Replication of human immunodeficiency virus type 1 (HIV-1) requires specific interactions of Tat protein with the trans-activation responsive region (TAR) RNA, a stem-loop structure containing two helical stem regions separated by a trinucleotide bulge. The Tat protein contains a basic RNA-binding region (amino acids 49–57) located in the carboxyl-terminal half of the protein, and peptides containing this basic domain of Tat protein can bind TAR RNA with high affinities. We synthesized a 31-amino acid Tat fragment (amino acids 42–72) containing the basic region and part of flanking regulatory core domain that formed a specific complex with TAR RNA. Upon UV irradiation (254 nm), this Tat fragment cross-linked covalently with TAR RNA. Sites of cross-links were determined on both the TAR RNA and Tat protein fragment by RNA and protein sequencing, respectively. These results revealed that guanosine 26 of TAR RNA was cross-linked with tyrosine 47 of the Tat peptide. Our results provide the first physical evidence for a direct amino acid-base contact in Tat-TAR complex. Recently, orientation of the Tat-(42–72) was determined in our laboratory by psoralen cross-linking* and trans-cleavage. RNA molecules can fold into extensive structures containing regions of double-stranded duplex, hairpins, internal loops, bulged bases, and pseudoknotted structures (1, 2). Due to the complexity of RNA structure, the rules governing sequence-specific RNA-protein recognition are not well understood. Recent structural studies have demonstrated that RNA-binding proteins interact with RNA in both the minor and major grooves. For example, two tRNA synthetases (alanine and glutamine) interact with the acceptor stems of their cognate tRNAs in the minor grooves (3, 4). Major groove recognition takes place between aspartyl-tRNA synthetase and its cognate tRNAs at a site of local distortion in the RNA helix (5). Bulge loops or bulges (unpaired nucleotides on one strand of a duplex) in RNA helices are potentially important in tertiary folding of RNA and in providing sites for specific RNA-protein interactions, as illustrated by TFIIIA of Xenopus (6) and the coat protein of phage R17 (7). In a recent report, interactions between U1 small nuclear RNA and the N-terminal domain of the human U1A protein were mapped by multidimensional heteronuclear NMR studies (8). These studies showed that protein-RNA contacts occur at the single-stranded apical loop of the hairpin and also in the major groove of the helical stem at neighboring U-G and U-U non-Watson-Crick base pairs (8). Crystal structure of the RNA-binding domain of the U1A splicing protein complexed with an RNA hairpin also revealed that the loop sequence (AUUGCAG) interacts with the surface of the four-stranded β-sheet (9). On the basis of NMR data, it has been shown that TAR RNA in HIV-1 changes its conformation upon arginine binding (10, 11). All of these studies suggest that the diversity of RNA structures plays a central role in their specific recognition by proteins.

The promoter of the human immunodeficiency virus type 1 (HIV-1), located in the U3 region of the viral long terminal repeat, is an inducible promoter that can be stimulated by the trans-activator protein, Tat (12). As in other lentiviruses, Tat protein is essential for transactivation of viral gene expression (13–16). In the absence of Tat, most of the viral transcripts terminate prematurely, producing short RNA molecules ranging in size from 60 to 80 nucleotides. Jeang et al. (17) reported that integrated HIV-1 promoters did not show a high rate of abortive transcription. Nonetheless, HIV-1 proviruses and integrated long terminal repeats respond efficiently to Tat (17). The Tat protein is a small, cystein-rich nuclear protein containing 86 amino acids and comprised of three important functional domains. HIV-1 Tat protein acts by binding to the TAR (trans-activation responsive) RNA element, a 59-base stem-loop structure located at the 5'-ends of all nascent HIV-1 transcripts (18–22). Upon binding to the TAR RNA sequence, Tat causes a substantial increase in transcript levels (23–27). The increased efficiency in transcription may result from preventing premature termination of the transcriptional elongation

The role of RNA-protein interactions is vital for many regulatory processes, especially in gene regulation where proteins specifically interact with binding sites found within RNA transcripts. RNA molecules can fold into extensive structures containing regions of double-stranded duplex, hairpins, internal loops, bulged bases, and pseudoknotted structures (1, 2). Due to the complexity of RNA structure, the rules governing sequence-specific RNA-protein recognition are not well understood. Recent structural studies have demonstrated that RNA-binding proteins interact with RNA in both the minor and major grooves. For example, two tRNA synthetases (alanine and glutamine) interact with the acceptor stems of their cognate tRNAs in the minor grooves (3, 4). Major groove recognition takes place between aspartyl-tRNA synthetase and its cognate tRNAs at a site of local distortion in the RNA helix (5). Bulge loops or bulges (unpaired nucleotides on one strand of a duplex) in RNA helices are potentially important in tertiary folding of RNA and in providing sites for specific RNA-protein interactions, as illustrated by TFIIIA of Xenopus (6) and the coat protein of phage R17 (7). In a recent report, interactions between U1 small nuclear RNA and the N-terminal domain of the human U1A protein were mapped by multidimensional heteronuclear NMR studies (8). These studies showed that protein-RNA contacts occur at the single-stranded apical loop of the hairpin and also in the major groove of the helical stem at neighboring U-G and U-U non-Watson-Crick base pairs (8). Crystal structure of the RNA-binding domain of the U1A splicing protein complexed with an RNA hairpin also revealed that the loop sequence (AUUGCAG) interacts with the surface of the four-stranded β-sheet (9). On the basis of NMR data, it has been shown that TAR RNA in HIV-1 changes its conformation upon arginine binding (10, 11). All of these studies suggest that the diversity of RNA structures plays a central role in their specific recognition by proteins.

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The abbreviations used are: TAR, trans-activation responsive region; HIV-1, human immunodeficiency virus, type 1.
Identification of a Specific Contact in Tat-TAR Complex

EXPERIMENTAL PROCEDURES

Buffers

All buffer pH values refer to measurements at room temperature. Trancription buffer contained 40 mM tris-HCl (pH 8.1), 1 mM spermine, 0.01% Triton X-100, 0.5 mM dithiothreitol. TBE buffer contained 9 mM Tris, 50 mM boric acid (pH 8.3), 1 mM EDTA. Sample loading buffer contained 9 mM urea, 50 mM Tris, 50 mM boric acid (pH 8.3), 1 mM EDTA, 0.25% bromophenol blue, 0.025% xylene cyan.

Peptide Synthesis

All Fmoc (N-(9-fluorenly)methoxy carbonyl) amino acids, piperidine, 4-dimethylaminopyridine, dichloromethane, N,N-dimethylformamide, 1-hydroxybenzotriazole, 2-(1H-benzotriazo-1-yl)-1,3,3-tetramethyluronium hexafluorophosphate, diisopropylethylamine, and HMP-linked polystyrene resin were obtained from the Applied Biosystems Division, Perkin-Elmer. Trifluoroacetic acid, 1,2-ethanediol, phenol, and thioanisole were from Sigma. Two Tat-derived peptides (from amino acids 42–72 and 48–72) were synthesized on an Applied Biosystems 431A peptide synthesizer using standard Fmoc protocols. Cleavage and deprotection of the peptide was carried out in 2 ml of Reagent K for 6 h at room temperature. Reagent K contained 1.75 ml of trifluoroacetic acid, 100 μl of thioanisole, 100 μl of water, and 50 μl of ethanedithiol (47). After cleavage from the resin by high pressure liquid chromatography on a Zorbax 300 SB-C8 column. The mass of fully deprotected and purified peptide was confirmed by fast atom bombardment mass spectrometry: calculated mass for Tat-(42–72) C123H219N53O35 = 3606.5, found 3606.5 (M + H); calculated mass for Tat-(48–72) C124H221N53O36 = 3000.4, found 3001.4 (M + H).

Base Hydrolysis of RNA Cross-linked with Peptide

Preparation of Cross-linked Complex on a Preparative Scale—The reaction mixture (10 ml) contained 4 μM 5'-end-labeled RNA, 6 μM peptide, 25 mM Tris (pH 7.4), and 50 mM NaCl. After UV irradiation, the mixtures were applied to a Mono Q HR 5/5 column (Pharmacia) equilibrated with 40 mM KCl, 25 mM Tris (pH 8.3) and eluted from the column with a salt gradient (0–8 min, 40–440 mM KCl; 8–16 min, 440–640 mM KCl; and 16–24 min, 640–1040 mM KCl) at a flow rate of 1 ml/min. RNA-peptide complex was eluted at approximately 480 mM KCl and free RNA at 550 mM KCl. The fractions containing RNA-peptide complex were identified by gel electrophoresis (SDS and urea gels) and autoradiography. The RNA-peptide complex was desalted on a Sep-Pak C18 cartridge and lyophilized.

Digestion and Peptide Sequencing—The 5'-end-labeled RNA-peptide complex was dissolved in 0.15 ml of H2O, 80 μl of 0.5 mM Tris, and 80 μl of 1% SDS. After heating at 70°C for 1 min and cooling to 37°C, 0.62 ml of H2O, 0.067 ml of 15 mM CaCl2, and then 0.25 μl of sequencing grade modified trypsin (Promega) were added. The samples were incubated at 37°C for 13 h. After phenol extraction and ethanol precipitation, the samples were dissolved in 70 μl of H2O and 70 μl of 1 M ammonium carbonate (pH 8.1), washed exhaustively with H2O on Sep-Pak C18 cartridge, and dried in a Savant Speedvac. The concentration of cross-linked RNA-peptide complex was determined based on RNA absorption at 260 nm. Amino acid sequencing was conducted at Yale University (New Haven, CT) on an Applied Biosystems sequencer.

RESULTS

Formation of Cross-linked RNA-Peptide Complex—Before performing the UV-induced cross-linking experiment, it was necessary to establish the binding affinities of the Tat fragment (residues 42–72) used in this study. The dissociation constant
The concentration of TAR RNA and the Tat peptide employed for cross-linking reactions were 0.25 \( \mu \text{M} \) and 1.9 \( \mu \text{M} \), respectively. Under these conditions, electrophoretic mobility shift assays revealed (data not shown) only one slow migrating RNA-peptide complex, indicating the absence of other nonspecific RNA-peptide complex formation (44, 48). TAR RNA labeled at the 5’-end with \( ^{32} \text{P} \) was incubated with the Tat peptide for 20 min in 25 \( \mu \text{M} \) Tris (pH 7.4) and 100 \( \mu \text{M} \) NaCl and UV-irradiated with 254-nm light (see “Experimental Procedures”). Products of the photoreaction were analyzed by denaturing 8 \( \mu \)M urea-polyacrylamide gel electrophoresis (Fig. 1). Irradiation of the RNA-peptide complex yields a new band with electrophoretic mobility less than that of TAR RNA (lane 4). Both the peptide and UV irradiation are required for the formation of a cross-linked RNA-protein complex. This is evidenced by the fact that no cross-linked products are observed when RNA is irradiated in the absence of Tat peptide (lane 2) or incubated with peptide in the dark without UV irradiation (lane 3). Further control experiments showed that no cross-linking was observed when RNA and peptide were irradiated separately and then mixed (lane 5). Digestion of the RNA-peptide cross-link with Proteinase K (5 units for 30 min at 37 °C) resulted in an RNA species with mobility similar to TAR RNA (lane 6). The products of irradiation were also analyzed on a denaturing SDS-15% polyacrylamide gel. Again, a photoproduct with electrophoretic mobility less than that of TAR RNA was observed that was dependent on the presence of RNA and peptide (data not shown). The photoproduct yield is ~5% as determined by a PhosphorImager analysis. Since the cross-linked RNA-peptide complex is stable to alkaline pH (9.5), high temperature (85 °C) and denaturing conditions (8 \( \mu \)M urea, 2% SDS), we conclude that a covalent bond is formed between TAR RNA and the peptide during the cross-linking reaction.

Dependence of the Cross-Linking Reaction on the Concentration of Peptide and Time of Irradiation—Formation of RNA-peptide photocross-link was dependent on the concentration of Tat peptide. Here, as in Fig. 1, the major cross-link product (XL1) has slightly lower electrophoretic mobility than TAR RNA. In Fig. 2, the efficiency of XL1 formation increased as the peptide concentration was raised from 0.13 \( \mu \text{M} \) to 1.25 \( \mu \text{M} \). At a peptide concentration higher than 1.25 \( \mu \text{M} \), a second minor cross-link product (XL2) with a lower electrophoretic mobility than that of XL1 was observed.

The photocross-linking reaction between the Tat peptide and TAR RNA was also dependent on time of irradiation. The yields of cross-linked RNA-peptide complex were increased with an increase in time of irradiation (Fig. 3). In this experiment, similar to that shown in Fig. 2, extended time of irradiation also resulted in the formation ofXL2 at 30 and 40 min. This second minor photoproduct could be the result of nonspecific binding of the peptide to RNA (at higher concentrations of peptide) or nonspecific association of photodamaged RNA and peptide after longer irradiation times. Further characterization of this minor photoproduct was not carried out in this study.

Specificity of the Cross-Link Formation—Specificity of the cross-linking reaction was established by competition experiments. Cross-linking reactions were performed in a 15-\( \mu \text{l} \) volume containing 0.25 \( \mu \text{M} \) of \( ^{32} \text{P} \)-5’-end-labeled TAR RNA and 1.9 \( \mu \text{M} \) Tat peptide and was UV irradiated as described under "Experimental Procedures." After the indicated irradiation times, aliquots were withdrawn and analyzed on 8 \( \mu \)M urea-polyacrylamide gel. XL1, cross-linked RNA-peptide complex, major photoproduct; XL2, cross-linked RNA-peptide complex, minor photoproduct.
Identification of a Specific Contact in Tat-TAR Complex

Fig. 4. Specificity of the cross-linking reaction determined by competition assays. Complexes were formed between 0.25 μM 32P-labeled TAR RNA and 1.9 μM of Tat-(42–72) in the presence of unlabeled wild-type TAR RNA (A) or bulgeless mutant TAR RNA (B). Concentrations of the competitor RNA in lanes 2, 3, 4, 5, 6, and 7 were 0, 0.25, 0.5, 2.5, 5, and 10 μM, respectively. Lane 1 was a control RNA-protein complex without UV irradiation. C, quantitative analysis of competition experiments. The fraction of RNA in RNA-protein cross-link was determined by PhosphorImager analysis as described under "Experimental Procedures." □, wild-type TAR RNA competitor; ▣, bulgeless mutant TAR RNA.

Fig. 5. Mapping of cross-linked base in the RNA-protein cross-links by alkaline hydrolysis. A, analysis of 5′-end-labeled TAR RNA and cross-link: B. cereus ladder of TAR RNA (lane 1); hydrolysis ladder of TAR RNA (lane 2); hydrolysis ladder of cross-linked RNA-peptide complex (lane 3). The sequence of TAR RNA from C9 to U25 is labeled. A gap in the sequence is obvious after the U25 residue, indicating that G26 is the cross-linked base. B, sequence and secondary structure of wild-type TAR RNA used in this study. TAR RNA spans the minimal sequences that are required for Tat responsiveness in vivo (21) and for in vitro binding of Tat-derived peptides (38). Wild-type TAR contains two non-wild-type base pairs to increase transcription by T7 RNA polymerase. U25 represents the nucleotide at which the hydrolysis of the 5′-end-labeled cross-linked RNA-peptide complex was stopped. The arrow indicates the location of guanosine 26, which is the cross-linked base in TAR RNA (shown in boldface).

5A, lane 2). Thus, we conclude that guanosine 26 of TAR RNA is the site at which cross-linking occurs (Fig. 5B).

Cross-linking Occurs at Tyr47 of Tat—To identify the amino acid(s) of Tat that are involved in specific cross-linking with TAR RNA, the cross-linked RNA-peptide complex (XL1) was prepared on a preparative scale (see "Experimental Procedures"), purified from noncross-linked TAR RNA by ion exchange fast protein liquid chromatography, and digested with trypsin. The tryptic digest products were purified by 8 M urea, 20% acrylamide denaturing gels and visualized by autoradiography. We recovered ~100 pmol quantities of a tryptic fragment of XL1 and subjected it to N-terminal sequencing. The amino acid sequencing data showed that it had a sequence of Ala-Leu-Gly-Ile-Ser-X-Gly-Arg-Lys-Lys. This sequence corresponds to the sequence encompassing amino acids 42–51 in HIV-1 Tat protein (Fig. 6). X at the 6th position represents a nonstandard amino acid instead of tyrosine 47 of the Tat peptide. Thus, cross-linking occurs at tyrosine 47 of the Tat peptide.

DISCUSSION

Ultraviolet-induced cross-linking of RNA to proteins is a widely used technique to study in vitro and in vivo RNA-protein interactions (49–51). UV irradiation with sufficient intensity generates a highly reactive species of RNA, which reacts with protein and organic molecules involved in making direct contacts with RNA (52, 53). To identify specific RNA-protein contacts, we irradiated TAR RNA and Tat-(42–72) protein complex with UV light and observed the formation of a covalent bond between RNA and protein. Formation of this covalently cross-linked product was dependent on the concentration of Tat peptide and irradiation time (Figs. 2 and 3). Our competition and control experiments showed that a specific RNA-protein complex formation between TAR RNA and Tat fragment was necessary for photo-crosslinking reactions (Fig. 4).

To locate the cross-link sites in TAR RNA and the Tat peptide, we prepared the RNA-protein cross-link on a preparative scale, purified the cross-link, and analyzed it by RNA and protein sequencing. Alkaline hydrolysis of 5′-end-labeled cross-links indicated that a single nucleotide, G26, in TAR RNA was involved in covalent interaction (Fig. 5A, A and B). The absence of bands in the hydrolysis ladder after U25 from the 5′-end of RNA indicates that the RNA fragments after U25 are covalently linked to the Tat peptide and migrate more slowly to create a gap in the standard hydrolysis ladder. Our results clearly demonstrate that cross-linking occurs at G26 in TAR RNA.

Peptide sequencing on a tryptic fragment of the cross-link complex was accomplished by Edman degradation chemistry. The sequencing data indicate that cross-linking occurred at Tyr47 of the Tat peptide. As shown in Fig. 6, peptide sequencing identified a nonstandard amino acid X at the 6th position of the cross-linked peptide, Ala-Leu-Gly-Ile-Ser-X-Gly-Arg-Lys-Lys. This sequence corresponds to the region encompassing amino acids 42–51 in HIV-1 Tat protein (Fig. 6). The nonstandard amino acid most likely corresponds to a photomodified tyrosine. Sequencing of proteins by Edman degradation chemistry requires unmodified amino and carbonyl groups in the backbone of the peptide. Evidence that the Edman sequencing reaction...
was able to continue through Tyr\textsuperscript{47} indicates that cross-linking does not occur at these locations (54). Therefore, we conclude that the aromatic side chain or C-atom in the peptide backbone of Tyr\textsuperscript{47} is involved in the covalent cross-link formation with TAR RNA.

It has been shown by a number of groups that Tat-derived peptides that contain the basic arginine-rich region of Tat are able to form in vitro complexes with TAR RNA (36, 38–44). Recently, Churcher et al. (44) published a detailed comparative study arguing that Tat peptides can mimic the binding affinity and specificity of Tat protein. Results from that study showed that the addition of amino acid residues from the core region of the Tat protein to the arginine-rich domain-containing peptides increased binding specificities (44). To achieve specific RNA binding by a Tat fragment, we used a Tat peptide, Tat-(42–72), that contained an RNA-binding domain and six amino acids from the core domain of the Tat protein. In this report, our cross-linking results have established that this Tat-(42–72) peptide forms a specific covalent photocross-link to TAR RNA where Tyr\textsuperscript{47} of the peptide contacts G\textsuperscript{26} of the RNA.

What is the biological relevance of these findings? A number of studies showed that the immediate stem nucleotide base pairs flanking the bulge region of TAR RNA are required for Tat binding and trans-activation (44, 55, 56). During a detailed mutational analysis of TAR RNA, it was reported that a change of the G\textsuperscript{26}C\textsuperscript{39} base pair to C\textsuperscript{26}G\textsuperscript{39} base pair resulted in only 12% trans-activation by HIV-1 Tat (56). These results strongly support our finding that G\textsuperscript{26} is directly involved in sequence-specific recognition and trans-activation by HIV-1 Tat protein. However, Tat protein mutants where Tyr\textsuperscript{47} was substituted with Ala or His were functional for trans-activation (57, 58). These data raise the possibility that Tyr\textsuperscript{47} is not essential for RNA recognition and that the cross-link formation between Tyr\textsuperscript{47} and G\textsuperscript{26} could be the result of close proximity and favorable photochemistry. To address this question, we carried out cross-linking experiments with a Tat fragment lacking Tyr\textsuperscript{47}, Tat-(48–72), which binds TAR RNA with high affinities (38, 39, 44). UV irradiation of TAR RNA complexed with Tat-(48–72) did not yield any specific RNA-protein cross-link products (data not shown). These results support our model of Tat-TAR interactions where the basic recognition sequence of Tat is located in the major groove of TAR RNA, bringing Tyr\textsuperscript{47} in close vicinity of G\textsuperscript{26} (Fig. 7). The cross-link formation between G\textsuperscript{26} and Tyr\textsuperscript{47} is likely the result of close proximity, favorable orientation, and photoactivity of tyrosine.

How does Tat interact with TAR RNA? Several lines of evidence suggest that Tat protein contacts TAR RNA in a widened major groove. In a recent study from our laboratory, we used a rhodium complex, bis(phenanthroline)(phenanthrenequinone diimine)-rhodium(III) (Rh(phen),phen\textsuperscript{2+}), to probe the effect of bulge bases on the major groove width in TAR RNA (59). This metal complex does not bind double helical RNA or unstructured single-stranded regions of RNA. Instead, sites of tertiary interaction that are open in the major groove and accessible to stacking are targeted by the complex through photocleaved cleavage (60). The sites targeted by the rhodium complex have been mapped to single nucleotide resolution on wild-type TAR RNA and on several mutants of the TAR RNA containing different numbers of mismatch bases in the bulge region (59). A strong cleavage at residues C\textsuperscript{39} and U\textsuperscript{40} was observed on the wild-type TAR RNA and in mutant TAR RNA containing two mismatch bases in the bulge. No cleavage at C\textsuperscript{39} and U\textsuperscript{40} was observed in a bulgeless TAR RNA and in a one-base bulge TAR RNA. Our studies establish two important factors involved in Tat-TAR recognition. (i) There is a correlation between major groove opening and Tat binding. At least a two-base bulge is required for major groove widening and other conformational changes to facilitate Tat binding. This cannot be accomplished by a single base bulge. (ii) The Tat fragment Tat-(42–72) occupies the major groove of TAR RNA and abolishes access of the rhodium complex. On the basis of chemical modification and gel mobility studies, a similar model was suggested earlier by Weeks and Crothers (55). Last, Hamy et al. (61) carried out site-specific modifications of functional groups on TAR RNA and showed that Tat forms multiple specific hydrogen bonds to a series of dispersed sites displayed in the major groove.

To determine the relative orientation of the nucleic acid and protein in the Tat-TAR complex, we have devised a new method based on psoralen photochemistry (48). We synthesized a 30-amino acid fragment containing the arginine-rich RNA-binding domain of Tat (residues 42–72) and chemically attached a psoralen to the amino terminus of the peptide (indicated in black) to demonstrate a close proximity between Tyr\textsuperscript{47} and G\textsuperscript{26}. As determined by psoralen-Tat cross-linking experiments, the amino terminus of Tat-(42–72) contacts, or is close to, uridine 42 in the lower stem of TAR RNA (48); the amino terminus of the peptide is labeled as NH\textsubscript{2}, and its proximal base, U\textsuperscript{42} of TAR RNA, is indicated in black. Structures of TAR RNA were visualized using Insight II software on an IRIS work station.

**Fig. 7. A schematic illustration to show a three-dimensional model of the HIV-1 Tat binding site of TAR RNA and the location of the amino terminus of Tat-(42–72) and interactions of Tyr\textsuperscript{47} of the peptide with G\textsuperscript{26} of the RNA.** Orientation of the Tat-(42–72) was determined in our laboratory by psoralen-Tat-(42–72) conjugate (48). The TAR RNA structure is based on NMR data (63). Ribonucleotide structure of TAR RNA is shown in five dark lines. The basic region of Tat-(47–57) is represented as a barrel positioned in the wide major groove, and the N-terminal region containing Tat-(42–46) is drawn as a line. Tyrosine 47 is shown directly above the G\textsuperscript{26} of TAR RNA (indicated in black) to demonstrate a close proximity between Tyr\textsuperscript{47} and G\textsuperscript{26}. As determined by psoralen-Tat cross-linking experiments, the amino terminus of Tat-(42–72) contacts, or is close to, uridine 42 in the lower stem of TAR RNA (48); the amino terminus of the peptide is labeled as NH\textsubscript{2}, and its proximal base, U\textsuperscript{42} of TAR RNA, is indicated in black. Structures of TAR RNA were visualized using Insight II software on an IRIS work station.
sequence into the enlarged major groove with an orientation where lysine 41 in the core domain of Tat contacts the lower sequence into the enlarged major groove with an orientation RNA recognition by Tat.

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