

HIV-1 Transcription: Activation Mediated by Acetylation of Tat

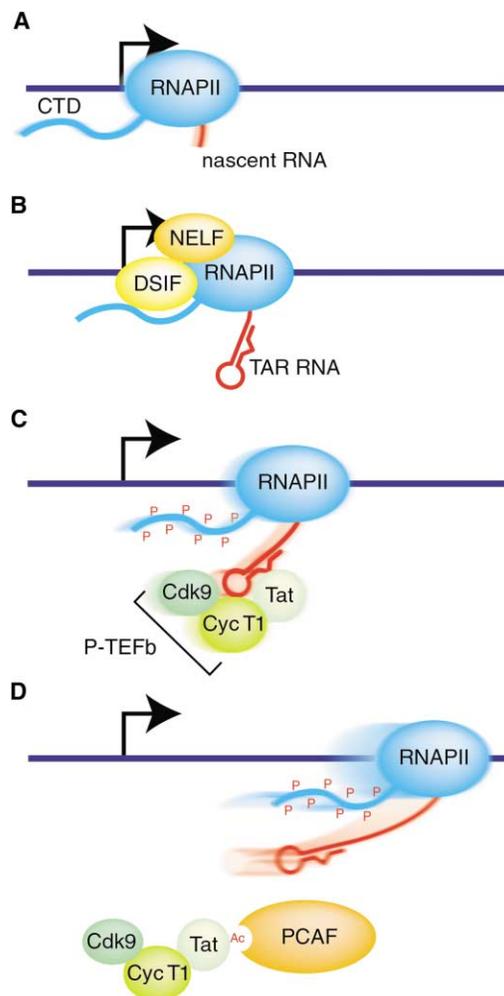
Acetylation of HIV-1 Tat stimulates transcriptional elongation by dissociating Tat from TAR, a transactivation response RNA element in nascent HIV-1 transcripts. In the March issue of *Molecular Cell*, Mujtaba et al. show that the bromodomain of PCAF acetylase specifically binds to acetylated Tat and leads to dissociation of Tat from TAR.

Transcription elongation is a critical rate-limiting step for transcription of proviral DNA of human HIV-1. In the HIV-1 promoter, RNA polymerase II (RNAPII) activity is stalled shortly after initiation of transcription (see Figure, panel A). Previous work showed that two cellular complexes, DSIF (DRB sensitivity-inducing factor) and NELF (negative elongation factor), function together to block elongation [1, 2]. DSIF is composed of hSPT4 and hSPT5 (human homologs of yeast factors which affect transcriptional elongation), while NELF is composed of at least five subunits, including WHSC2 (a factor which appears to be targeted by the viral elongation-stimulating factor, hepatitis delta antigen) and RD (a putative RNA binding protein).

Although binding of DSIF and NELF to RNAPII shortly after initiating RNA synthesis leads to inhibition of elongation, this step is regulated by the phosphorylation of CTD (carboxy-terminal domain) of the largest subunit of RNAPII (see Figure, panel B). Importantly, in a nuclear extract, DSIF and NELF tightly bind to hypophosphorylated RNAPII, but not to the hyperphosphorylated form, suggesting that phosphorylation of CTD causes dissociation of these negative factors from RNAPII.

How is repression by DSIF and NELF counteracted in HIV-1 transcription? HIV-1 Tat plays a key role in such derepression. First, Tat forms a complex with the positive elongation factor P-TEFb, which is composed of cyclin T1 and CDK9 (see Figure, panel C). Then, the Tat/P-TEFb complex is tethered to the RNA element in nascent HIV-1 transcripts, called TAR. Accordingly, P-TEFb phosphorylates CTD. Thus, recruitment of the CTD kinase P-TEFb on TAR RNA via Tat could be a key step to form a full-length transcript [3].

As is well known, histone acetylases, by acetylating histone tails, contribute to transcriptional activation in chromatin contexts [4, 5]. Similarly, the p300/CBP and PCAF histone acetylases help transactivation of HIV-1. These acetylases form a substoichiometric complex and act together in many promoters, such as p53-responsive promoters. p300/CBP and PCAF have distinct substrate specificities in nucleosomes: while p300/CBP acetylates mostly lysine residues which are acetylated in vivo, PCAF acetylates only Lys14 on histone H3. Although exactly how histone acetylation contributes to transactivation is still unclear, acetylated histones likely serve as



Stimulation of Transcriptional Elongation by HIV-1 Tat

(A and B) Shortly after initiation of transcription, DSIF and NELF bind to hypophosphorylated RNAPII to block elongation. In addition, nascent transcripts, DNA elements, and/or chromatin structure might be involved in stalling RNAPII. At this stage, CTD might be partially phosphorylated by the general transcription factor TFIIH. (B) Tat, by forming a complex with the positive elongation factor P-TEFb, is recruited on the RNA element in HIV-1 nascent transcripts called TAR.

(C) P-TEFb phosphorylates CTD, leading to dissociation of DSIF and NELF from RNAPII. This step is stimulated by the acetylation of Lys28 of Tat with PCAF.

(D) Acetylation of Lys50 by p300/CBP leads to dissociation of Tat from TAR. This step involves binding of Lys50-acetylated Tat with the PCAF bromodomain. Note that PCAF may exist in the elongation complex.

“flags” that are recognized by other factors responsible for transactivation.

The bromodomain is a potential target for acetylated histones. The double bromodomain module of TAF_{II}250 has been shown to interact with the histone H4 tail in an acetylation-dependent manner [6], although it is still

unclear how this interaction contributes to activation. While p300/CBP and PCAF could activate HIV-1 transcription by acetylating nucleosomal histones, they also acetylate Tat [7, 8]. Interestingly, p300/CBP and PCAF appear to contribute to HIV-1 activation via alternative mechanisms by acetylating distinct residues of Tat. Acetylation of Lys28 by PCAF has been shown to stimulate interaction of Tat with P-TEFb, whereas acetylation of Lys50 by p300/CBP leads to dissociation of Tat from TAR.

Now, in the March issue of *Molecular Cell*, Mujtaba et al. [9] explain how acetylation of Lys50 of Tat contributes to HIV-1 activation at the structural level. They demonstrate that the bromodomain of PCAF recognizes Lys50-acetylated Tat, but not Lys28-acetylated Tat. In contrast, the bromodomain of CBP recognizes neither Lys28- nor Lys50-acetylated Tat, suggesting that Lys50-acetylated Tat by p300/CBP is recognized by the bromodomain of PCAF (see Figure, panel D). Moreover, they determined the NMR structure of the PCAF bromodomain bound to the Lys50-acetylated Tat peptide. Interestingly, the peptide induces local conformational alterations around its binding sites on the PCAF bromodomain, producing a hydrophobic cavity that accommodates the Lys50-acetylated Tat peptide. Moreover, TAR RNA does not significantly affect the conformational alterations of the PCAF bromodomain induced by the acetylated Tat, indicating that the affinity of Lys50-acetylated Tat with the PCAF bromodomain is much higher than that with TAR RNA. These data support the previous model that Lys50 acetylation of Tat leads to dissociation of Tat from TAR RNA, contributing to stimulation of transcriptional elongation of HIV-1 [7, 8].

This work has significantly advanced our knowledge of how acetylation contributes to gene activation. Nevertheless, an additional issue must be addressed in the

near future. Jones and colleagues demonstrated that binding of cyclin T1 (a subunit of P-TAFb) to Tat significantly increases the affinity and specificity of the interaction between Tat and TAR RNA: the Tat/cyclin T1 complex recognizes sequences in the loop of TAR RNA, while Tat alone does not [10]. Therefore, it is important to demonstrate whether the PCAF bromodomain dissociates Tat from the Tat-P/TAFb complex bound to TAR RNA.

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Selected Reading

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A Glimpse of the Catalytic Core of a Group II Intron

A paper in a recent issue of *Science* describes the first high-resolution structure of part of the catalytic core of a group II intron that will allow more detailed comparisons between the excision of introns by self-splicing group II introns and by nuclear pre-mRNA introns.

The processes by which self-splicing group II introns and nuclear pre-mRNA introns are excised have long been compared and contrasted. The former occurs by an RNA-only mechanism, and the latter within a large RNP assemblage, the spliceosome. Yet both systems share strikingly similar structural features within their cores. So far, however, no high-resolution structural information has existed. Now, a new structure of core

components of a group II intron and new evidence of bound metal ions will allow for more detailed future comparison [1–4].

Group II introns are found in eubacteria and in the organellar genomes of fungi and plants. The best characterized of these, ai5 γ , excises itself from pre-mRNA by several related RNA-catalyzed pathways: two *cis* pathways, branching or hydrolysis, and a newly recognized *trans* pathway. The branching pathway is chemically identical to the excision of nuclear pre-mRNA introns by the U2- and U12-dependent spliceosomes, involving two sequential transesterification reactions (Figure 1A, pathway 1): (i) nucleophilic attack by an adenosine 2'OH within the intron (the branch site) at the 5' splice site phosphate, forming a free exon 1 intermediate and a lariat intron, followed by (ii) attack of the free exon 1 3'OH at the 3' splice site, resulting in exon-exon ligation and release of the intron. The hydrolysis pathway uses water or hydroxide as the first step nucleophile, followed by a second step chemically identi-