Activation of HIV transcription by Tat

Alan D. Frankel

Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA

Recent studies suggest that the human immunodeficiency virus transactivator, Tat, increases expression of viral genes primarily by enhancing the efficiency of transcriptional elongation. The degree to which Tat influences elongation may depend on the rate of transcriptional initiation. Current models in which Tat interacts with the transcription complex suggest directions for future studies.

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Introduction

Replication of human immunodeficiency virus (HIV) is critically dependent on two viral regulatory proteins, Tat and Rev. Tat is required early in the viral life cycle to increase the rate of transcription from the viral long terminal repeat (LTR). As the level of transcription first rises, spliced mRNAs that encode Tat and Rev are made. Tat further stimulates transcription, and then Rev, after reaching a critical level, acts to suppress splicing and help transport unspliced viral mRNAs to the cytoplasm. These unspliced mRNAs encode the viral structural proteins, which are translated and assembled into virus particles.

The mechanism by which Tat acts has been the subject of controversy. Most researchers now agree that its primary role is to stimulate HIV transcription. Although other roles have been proposed, including control of translation, stimulation of cell growth, and inhibition of T-cell responses, none have yet been shown to be functionally important *in vivo*. Therefore, this review will focus exclusively on the function of Tat in transcriptional activation.

Tat is a transcription factor

Several early studies using heterologous reporter plasmids showed that Tat increased the steady-state level of RNAs transcribed from the HIV LTR approximately 20–50-fold. Recent experiments examining Tat transactivation in the natural context of the virus have shown a similar increase in transcription and suggest that transcriptional regulation is the primary function of Tat during normal viral growth [1••]. Some of the earlier work had suggested that Tat may also control post-transcriptional events, however, the effects observed may reflect nuances of particular reporter systems rather than true activities of Tat [1••,2•].

The HIV promoter and TAR

Transcription from the HIV LTR is controlled through several upstream regulatory elements, including TFIID-, Sp1-, and NF-κB-binding sites (Fig. 1). An additional element, the *trans*-acting responsive element (TAR), is located just 3′ to the start of transcription and is required for Tat transactivation. Unlike a traditional enhancer element, the natural position and orientation of TAR must be maintained. TAR forms a stable RNA hairpin at the 5′ end of the nascent viral transcripts [3], and it is now clear that Tat binding to TAR RNA is essential for Tat function [4,5,6•].

The RNA-binding domain of Tat is nine amino acids long and contains six arginines and two lysines. Short peptides spanning just this region bind specifically to TAR [6•,7,8]. The RNA-binding domain is unusually flexible both in amino acid sequence and in structure [6•], with a single arginine residue providing the only sequence-specific RNA contact [9••]. In contrast to the protein, the structural requirements for TAR are more rigid. Mutagenesis experiments have shown that a three-nucleotide bulge and particular nucleotides surrounding the bulge (Fig. 1) are essential for Tat binding and transactivation [4,5,10••,11]. Modification of two phosphates at the base of the bulge interferes with binding [9••], leading to a model in which arginine forms a network of hydrogen bonds with two structurally distinct phosphates. Another model has been proposed, based on chemical accessibility [10••], in which the RNA major groove is widened near an A–U base pair above the bulge, exposing specific groups for recognition. It is interesting that this particular adenine is susceptible to deamination in *Xenopus* oocytes [12]. Structural studies will be needed to determine the details of Tat–TAR recognition.

In addition to Tat binding, it appears that TAR also binds cellular proteins whose function may be to stabilize Tat binding or to modulate Tat activity. Mutations within a six-nucleotide loop in TAR (Fig. 1) reduce transactivation but do not directly affect Tat binding [4,5,6•,8]. Several

Abbreviations

HIV—human immunodeficiency virus; **LTR**—long terminal repeat; **TAR**—*trans*-acting responsive element.

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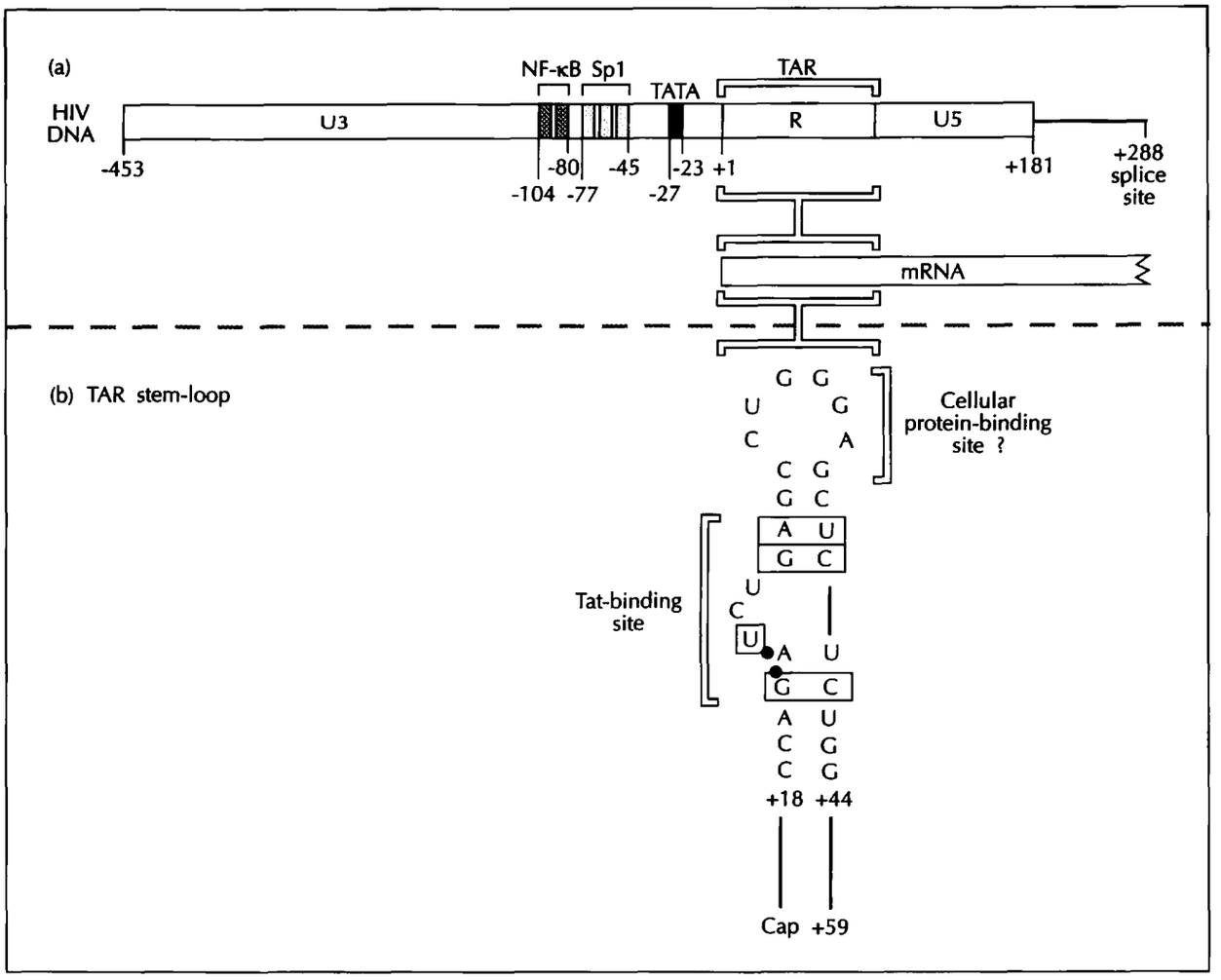

Fig. 1. Structure of the HIV long terminal repeat and the *trans*-acting responsive element (TAR). (a) Regulatory sites upstream from the start of transcription (+1) include two NF-κB sites (between -104 and -80), three Sp1 sites (between -77 and -45), and a TFIID site (the TATA box between -27 and -23). The transcribed region from +1 to +288 (including the TAR site) is present on all spliced and unspliced viral mRNAs. (b) The upper part of the TAR stem-loop (nucleotides +18 to +44) is required for Tat transactivation. The nucleotides and base pairs required for Tat binding are boxed. Black dots indicate the positions of two phosphates the modification of which interferes with Tat binding. (Note that these phosphates were initially assigned incorrectly as the two phosphates surrounding the U at position 23 (J Tao, AD Frankel, unpublished) [9••]. The six-nucleotide loop of TAR may bind a cellular protein.

proteins have been shown to bind to the loop and some partially purified proteins have been shown to stimulate HIV transcription *in vitro* [13–15]. Their role in transactivation has, however, not yet been firmly established and the function and identity of cellular TAR-binding proteins remains an important question.

A role in transcriptional elongation

The unusual location of TAR and the fact that TAR is an RNA element suggested that Tat might not act as a 'typical' transcriptional activator. Most activators are thought to increase the rate of initiation. However, analysis of steady-state transcripts produced in the presence or absence of Tat revealed that Tat increased promoter-distal but not promoter-proximal transcription, suggesting that Tat may affect elongation rather than initiation [16]. Nuclear run-on experiments, in which the distribution of transcription complexes along a transcript can be measured by extending the nascent transcripts in isolated nuclei, have confirmed that Tat increases the efficiency of elongation [1••,2•,17]. In the absence of Tat, a strong polarity to transcription was observed, with transcription terminating gradually over the first several hundred nucleotides. In the presence of Tat, no polarity was observed and virtually all initiated transcripts were efficiently completed.

In an *in vitro* transcription system, Tat shows the same effect on elongation as seen *in vivo* [13]. In the absence of Tat, most transcripts terminate within a few hundred nucleotides from the transcription start site whereas in the presence of Tat most transcripts are efficiently completed. It had initially been proposed that the TAR stem-loop might be a transcriptional terminator [16], however,

no specific termination site has been observed either *in vivo* or *in vitro*. Furthermore, it is known that deleting TAR does not increase transcription [18], suggesting that poor elongation is determined by the promoter rather than resulting from a discrete termination event. How interactions at the promoter determine the subsequent efficiency of elongation complexes is unclear, however, recent *in vitro* transcription experiments suggest that there may be two classes of elongation complexes, a 'less-processive' and a 'more-processive' form, and that Tat may increase the proportion of 'more-processive' complexes [19•].

Tat can also enhance transcriptional elongation from a heterologous promoter. Transcription from the U2 small nuclear RNA promoter normally terminates at a specific 3′ termination signal, but when TAR was placed downstream of the start site and Tat was added, transcription proceeded through the terminator [20•]. In these same experiments, it was also observed that inserting the TAR region increased the rate of initiation, but that the resulting transcripts were incompletely elongated in the absence of Tat [20•]. Thus, the inserted TAR-containing region (in HIV, nucleotides −5 to +82) may encode two functions: an activation element that stimulates initiation; and the Tat-binding site, TAR, which is needed, along with Tat, to efficiently elongate the increased number of initiated transcripts. One possible interpretation, in light of the recent *in vitro* transcription results [19•], is that the additional initiation complexes formed are of the 'less-processive' class and that Tat is required to convert them to the 'more-processive' class.

Elongation versus initiation

Although the results seen in the virus, in some of the reporter systems, and *in vitro* suggest that Tat works primarily by enhancing transcriptional elongation, it is difficult to rigorously separate effects on elongation from effects on initiation. There are technical problems, especially *in vivo*, in monitoring the addition of just the first few nucleotides to the nascent transcript. There are also theoretical problems, in that initiation and elongation may be coupled processes. Both the kinetics of initiation complex assembly and the particular components assembled during initiation may determine the ultimate efficiency of an elongation complex. Furthermore, it may be difficult to distinguish effects on initiation from 'traffic jams', in which slowly moving or 'less-processive' elongation complexes back up to the promoter, indirectly affecting the rate of initiation. With these problems in mind, there is evidence that in addition to elongation Tat can stimulate transcriptional initiation.

It has been proposed that TAR may function as an RNA enhancer, recruiting Tat to the HIV promoter and positioning Tat to interact with the initiation machinery [21]. This is consistent with experiments showing that Tat can transactivate through heterologous RNA-binding sites when Tat is fused to the corresponding RNA-binding protein [22,23]. Tat can also function through GAL4 DNA-binding sites placed upstream of the transcription start site, provided that Tat is fused to GAL4 and that other regulatory elements in the HIV promoter are present, particularly sites for Sp1 [24•,25•]. These results have been interpreted to support increased initiation, based on analogy to other transcriptional activators, with the provision that Tat works only in synergy with other upstream regulatory factors. An alternative interpretation is that Tat enhances elongation only when the rate of initiation is high (thus the dependence on Sp1). This would be consistent with the U2 promoter experiments, in which another element, the -5 to +82 region of the HIV promoter, increases the rate of initiation.

The nuclear run-on experiments using heterologous reporter systems have suggested that Tat may effect initiation as well as elongation [2•,17]. Interestingly, when basal transcription was increased by either adenovirus E1A or phorbol ester stimulation, Tat's effect on elongation was more pronounced [2•]. This is consistent with the idea that Tat may enhance elongation only when the rate of initiation is high. Experiments with reporter plasmids, in which the location of the SV40 origin of replication was shown to influence the rate of initiation from the HIV promoter, further demonstrate that a high rate of initiation is needed for Tat to exert a strong effect on elongation [26]. Because the nuclear run-on experiments in the virus showed no effect of Tat on initiation [1••], it is possible that basal transcription in the natural setting of the LTR is always high enough for Tat to enhance elongation. It would be of interest to study whether there are particular cell types, or growth conditions *in vivo*, that suppress initiation from the HIV promoter, perhaps allowing Tat to function in initiation.

A model of Tat transactivation

A current working model to explain Tat's effect on elongation is presented in Figure 2. In the absence of Tat, the HIV promoter initiates transcription at a high rate, as suggested by experiments in the virus [1••], but few transcripts are completely elongated. When Tat is present, the same high rate of initiation is observed, but virtually all initiated transcripts are completed. The simplest model would suggest that Tat is targeted to the HIV promoter by binding to TAR and interacts directly with the transcription machinery to enhance elongation efficiency. Thus, Tat may act as a gene-specific elongation factor. Tat itself might remain bound to the transcribing complex or it might modify the complex (for example, by causing dissociation or association of other proteins) to create a 'more-processive' complex.

Why is elongation from the HIV promoter inefficient? The current data do not provide an obvious answer but they do suggest that the ability to elongate may be related to the rate of initiation, perhaps because elongation-determining events occur during initiation complex assembly. Because Tat transactivation is more efficient at higher levels of basal transcription [2•,20•,24•,25•,26], it is possible that the elongation-determining step for the HIV pro-

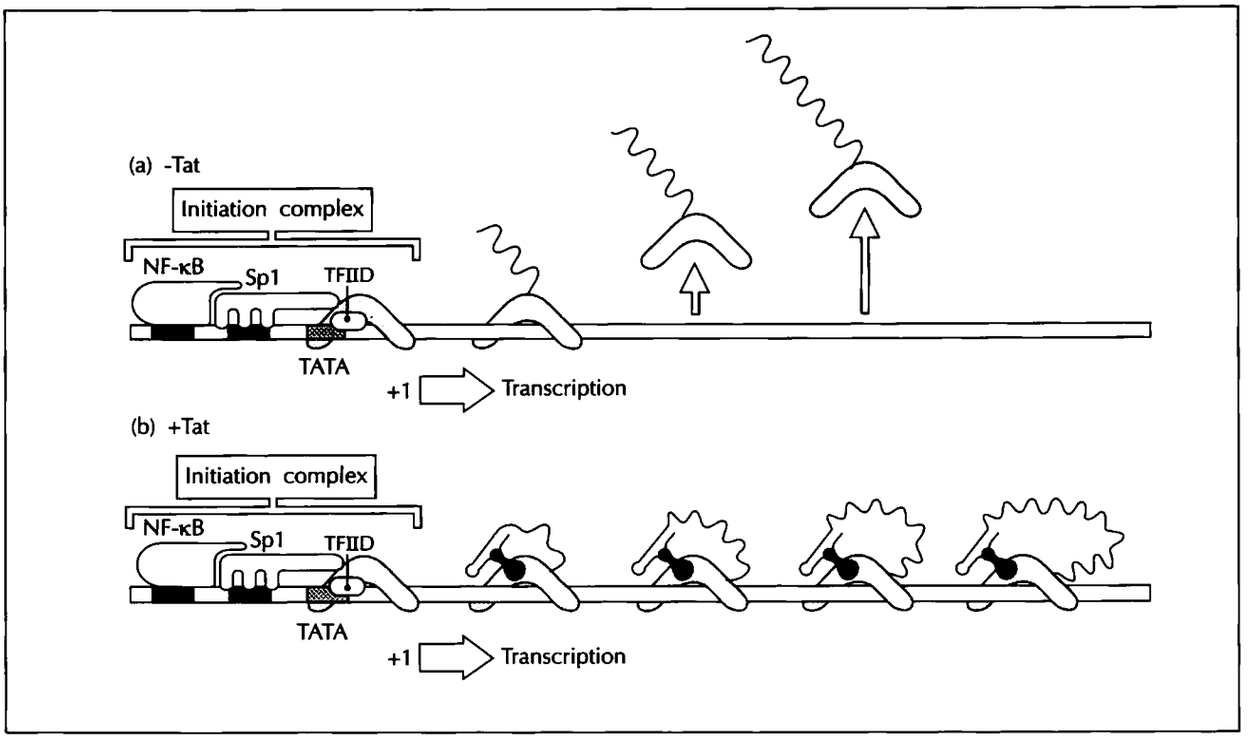

Fig. 2. A current model of Tat transactivation. (a) In the absence of Tat (-Tat), the rate of initiation of transcription from the HIV promoter (indicated by the large white arrow), which has upstream binding sites for several regulatory factors including NF-κB, Sp1 and TFIID (see Fig. 1), is high, but most elongating transcription complexes terminate within several hundred nucleotides of the transcription start site (+1). Only rarely is the 10 kb viral transcript completed. (b) In the presence of Tat (+Tat), virtually all initiated transcripts are completed. In the particular model shown, Tat (black dumbell) interacts with both TAR and the transcription complex and remains bound during elongation. (There is currently no direct evidence that Tat does indeed interact with the transcription complex or remains bound during elongation.) Cellular TAR-binding proteins that may interact with the loop and stabilize Tat binding or otherwise modulate transactivation are not shown. DNA polymerase II is shown as a grey boomerang.

moter may be slow relative to initiation complex assembly, resulting in the formation of 'less-processive' elongation complexes when the promoter is rapidly initiating. Tat might increase the formation of 'more-processive' complexes by increasing the rate of the slow elongation-determining step during initiation or by modifying the transcription complex after initiation. Other promoters that initiate transcription at a high rate may also assemble 'less-processive' elongation complexes [19•]. Not all strong promoters show inefficient processivity, suggesting that the particular array of regulatory factors bound to any given promoter may, in addition to influencing initiation complex assembly, also influence elongation complex assembly.

Future directions: cellular factors

Clearly, Tat does not act alone. In addition to binding TAR, Tat must interact, either directly or indirectly, with the transcription apparatus. Thus, a major direction for future work will be the identification of cellular proteins that interact with Tat. One such protein has been found by screening an expression library with Tat [27], although it is not yet known if this protein is involved in transactivation. Studies of hybrid-chromosome cell lines suggest that the human chromosome 12 may encode a protein that enhances Tat transactivation [28,29]. The existence of Tat-binding proteins is further suggested by a transdominant Tat mutant in which the TAR RNA-binding domain was deleted [30]. It is presumed that this mutant is transdominant because it competes with Tat for binding to cellular proteins.

The level of Tat transactivation can differ between cell types [31,32], suggesting that factors that interact with Tat may be present in varying amounts or that the amount or type of basal transcription factors (and thus rates of initiation) may differ. After a period of activity, transactivation by Tat may be downregulated, a process perhaps mediated by cellular factors [33]. It has been reported that, under some conditions, Tat can transactivate in the absence of TAR [34]. Understanding the differences in these cellular environments will undoubtedly provide clues about the mechanism of Tat activity.

Cellular TAR-binding proteins might exist that provide additional specificity for the Tat–TAR interaction, which on its own displays relatively modest specificity [9••,10••]. Further specificity might come from interactions with other cellular proteins, such as components of the transcription apparatus. It would be interesting to elucidate whether expression of TAR- or Tat-binding proteins

is regulated, thus providing a potential means for controlling Tat activity. Whatever components are involved, it is clear that identifying interacting proteins and demonstrating their functional significance will be the next critical steps in understanding the mechanism of transactivation by Tat.

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- • of special interest
- •• of outstanding interest

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AD Frankel, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA.