HIV-1 TAR RNA Enhances the Interaction between Tat and Cyclin T1*

Jing Zhang,‡ Natarajan Tamilarasu,‡ Seongwoo Hwang,‡ Mitchell E. Garber,¶ Ikramul Huq,‡
Katherine A. Jones,¶ and Tariq M. Rana‡§

‡Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane,
Piscataway, New Jersey 08854; and ¶Regulatory biology Laboratory, The Salk Institute for
Biological Studies, La Jolla, California 92037.

Running Title: RNA-induced protein:protein interactions

§To whom correspondence should be addressed.

Phone: (732) 235-4082 Fax: (732) 235-3235
E. Mail: rana@umdnj.edu

* This work was supported by an NIH grant AI 41404 to T.M.R. T. M. R. is a recipient of a Research Career Development Award from NIH.
SUMMARY

HIV-1 Tat activates elongation of RNA polymerase II transcription at the HIV-1 promoter through interaction with the Cyclin T1 (CycT1) subunit of the positive transcription elongation factor complex, P-TEFb. Binding of Tat to CycT1 induces co-operative binding of the P-TEFb complex onto nascent HIV-1 TAR RNA. Here the specific interaction between Tat protein, human Cyclin T1, and HIV-1 TAR RNA was analyzed by fluorescence resonance energy transfer, using fluorescein-labeled TAR RNA and a rhodamine-labeled Tat protein synthesized through solid-phase chemistry. We find that CycT1 remodels the structure of Tat to enhance its affinity for TAR RNA, and that TAR RNA further enhances the interaction between Tat and CycT1. We conclude that TAR RNA nucleates the formation of the Tat:P-TEFb complex through an induced fit mechanism.
INTRODUCTION

The human immunodeficiency virus (HIV-1) encodes a transcriptional activator protein, Tat, which increases the processivity of RNA polymerase II (for reviews, see ref. (1-3)). Tat activates transcription through binding to the upper stem and bulge region of TAR, a structured element in the nascent viral RNA, and controls a DRB-sensitive step early in RNAPII transcription elongation that results in hyperphosphorylation of the carboxy-terminal domain (CTD) of RNA polymerase II. In nuclear extracts, HIV-1 Tat associates tightly with the CDK9-containing positive transcription elongation factor complex, P-TEFb (4-6). Recent studies indicate that Tat binds directly through its trans-activation domain to the cyclin subunit (CycT1) of the P-TEFb complex and induces loop sequence-specific binding of the P-TEFb complex to TAR RNA (7-9). Neither CycT1 nor the P-TEFb complex bind TAR RNA in the absence of Tat, and thus the binding is highly co-operative for both Tat and P-TEFb (7,9). The Tat:CycT1 interaction requires zinc as well as cysteine residues in each protein, and therefore may represent a metal-linked heterodimer (8). Tat appears to contact residues in the carboxy-terminal boundary of the CycT1 cyclin domain which are not critical for binding of cyclinT1 to CDK9 (8,10-14), and basic residues in CycT1 (R251, R254) further stabilize the Tat:P-TEFb: TAR RNA complex (8). Thus the assembly of this complex appears to involve a series of adaptive interactions between the trans-activation and arginine-rich motif (RNA-binding) domains of Tat and their respective protein (CycT1) and nucleic acid (TAR) partners during transcription.

These studies have raised the possibility that at least two separate events may govern the assembly of functional P-TEFb:Tat:TAR complexes: 1) the interaction of Tat with CycT1 induces a conformational change in Tat that enhances its affinity and kinetic stability for TAR RNA, and 2) TAR RNA may enhance the affinity between CycT1 and Tat, through an "induced fit" mechanism. These two events would not necessarily be mutually exclusive, and both could contribute
significantly to the assembly of a stable ternary complex necessary to position P-TEFb at the RNA exit channel in a location favorable for phosphorylation of the RNAPII CTD. To test these hypotheses, we developed a fluorescence resonance energy transfer (FRET) system containing TAR RNA and Tat protein uniquely labeled with donor and acceptor dye molecules (Figure 1). FRET, in which a fluorescent donor molecule transfers energy via a non-radiative dipole-dipole interaction to an acceptor molecule (which is usually also a fluorescent molecule) is a standard spectroscopic technique for measuring distances in the 10-70 Å range (15,16). The donor's lifetime and quantum yield are reduced upon energy transfer, and the acceptor fluorescence is increased, or sensitized. Quantification of the efficiency of energy transfer allows determination of the distance between the two fluorophores. We used a well-characterized donor-acceptor dye-pair, fluorescein-rhodamine, for FRET experiments. Our results demonstrate that CycT1 enhances the affinity and kinetic stability of Tat-TAR complex formation. In addition, it was discovered that TAR RNA enhances CycT1 and Tat interaction showing how a small RNA hairpin can provide a platform for protein-protein interactions.

**EXPERIMENTAL PROCEDURES**

**RNA Synthesis**

RNAs were synthesized by chemical and enzymatic methods. Modified TAR RNA was synthesized on an Applied Biosystems Model 392 DNA/RNA synthesizer using 2-cyanoethyl phosphoramidite chemistry. All the monomers of (2-cyanoethyl)phosphoramidites were obtained from Glen Research (Sterling, Virginia). TAR RNA was chemically synthesized on a fluorescein-containing CPG 500 support. RNA (1 µmole) containing fluorescein was deprotected by treatment with NH₃ saturated methanol (2 mL) at 25°C for 17 h. Product was filtered and dried in Speedvac. To deprotect 2’-OH silyl groups, the red pellet was dissolved in 50% triethylamine trihydrofluoride in dimethyl sulfoxide (0.5 mL) and left at room temperature for 16 h. Deprotected
RNA was precipitated by the addition of 2 mL of isopropyl alcohol. After deprotection, RNA was purified and characterized as described previously (17-19).

Wild-type and mutant TAR RNAs were prepared by *in vitro* transcription (20,21). Enzymatically transcribed RNAs were 5' dephosphorylated by incubation with calf intestinal alkaline phophatase (Promega) for one hour at 37°C in 50 mM Tris-Cl, pH 9.0, 1 mM MgCl₂, 0.1 mM ZnCl₂, 1 mM spermidine. The RNAs were purified by multiple extractions with Tris saturated phenol and one extraction with 24:1 chloroform:isoamyl alcohol followed by ethanol precipitation. Chemically synthesized RNA contains only free 3'-OH groups and does not require dephosphorylation procedures. The RNAs were 5' end-labeled with 0.5 µM $[^{32}\mathrm{P}]\mathrm{ATP}$ (6000 Ci/mmol) (ICN) per 100 pmoles RNA by incubating with 16 units T4 polynucleotide kinase (New England Biolabs) in 70 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 5 mM DTT (21,22). The RNAs were labeled at the 3'-end by ligation to cytidine 3',5'-[5'-32P]bisphosphate ($[^{32}\mathrm{P}]\mathrm{pCp}$) using T4 RNA ligase. Reaction mixtures (50 µL) contained 250 pmol RNA, 65 µCi $[^{32}\mathrm{P}]\mathrm{pCp}$ (3000 Ci/mmol, NEN™, Boston, MA), and 40 units T4 RNA ligase (New England Biolabs) in a buffer containing 50 mM Tris-HCl (pH 8.0), 3 mM DTT, 10 mM MgCl₂, 25 mM NaCl, 50 mM ATP, 25 µg/mL bovine serum albumin, and 10% dimethylsulfoxide (v/v). After incubation at 4°C overnight, the labeled RNAs were purified by phenol-chloroform extraction and ethanol precipitation. 3'- and 5'-end-labeled RNAs were gel purified on a denaturing gel, visualized by autoradiography, eluted out of the gels, and desalted on a reverse-phase cartridge.

The sequence of RNAs was determined by base hydrolysis and nuclease digestion. Alkaline hydrolysis of RNAs was carried out in hydrolysis buffer for 8-12 minutes at 85°C. RNAs were incubated with 0.1 units RNAse from *B. cereus* (Pharmacia) per pmole RNA for 4 minutes at 55°C in 16mM sodium citrate, pH 5.0, 0.8 mM EDTA, 0.5 mg/ml yeast tRNA (Gibco-BRL).
This enzyme yields U and C specific cleavage of RNA. Sequencing products were resolved on 20% denaturing gels and visualized by phosphor image analysis.

**Tat and CyclinT1 Proteins**

Tat protein (aa1-72) was chemically synthesized using standard F-moc amino acids and 5-carboxytetramethylrhodamine was incorporated at K19 position. Details of Tat-Rhodamine synthesis will be described elsewhere (Tamilarasu and Rana, unpublished data). Rhodamine-labeled Tat (aa1-72) was purified by HPLC and characterized by mass spectrometry. In vitro transcription assays and electrophoretic mobility shift experiments showed that the rhodamine labeling did not significantly alter the structure of Tat which can interfere with its function (data not shown).

Human CycT1 (aa 1-303 and aa 1-254) was expressed as GST-fusion protein, purified, and characterized as described previously (7,8).

**Fluorescence Resonance Energy Transfer Measurements (FRET)**

The fluorescence measurements were performed on PTI fluorescence spectrophotometer controlled by Felix software. The excitation wavelength was 490 nm and slits width was set 3.5 nm for both excitation and emission. TAR-Fluorescein (200 µL) samples were excited at 490 nm and the emission intensity was measured at 512 nm and 575 nm. Measurements were performed in a 200 µL cuvette to reduce the inner filter effect. The concentrations of TAR RNA-Fl, TAT-Rh and CycT1 (aa1-303)/Zn\(^{2+}\) were 8.0 nM, 16.5 nM, and 8.0 nM, respectively. The final concentration of Zn\(^{2+}\) was 0.18 mM. The absorbance of dye-labeled samples were maintained below 0.002 to avoid the inner filter effects. All samples were corrected for the light source excitation effect and for the background intensity of buffer fluorescence as well as for dilution factors. All experiments were done at room temperature and the following buffer conditions were maintained: 50 mM Tris-HCl, pH 7.4 at 25°C, 20 mM KCl and 1 mM β-mercaptoethanol.
RESULTS AND DISCUSSION

For quantitative application of FRET, it is necessary to label RNA and protein stoichiometrically at unique sites while retaining the functional properties of biological molecules. Fluorescein labeled TAR RNA (TAR RNA-Fl) and rhodamine labeled Tat (Tat-Rh) protein were chemically synthesized by solid-phase methods. TAR RNA was labeled with fluorescein at its 3'-end and rhodamine was incorporated at K19 in the Tat sequence. We chose K19 in the activation domain of Tat because it is located in the CycT1-interacting region of Tat and can be replaced with other amino acids without affecting the function of Tat.

We examined FRET between Tat and TAR in the absence and presence of cyclinT1. Emission scans were recorded from 500 to 650 nm during the excitation at 490 nm of TAR RNA-Fl, TAR RNA-Fl with Tat-Rh, and TAR RNA-Fl, Tat-Rh and CycT1 (aa1-303) with Zn$^{2+}$ (Figure 2A). TAR RNA-Fl has an emission maximum around 512 nm (black), whereas upon adding Tat-Rh (red), this peak is decreased and another peak at 575 nm is increased. This reduction in fluorescein emission and the corresponding increase in rhodamine emission were due to the resonance energy transfer from TAR RNA-Fl to Tat-Rh. However, the presence of CycT1 (aa1-303) with Zn$^{2+}$ (blue) results in a smaller decrease in the donor fluorescence emission at 512 nm and a smaller increase at 575 nm in the acceptor fluorescence emission as compared to TAR RNA-Fl and Tat-Rh. The decrease in the efficiency of energy transfer in the presence of CycT1 (aa1-303) and Zn$^{2+}$ shows that the distance between the two fluorophores has increased, indicating that CycT1 induces a conformational change in Tat structure in a cyclinT1-Tat-TAR ternary complex.

Several control experiments further support these observations and demonstrate the specificity of CycT1-Tat-TAR interactions. First, CycT1 (aa1-303) alone has no effect on the emission spectrum of TAR RNA-Fl (data not shown), an indication of no interaction between these
two molecules. Second, in the presence of a truncated CycT1 (aa1-254) which does not contain Tat-interacting sequences, no specific quenching was observed on either Tat protein or Tat-TAR complex (data not shown). Finally, fluorescence energy transfer was not observed when TAR RNA-Fl was incubated with either free rhodamine dye or unlabeled Tat protein. Wild type unlabeled TAR RNA successfully competed with the labeled TAR RNA bound to Tat (data not shown), indicating that the fluorescence energy transfer between TAR RNA-Fl and TAT-Rh is due to their specific interactions. These results demonstrate that we have established a specific FRET-based system to study cyclinT1-Tat-TAR ribonucleoprotein complex.

To determine the effect of cyclinT1 on the affinity of Tat for TAR RNA, we examined fluorescence quenching of TAR RNA-Fl at several different Tat-Rh concentrations in the absence and presence of CycT1 (aa1-303). Results of these experiments are shown in Figure 2B. K_D values were calculated by fitting data to quadratic equation 1.

\[ F = F_{\text{min}} - \frac{(F_{\text{max}} - F_{\text{min}})((R + P + K) - ((R + P + K)^2 - 4RP)^{1/2})}{2R} \]  

Where F is the relative fluorescent intensity, F_{\text{min}} is the fluorescent intensity at the start of the titration, F_{\text{max}} is the fluorescent intensity at saturating concentration of protein, P. R is the total concentration of the RNA. K is the dissociation constant of the RNA and protein and can be obtained by fitting data to this equation. The dissociation constants (K_D) of Tat(aa1-72)-TAR complex in the presence and absence of CycT1(1-303) were 0.8±0.4 and 8.2±0.8 nM, respectively. The 10-fold increase of the affinity between TAR RNA and Tat in the presence of CycT1 demonstrates that CycT1 has a significant effect on the formation of high affinity Tat-TAR complex.

What is the affinity of CycT1-Tat interactions and does TAR RNA play any role in this interaction? To address this question, we measured rhodamine fluorescence quenching at 575 nm by titration of CycT1 (aa1-303) with Tat-Rh. Figure 3A shows the best fit of the data using
equation 1, and a dissociation constant of 9.1 nM for Tat and CycT1 (aa1-303) complex was obtained. To determine the effect of TAR RNA on cyclinT1-Tat interactions, the same experiments were performed in the presence of equal molar of unlabeled TAR RNA to TAT-Rh (Figure 3B). The dissociation constant for CycT1 (aa1-303) and Tat in the presence of TAR was 0.4 nM. These results show that TAR RNA greatly enhances protein-protein interactions between CycT1 and Tat.

An important test of biological significance of ribonucleoprotein complexes is their kinetic stability, especially in the context of a vast excess of nonspecific RNA in the cell. The kinetic stability of the cyclinT1-Tat-TAR complex was determined by forming a complex between TAR RNA-Fl, Tat-Rh, and CycT1 (aa1-303), challenging the complex with an excess of unlabeled competitor, and measuring the amount of remaining complex at different time intervals. Unlabeled TAR RNA 40-100 fold excess over labeled TAR RNA-Fl was used for the competition. As shown in Figure 2A, TAR RNA-Fl fluorescence is quenched by Tat-Rh in a Tat-TAR complex, therefore, addition of unlabeled competitor would displace Tat-Rh from the complex and would result in an increase in fluorescence signal of TAR RNA-Fl at 512 nm. Therefore, the amount of remaining CycT1-Tat-TAR complex can be assayed by measuring the fluorescence intensity of TAR RNA-Fl at 512 nm. Figure 4A shows the fraction of the remaining CycT1-Tat-TAR complex as a function of time when 70-fold excess of unlabeled TAR RNA competitor was added. The dissociation rate constant ($k_{\text{off}}$) can readily be obtained by fitting data using equation 2.

\[
\frac{[\text{Complex}]}{[\text{Complex}]_{t=0}} = A \exp(-K_{\text{off}} t) \quad (2)
\]

where $[\text{Complex}]_t$ and $[\text{Complex}]_{t=0}$ are the concentration of the complex at time $t$ and $t=0$, respectively. $A$ is the fitting parameter which represents the ratio of $[\text{Complex}]_t/[\text{Complex}]_{t=0}$ at $t=0$. $K_{\text{off}}$ is the dissociation rate constant.

In order to test the relationship between $k_{\text{off}}$ and the concentration of competitor, 40- to 100-fold excess of unlabeled TAR RNA over labeled TAR RNA were used. The $K_{\text{off}}$ values for
CycT1-Tat-TAR complex in the presence of 40-, 70-, and 100-fold competitor RNA were 0.021, 0.034 and 0.09 min⁻¹, respectively. A linear increase of the dissociation rates of the complex with the increase of the total competitor concentration was observed (Figure 4B), which indicates that the dissociation of the complex is facilitated by competitor RNA (23). Similar experiments were performed on Tat-Rh and TAR RNA-Fl complex in the absence of CycT1 which showed that the complex dissociated with a $K_{\text{off}}$ of 0.2 min⁻¹ in the presence of 40-fold competitor RNA (data not shown). The lifetime of Tat-TAR complex is 5 minutes while under similar conditions CycT1-Tat-TAR complex has a lifetime of 48 minutes. Therefore, these data indicate that CycT1 enhances the kinetic stability of the Tat-TAR interactions by ~ 10 fold. The result that CycT1 modulates kinetic stability of Tat-TAR interactions may have great biological implications because a functional ribonucleoprotein complex must be stable to an overwhelming excess of nonspecific RNA in the cell.

To determine the conformational changes caused by CycT1 in Tat-TAR complex, we formed a complex between TAR RNA-Fl and Tat-Rh and calculated the distance between two fluorophores, rhodamine at K19 of Tat and fluorescein at 3’-end of TAR RNA. The distance (R) between donor, TAR RNA-Fl, and acceptor, TAT-Rh, can be measured spectroscopically by Förster energy transfer (24). The distance R was determined from equation: $R = R_0 (E^{-1} - 1)^{1/6}$, where E is the efficiency of nonradiative transfer. E was calculated from the quenching of fluorescence emission maximum at 512 nm, i.e., $E = (1 - I_{DA}/I_D)$, $I_{DA}$ and $I_D$ are the fluorescence intensities at 512 nm in the presence and absence of acceptor, respectively. $R_0 = 9786(k^2n^{-4}Q_dJ)^{1/6}$ A. The donor quantum yield $Q_d$ (0.26) was determined using disodium fluorescein in 0.1 M NaOH (25), where k represents the relative orientation of the two fluorophors. Considering a random orientation of donor and acceptor transition dipoles, k was assumed to be 2/3 (24). The refractive index of the medium, n, was taken as 1.4 for protein in water. $J$ (4.46 x 10⁻¹³ cm⁶/mol) is
the overlap of the integral and was calculated from the overlap between the donor emission and acceptor absorbance. \( J \) can be calculated from the sum of the wavelength of equation \( J = \sum (\varepsilon_A(\lambda) F_D(\lambda) \lambda^4 \Delta \lambda) / \sum F_D(\lambda) \), where \( \varepsilon_A(\lambda) \) and \( F_D(\lambda) \) are the extinction coefficient of acceptor and the intensity of fluorescence emission of donor, respectively. The fluorescence intensity of the TAR RNA-Fl in the presence of TAT-Rh was used to calculate the distance between the two fluorophores. The efficiency of energy transfer (E) is shown in Figure 5. The efficiency of transfer between TAR-Fl and TAT-Rh was 25%. However, in the presence of CycT1 (aa1-303) and 0.18 mM \( \text{Zn}^{2+} \), the efficiency of energy transfer was decreased to 15%. The distance (R) between two fluorophores was determined from \( R_0 \) and E which showed that K19 rhodamine in Tat is 57.2 Å apart from fluorescein at the 3’-end of TAR RNA and this distance was changed to 63.6 Å in the presence of CycT1 (1-303). Since K19-rhodamine is located in the CycT1-interacting region of Tat, these results indicate that Tat goes through a structural reorganization upon CycT1 binding (Figure 5).

Interestingly, when \( \text{Zn}^{2+} \) was removed from the binding reactions, no energy transfer was detected. This result supports a previously proposed model for cyclinT1-Tat-TAR interactions suggesting that Tat forms a metal-linked heterodimer with cyclinT1 (8).

We find that Tat (aa1-72) binds TAR RNA with a \( K_D \) of 8.2 nM, and that this affinity is enhanced ten-fold in the presence of CycT1 (aa1-303). Taken together with the changes in the relative location of Tat on TAR when bound to CycT1, we infer that CycT1 enhances the affinity and stability of the Tat:TAR complex in a manner that is accompanied by a significant conformational change in the structure of the Tat protein. In addition, a Tat peptide containing only the arginine rich motif, Tat (aa49-57), binds TAR with a \( K_D \) of 1 nM while a longer peptide containing additional amino acids from the core domain, Tat(aa38-72), has a lower affinity for TAR RNA with a \( K_D \) of 5.7 nM (Zhang, Tamilarasu, Hwang, and Rana, unpublished observations).
The observation that the isolated arginine-rich motif of Tat binds much more tightly to TAR RNA than Tat proteins containing the activation domain has also been observed with the native full-length (86aa) HIV-1 and (130aa) HIV-2 Tat proteins (9), and suggests an autoinhibitory mechanism in which intramolecular interactions involving residues that overlap the activation domain effectively block binding to TAR RNA in the absence of CycT1. Interaction with CycT1 overcomes this inhibitory effect and permits high affinity binding of residues in the arginine-rich motif to TAR RNA. Although intramolecular masking is a well-characterized feature of DNA-binding enhancer factors such as Ets-1 and p53 (26-28), to our knowledge it has not been reported previously in sequence-specific RNA-binding proteins. Autoinhibition could serve an important mechanism to ensure that Tat will not bind TAR RNA without first interacting with CycT1 in the P-TEFb complex, and free uncomplexed Tat protein may be unstable and subject to rapid turn-over by cellular proteases, as has been suggested for other unstructured transcriptional regulators (reviewed in ref. (29)), thus providing the virus a mechanism to restrict the level of nonfunctional Tat in infected cells.

Importantly, we also find that TAR RNA strongly enhances the interaction between Tat and CycT1. RNA-induced protein:protein interactions has been most clearly documented with the lambda N protein, which binds to a site in the boxB RNA to mediate transcriptional anti-termination (30-33). Recent models suggest that the N protein induces a restructuring of residues in the loop of the boxB RNA hairpin loop to provide a platform for the subsequent loading of the lambda NusA protein (31-33), suggesting that the interaction of N with boxB RNA enhances the weak protein:protein interaction that is otherwise observed between N and NusA (reviewed in ref. (29)). The regulation of protein interaction through structural alterations in the RNA could be an important mechanism for controlling the order of assembly of the Tat:P-TEFb:TAR complex both to ensure that Tat will not commit to TAR in the absence of CycT1(P-TEFb) and, similarly, to
ensure that P-TEFb is preferentially utilized at the viral promoter, since cellular genes do not express TAR RNA. The ability to remodel a transcriptional activator through interaction with protein and nucleic acids partners could also be important for exchanging Tat protein with other possible partners. Thus, after phosphorylation of the RNAPII CTD, Tat has been shown to interact with RNA polymerase II rather than TAR (34). The mechanism that controls the disassembly of the stable Tat:P-TEFb:TAR complex is unknown, but may rely on the ability of the Tat protein to model itself to available surfaces on the RNA polymerase elongation complex. These findings with the Tat and lambda N proteins highlight the versatility of RNA as an enhancer of specific protein:protein interactions.
REFERENCES


FIGURE LEGENDS

Figure 1

(A) Secondary structure of TAR RNA used in this study. TAR RNA spans the minimal sequences that are required for Tat responsiveness in vivo (35) and for in vitro binding of Tat-derived peptides (36). (B) Regions of the HIV-1 Tat (aa1-72) protein and the position of modification with rhodamine.

Figure 2

(A) Fluorescence spectra of TAR RNA-Fl (black), TAR RNA-Fl with TAT-Rh (red) and TAR RNA-Fl with TAT-Rh and CycT1 (aa1-303) /Zn^{2+} (blue). (B) Fluorescence titration of TAT-Rh with 8.0 nM TAR RNA-Fl in the absence (squares) and presence (triangles) of 8.0 nM CycT1 (aa1-303) with 0.18 mM Zn^{2+}. Each addition of TAT-Rh was followed by 2-min equilibration before the fluorescence signal was measured. The solid lines represent the best fits of the data by nonlinear regression to quadratic equation 1.

Figure 3

Fluorescence quenching of TAT-Rh at 575 nm as a function of the total CycT1 (aa1-303) concentration in the absence (A) and (B) presence of 3.0 nM unlabeled TAR RNA. The solid lines represent the best fit of the data by nonlinear regression to quadratic equation 1. The dissociation constant of 9.1 ± 2.0 nM and 0.4 ± 0.2 nM between Tat and CycT1 (aa1-303) complex in the absence and presence of TAR RNA were obtained, respectively. (C) A schematic model of CycT1:Tat:TAR interactions indicating binding affinities between Tat:TAR and CycT1:Tat in this ternary complex.

Figure 4

Kinetic stability of the TAR RNA, Tat and CycT1 (aa1-303) complex. (A) The fractions of remaining CycT1-Tat-TAR complexes were plotted as a function of time. The solid line is the best
fit of the data using equation 2 described in the text. $K_{\text{off}} = 0.034 \text{ min}^{-1}$ was obtained. (B)
Dissociation rates of the CycT1-Tat-TAR complex are in linear increase with the increase of the total concentration of competitor unlabeled RNA.

**Figure 5**

(A) Efficiency of energy transfer as a function of total Tat-Rh concentration in the absence (squares), presence of 8.0 nM CycT1 (aa1-303) without Zn$^{2+}$ (crosses) and of 8.0 nM CycT1 (aa1-303) with 0.18 mM Zn$^{2+}$ (triangles). Buffer conditions: 50 mM Tris-HCl, pH 7.4 at 25°C, 20 mM KCl and 1 mM β-mercaptoethanol. (B) Schematic representation of FRET results showing the conformational change in Tat upon CycT1 (aa1-303) binding and the lifetimes of ribonucleoprotein complexes.
Table I. Distance between the fluorescein-labeled TAR RNA and rhodamine-labeled Tat in the presence and absence of Cyclin T1

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>$Q_d$</th>
<th>$J_{DA}$ (cm$^2$/mol)</th>
<th>$R_0$ (Å)</th>
<th>$E$ (%)</th>
<th>$R$ (Å)</th>
<th>Cyclin T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAR-Fl</td>
<td>Tat-Rh</td>
<td>0.26</td>
<td>$4.46 \times 10^{-13}$</td>
<td>47.6</td>
<td>25±3</td>
<td>57.2</td>
<td>-</td>
</tr>
<tr>
<td>TAR-Fl</td>
<td>Tat-Rh</td>
<td>0.26</td>
<td>$4.46 \times 10^{-13}$</td>
<td>47.6</td>
<td>15±3</td>
<td>63.6</td>
<td>+</td>
</tr>
</tbody>
</table>
Fig. 4

A

\[
\frac{[\text{Complex}]_t}{[\text{Complex}]}_0
\]

Time (min)

B

\[K_{\text{off}} \text{ (min}^{-1}\text{)}\]

Unlabeled TAR RNA / TAR RNA-FI
Figure 5

A

![Graph showing the efficiency of energy transfer (E) versus TAT-Rh concentration (nM).](image)

B

**TAR**

- Acceptor
- R
- Tat

---

**FRET**

- Donor
- Lifetime = 5 min

---

- Cyclin T1
- 63.6 Å
- Lifetime = 48 min

- Tat
- 57.2 Å
HIV-1 TAR RNA enhances the interaction between Tat and cyclin T1
Jing Zhang, Natarajan Tamilarasu, Seongwoo Hwang, Mitchell E. Garber, Ikramul Huq, Katherine A. Jones and Tariq M. Rana

J. Biol. Chem. published online August 15, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M006804200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/early/2000/08/15/jbc.M006804200.citation.full.html#ref-list-1