

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

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Human immunodeficiency virus, type 1 (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 cooperative 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.

The human immunodeficiency virus (HIV-1)¹ encodes a transcriptional activator protein, Tat, that increases the processivity of RNA polymerase II (for reviews see Refs. 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 carboxyl-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 cooperative 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 carboxyl-terminal boundary of the CycT1 cyclin domain which are not critical for binding of cyclin T1 to CDK9 (8, 10–14), and basic residues in CycT1 (Arg-251 and Arg-254) 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. 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 (Fig. 1). FRET, in which a fluorescent donor molecule transfers energy via a non-radioactive 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 lifetime and quantum yield of the donor 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, VA). TAR RNA was chemically synthesized on a fluorescein-containing CPG 500 support. RNA (1 μmol) 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 depro-

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¹ The abbreviations used are: HIV-1, human immunodeficiency virus, type 1; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; aa, amino acid(s); CTD, carboxyl-terminal domain; CycT1, cyclin T1; FRET, fluorescence resonance energy transfer.

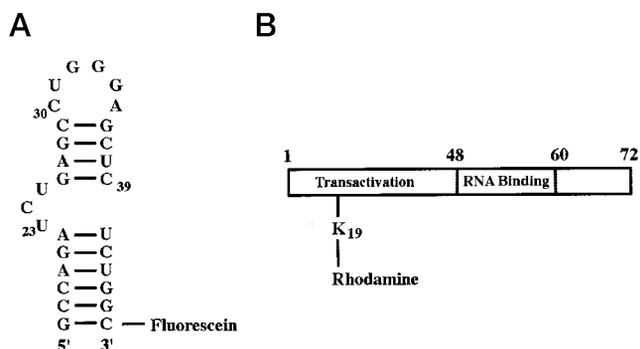


FIG. 1. **A**, secondary structure of TAR RNA used in this study. TAR RNA spans the minimal sequences that are required for Tat responsiveness *in vivo* (35) and for *in vitro* binding of Tat-derived peptides (36). **B**, regions of the HIV-1 Tat (aa 1–72) protein and the position of modification with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

tection, 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 phosphatase (Promega) for 1 h 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 [γ -³²P]ATP (6000 Ci/mmol) (ICN) per 100 pmol of RNA by incubating with 16 units of T4 polynucleotide kinase (New England Biolabs) in 70 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 5 mM dithiothreitol (21, 22). The RNAs were labeled at the 3'-end by ligation to cytidine 3',5'-(5'-³²P)bisphosphate [³²P]pCp) using T4 RNA ligase. Reaction mixtures (50 μl) contained 250 pmol of RNA, 65 μCi of [³²P]pCp (3000 Ci/mmol, PerkinElmer Life Sciences), and 40 units of T4 RNA ligase (New England Biolabs) in a buffer containing 50 mM Tris-HCl, pH 8.0, 3 mM dithiothreitol, 10 mM MgCl₂, 25 mM NaCl, 50 mM ATP, 25 μg/ml bovine serum albumin, and 10% dimethyl sulfoxide (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 min at 85 °C. RNAs were incubated with 0.1 unit of RNase from *Bacillus cereus* (Amersham Pharmacia Biotech) per picomole of RNA for 4 min at 55 °C in 16 mM sodium citrate, pH 5.0, 0.8 mM EDTA, 0.5 mg/ml yeast tRNA (Life Technologies, Inc.). This enzyme yields U- and C-specific cleavage of RNA. Sequencing products were resolved on 20% denaturing gels and visualized by phosphorimager analysis.

Tat and Cyclin T1 Proteins—Tat protein (aa 1–72) was chemically synthesized using standard Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) amino acids, and 5-carboxytetramethylrhodamine was incorporated at Lys-19. Details of Tat-Rhodamine synthesis will be described elsewhere.² Rhodamine-labeled Tat (aa 1–72) was purified by high pressure liquid chromatography 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 that can interfere with its function (data not shown).

Human Cyt1 (aa 1–303 and aa 1–254) was expressed as a glutathione *S*-transferase fusion protein, purified, and characterized as described previously (7, 8).

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 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 Cyt1 (aa

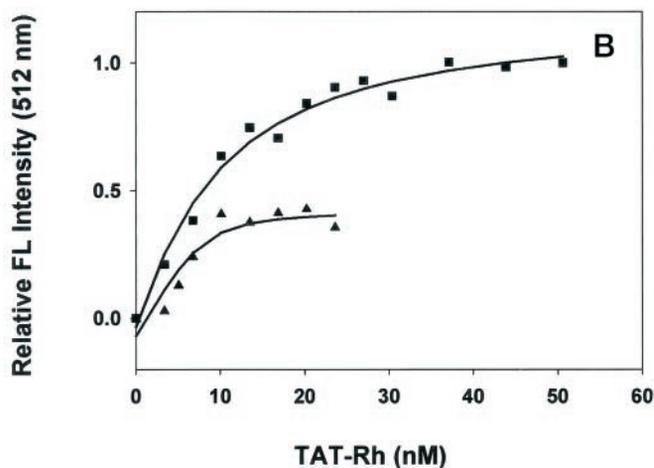
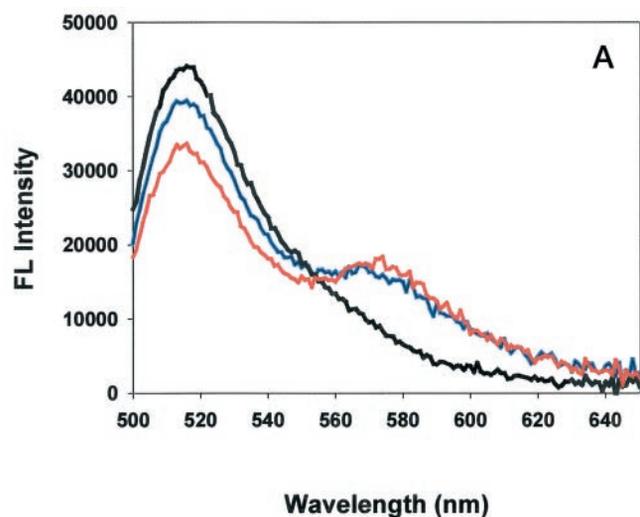


FIG. 2. **A**, fluorescence spectra of TAR RNA-Fl (black), TAR RNA-Fl with TAT-Rh (red), and TAR RNA-Fl with TAT-Rh and Cyt1 (aa 1–303)/Zn²⁺ (blue). **B**, fluorescence titration of TAT-Rh with 8.0 nM TAR RNA-Fl in the absence (squares) and presence (triangles) of 8.0 nM Cyt1 (aa 1–303) with 0.18 mM Zn²⁺. Each addition of TAT-Rh was followed by a 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.

1–303)/Zn²⁺ were 8.0, 16.5, and 8.0 nM, respectively. The final concentration of Zn²⁺ 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 Lys-19 in the Tat sequence. We chose Lys-19 in the activation domain of Tat because it is located in the Cyt1-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

² N. Tamilarasu and T. M. Rana, unpublished data.

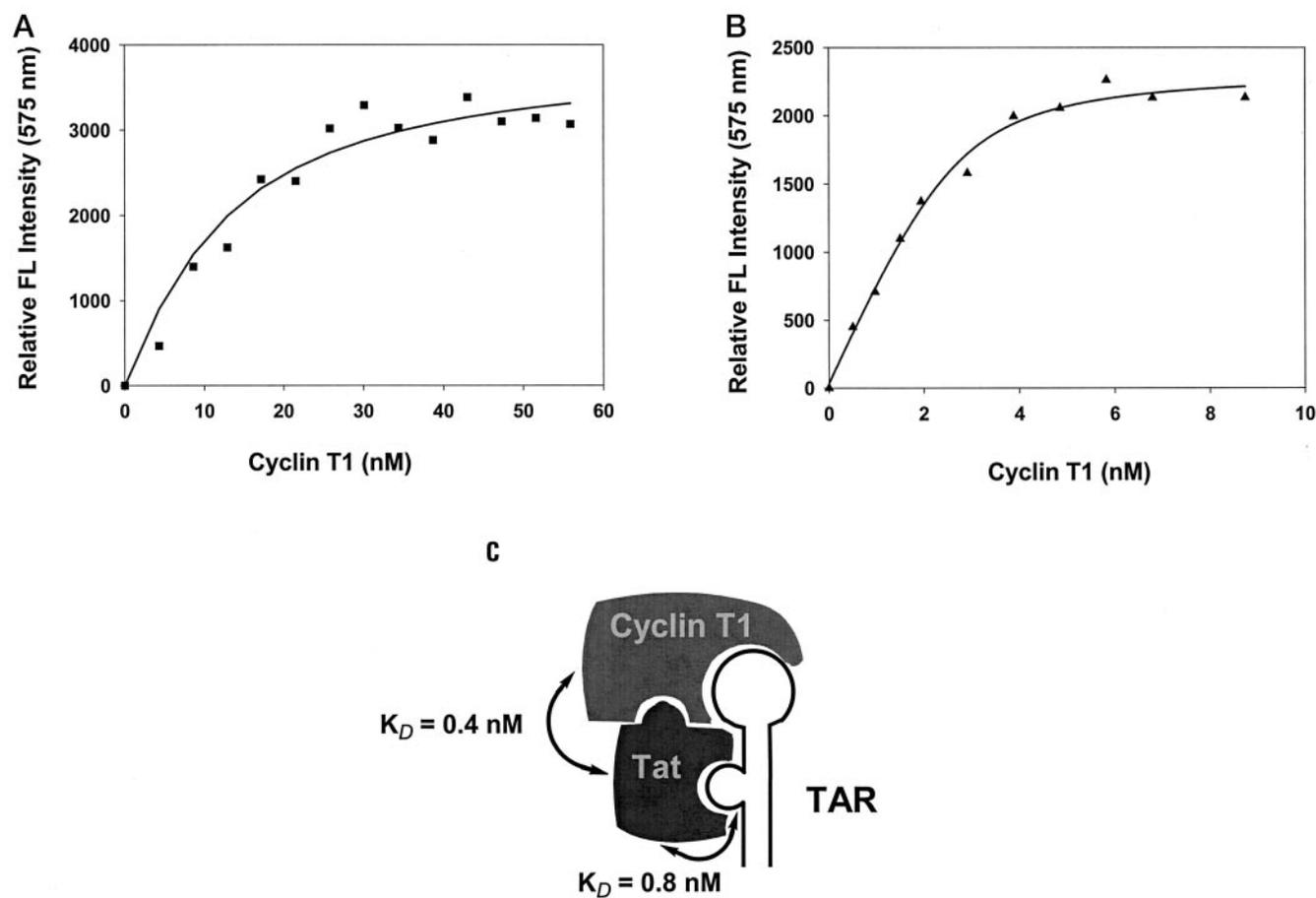


FIG. 3. Fluorescence quenching of TAT-Rh at 575 nm as a function of the total CycT1 (aa 1–303) concentration in the absence (A) and presence (B) of 3.0 nM unlabeled TAR RNA. The solid lines represent the best fit of the data by nonlinear regression to quadratic Equation 1. The dissociation constant of 9.1 ± 2.0 and 0.4 ± 0.2 nM between Tat and CycT1 (aa 1–303) 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.

and presence of cyclin T1. 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 (aa 1–303) with Zn^{2+} (Fig. 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 (aa 1–303) 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 with TAR RNA-Fl and Tat-Rh. The decrease in the efficiency of energy transfer in the presence of CycT1 (aa 1–303) 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 cyclin T1-Tat-TAR ternary complex.

Several control experiments further support these observations and demonstrate the specificity of CycT1-Tat-TAR interactions. First, CycT1 (aa 1–303) 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 (aa 1–254), 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 cyclin T1-Tat-TAR ribonucleoprotein complex.

To determine the effect of cyclin T1 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 (aa 1–303). Results of these experiments are shown in Fig. 2B. K_D values were calculated by fitting data to quadratic Equation 1,

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

where F is the relative fluorescent intensity; F_{\min} is the fluorescent intensity at the start of the titration; F_{\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(aa 1–72)-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

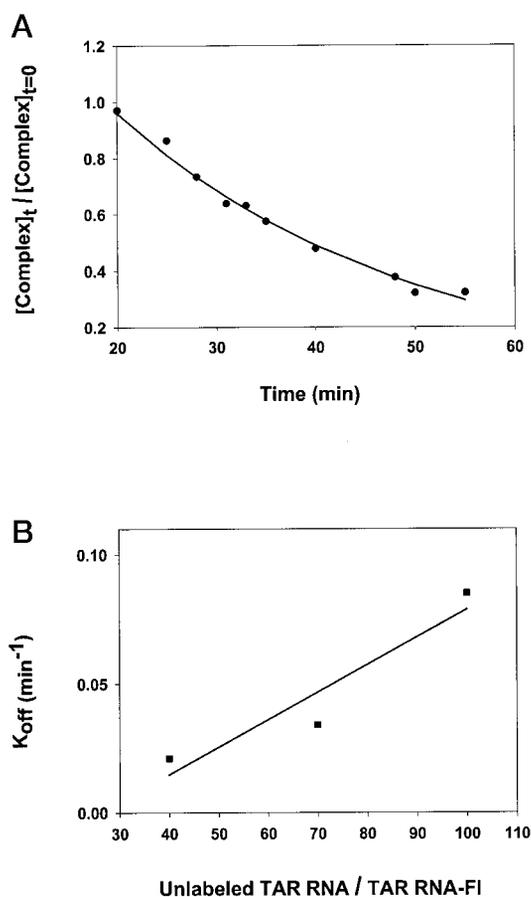


FIG. 4. Kinetic stability of the TAR RNA, Tat, and CycT1 (aa 1-303) complex. A, the fractions of remaining CycT1·Tat·TAR complexes were plotted as a function of time. The solid line is the best fit of the data using Equation 2 described in the text. $k_{\text{off}} = 0.034 \text{ min}^{-1}$ was obtained. B, dissociation rates of the CycT1·Tat·TAR complex are in linear increase with the increase of the total concentration of competitor unlabeled RNA.

titration of CycT1 (aa 1-303) with Tat-Rh. Fig. 3A shows the best fit of the data using Equation 1, and a dissociation constant of 9.1 nM for Tat and CycT1 (aa 1-303) complex was obtained. To determine the effect of TAR RNA on cyclin T1-Tat interactions, the same experiments were performed in the presence of equal molar of unlabeled TAR RNA to TAT-Rh (Fig. 3B). The dissociation constant for CycT1 (aa 1-303) 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 cyclin T1·Tat·TAR complex was determined by forming a complex between TAR RNA-FI, Tat-Rh, and CycT1 (aa 1-303), 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-FI was used for the competition. As shown in Fig. 2A, TAR RNA-FI 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-FI at 512 nm. Therefore, the amount of remaining CycT1·Tat·TAR complex can be assayed by measuring the fluorescence intensity of TAR RNA-FI at 512 nm. Fig. 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_{off}) can readily be obtained by fitting data using Equation 2.

$$[\text{complex}]_t / [\text{complex}]_{t=0} = A \exp(-k_{\text{off}} t) \quad (\text{Eq. 2})$$

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

In order to test the relationship between k_{off} and the concentration of competitor, 40-100-fold excess of unlabeled TAR RNA over labeled TAR RNA were used. The k_{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 (Fig. 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-FI complex in the absence of CycT1 which showed that the complex dissociated with a k_{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 min, while under similar conditions CycT1·Tat·TAR complex has a lifetime of 48 min. 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-FI and Tat-Rh and calculated the distance between two fluorophores, rhodamine at Lys-19 of Tat and fluorescein at 3'-end of TAR RNA. The distance (R) between donor, TAR RNA-FI, and acceptor, TAT-Rh, can be measured spectroscopically by Förster energy transfer (24). The distance R was determined from the following equation: $r = R_0 (E^{-1} - 1)^{1/6}$, where E is the efficiency of nonradioactive 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. $R_0 = 9786(k^2 n^{-4} Q_d J)^{1/6} A$. 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 fluorophores. 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. $J(4.46 \times 10^{-13} \text{ cm}^6/\text{mol})$ 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 $J = \sum(\epsilon_A(\lambda) F_D(\lambda) \lambda^4 \Delta\lambda) / \sum F_D(\lambda)$, where $\epsilon_A(\lambda)$ 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-FI 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 Fig. 5. The efficiency of transfer between TAR-FI and TAT-Rh was 25% (Table I). However, in the presence of CycT1 (aa 1-303) and 0.18 mM Zn^{2+} , the efficiency of energy transfer was decreased to 15% (Table I). The distance (R) between two fluorophores was determined from R_0 and E that showed that Lys-19 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 Lys-19-rhodamine is located in the CycT1-interacting region of Tat, these results indicate that Tat goes

FIG. 5. *A*, efficiency of energy transfer as a function of total Tat-Rh concentration in the absence (*squares*), presence of 8.0 nM CycT1 (aa 1–303) without Zn^{2+} (*crosses*), and of 8.0 nM CycT1 (aa 1–303) with 0.18 mM Zn^{2+} (*triangles*). Buffer conditions are as follows: 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 (aa 1–303) binding and the lifetimes of ribonucleoprotein complexes.

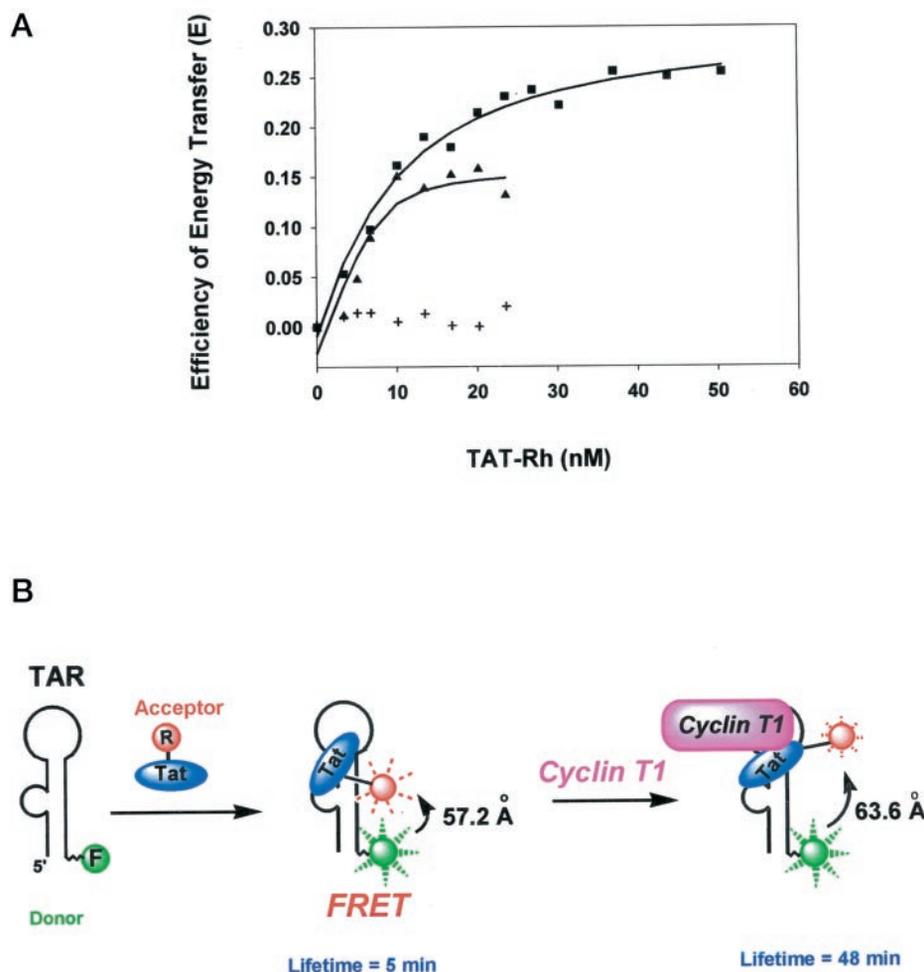


TABLE I
Distance between the fluorescein-labeled TAR RNA and rhodamine-labeled Tat in the presence and absence of cyclin T1

Donor	Acceptor	Q_d	J_{DA}	R_0	E	R	Cyclin T1
			cm^6/mol	\AA	%	\AA	
TAR-FI	Tat-Rh	0.26	4.46×10^{-13}	47.6	25 ± 3	57.2	–
TAR-FI	Tat-Rh	0.26	4.46×10^{-13}	47.6	15 ± 3	63.6	+

through a structural reorganization upon CycT1 binding (Fig. 5).

Interestingly, when Zn^{2+} was removed from the binding reactions, no energy transfer was detected. This result supports a previously proposed model for cyclin T1-Tat-TAR interactions suggesting that Tat forms a metal-linked heterodimer with cyclin T1 (8).

We find that Tat (aa 1–72) binds TAR RNA with a K_D of 8.2 nM and that this affinity is enhanced 10-fold in the presence of CycT1 (aa 1–303). 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 (aa 49–57), binds TAR with a K_D of 1 nM, whereas a longer peptide containing additional amino acids from the core domain, Tat(aa 38–72), has a lower affinity for TAR RNA with a K_D of 5.7 nM.³ 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 (86 aa) HIV-1 and (130 aa) 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 turnover 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 have been most clearly documented with the λ N protein, which binds to a site in the box B RNA to

³ J. Zhang, N. Tamilarasu, S. Hwang, and T. M. Rana, unpublished observations.

mediate transcriptional anti-termination (30–33). Recent models suggest that the N protein induces a restructuring of residues in the loop of the box B RNA hairpin loop to provide a platform for the subsequent loading of the λ NusA protein (31–33), suggesting that the interaction of N with box B 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 λ N proteins highlight the versatility of RNA as an enhancer of specific protein-protein interactions.

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