HIV-1 Tat: Structure and Function

Kuan-Teh Jeang

Chief, Molecular Virology Section
LMM, NIAID, National Institutes of Health, Bethesda, MD 20892-0460

INTRODUCTION

Most viruses encode functions used to regulate genome transcription. Examples include the SV40 T-antigen, the adenovirus E1a protein, and the herpes virus immediate-early proteins. For the human immunodeficiency viruses (HIV), Tat functions similarly, though not identically, to those above. Over the past decade, we have learned much about Tat, both in structure and in function. While a goal of this Los Alamos database is to completely compile relevant tat sequences, the intent of this brief accompanying overview is not similarly archival, but rather to "add flavor" to raw data. It is written for the purpose of apprising, in a short format, the readers on some of the current thoughts about Tat. For more in-depth discussions, complete subject reviews can be found elsewhere (e.g. Felber and Pavlakis, 1993; Jeang and Gatignol, 1994).

TAT FUNCTION

Tat is a small nuclear protein of 86 to 101 amino acids in size (depending on the viral strain) which is encoded from two separate exons (see Section I). Analyses of "full-length" Tat have been performed commonly using the 86 amino acid version. However, it should be noted that while many laboratory strains (e.g. HXB2 and NL4-3) have the smaller Tat (86 aa) most HIV-1s have the 101 aa protein (see compendium Part II).

A. Transcription. Despite intensive efforts, the mechanism of Tat action remains incompletely understood. It is, however, accepted that Tat is required for optimal HIV viability (Fisher et al., 1986; Dayton et al., 1986). Tat's role in critically directing transcription (Peterlin et al., 1986; Rice and Mathews, 1988; Laspia and Mathews, 1989) from the HIV LTR is one indispensable function suggested for this protein. However, increasingly there is evidence that Tat has other important effects on the virus and on the host cell (see Concluding Perspectives, below).

In activating transcription from the LTR, Tat differs from other prototypic viral transcription trans-activators in requiring a bipartite responsive element consisting of DNA and RNA. To our knowledge, Tat is the first characterized eukaryotic transcription factor that binds to a nascent leader RNA, TAR (Berkhout et al., 1989; Dingwall et al., 1989; Cordingley et al., 1990; Roy et al., 1990; Calnan et al., 1991), and then influences formative events at the TATAA-enhancer-promoter (Berkhout et al., 1990; Selby and Peterlin, 1990; Southgate et al., 1990; reviewed in Jeang et al., 1991; see Fig. 1A). TAR RNA has extensive secondary structure containing a stem, a bulge, and a loop (Muesing et al.,1987; Berkhout and Jeang, 1989; Roy et al., 1990c; see Fig. 1B). Earlier, studies have indicated that the specific UCU sequence of the bulge is critical for binding by Tat (Dingwall et al. 1989,; Roy et al. 1990a, Calnan et al., 1991; Cordingley et al., 1990). In comparison, the structure of the stem, but not its specific sequence, was proposed to be important for function. However, more recent findings reveal that both for Tat binding and for transcription there are sequence specific requirements for the immediate stem nucleotide pairs that flank the bulge (Weeks and Crothers, 1991; Berkhout and Jeang, 1991; Churcher et al., 1993). The loop of TAR RNA is an important binding site for cellular factor(s) that cooperate with Tat in the activation of the LTR (Sheline et al., 1991; Wu et al., 1991). A more extensive discussion of the role of cellular factors that bind TAR RNA (Gatignol et al., 1989; Gaynor et al., 1989; Gatignol et al., 1991) is presented in the compendium Part IV.

While it is clear that Tat binds TAR RNA and interacts with enhancer-promoter-binding factors (Berkhout and Jeang, 1992), the mechanism by which these physical events influence transcription

are not wholly evident. A number of models that attempt to explain the transcription function of Tat have been proposed. These include i) anti-terminating (Kao et al., 1987) stalled RNA polymerase II (RNAP II), ii) increasing processivity/elongation of transcribing RNAPII complex (Laspia et al., 1989; Marciniak et al., 1990), and iii) facilitating initiation of RNAPII complexes at the promoter (Laspia et al., 1989, Jeang and Berkhout, 1992; Jeang et al., 1993a; reviewed in Cullen, 1993). Currently, there are conflicting findings that support each of the models. It is possible that Tat, like basal transcription factor TFIIF (Buratowski, 1994), functions simultaneously as an initiation and as an elongation factor. Alternatively, conflicting attributions could arise from differences in the rate-limiting events (Jeang et al., 1993a) for the various experimental systems used to define Tat action.

A couple of observations indicate that Tat is not a typical transcriptional trans-activator. First, unlike other activators (Tjian and Maniatis, 1994), a modular activation domain has been difficult to define for HIV-1 Tat (see below, Activity domains of Tat). Second, in different settings, neither Tat nor Tat-chimera, in contrast to modular activators, is able to directly activate transcription from a minimal TATA-promoter (Southgate and Green, 1991; Berkhout and Jeang, 1992; Kamine and Chinnadurai, 1992). Instead for physiological activity, Tat requires the presence of an upstream enhancer-binding factor such as Sp1 (Harrich et al., 1989; Kamine et al., 1991;; Southgate and Green, 1991; Zimmermann et al., 1991; Jeang and Berkhout, 1992; Jeang et al., 1993b). Thus it appears that Tat is more aptly classified with transcriptional co-activators (Dynlacht et al., 1991) in bridging conformationally a stereospecific architecture (Tjian and Maniatis, 1994) between Sp1 (Jones and Tjian, 1985; Jones et al., 1986) and TATA-binding protein (TBP; Jeang et al., 1993b; Huang et al., 1993; Kashanchi et al., 1994).

B. Translation. There is evidence that Tat also functions in regulating translation (Rosen et al., 1986; Cullen, 1987). Indeed there are findings that TAR RNA can inhibit translation (Parkin et al., 1988; SenGupta and Silverman, 1989) of HIV-1 mRNAs, most likely through activation of double-stranded RNA-dependent protein kinase and 2-5A synthetase (SenGupta and Silverman, 1989; Edery et al., 1989). Addition of Tat was found to reverse this translational inhibition (SenGupta et al., 1990; Braddock et al., 1990).

ACTIVITY DOMAINS IN TAT

Tat is synthesized from an mRNA joined from two coding exons. The first exon codes for amino acids 1–72, while (in most strains of HIV-1) the second exon codes for amino acids 73–101 (see Fig. 2). In most functional assays, the first 72 amino acids of Tat fully effect transcriptional trans-activation of the LTR. In fact, a truncated 58 amino acid form of Tat is virtually completely active in co-transfection assays (Seigel et al., 1986; Garcia et al., 1988; Kuppusway et al., 1989).

A. First coding exon. The combined results from many laboratories have permitted an arbitrary demarcation of "domains" in Tat (Kuppuswamy et al., 1989). For instance, the N-terminus of Tat (domain 1; Fig. 2) has 13 amino acids with amphipathic characteristics. Mutations that alter the acidic composition of this region were felt originally to affect trans-activation (Rappaport et al., 1989); however, results from a later study conflicted with this interpretation (Tiley et al., 1990).

Amino acids 22 to 37 (domain 2, Fig. 2) contain seven cysteines and are highly conserved between different isolates of HIV-1s, group O as well as group M. Individual mutation in six of the seven cysteines abolish Tat function (see Table I). Although originally proposed as a metal-chelating dimerization domain (Frankel et al., 1988), this region was recently shown to be used for intramolecular disulfide bond formation in monomeric Tat proteins found within cells (Koken et al., 1994). Currently, it is believed that Tat is active functionally as a monomer rather than a dimer (Rice and Chan, 1991; Koken et al., 1994).

Domain 3 (amino acids 40 to 48) contains a RKGLGI motif that is conserved between HIV-1, HIV-2 and SIV Tat. This region, in conjunction with the amino terminus and the cysteine domain, has been suggested to circumscribe the minimal activation domain of HIV-1 Tat (Carroll et al., 1991; Derse et al., 1991). Domain 4 (amino acids 49-72) contains a basic RKKRRQRRR motif. These amino acids confer TAR RNA-binding properties to Tat (Dingwall et al., 1989; Roy et al., 1990;

Weeks et al., 1990; Chang and Jeang, 1992) and are important for nuclear localization of the protein (Ruben et al., 1989; Hauber et al., 1989). However, recent studies suggest that this short basic domain is insufficient in determining the entire specificity of Tat-TAR binding since amino acids outside of the domain also contribute to this interaction (Churcher et al., 1993; Luo et al., 1993).

Table I summarizes 75 point mutations in the first coding exon of Tat collated from the work of eight laboratories (Garcia et al., 1988; Sadaie et al., 1989; Kuppuswamy et al., 1989; Ruben et al., 1989; Hauber et al., 1989; Meyerhans et al., 1989; Rice and Carlotti, 1990a; Rice and Carlotti, 1990b; Siderovski et al., 1992). As alluded to above, since the first 58 amino acids of Tat recapitulate well the trans-activation function of the whole protein, it is not surprising that most of the mutations are clustered within amino acids 1 to 58. In many cases, individual amino acids have been changed to more than one counterpart. This heightens the validity of the resulting phenotype. Examination of these mutants reveals that the region spanning amino acids 1–21 is remarkably tolerant of changes. In contrast, changes in amino acids 22 through 40 were generally deleterious for trans-activation. Finally, although the basic domain (amino acids 49–57) as a unit is necessary for Tat function, individual amino acid changes do not significantly affect activity.

B. Second coding exon. Less information is available about the second coding exon of Tat. It is clear that in routine transfection assays of HIV-1 Tat, absence of the second exon does not alter greatly Tat activity. However, findings from HIV-2 and SIV Tat are quite clear in demonstrating that this exon contributes towards optimal trans-activation (Viglianti and Mullin, 1988; Tong-Starksen et al., 1993). Recently, there have been suggestions that the second exon of HIV-1 Tat, in specific assays, is important for trans-activation (Jeang et al., 1993b) and for trans-repression (Howcroft et al., 1993).

There are two short motifs in the second exon of HIV-1 Tat that could have functional importance (see Fig. 2). The first is an RGD sequence that is used as a cell adhesion signal for binding to cellular integrins (Brake et al., 1990). This RGD motif is not found, however, in HIV-2 or SIV Tat proteins. In addition, a ESKKKVE motif is conserved across different HIV-1 Tat proteins and is partially preserved in HIV-2 and SIV Tats. However, because this motif falls beyond amino acid 86, commonly regarded as the C-terminal boundary of full-length Tat, its functional significance has not been examined.

CONCLUDING PERSPECTIVES

While research on Tat has been narrowly focused upon its trans-activation properties for the HIV LTR-promoter, there is increasing evidence that Tat has other pleiotropic effects on cellular genes, host cell metabolism, and viral infectivity/pathogenesis (see e.g. Drysdale and Pavlakis, 1991). For instance, Tat is reported to function as a secreted growth factor in stimulating the growth of Kaposi-like cells (Ensoli et al., 1990; Ensoli et al., 1993; Barillari et al., 1993). It can affect the organization of neurons and astrocytes (Kolson et al., 1993) and is neurotoxic at low concentrations (Sabatier et al., 1991). Some of these findings are partly explained by the ability of Tat to modulate expression of cellular genes (Roy et al., 1990b; Buonaguro et al., 1994; Scala et al., 1994), to perturb ambient levels of cytokines (Rautonen and Rautonen, 1992), and to protect cells from programmed cell death (Zauli et al., 1993).

Because the viral functions of Tat have been largely extrapolated from subgenomic experiments, one is unsure whether these findings reflect well the complete role of Tat in the setting of the whole virus. The limited number of experiments performed on Tat function in the context of infectious virions suggests that Tat has additional roles, beyond transcription, in affecting viral pathogenicity (Cheng-Mayer et al., 1991; Sakai et al., 1992; Huang et al., 1994).

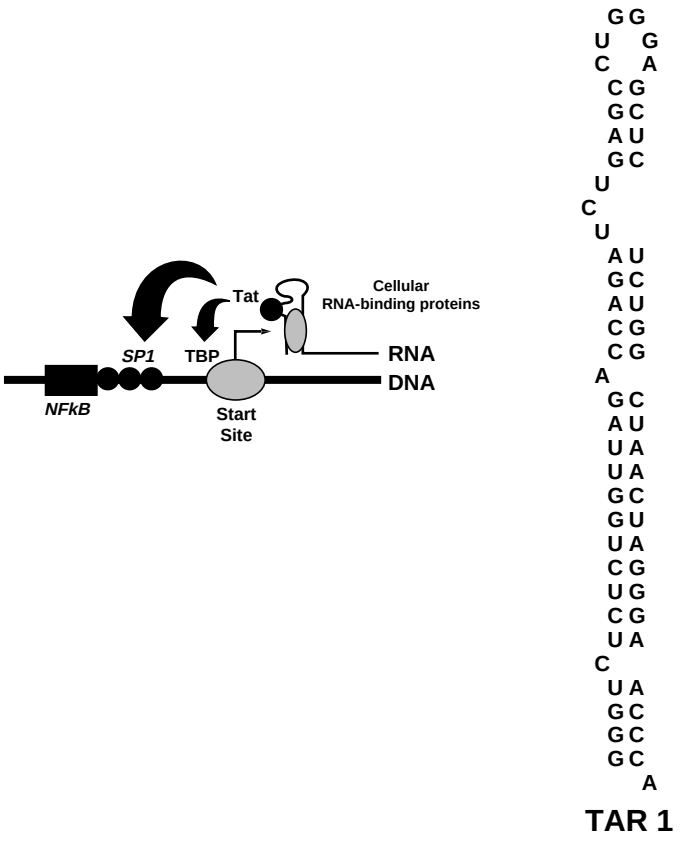

Fig. 1. Interaction of Tat with DNA and RNA targets in the HIV-1 LTR. A) A schematic representation of the functional interactions between Tat, TAR-RNA-binding proteins and promoter elements. Biochemical evidence exists that Tat contacts directly SP1 (Jeang et al., 1993b) and TATAA-binding protein (TBP; Kashanchi et al., 1994). B) Secondary structure of TAR RNA. The crucial trinucleotide bulge and hexanucleotide loop elements are boxed.

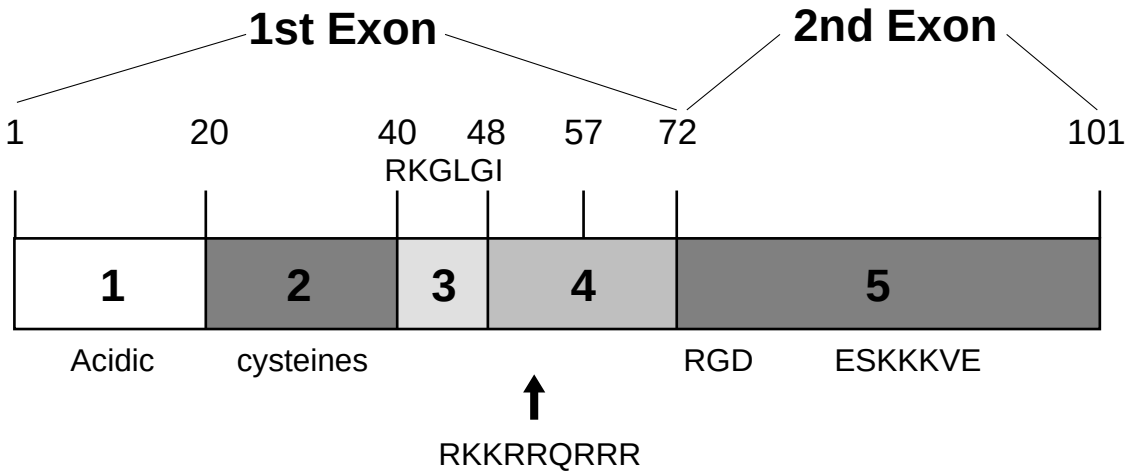

Fig. 2. Domain classifications of Tat protein. The demarcation of domains is somewhat arbitrary. The first exon includes amino acids 1–72, while the second exon includes 73-101. Motifs and characteristics of each “domain” are indicated above or below each region.

Table I. Point mutations in Tat

Original Amino Acids(s) and Their Position(s)	Mutant Amino Acid(s)	Resulting Activity	Consensus Amino Acid(s)
Q2	A	++	E
P3	A	++	P
P3	Q	++	P
V4	A	++	V
D5	A	+	D
P6	A	++	P
P6	S	++	P
Δ3–6		+	
P6P10	LL	++	
R7	A	++	R
L8	A	++	L
E9	A	++	E
P10	A	++	P
P10P13	LL	++	
W11	A	++	W
K12	A	++	K
K12	N	++	K
P18	A	++	P
K19	R	++	K
A21	D	++	A
A21T23	VA	+	
T23	A	++	T
C22	S	–	C
C22	G	–	C
N23	T	++	T
N24	A	++	N
N24	K	++	N
C25	R	–	C
C25	G	–	C
Y26	A	+	Y
Y26	F	++	Y
C27	S	–	C
C27	G	–	C
K28K29	AA	+	KK
K28K29	EA	–	KK
C30	G	–	C
C31	S	++	C
C31	E	–	C
C31	G	++	C
F32	A	+	F
H33	A	–	H
C34	G	–	
C34	S	–	C
G35	A	+	Q

Key: Column 3, ++ > 50% wild type activity; + > 10% wild type activity; +/– or – indicate <10% wild type activity.

Table I. (cont.) Point mutations in Tat

Original Amino Acids(s) and Their Position(s)	Mutant Amino Acid(s)	Resulting Activity	Consensus Amino Acid(s)
C37	G	–	C
C37	S	–	C
F38	A	–	F
F38	L	++	F
K40	D	–	T
K40	T	++	T
K41	A	–	K
K41*	T	–	K
K41*	T	++	K
L43	F	+	L
G44	S	++	G
S46	A	++	S
S46	P	–	S
Y47	H	++	Y
Y47	A	++	Y
G48	S	++	G
G48R49†	SG	++	
R49	T	++	R
K50	stop	–	K
K50K51	Y50Y51	+	KK
K50K51	S50G51	+/+	
K50	E	++	K
K50	T	++	K
R52	E	++	R
R53	I	++	R
Q54	N	++	Q
R55	G	+	R
R55R56	L55T56	+	
R56	E	++	R
R57Q63	SE	++	RQ
L69	I	++	L

Key: Column 3, ++ > 50% wild type activity; + > 10% wild type activity; +/– or – indicate <10% wild type activity.

* Different results reported for the same mutation from Kuppuswamy et al., 1989, and Myerhans et al., 1989.

† Amino acids beyond position 59 completely changed.

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HIV-1 Tat Consensus Sequences

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                                                   \/3'sj \/3'sj
                                                rev cds ->
CONSENSUS.A   M?PVDPnLEPWnHPGSqPTTaCskCYCK?CCwHCqlCFLnKGLGISYGrKKR..r?RRgtPQ.s?kDhQnp     64
CONSENSUS.B   -e----r----k------kt-ctn----k--f---v--tt-g-g--------..-Q-rrapq.dSqt--vs     68
CONSENSUS.C   m--v--?--------s--K---t--yc-k-sY--lV--qt------------..-q--sa-?.-SE-----     65
CONSENSUS.D   md-v---l-p--------r-p-N--?--K--Y---v--it------------..-Q--rppq.g-Qa--v-     66
CONSENSUS.O   -D----E?P--H----?-Q?P-NN----R--Y--YV--??------?-----....?-PAAA?--P-?KD-     57
CONSENSUS.CPZ -D-?-????--?--???-?-?-NN-------Y--??--TK----------?-???---T???.?S?NN-D?     45

             exon\/exon
CONSENSUS.A   ipKQplPqtqg??ptgpkESkKkVeSKteTDrf?                                          95
CONSENSUS.B   Ls-q-?s-pr-D.-----es-k---rE--tdP?dQ                                        100
CONSENSUS.C   -s----p--r-d.----Ee----------t-p-D                                          98
CONSENSUS.D   --k--SS-pR-d------eQ------kA-t-p-Dw                                        100
CONSENSUS.O   V-?-S???-?RK.Q?RQE-QE??--K??GP?G?P????SC??CTR?S?Q                           85
CONSENSUS.CPZ ??-?????SR?-.?????K-?-?-??--????G-C                                          56
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HIV-2/SIV Tat Consensus Sequences

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                                                                <- vpR
CONSENSUS.A METpLKaPEsSL?syNEPsS?TSeqdv??QelakqGeEiLsQLyrPLEaCtNsCyCK?CcyhCQlCFlkKGLG     68
CONSENSUS.B --I--qEQ----k-Ssep--S---pV-NT-G-DN-------------k--d-t----K---------------     73
CONSENSUS.D -----rEQ-n--e-sner--cis-a-a-tp-s-nlg---------pl---y-tc---k------f--------     72

      rev cds ->                exon \/ exon
CONSENSUS.A IwY?RkgRRRRtPkKtK?hsssasD.KSISTRTg?SQptKkQKKTle?tvetd?glGr                  120
CONSENSUS.B -c-drS..-k-Ss-RA-tTa---pd??-L-Ar--D--------keV-T-g?--l-P--SNTSTSRFA          135
CONSENSUS.D -c-Eqsr?----p--a-ant----n?k?isn-tRhc--k-ak-etV--a-a-ap--g-                  126
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