Minireview

Multifaceted Activities of the HIV-1 Transactivator of Transcription, Tat*

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Human immunodeficiency virus, type 1 (HIV-1) is the etiological agent for the acquired immunodeficiency syndrome (AIDS). HIV-1 is a retrovirus that encodes a small nuclear transcriptional activator protein, Tat (Fig. 1). In vivo, Tat is required for virus replication and is conserved in the genomes of all primate lentiviruses (1). Over the past decade, the transcriptional function(s) of Tat (reviewed in detail several years ago (2)) has been intensely investigated. It has become clear that a primary role for Tat is in regulating productive and processive transcription from the HIV-1 long terminal repeat (LTR). Tat also has other activities; some are consistent with that of a secreted growth factor (3–5) and a potent inhibitor of reverse transcription (6). Here we review recent insights into the multifaceted activities of Tat.

Domains of the 101-Amino Acid RNA-binding Tat Protein

Tat is a transcriptional activator that binds to a short nascent stem-bulge-loop leader RNA, TAR (trans-activation responsive (7–10)), for its activity. The 101-amino acid Tat protein, with residues 1–72 encoded by a first exon and residues 73–101 encoded by a second exon, can be arbitrarily considered as containing several "domains" (11) (Fig. 1). Of interest, it should be noted that whereas an 86-amino acid form of Tat, which exists for a few laboratory-passaged virus strains (10), for its activity. The 101-amino acid Tat protein, with residues 1–72 encoded by a first exon and residues 73–101 encoded by a second exon, can be arbitrarily considered as containing several "domains" (11) (Fig. 1). Of interest, it should be noted that whereas a 96-amino acid form of Tat, which exists for a few laboratory-passaged virus strains (e.g. LAI, HXB2, pNL4–3) (Fig. 1), has been frequently used; this version represents a truncated and not naturally full-length protein. Indeed, a single nucleotide change in LAI, HXB2, and/or pNL4–3 at putative residue 87 unmasks in these respective genomes the conserved 101 amino acids (Fig. 1) of Tat that are found in most in vivo isolates of virus. This suggests that the premature termination codon that exists in laboratory isolates at position 87 conceivably arose artifically during tissue culture passaging (12). Thus, that more than 90% of the more than 100 extant independently characterized HIV-1 Tat proteins maintain the 101 (and not the 86) amino acid configuration (1) is consistent with this interpretation. Hence, although residues 87–101 of Tat might not contribute greatly to the ex vivo propagation of HIV-1, their conservation in viruses that replicate in vivo provides a good indication of their biological importance. In this regard, the second coding exon of Tat, which in many studies has been frequently not considered, has been shown to be significant in several biological assays (13–19).

Over the past decade, a detailed structure-function analysis of Tat has emerged, in part through the generation of an extensive collection of point mutants (Table I) produced from 11 laboratories (11, 12, 20–29). From these mutants, one notes that single residue changes in domain 1 of Tat (amino acids 1–20) are well tolerated. By contrast, changes in six of the seven highly conserved cysteines in amino acids 21–40 (Fig. 1, domain 2) abolish function (see Table I). Domain 3 (amino acids 41–48) contains a common RKKGLGI motif found in HIV-1, HIV-2, and SIV Tat. Amino acids 1–48 together circumscribe a minimal activation domain for HIV-1 Tat (30, 31).

Perhaps the best studied region of Tat resides in amino acids 49–72 (domain 4), which contain a basic RKKRRKQRRR motif. This peptide motif confers TAR RNA binding properties to Tat (32–35) and is important for nuclear localization of the protein (22, 23) and uptake of Tat by cells (3). For association with TAR, the short basic motif contributes importantly to affinity but dictates insufficiently specificity of binding. Flanking amino acids outside this basic domain influence significantly the specificity of Tat-TAR interaction (36, 37). A recent detailed review of Tat-TAR RNA interaction is available elsewhere (38).

Role of Tat in HIV-1 LTR-directed Transcription

Transcription from the HIV-1 LTR is several hundred-fold higher in the presence of Tat than in its absence. Thus, Tat must resolve a rate-limiting step at this promoter. Optimal Tat action requires, in addition to TAR RNA, basal (TATA and initiator sequence) and upstream promoter elements (i.e. Sp1) (39) (Fig. 2). Recent experimental findings have added to our understanding of the mechanism(s) through which Tat acts through these elements.

In considering Tat action, one understands that two operationally defined events occur for each round of transcription at virtually all promoters. These are: (i) recruitment of an RNA polymerase II (RNAP II) complex to the promoter and (ii) the escape of that complex from the promoter into productive elongation. A typical role proposed for transcriptional activator proteins is that of facilitating a rate-limiting step in the recruitment of TBP-bound RNAP II to the promoter (40, 41). Although Tat is not a typical activator
protein, it does possess the capacity to bind directly several general transcription factors including TFIIID (42), TFIIIB (43), TFIIH (44), and RAP II (45). Thus, an attractively simple mechanism of how Tat might accelerate the rate of transcription from the HIV-1 LTR would be if it increased the recruitment of TBP/RNAP II to the viral promoter. This hypothesis was directly examined; and in such a study, it was found that Tat unlikely functions at recruiting TBP/RNAP II to the viral promoter. Indeed, the rate-limiting event(s) resolved by Tat occurs at a step(s) post-TBP recruitment to the viral promoter. SF1 has been suggested as an additional contributory event to the recognition of positive (P-TEF) transcription elongation factor (50). A recent breakthrough in understanding Tat function came with the integration of positive transcription elongation factor, P-TEFb, into HIV-1 LTR-directed transcription. This recognition culminated from several preceding observations. First, it was found that a Tat-associated kinase (TAK (51)) could phosphorylate the carboxyl-terinal domain (CTD) of the large subunit of RNAP II. Next, phosphorylation of the RNAP II-CTD was correlated with Tat activation of transcription (52, 53). Subsequently, TAK was elucidated to be the P-TEFb complex of proteins, which included the cyclin-dependent kinase, cdk9 (54–56). In the P-TEFb complex, cdk9 was shown to be bound to one of several forms of cyclin T (T1, T2a, T2b (58, 59)). The cdk9-cyclin T complex (P-TEFb) was found to associate directly with Tat. This association with P-TEFb facilitates a high affinity binding of Tat for TAR RNA (57, 58). In the P-TEFb complex, cdk9 was shown to bind to one of several forms of cyclin T (51). Tat/SF1, resulting in its phosphorylation (57). This phosphorylation of Tat/SF1 has been suggested as an additional contributory event to the role of P-TEFb in HIV-1 LTR transcription (57).

Schematically, a simplified view of Tat/P-TEFb/TAR/RNAP II interaction can be represented by the illustration in Fig. 2A. Association of Tat and P-TEFb (TAK) with TAR leads to phosphorylation of the RNAP II-CTD. Phosphorylation of RNAP II-CTD ren-

### TABLE I

**Multifaceted Activities of Tat**

| Amino acid changes | From | To | Activities |
|--------------------|------|----|------------|
| Q2                 | A    | +  | ++         |
| E2D3E9             | GGG  | (−) | −/−        |
| P3                 | A    | +  | ++         |
| P3                 | G    | +  | +/−        |
| P3                 | L    | (+/−) | −/−        |
| V4                 | A    | +  | ++         |
| D5                 | A    | +  | +/−        |
| D5                 | N    | −  | −/−        |
| P6                 | A    | +  | ++         |
| P6                 | S    | +  | ++         |
| Δ5–6               | +    |    |            |
| P6P10              | LL   | +  | −/−        |
| R7                 | A    | +  | ++         |
| L3                 | A    | +  | ++         |
| E9                 | A    | +  | ++         |
| P10                | A    | +  | ++         |
| P10P14             | LL   | +  | −/−        |
| W11                | A    | +  | ++         |
| K12                | A    | +  | ++         |
| K12                | N    | +  | ++         |
| P18                | A    | +  | ++         |
| K19                | R    | +  | ++         |
| A21                | D    | +  | ++         |
| A21P23             | VA   | +  | ++         |
| T23                | A    | +  | ++         |
| C22                | S    | –  | −/−        |
| C22                | G    | –  | −/−        |
| N23                | T    | +  | ++         |
| N24                | A    | +  | ++         |
| N24                | K    | +  | ++         |
| C25                | R    | –  | −/−        |
| C25                | G    | −  | −/−        |
| Y26                | A    | +  | ++         |
| Y36                | F    | +  | ++         |
| C27                | S    | −  | −/−        |
| K23K29             | AA   | +  | ++         |
| K23K29             | EA   | −  | −/−        |
| C30                | G    | −  | −/−        |
| C31                | S    | +  | ++         |
| C31                | E    | –  | −/−        |
| C31                | G    | +  | ++         |

| Activities | From | To | |
|------------|------|----|---|
| ++         |      | G  | −/− |
| −/−        |      | A  | −  |
| −/−        |      | −  | −  |
| +/−        |      | +  | −  |
| −/−        |      | −  | +  |
| +/−        |      | +  | −  |
| −/−        |      | −  | −  |

### TABLE 1

| Amino acid changes | From | To | Activities |
|--------------------|------|----|------------|
| F32                | A    | +  | +          |
| H83                | A    | –  | −/−        |
| C34                | G    | –  | −/−        |
| C34                | S    | –  | −/−        |
| G35                | A    | +  | ++         |
| C37                | G    | –  | −/−        |
| C37                | S    | –  | −/−        |
| F38                | A    | −  | −/−        |
| F38                | L    | +  | ++         |
| F38R40            | LK   | −  | −/−        |
| K40                | D    | –  | −/−        |
| K40                | T    | +  | ++         |
| K41               | A    | −  | −/−        |
| K41               | T    | +  | ++         |
| K41               | F    | +  | ++         |
| K41               | F    | +  | ++         |
| K44                | S    | +  | ++         |
| K44                | D    | −  | −/−        |
| S46                | A    | +  | ++         |
| S46                | F    | –  | −/−        |
| Y47                | H    | +  | ++         |
| Y47                | A    | +  | ++         |
| G44                | S    | +  | ++         |
| G44                | S    | +  | ++         |
| G44R49            | SG   | +  | ++         |
| R49                | T    | +  | ++         |
| K50                | Stop | −  | −/−        |
| K50K51            | YY   | +  | ++         |
| K50K51            | SG   | +  | ++         |
| K50                | E    | +  | ++         |
| K50                | T    | +  | ++         |
| R52                | E    | +  | ++         |
| R53                | I    | +  | ++         |
| Q54                | N    | +  | ++         |
| R55                | N    | +  | ++         |
| R55R56            | LT   | +  | ++         |
| R56                | E    | +  | ++         |
| R57Q63            | SE   | ++ | ++         |
| L69                | I    | +  | ++         |
| Q72                | Stop | +  | ++         |
| P91                | fi   | −  | −/−        |
| K90                | R    | −  | −/−        |

| Activities | From | To | |
|------------|------|----|---|
| ++         |      | G  | −/− |
| −/−        |      | A  | −  |
| −/−        |      | −  | −  |
| +/−        |      | +  | −  |
| −/−        |      | −  | −  |
| +/−        |      | +  | −  |
| −/−        |      | −  | −  |

### Notes

a Letter(s) indicates original amino acid(s). Number indicates position of amino acid in Tat.
b Letter(s) indicates the resulting amino acid(s).
c +, >50% wild type; +, +10% wild type activity; −/−, −10% or <10% wild type activity.
d Measurement of contribution by Tat to reverse transcription (29).
e Measurement is based not upon trans-activation of a reporter plasmid but on delayed replication of an HIV-1 molecular clone in T-cell lines (12, 28).
f Different results reported for the same mutation from Refs. 11 and 24.
g Amino acids beyond position 59 completely changed.
h Frameshift of amino acids beyond position 81.
entered a promoter-upstream locale by direct contact with DNA-bound Sp1 transcription complex (76, 78). It remains to be determined if upstream promoter-ovals. TAR-bound proteins serves to facilitate promoter clearance (46). The model in which Tat (the promoter. is suggested that TAK acts on a paused RNAP II molecule which has cleared (RNAP II that is yet docked at the promoter, converting a non-productive non-processive (TAK is shown to phosphorylate RNAP II in its CTD domain converting a productive (A)).

Recent biochemical evidence that Tat can exist in a TAR-RNA

A

NF-kB Sp1 TATAAA

B

NF-kB Sp1

C

NF-kB Sp1

FIG. 2. Schematic models of Tat transactivation. A simplified representation of the HIV-1 promoter containing two (small yellow rectangles) NF-kB-binding sites and three (small yellow ovals) Sp1-binding sites. Large ovals represent RNAP II complexes that overlie the TATAAA box and transcribe a promoter-proximal stem-bulge-loop TAR RNA. Tat (gray) binds the bulge of TAR, whereas TAK (purple) binds the loop of TAR. In A, loop-bound TAK is shown to phosphorylate RNAP II in its CTD domain converting a non-processed (red) to a processively elongating (green) polymerase. Here, it is suggested that TAK acts on a paused RNAP II molecule which has cleared the promoter. B diagrams an alternate view whereby protein(s) bound to the TAR loop of an early elongating RNAP II affects the activity of a subsequent RNAP II that is yet docked at the promoter, converting a non-productive (red) to a productive (green) complex. In this perspective, an activity of TAR-bound proteins serves to facilitate promoter clearance (46). The activities in A and B need not be mutually exclusive. C illustrates a speculative model in which Tat (gray) either dissociated from TAR RNA or in a free form entered a promoter-upstream locale by direct contact with DNA-bound Sp1 (blue). Through protein-protein interaction with Sp1 (67), it is envisioned that some Tat protein could form an early promoter-DNA-associated preinitiation complex (76, 78). It remains to be determined if upstream promoter-bound Tat could influence RNAP II (re-initiation events (cluster of green ovals) at the TATAAA box.

der otherwise non-processive RNAP II into productively elongating molecules (60, 61).

If P-TEFb explains Tat transactivation, then are there roles to be played by other Tat-associated cellular factors (49, 62–68)? In considering this question, one notes that although the requirement for presynthesized TAR RNA is consistent with a Tat effect on RNAP II elongation, it is equally compatible with a mechanism affecting reinitiation of transcription (Fig. 2B). Intracellularly, the ability to achieve continuous and rapid reinitiations, rather than simple initiations, represents the major determinant of the strength (defined by the amount of transcripts produced over time) of a eukaryotic promoter (69). Hence, although extant cell-free transcription studies (70–74) have analyzed well the effect of Tat on the processivity of initiated polymerases, they do not address potential contributions to reinitiations. If Tat does contribute significant to reinitiations, this could possibly explain the rather large discrepancies in the magnitudes measured for its cell-free elongation effects (reported variously as inductions of 3-fold (75) to 10-fold (54)) versus its intracellular effect on steady state transcription, which has been quantified as several hundred-fold. Indeed, recent biochemical evidence that Tat can exist in a TAR-RNA independent complex at the promoter (76, 77) and findings that this TAR-independent complex may directly contact DNA-bound Sp1 (78, 79) suggest that some Tat moieties might not necessarily be tethered to an elongating RNAP II and migrate away from the promoter (71, 75). In principle, TAR-RNA independent protein-protein contact of Tat with Sp1 (67, 79) implies that some Tat protein could be stably docked at a promoter proximal locale. If so, promoter-associated Tat (Fig. 2C) could influence transcriptional reinitiations in a fashion similar to promoter-bound TBPs/TAFs/TFIIA (80). It remains to be directly tested whether such a mechanistic function could exist for Tat.

HIV-1 has an additional transcriptional complexity that is not found for non-integrating viruses. For large portions of its life cycle, HIV-1, like other retroviruses, exists as chromosomally integrated proviral DNA (81) in infected cells. In its integrated form, the HIV-1 LTR is bound with histone proteins in a nucleosome-organized format. In this regard, access by RNAP II to these histone-packaged LTRs is a step that is not well reflected by the existing cell-free transcription assays for Tat function. Four recent reports, however, have addressed this issue from an intracellular context. Collectively, these reports firmly demonstrate a role for a Tat-associate histone acetyltransferase (TAH) in transcriptional access of RNAP II to integrated proviral LTRs. TAH activity was shown to be redundantly provided by the p300/CBP, PCAF, and/or TAF250 (82–85) proteins.

Additional Activities of the Tat Protein

Large viruses, such as those from the herpes family, have genome sizes of 200 or more kilobase pairs. By contrast, the HIV-1 genome is less than one-twentieth in size. For purposes of viral replication, the smaller HIV-1 genome must provide functions similar to those encoded within the genomes of larger viruses. A possible consequence of genome size constraint is that each HIV-1 open reading frame might have been selected to evolve multiple functions. Indeed, based on genetic inferences, Tat was shown to have activities in addition to its transcriptional function for the viral LTR (86). Some of these additional functions have been described from several independent laboratories and include the activation of quiescent T-lymphocytes (19), the induction of cellular apoptosis (87), and the modulation of cellular gene expression such as that for manganese-dependent superoxide dismutase (88). Tat also has functions consistent with an extracellular chemokin (5) and/or growth factor (4). There is evidence that Tat might additionally affect gene expression through post-transcriptional (18) and/or reverse transcription (20) steps. Many of these non-transcriptional activities for Tat have not been studied in sufficient detail; their physiological relevance remains to be verified in future investigations.

Concluding Remarks

Studies on Tat over the last decade have yielded important biological and virological insights. Early work established Tat as a lead example of an RNA-binding protein that functions in eukaryotic transcription. Later, in studying the transcriptional activity of Tat, new understandings of general controls of transcriptional elongation and processivity and activation of chromosomally integrated promoters were gained. From the latter arena we have learned how this RNA-binding protein can cooperate with RNAP II CTD domains and histone acetylases in modulating expression from the LTR promoter. In studies unrelated to transcription, Tat has provided an important paradigm for how highly charged proteins can be specifically taken up into cells (89); and in aspects related to AIDS pathogenesis, there is emerging evidence that HIV-1 virulence (90) and a potentially useful approach for a viral vaccine (91) can be specifically taken up into cells (89); and in aspects related to AIDS pathogenesis, there is emerging evidence that HIV-1 virulence (90) and a potentially useful approach for a viral vaccine (91).

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