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Identification and characterization of a HeLa nuclear protein that specifically binds to the trans-activation-response (TAR) element of human immunodeficiency virus

(RNA-binding proteins/UV cross-linking)

ROBERT A. MARCINIAK*†, MARIANO A. GARCIA-BLANCO*, AND PHILLIP A. SHARP*‡

*Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, and †Harvard–MIT Division of Health Sciences and Technology, Cambridge, MA 02139

Contributed by Phillip A. Sharp, February 21, 1990

ABSTRACT **Human immunodeficiency virus type 1 RNAs contain a sequence, trans-activation-response (TAR) element, which is required for tat protein-mediated trans-activation of viral gene expression. We have identified a nuclear protein from extracts of HeLa cells that binds to the TAR element RNA in a sequence-specific manner. The binding of this 68-kDa polypeptide was detected by UV cross-linking proteins to TAR element RNA transcribed *in vitro*. Competition experiments were performed by using a partially purified preparation of the protein to quantify the relative binding affinities of TAR element RNA mutants. The binding affinity of the TAR mutants paralleled the reported ability of those mutants to support tat trans-activation *in vivo*. We propose that this cellular protein moderates TAR activity *in vivo*.**

Expression of human immunodeficiency virus type 1 (HIV-1) genes exhibits complex and multifactorial regulation, involving both viral and cellular control elements (1, 2). One of the viral gene products that plays a central role in virus replication is tat protein.

Tat is a powerful trans-activator of gene expression at one or more control levels (3–5) and is required for efficient virus growth (6, 7). The specificity of genes trans-activated by tat protein is conferred by the TAR (trans-activation-response) element. Several properties of TAR element distinguish it from typical transcription factor-binding sites: (*i*) It is located immediately downstream of the site of transcription initiation by RNA polymerase II (8–11). (*ii*) It is orientation dependent; inversion of TAR element so that the complementary strand is transcribed into RNA eliminates its activity (12). (*iii*) Its activity does not require specific sequences upstream of the site of initiation. (*iv*) TAR element RNA sequences exhibit extensive intramolecular base pairings and can fold into a hairpin structure *in vitro* (9). (*v*) Introducing mutations that destabilize the predicted hairpin structure causes loss of the ability of the TAR element to respond to tat protein *in vivo* (11, 13). Introducing compensatory mutations that restore the stem-loop structure restores the ability to respond to tat protein (13). Recently, it has been directly demonstrated that the formation of the TAR stem loop is necessary for tat protein-mediated trans-activation (14).

There has been considerable difficulty in demonstrating a direct and specific interaction between tat protein and TAR element RNA (ref. 15 and R.A.M., unpublished work). When binding of tat protein to TAR element RNA was seen, the binding was not affected by mutations in the TAR element loop sequences (16). *In vivo*, it has been demonstrated that the sequence content of the TAR loop is critical for tat protein trans-activation (11, 13, 17, 18). These results indicate that cellular factors may be responsible for much of the specific recognition of the TAR element RNA in tat protein trans-activation. Here we report the identification and partial purification of a nuclear protein that exhibits high-affinity, sequence-specific binding to the TAR element RNA.

MATERIALS AND METHODS

Plasmid Constructs and the Labeling of RNAs. The plasmid pBS-αTAR was constructed by ligation of the 99-base-pair (bp), *Eco*RI–*Hin*dIII fragment of pSP-2 (provided by M. Martin of the National Institutes of Health, Bethesda, MD; this fragment corresponds to the *Pvu* II [436]–*Hin*dIII [531] fragment of pNL4-3 of ref. 19 and GenBank HIVNL43/M19921) to the 3419-bp *Eco*RI–*Hin*cII vector fragment of pBS M13(−) (Stratagene). The plasmid pT7TAR was constructed from pBS-αTAR by using a 37-mer oligonucleotide containing the sequence of the T7 promoter joined to the HIV genomic transcript sequences +1 to +20 (numbering is relative to the HIV transcript start): 5′-TGGTCTAACCAGAGAGACCC-TATAGTGAGTCGTATT-3′. pT7TAR, contains the T7 promoter directly ligated to the HIV sequences from +1 to +82.

pT7Δ+35/+38 was produced by cutting pT7TAR with *Sac* I, removing the resulting 4-base 3′ overhang with Klenow fragment, and re-ligating. pT7Δ+44/+77 and pT7Δ+45/+77 were constructed by ligating the *Bgl* II–*Nru* I fragments of pΔ+44/+77 and pΔ+45/+77 (10) containing the HIV TAR sequences with the *Bgl* II–*Hin*cII fragment of pT7TAR. pT7TAR(G/+29,C/+36), pT7TAR(U/+34), pT7TAR(G/+29), and pT7TAR(+31/+34) were constructed by replacing the 62-bp *Bgl* II–*Hin*dIII fragment of pT7TAR with the homologous *Bgl* II–*Hin*dIII fragments from pHIVCAT8, pHIVCAT5, pHIVCAT7 (13), and pJGFCAT18(+31/+34) (18). The final sequence of all constructs was verified by dideoxynucleotide chain-termination sequencing.

RNAs were synthesized from the DNA templates by using T7 or T3 RNA polymerase as described (20, 21). RNAs for direct UV cross-linking were labeled at 6300 Ci/mmol (1 Ci = 37 GBq) with [α-^{32}P]ATP, [α-^{32}P]UTP or [α-^{32}P]GTP. RNAs for competitions were labeled at 10,000-fold lower specific activity.

***In Vitro* Assays.** HeLa cell nuclear extracts were prepared by using the procedure of Dignam *et al.* (22). For RNA–protein binding in the HeLa nuclear extract, the conditions used were similar to those used for splicing *in vitro*: 20 mM Hepes·KOH (pH 7.9)/62 mM KCl/2 mM $MgCl_2$/150 μM dithiothreitol/6% (vol/vol) glycerol containing labeled RNA (1×10^6 cpm, 2 ng) and 7.5 to 45 μg of HeLa nuclear extract protein, in a 25-μl reaction volume. The mixture was incu-

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Abbreviations: HIV-1, human immunodeficiency virus type 1; TAR element, trans-activation-response element.

‡To whom reprint requests should be addressed.

bated at 30°C for 10 min and transferred to ice; heparin (Sigma) was then added to a final concentration of 5 mg/ml. After further incubation for 5 min on ice, the samples were processed for UV cross-linking as described below.

For column fraction assays and competitions, the reaction volume was decreased to 12.5 μl, the incubation at 30°C was eliminated, and heparin at 20 μg/ml was added at the start of the 0°C incubation.

UV Cross-Linking Assays. The reaction mixtures were transferred to ice and irradiated with a UV light [maximum intensity at 254 nm of 10 mW/min measured at the surface of the lamp, model UVG-54 from UVP (San Gabriel, CA)] at 4.5 cm from the surface of the lamp for 10 min (23, 24). The samples were diluted 1:1 (vol/vol) with water, followed by addition of RNase A to 1 mg/ml. After incubation at 37°C for 15 min, SDS/PAGE sample buffer was added, and the incubation was continued at 65°C for 5 min. The cross-linked proteins were then resolved on discontinuous 12.5% SDS/polyacrylamide gels (0.75 mm thickness) (25).

Purification of the p68 Cross-Linked Product. Fifteen milliliters of HeLa nuclear extract (202 mg of total protein) in buffer D/100 mM KCl [20 mM Hepes·KOH, pH 7.9/200 μM EDTA/0.5 mM dithiothreitol/20% (vol/vol) glycerol/100 mM KCl] was diluted to a final concentration of 50 mM KCl by addition of an equal volume of buffer D/0 mM KCl. This material was clarified by centrifugation at 15,000 rpm for 20 min at 4°C in a Sorvall SS-34 rotor. The supernatant was loaded on a DEAE-Sephacel (Pharmacia) matrix column (cross-sectional area, 4.9 cm^2; height, 4 cm; vol, 19.6 cm^3) at a flow rate of 10 ml/hr·cm^2. The matrix was washed with 60 ml of buffer D/50 mM KCl at the same flow rate. The bound p68 was eluted from the matrix by washing the column with 50 ml of buffer D/150 mM KCl. Fractions from the load, flowthrough, 50 mM KCl wash, and 150 mM KCl wash were collected and assayed for RNA-binding proteins using the UV cross-linking assay, for total protein using the Bio-Rad assay and by monitoring the absorbance at 280 nm, and for conductivity. Twenty-two and one-half milliliters of the pooled, 150 mM KCl protein peak fractions (42 mg of total protein) were diluted 1:1 (vol/vol) with buffer D/0 mM KCl and were loaded on a heparin-Sepharose Cl-6B (Pharmacia) matrix column (area, 0.79 cm^2; height, 2.5 cm; vol, 2.0 cm^3) at a flow rate of 8 ml/hr·cm^2. The matrix was washed with 7 ml of buffer D/50 mM KCl and developed with a 12-ml gradient of KCl concentration from 50 mM to 500 mM KCl in buffer D at the same flow rate. The load, flowthrough, wash, and gradient fractions (0.5 ml) were assayed as above.

RESULTS

A Nuclear Protein in HeLa Cells Specifically Binds to the HIV-1 TAR Element RNA. Cellular proteins bound to the TAR element RNA were detected by using a UV cross-linking protocol (26). The TAR element RNAs used in the *in vitro* binding studies maintain the *in vivo* positioning of the TAR element relative to the cap site (Fig. 1*A*). By minimizing non-HIV derived sequences, background UV cross-linking and the potential for the formation of nonnative alternative RNA structures were reduced.

In vitro synthesized TAR element RNA and TAR element RNA mutants were incubated with nuclear extracts, and proteins were cross-linked to RNA by irradiation at 254 nm. After digestion of the RNA by nuclease, individual RNA–protein adducts were resolved by SDS/PAGE (Fig. 2). Many proteins cross-linked to the TAR element RNA in the absence of competitor (Fig. 2*A*, lane 4). Heparin was added as a nonspecific competitor for RNA-binding proteins. As the concentration of heparin was increased, a protein of 68 kDa became the predominant cross-linked product (lanes 1 and 2). On higher resolution gels, the UV cross-linked product at 68 kDa was resolved into a doublet. No difference in the specificity of cross-linking between these two bands has been detected. The proteins giving rise to the bands of the doublet

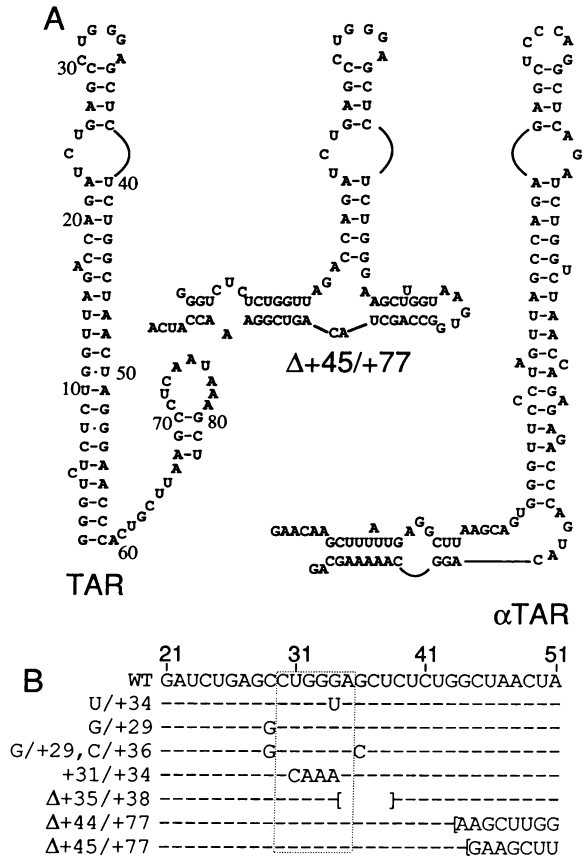

FIG. 1. Computer-predicted secondary structure of TAR element and TAR element RNA mutants. The method used for determining the structure is that of Zuker and coworkers (27). (*A*) Entire sequence of the RNAs synthesized *in vitro*. Free energies of folding for the structures (in kcal/mol; 1 cal = 4.184 J) are as follows: TAR element, −41.7; Δ+45/+77, −18.5; αTAR, −44.2. The Δ+45/+77 sequence shown is not the structure with lowest predicted free energy. The predicted lowest free energy structure (−22.2 kcal/mol) has the TAR loop sequences paired with nucleotides 11–6. (*B*) Sequence of other TAR mutants. The loop sequences of the TAR element are boxed.

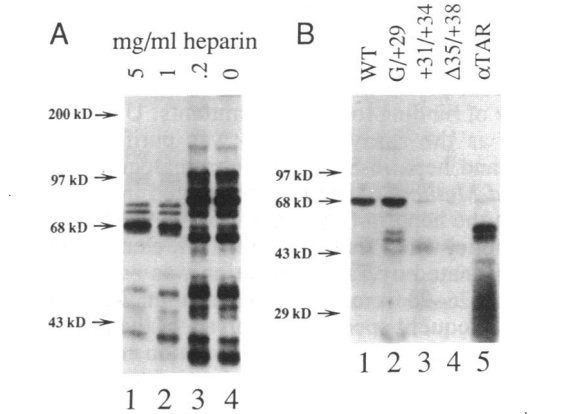

FIG. 2. UV cross-linking of TAR element RNA with HeLa nuclear extract. In all experiments, ≈10^6 cpm of [^{32}P]ATP-labeled RNA was incubated with 2 μl (30 μg) of HeLa nuclear extract in a 25-μl reaction volume. (*A*) Heparin titration. Cross-linking was performed in the presence of heparin at 0–5 mg/ml (Sigma). (*B*) Cross-linking of TAR elements and TAR mutants to HeLa nuclear extract. kD, kDa.

can be chromatographically separated on DEAE-Sepharose, indicating that they are distinct, independently binding polypeptides. The predominant cross-linked bands detected in the absence of heparin did not bind TAR element RNA with biological specificity, as judged by UV cross-linking to TAR mutants (data not shown).

Generation of distinct bands depended on the addition of HeLa cell nuclear extract and UV irradiation. Cross-linked products are protein–RNA adducts as shown by protease and nuclease digestion studies (data not shown). The binding of the p68 protein was not affected by the addition of ATP to the binding reaction. Binding was also unaffected by the incorporation of cap analogue (7-methylguanosine triphosphoguanosine) in the TAR element RNA. Binding was equally efficient under $MgCl_2$ concentrations from 1–16 mM and under KCl concentrations up to 200 mM. The 68-kDa protein cross-linked efficiently when the RNA was labeled with either [α-^{32}P]ATP, [α-^{32}P]UTP, or [α-^{32}P]GTP; [α-^{32}P]CTP was not tested (data not shown). Pure tat protein (15) up to a concentration of 10 μM was added to nuclear extract cross-linking reactions, causing no change in the pattern of cross-linked proteins detected. Purified tat protein did not UV cross-link to the TAR element RNA under these standard conditions (data not shown).

In initial tests of the specificity of UV cross-linking, wild-type TAR element RNA, but not TAR element RNA truncated at position +27, an adenovirus pre-mRNA, or unrelated pBlueScribe sequences, efficiently labeled a 68-kDa protein. To examine more closely the sequence requirements for the TAR element RNA–p68 interaction, we next tested by direct cross-linking minor TAR element RNA sequence mutants (Fig. 2*B*). The latter comparison indicated that of the proteins labeled in the UV cross-linking assay, only the 68-kDa protein showed specificity for the wild-type TAR sequence; the other bands of cross-linked polypeptides were also generated with different mutant RNAs (Fig. 2*B*, lanes 1–5). Very little cross-linked protein was observed with either the +31/+34 or Δ+35/+38 mutants, both of which are negative for ability to be trans-activated by tat protein (10, 18). G/+29 creates a G·G bp at the top of the TAR stem and exhibited diminished tat response *in vivo* (13). At the RNA concentration used in the experiment shown in Fig. 2, this mutant cross-linked efficiently (lane 2). When the experiment was repeated at lower RNA concentration, G/+29 was observed to have a slightly diminished direct cross-linking efficiency (data not shown) and also a lower competition efficiency (see Fig. 5). Anti-sense TAR element RNA generated little direct cross-linking to p68.

Purification of the 68-kDa TAR Element Binding Protein. The 68-kDa TAR element binding protein p68 was partially purified from HeLa nuclear extract to better quantify the specificity of binding to the TAR mutants. UV cross-linking was used as the assay, and p68 was purified by DEAE-Sephacel and heparin-Sepharose chromatography (see *Materials and Methods*). The material eluted in the peak fractions from the heparin-Sepharose column yielded one cross-linked product when analyzed with TAR element RNA (Fig. 3). The estimated overall purification was ≈10 fold.

Fractions 26–28 from the heparin column were pooled and used in subsequent specificity analysis of the p68 binding to TAR element. To estimate the dissociation constant for p68, a saturation binding curve was determined (Fig. 4). UV cross-linking to p68 was saturated at concentrations of TAR element RNA < 10 nM and reached half saturation at ≈2 nM. Because the concentration of p68 in the pooled column fractions was unknown, 2 nM would be the upper limit on the dissociation constant for p68.

Competition Analysis. Competition experiments were performed to quantify the differences in p68 direct cross-linking efficiency to the mutant RNAs (Fig. 5). The competition

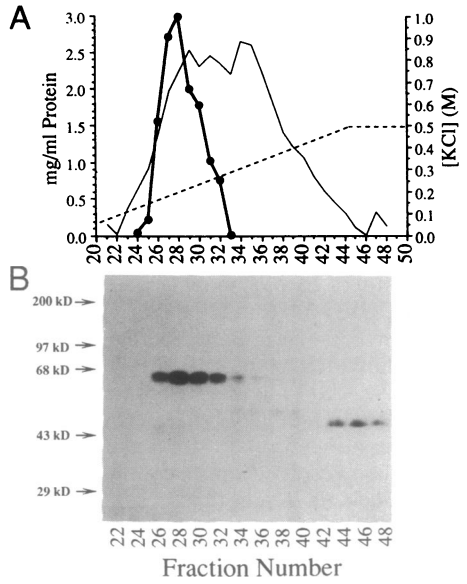

FIG. 3. Heparin-Sepharose chromatography of the 68-kDa TAR element binding protein. (*A*) Protein–activity profile of the KCl gradient of heparin-Sepharose chromatography. ●, Activity; —, mg/ml of protein; ---, [KCl]. (*B*) Two microliters of every other gradient fraction was assayed by cross-linking to wild-type TAR element RNA. kD, kDa.

efficiencies are a measure of the relative binding affinities of the RNAs and are not affected by possible differences in their photochemical reactivities. In all experiments, a constant concentration of ^{32}P-labeled wild-type TAR element RNA was premixed with increasing amounts of competitor RNA before adding purified p68 fraction.

Wild-type TAR element RNA was a more efficient competitor than either double-stranded or single-stranded RNA (Fig. 5*A*). The apparent affinity for double-stranded RNA was greater than that of single-stranded RNA. Competition by wild-type TAR element was also more efficient than either the Δ+44/+77 or Δ+45/+77 RNAs (Fig. 5*B*). These mutants contain 3′ deletions of the TAR stem structure but differ in the extent of the deletion by only 1 nucleotide (see Fig. 1*B*). This pair of TAR mutants defines the 3′ boundary of the TAR element necessary for tat response. When tested for their ability to respond to the presence of tat protein in a cotransfection assay in COS cells, the Δ+45/+77 mutant retained

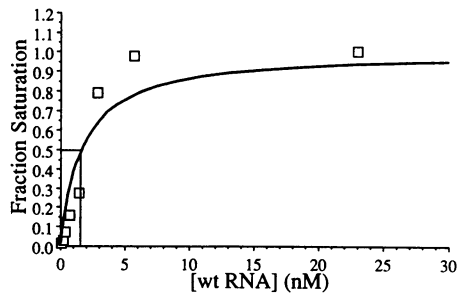

FIG. 4. Saturation binding curve of the 68-kDa TAR binding protein. One and one-half microliters of pooled heparin-Sepharose column fractions 26–28 was cross-linked to increasing amounts of ^{32}P-labeled TAR element RNA. Fraction saturation is defined here as the ratio of 68-kDa cross-linked product at each concentration to the maximum cross-link signal and is a measure of the fractional saturation of binding sites on the protein. The curve drawn is determined by the best fit of the equation: fraction saturation = [RNA]/(K_d + [RNA]). The calculated dissociation constant for the line is 1.6×10^{-9} M. The point of 50% fractional saturation is indicated.

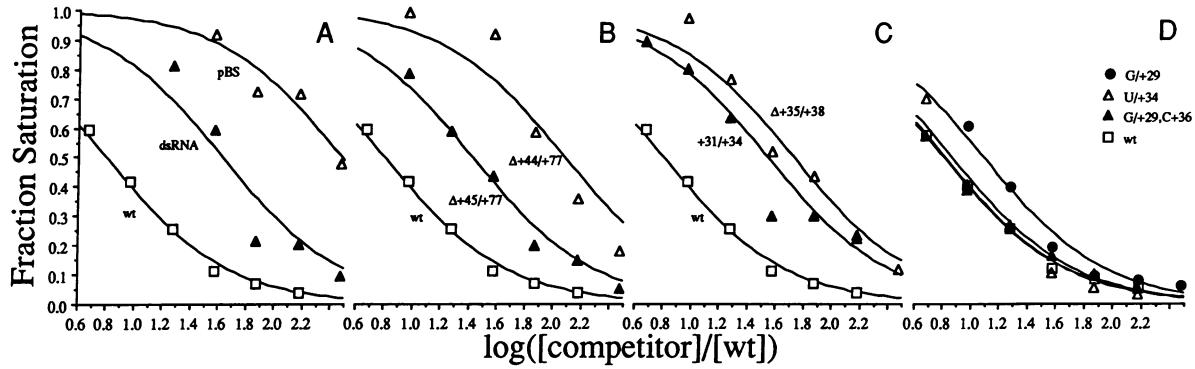

FIG. 5. Competition analysis of TAR-binding protein specificity. TAR element RNA (specific activity, 6300 Ci/mmol, 10^6 cpm) was first mixed with various amounts of competitor RNAs, incubated with 1.5 μl of pooled heparin-Sepharose fractions 26–28, and analyzed by UV cross-linking. Fraction saturation is defined here as the amount of cross-linked product produced in the presence of competitor RNA normalized to the amount of cross-linked product in the absence of any added competitor RNA. This is a measure of the fraction of RNA-binding sites on the protein occupied by the wild-type TAR element RNA. (*A*) □, Wild-type TAR; ▲, double-stranded RNA; △, pBS. pBS is the T7 polymerase produced, *Xho* I runoff of pBlueScript SK(−) (Stratagene), and double-stranded RNA is the pBS transcript hybridized to the T3 polymerase transcript of the same plasmid linearized with *Bam*HI. (*B*) □, Wild-type TAR element; ▲, Δ+45/+77; △, Δ+44/+77. (*C*) □, Wild-type TAR element; ▲, +31/+34; △, Δ+35/+38. (*D*) □, Wild-type TAR element; ▲, G/+29,C/+36; △, U/+34; ●, G/+29. Concentration ratio graphed for *A* is the base ratio of the RNAs; concentration ratio graphed for *B–D* is the molar concentration ratio. The curves drawn are the best fit to the experimental data of the function: fraction saturation = $[1 + (K_d^{wt}/K_d^{comp})([comp]/[wt])]^{-1}$. wt, wild type; comp, competitor RNA.

40% of wild-type trans-activation activity, but the Δ+44/+77 mutant was nonresponsive (28). Similarly, the Δ+45/+77 RNA competed more effectively than the Δ+44/+77 RNA in our binding assay. The Δ+44/+77 RNA competed only slightly more effectively than the control pBS RNA (Fig. 5*B* and Table 1). These mutants should only form part of the TAR stem; the Δ+45/+77 mutant has a predicted free energy of folding of −22 kcal/mol, as opposed to −42 kcal/mol for the wild-type TAR sequence. It is important to note that the Δ+45/+77 mutant retained substantial binding affinity even though more than half of the TAR stem was deleted.

TAR element RNA also competed more efficiently than either +31/+34 or Δ+35/+38 RNAs (Fig. 5*C*). The +31/+34 RNA contains nucleotide substitutions only in the single-stranded region of the TAR element loop (Fig. 1*B*) and has the same predicted free energy of folding as the wild-type TAR element RNA; yet it competed at an efficiency no greater than double-stranded RNA (Table 1). In the Δ+35/+38 RNA only 4 bases of TAR sequence are deleted; it has a predicted free energy of folding of −41.7 kcal/mol and a predicted 22 bp of the TAR stem remaining. The Δ+35/+38 RNA competed no better than double-stranded RNA for p68 binding. Thus, p68 exhibits sequence-specific binding for the loop sequences in TAR element RNA.

Table 1. Relative competition efficiencies of TAR mutants

RNA	Relative competition efficiency
wt TAR	1.0
G/+29,C/+36	1.0
U/+34	0.87
G/+29	0.52
Δ+45/+77	0.24
+31/+34	0.19
dsRNA	0.15
Δ+35/+38	0.12
Δ+44/+77	0.053
pBS	0.021
Yeast tRNA	0.017
Total *E. coli* RNA	<0.01

The relative competition efficiency is defined as the ratio of the dissociation binding constant for the competitor, as determined by the best-fit curve shown in Fig. 5, to the dissociation binding constant for the wild-type (wt) TAR element RNA, as determined by the wild-type TAR element RNA competition curve in Fig. 5. The competition curves for yeast tRNA and total *E. coli* tRNA were determined in the same manner as pBS (data not shown).

G/+29 RNA has a single base change that introduces a G·G mismatch at the top of the TAR stem (see Fig. 1). G/+29,C/+36 regenerates a C·G base pair at this position for the G·C base pair found in the wild-type TAR element structure. In the study of Feng and Holland (13), the G/+29 mutation had a decreased ability to respond to tat protein *in vivo*, whereas response of the G/+29,C/+36 mutant was indistinguishable from wild-type TAR element. G/+29 RNA yielded a slight, but significant, decrease in p68 binding affinity, as measured by competition efficiency, whereas the competition efficiency of the G/+29,C/+36 RNA was indistinguishable from wild-type TAR element (Fig. 5*D* and Table 1).

U/+34 RNA contains a single-base change in the loop sequence of the TAR element RNA (Fig. 1*B*). Under the conditions used for *in vitro* binding, the competition efficiency of the U/+34 RNA was indistinguishable from wild-type TAR element RNA. This mutant has tested as showing 17% of wild-type response to tat protein *in vivo* (17).

DISCUSSION

We have identified and partially purified a protein from HeLa nuclei that exhibits high-affinity, sequence-specific binding to the TAR element RNA of HIV-1. The potential biological importance of this binding emerged from a strong correlation between the p68 binding affinity for mutants of TAR element RNA and the reported ability of these mutants to support tat trans-activation *in vivo*. Recognition of the nascent TAR element RNA by this cellular protein may account for the observed sequence requirements for tat action in mammalian cells.

Binding of p68 to the TAR element was detected by UV cross-linking of labeled RNA to proteins in a nuclear extract of HeLa cells. The specificity of binding was initially determined by comparing the relative efficiencies of direct UV cross-linking of the wild-type TAR element RNA and TAR element RNA mutants. After partial purification of the protein, the specificity of binding was additionally tested by comparing the efficiency of competitions of the various RNAs for formation of the cross-linked product.

Most significantly, the binding of p68 to TAR element RNA mutants closely paralleled the ability of those mutants to support tat transactivation. Sequence alterations restricted to the TAR loop, which had no effect on the predicted secondary structure of the RNA, greatly diminished p68 binding. In addition, 3′ terminal deletions demonstrated that the extent of TAR element RNA necessary for p68 binding was the same as that necessary for tat response (10). When the loop length was increased two nucleotides by creating a mismatch at its base, p68 binding was slightly diminished. This change eliminated the ability to respond to tat trans-activation (13). When the loop length, but not the sequence of the final stem base-pair, was restored, p68 binding was restored to wild-type levels. This change also restored the ability to respond to tat protein (13).

In only one case, that of a single-base change at position +34 in the TAR loop, did an RNA that showed decreased ability to respond to tat protein show no decrease in binding to p68. This RNA, when tested *in vivo*, exhibited 17% of wild-type trans-activation (17). This lack of correlation may reflect the conditions used for testing the *in vitro* competition efficiency, as these conditions were optimized for sensitivity of detection of the interaction of p68 and TAR element RNA and were not optimized for selectivity. The intracellular environment for a potential p68–TAR element RNA interaction is probably quite different than that in the binding reaction containing the partially purified p68 protein.

The 68-kDa, double-stranded RNA-dependent protein kinase (dsI) can interact with the TAR element RNA (29, 30). Despite this similarity, the p68 cross-linked protein analyzed in this study is most likely not dsI. Alterations of sequences at the base of the TAR stem eliminates TAR element activation of dsI (30), whereas deletion of over half of the TAR stem in the Δ+45/+77 RNA did not eliminate p68 binding. dsI is predominantly a cytoplasmic protein, whereas the p68 protein was exclusively detected in nuclear extracts. Furthermore, dsI activity was not detected in the HeLa nuclear extracts used in this study but was easily detected in S-100 cytoplasmic preparations from the same cells (data not shown). Finally, antiserum to mouse dsI (31) did not immunoprecipitate the p68 cross-linked product, whereas the same antiserum could immunoprecipitate dsI derived from HeLa S-100 preparations (data not shown).

It has been reported that HeLa proteins interact with the TAR element RNA (32, 33). Using a gel-mobility retardation/UV cross-linking assay, Gatignol *et al.* (32) identified three proteins of approximate molecular masses of 100, 62, and 46 kDa that showed TAR element RNA-binding affinity. When HeLa nuclear extract was analyzed for UV cross-linking to the TAR element RNA without heparin (Fig. 2*A*, lane 4), among the prominent bands detected were those of ≈100-, 62-, and 46-kDa. It is unlikely that p68 corresponds to one of these proteins, as the addition of heparin to the standard assay reduced cross-linking of these proteins permitting detection of p68.

We had proposed that trans-activation by tat protein involves recognition of the TAR element in a role similar to an enhancer of transcription (2). For example, proteins binding the newly synthesized TAR element RNA would stimulate initiation of transcription by polymerase at the immediately upstream promoter. Tat protein could stimulate this process by modifying the activity of a cellular protein binding to TAR element or by directly binding the TAR element. We suggest, on the basis of the specificity of interaction between p68 and TAR element RNA, that this cellular protein moderates TAR activity *in vivo*.

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