing that deacetylases are targeted to specific promoter regions by virtue of their interactions with specific transcription factors (Fig. 1B) [44–47]. Several factors binding to the HIV-1 LTR represent good candidates for the specific targeting of deacetylases to nuc-1, causing transcription suppression (see Section 4).

The repressive role of nuc-1 in latency remains to be determined. Nuc-1 could block the binding of a transcription factor necessary for the assembly of the initiation complex. Alternatively, nuc-1, by its position immediately after the transcription start site, could impede the progression of RNA polymerase II (RNAPII) (by accentuating a natural pausing site), resulting in inefficient elongation [48–50] and in the accumulation of short attenuated transcripts detected *in vivo* [51]. Thus, during transcriptional activation, nuc-1, which seems to be a major hurdle for transcription, has to be disrupted. This disruption could occur either by inhibition of the recruited deacetylases at the level of the viral LTR or by recruitment of acetyltransferases (Fig. 1C). Consistent with this model, it is now well established that acetyltransferases are targeted to specific promoter regions by interactions with specific transcription factors. Several cellular factors binding to the HIV-1 LTR (see Section 4) and the viral *trans*-activator Tat (see Section 7) represent good candidates for the specific recruitment of acetyltransferases to the HIV-1 promoter region.

4. Deacetylase(s) and acetyltransferase(s) recruitment to the HIV-1 promoter

The 5′ LTR has been extensively characterized *in vitro*, and binding sites for several cellular transcription factors have been identified using *in vitro* DNase I footprinting and gel retardation assays (Fig. 1A) (see for review [37,38,52]).

On one hand, several of these factors, including AP-1 [53,54], ligand-bound nuclear hormone receptors (able to bind NRRE [55]) [56–58], c-Myb [59,60], glucocorticoid receptor (GR) [61], NF-AT [62], E-box binding proteins [63,64], Ets-1 [65], TCF-1α/LEF-1 [66–68], NF-κB [69–73], Sp1 [74–76], IRF [77] and the HIV-1 *trans*-activator Tat [78–80] have been shown to interact with acetyltransferases. On the other hand, several transcription factors that bind to the LTR, including unliganded nuclear hormone receptors (able to bind NRRE [55]) [56–58], GR [81], E-box binding proteins [46,47], YY1 [82], Sp1 [83], TCF-1α/LEF-1 [84], NF-κB ([85,86], unpublished data from our laboratory) have been shown to interact with deacetylases. These factors therefore represent good candidates for the specific targeting of acetyltransferases and deacetylases, respectively, to the HIV-1 promoter, thereby regulating the acetylation level of histones (in particular the histones of nuc-1).

4.1. Yin Yang-1

Recently, Coull *et al.* [87] has demonstrated for the first time a link between deacetylase recruitment and inhibition of HIV-1 expression by YY1 and LSF, in the natural context of a viral promoter integrated into chromosomal DNA. LSF would allow YY1 to recognize a site on the LTR that YY1 could not bind by itself [88]. Therefore, LSF would act as a docking molecule for YY1, which in turn recruits the deacetylase HDAC1 [87]. In this model, YY1 may be a limiting factor for LTR repression, required for the recruitment of HDAC1 to the viral promoter. This recruitment might maintain the nucleosome nuc-1 in a deacetylated state, thereby inhibiting HIV-1 expression.

4.2. E-box binding proteins

The LTR also possesses four E-box motifs [89–92]. The function of these motifs in HIV-1 regulation is poorly characterized and is under investigation in our laboratory. Generally, E-box motifs are able to recruit broad families of factors: the basic Helix–Loop–Helix (bHLH) and the bHLH-Leucine Zipper (bHLH-LZ) factors, including Mad-1, -2 (Mxi1), -3, -4, Max, c-Myc, Mnt/Rox, AP-4, USF, TFE3, TFEB, HTF4, E-47, E2A. The factors c-Myc, Mad, Max are members of a particular network. Although the binding of the factors of this network has not been demonstrated in the context of the HIV-1 LTR, such factors represent good candidates for the targeting of acetyltransferases and deacetylases to the HIV-1 promoter region. The central component of the c-Myc/Mad/Max network is Max. Max heterodimerizes either with Mad or with c-Myc. The roles of these heterodimers in the process of transcription are opposite: Mad–Max represses transcription, whereas c-Myc–Max actives it. Mad represses transcription by recruitment of HDACs *via* the co-repressor Sin3, whereas c-Myc activates transcription probably by recruiting the transcriptional co-activator TRRAP (transcription/transformation domain associated protein), which tethers the acetyltransferase hGCN5 (reviewed in [93]). Thus, in absence of HIV-1 activation, the E-boxes could be occupied by Mad–Max heterodimers, maintaining nuc-1 hypoacetylated. Following induction by TNF-α or TPA, a switch between the Mad–Max and c-Myc–Max heterodimers could take place, thereby allowing the recruitment of an acetyltransferase and the acetylation-dependent disruption of nuc-1.

4.3. Nuclear factor-κB (NF-κB)

An important candidate for the specific targeting of deacetylases and acetyltransferases is the transcription factor NF-κB. NF-κB plays a central role in the activation pathway of the HIV-1 provirus reviewed in [94,95]. Various studies have reported that the two NF-κB-binding sites in the HIV-1 enhancer [96,97] as well as the NF-κB

proteins [98,99] are critical for LTR promoter activity and important for optimal HIV-1 replication [100–103]. Recently, Karn and coworkers have shown that the p65 subunit of NF-κB stimulates transcriptional elongation from the HIV-1 promoter [104]. Moreover, two groups have separately reported the interaction of p65 either with HDAC1 [86] or with HDAC3 [85]. These HDACs could repress expression of NF-κB-regulated genes by maintaining histones in a deacetylated state. Moreover, NF-κB-dependent gene expression requires the function of transcriptional co-activator proteins, including CBP/p300, P/CAF, SRC-1, which possess acetyltransferase activity [69–73]. Finally, there is some evidence to suggest that deacetylase inhibitors may function to positively regulate NF-κB transcriptional activity [81,105]. We have recently demonstrated that deacetylase inhibitors (such as TSA and NaBut) synergize with both ectopically expressed p50/p65 and TNF-α-induced NF-κB to activate the HIV-1 promoter (see footnote 1). The major role of NF-κB in HIV-1 transcriptional activation and its ability to interact with deacetylases and acetyltransferases highlights the importance to investigate the potential role of NF-κB in the regulation of nuc-1-dependent HIV-1 transcriptional repression.

4.4. Stimulatory protein-1 (Sp1)

The ubiquitous transcription factor Sp1 is critical for both basal and Tat-induced transcription of the HIV-1 LTR [106,107]. Sp1 could also play an important role in the nuc-1-dependent regulation of HIV-1. Indeed, Sp1 has been demonstrated to interact with the acetyltransferase p300 and to act as a co-activator for Sp1-mediated transcriptional activation [74–76]. Sp1 has also been shown to directly interact with HDAC1 [83]. Whether these interactions have functional implications in the context of the HIV-1 promoter remains however to establish.

5. Possible role of transcription factor acetylation in HIV-1 regulation

It is important to note that histones are not the unique protein targets for reversible acetylation. In the case of HIV-1, acetylation could therefore also modulate the activity of some components of the basal transcription machinery as well as transcription factors important for optimal HIV-1 expression. Thus, acetyltransferase and deacetylase activities that are recruited to the LTR could modify the acetylation status not only of histones but also of transcription factors, thereby modulating their activity.

A growing list of factors has been demonstrated as substrates for acetyltransferases [11]. Binding sites for some of these factors are present in the HIV-1 5′ LTR: c-Myb, Sp1, IRF, TFIIEβ and TFIIF and the p65 and p50 subunits of NF-κB.

For Sp1, IRF, TFIIEβ and TFIIF, the functional role of their acetylation has not been reported [75,77,108]. For c-Myb, the substitution of acetylatable lysine residues into alanines increases c-Myb DNA binding activity and c-Myb *trans*-activating potential [59]. Moreover, acetylation of c-Myb by CBP increases its *trans*-activating capacity by enhancing its association with CBP [109]. Recently, Chen *et al.* [85] have reported that the p65 subunit of NF-κB is subject to a weak TNF-α-inducible acetylation and that the acetylated form of p65 interacts with reduced affinity, if at all, with its inhibitor IkBalpha. We also demonstrated a very weak acetylation of p65, however, in contrast to this latter study, we observed this acetylation independently of the presence of TNF-α.[2]

Interestingly, acetylation by p300/CBP of the p50 subunit of NF-κB in its DNA-binding domain increases its binding activity to the HIV-1 LTR, and this coincides with an increase in the rate of viral transcription [110]. Moreover, the acetylation of p50 is completely dependent on the presence of the viral *trans*-activator Tat [110]. This opens the possibility that Tat alters the specificity of p300/CBP activity in favor of another set of factors.

Eventhough binding sites for these acetylatable transcription factors are present in the HIV-1 5′ LTR, the precise significance of their acetylation in the context of HIV-1 transcriptional regulation remains undetermined.

6. Does protein acetylation modulate transduction signaling pathways?

Since protein acetylation affects an increasing number of substrate proteins and regulates them in various manners, it seems logical that acetylation as phosphorylation is involved in specific regulation pathways. Moreover, it seems likely that acetyltransferase activities should be regulated. This regulation will act at two levels: induction of acetyltransferase activity and substrate specificity. To date, very little is known about the regulation of acetyltransferase activity. Nevertheless, some reports show that CBP and hGCN5 acetyltransferase activities are modulated by phosphorylation [111,112] and that p53 phosphorylation stimulates acetylation, probably by increasing the association of p53 with CBP/p300 [113,114], indicating the existence of an intimate cross-talk between phosphorylation and acetylation. However, there is no evidence as yet for an acetylation cascade, i.e. an acetyltransferase modifying the enzymatic activity of a second acetyltransferase in order to transduce a biological signal (see [12] for extended discussion).

[2] Adam E, Quivy V, Nguyên TLA, Chariot A, Vanhulle C, Bex F, Schoonbroodt S, Goffin V, Collette Y, de Launoit Y, Burny A, Bours V, Piette J, Van Lint C. Potentiation of TNFalpha-induced NF-kappaB activation by deacetylase inhibitors is associated with a delay in the cytoplasmic reappearance of IkappaB-alpha, manuscript submitted for publication.

Recent results from our laboratory indicate that deacetylase inhibitor TSA activates HIV-1 transcription synergically with both ectopically expressed and TNF-α-induced NF-κB (see footnote 1). Mechanistically, we have shown that TSA delays the cytoplasmic reappearance of the NF-κB inhibitor IkBalpha. This coincides with a prolonged intranuclear presence and DNA binding activity of NF-κB on the HIV-1 LTR (see footnotes 1 and 2). Our results suggest a regulation of the NF-κB/IkBalpha signaling pathway by acetylation/deacetylation events. Therefore, in the context of the HIV-1 promoter, in addition to recruitment of acetyltransferases and deacetylases and to direct acetylation of transcription factors binding to the LTR, another level of acetylation-dependent regulation could intervene: modulation of a signaling pathway itself by deacetylase inhibitors.

7. The *trans*-activator Tat of HIV-1

Transcription of the HIV-1 provirus is characterized by an early, Tat-independent phase and a late, Tat-dependent phase. During the Tat-independent phase, the HIV-1 promoter is strictly under the control of the local chromatin environment and cellular transcription factors binding to *cis*-acting elements in the viral promoter region. Thus, as already described, this phase could be strongly inhibited through deacetylation and induced following acetylation of histones as well as of transcription factors. Additionally, acetylation is also involved in the Tat-dependent transcriptional phase.

The viral *trans*-activator protein Tat is an atypical transcriptional activator that functions through binding, not to DNA, but to a short leader RNA, called TAR (for *trans*-activation response) and located at the 5′-end of all viral transcripts ([115,116], reviewed in [117]). Tat activates transcription both at the level of initiation by augmenting the rate at which the cellular RNAPII starts transcription and at the level of elongation by increasing the processivity of the polymerase [117,118]. The role of Tat in transcriptional elongation can be ascribed to its specific interaction with protein complexes possessing protein kinase activity and being able to phosphorylate the carboxyl-terminal domain (CTD) of the largest subunit of RNAPII. This process is known to occur as RNAPII converts from an initiating (RNAPIIa) to an elongating enzyme (RNAPIIo). Those kinases are named Tat-associated kinases (TAKs) and include the RNAPII CTD kinases, TFIIH [119–121] and CDK9/P-TEFb ([122–124], reviewed in [125]).

Moreover, Tat is known to interact with different acetyltransferases: Tip60 [126,127], TAFII250 [128], hGCN5 [129], p300/CBP and P/CAF [78–80]. Although the targeting of acetyltransferases could be a general mechanism of Tat activity, the functional consequences of these interactions could be different for each acetyltransferase. Tat–Tip60 and Tat–TAFII250 interactions do not affect transcription from the HIV-1 LTR but repress transcription of cellular genes such as the manganese-dependent superoxide dismutase gene [130] and the major histocompatibility class I genes [128]. In contrast, p300/CBP, P/CAF and hGCN5 activate the HIV-1 LTR transcriptional activity. This activation could be mediated through acetylation of histones. This acetylation could derepress the chromatinized viral promoter and thus explain, at least in part, how Tat participates in initiating access and formation of the RNAPII complex. Consistent with this model, el Kharroubi *et al.* have shown a chromatin remodeling in the region of nuc-1 upon activation of the LTR by Tat [13]. Additionally, components of the basal transcription machinery (includ-

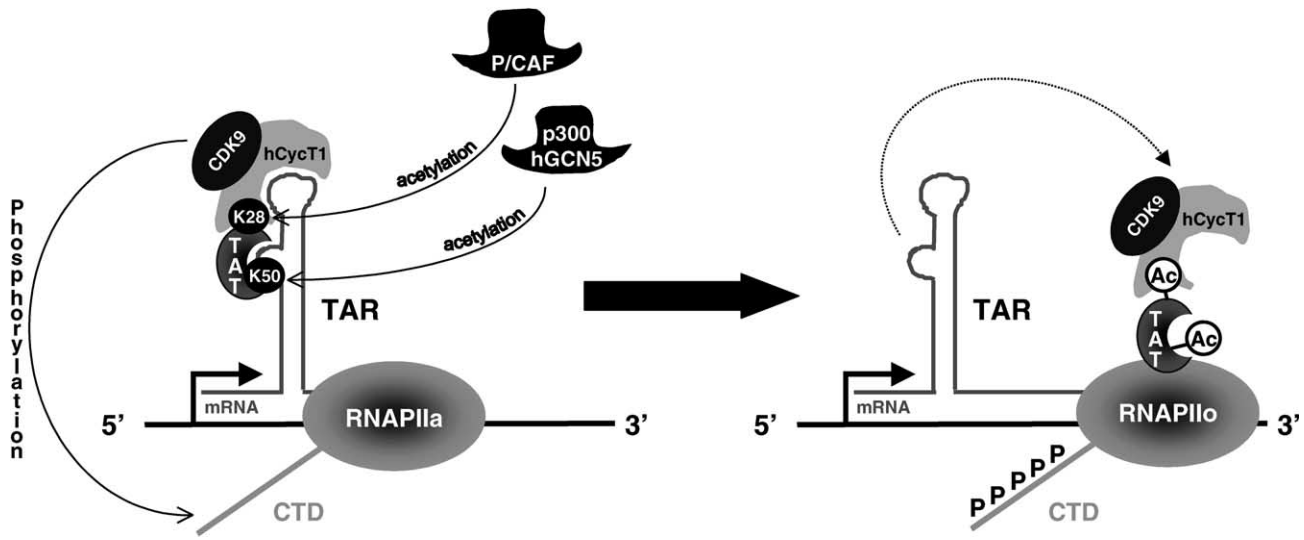

Fig. 2. Model for the acetylation-dependent regulation of Tat by p300, hGCN5 and P/CAF. P/CAF acetylates Lys28 in the *trans*-activation domain of Tat and this enhances the recruitment of hCycT1/CDK9. This would enhance CTD phosphorylation and transcriptional elongation. p300 and/or hGCN5 acetylate Lys50 in the RNA-binding domain of Tat and this promotes dissociation of Tat from TAR RNA.

ing TFIIEβ and TFIIF) and transcription factors required for an optimal HIV-1 expression (including Sp1 and NF-κB) could also have their activity modulated by acetylation through the recruitment of p300 by Tat. Moreover, we and others have demonstrated that Tat itself is modified by direct acetylation. The p300/CBP and hGCN5 acetylate lysine 50 and weakly lysine 51 in the TAR RNA binding domain of Tat [15,129,131,132], whereas P/CAF acetylates lysine 28 in the activation domain of Tat [15]. Mechanistically, acetylation at Lys28 by P/CAF enhances the recruitment of the cyclinT1(hCycT1)/CDK9 complex by Tat [15]. This would result in enhanced CTD phosphorylation by this Tat-associated kinase complex and in enhanced transcriptional elongation [15] (Fig. 2). Acetylation at lysine 50 of Tat promotes the dissociation of Tat from TAR RNA, dissociation that occurs during early HIV-1 transcription elongation [133,134] (Fig. 2). This would recycle functional Tat protein and enhance its apparent concentration in a manner advantageous for function. This could also explain the uncharacterized mechanism for the release of Tat from TAR and its presence in the elongation complex (Fig. 2). The use of different cellular acetyltransferases (p300/CBP and hGCN5) by Tat to acetylate the same residues (lysines 50 and 51) highlights the importance of this post-translational modification for the activity of Tat. Thus, acetylation of Tat regulates two discrete and functionally critical steps in HIV-1 transcription: binding of Tat to the RNAPII CTD-kinase and release of Tat from TAR RNA.

8. Conclusions

The HIV-1 provirus belongs to a family of genes whose transcriptional activation is modulated by acetylation/deacetylation. It is likely that the HIV-1 promoter will prove to be dependent on the activity of several specific deacetylases/acetyltransferases. Such specificity could be conferred by interactions of a given set of deacetylases/acetyltransferases with a distinct set of DNA-binding factors. In addition to the specific targeting of their enzymatic activities, these deacetylases/acetyltransferases have specificity in their choice of substrates: both in terms of which protein they target and in terms of which specific lysine residues are modified on each protein.

Increasing evidence shows that deacetylases and acetyltransferases intervene in transcriptional silencing and activation of the HIV-1 provirus, respectively. Therefore, in order to increase our understanding of HIV-1 latency and reactivation, the next challenge will be to identify how and which deacetylase(s)/acetyltransferase(s) are involved in viral transcriptional regulation. The knowledge of these should define new targets for drugs design and therapeutic intervention aimed at interfering with HIV-1 replication. There are two main opposite strategies: first, maintain cells in the latent state by acetyltransferase inhibition in order to improve highly active anti-retroviral therapy (HAART), and second, force virus expression in the resting cells by deacetylase inhibition in order to purge the latent HIV-1 reservoirs in presence of a continuous and efficacious HAART treatment.

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