Two distinct nuclear transcription factors recognize loop and bulge residues of the HIV-1 TAR RNA hairpin

Christian T. Sheline, Lawrence H. Milocco, and Katherine A. Jones

The Salk Institute, La Jolla, California 92037 USA

Transcriptional activation by the HIV-1 Tat protein requires specific residues in the hexanucleotide loop and trinucleotide bulge of the TAR RNA stem–loop structure found in the 5’-untranslated leader of all viral transcripts. Tat directly contacts residue U22 in the bulge and is thought to act in concert with cellular factors bound to the loop. We find that HeLa nuclear extracts contain two specific TAR RNA-binding proteins, designated TRP-1 and TRP-2, which compete for binding to the upper portion of the TAR hairpin. Analysis of point mutants in TAR RNA reveals that TRP-1 contacts residues in the loop that are important for trans-activation, whereas TRP-2 contacts the bulge, including the same residue (U22) that is required for the Tat–TAR interaction. Glycerol gradient sedimentation and UV cross-linking experiments indicate that TRP-1 is a large heteromeric complex containing a 185-kD RNA-binding protein, whereas TRP-2 activity derives from a family of 110- to 70-kD proteins. Interestingly, both TRP-1 and TRP-2 promote TAR-dependent transcription in vitro in the presence of Tat, although mixing experiments indicate that each of the three proteins must bind independently to TAR RNA. These findings suggest that the TAR element is recognized by two different nuclear RNA-binding proteins that affect transcriptional regulation by Tat.

[Key Words: HIV-1 Tat protein; TAR RNA; transcriptional activation]

Received September 16, 1991; revised version accepted October 23, 1991.

The HIV-1 regulatory protein, Tat, activates transcription through a stable RNA hairpin [trans-activating region (TAR)] that forms in the 5’-untranslated leader of nascent viral transcripts. Although the TAR element resembles an enhancer in the RNA [Sharp and Marciniak 1989], it cannot function from distal downstream locations but, rather, must be juxtaposed to the viral promoter [Muesing et al. 1987; Selby et al. 1989]. Nuclear run off experiments have shown that Tat functions primarily to promote efficient elongation of transcription in vivo and also enhances the RNA initiation rate [Kao et al. 1987; Laspia et al. 1989]. Purified Tat protein has been shown to activate elongation of HIV-1 transcription in a TAR RNA-dependent manner in vitro [Marciniak et al. 1990a]. An early proposal that Tat could act as an antiterminator of transcription was subsequently revised to accommodate the fact that a specific termination signal does not exist within TAR [Kao et al. 1987; Selby et al. 1989]. Instead, a gradual decline in the population of elongation-competent RNA polymerase II complexes is observed at increasing distances from the promoter [Laspia et al. 1989]. Analysis of promoter mutants in vivo and in vitro indicates that the elongation defect is not the result of pausing induced by the TAR RNA hairpin but, rather, originates at the promoter [Laspia et al. 1989; Selby et al. 1989; Toohey and Jones 1989] and may reflect the activity of deficient (nonprocessive) RNA polymerase II transcription complexes that are assembled in the absence of Tat.

Tat is targeted to the promoter through the TAR RNA element and can bind directly to the TAR hairpin in vitro [Dingwall et al. 1989, 1990; Roy et al. 1990a; Weeks et al. 1990]. Tat interacts with the bulge and the upper stem of TAR and directly contacts an unpaired residue [U22] in the bulge. In contrast, TAR loop sequences, which are critical for trans-activation in vivo [Feng and Holland 1988; Berkhout and Jeang 1989; Roy et al. 1990b], are not contacted by Tat and therefore appear to represent the target for a cellular sequence-specific RNA-binding protein. It has been proposed that the role of such a factor would be to stabilize or facilitate the interaction of Tat with TAR, as Tat has been reported to activate gene expression independently of TAR (and its associated cellular proteins) when it is fused to the RNA-binding domain of a heterologous protein [Selby and Peterlin 1990; Southgate et al. 1990]. The Tat–MS2 coat protein, however, was also found to be significantly less active on a heterologous promoter, in which the MS2 coat protein-binding sequence replaced TAR, than on the wild-type HIV-1–TAR promoter [Selby and Peterlin 1990]; consequently, these studies do not rule out the

1Corresponding author.
TAR RNA-binding transcription factors

The possibility that cellular TAR RNA-binding proteins play a different, more direct role in the trans-activation process.

In addition to its role in transcription, Tat can act post-transcriptionally in Xenopus oocytes to overcome a block to translation of RNAs that contain the TAR RNA hairpin (Braddock et al. 1990). This process appears to involve a covalent modification of TAR RNA that occurs in the nucleus in the presence of Tat, which is proposed to alleviate cytoplasmic translational repression (Braddock et al. 1991). Interestingly, mutations in the loop of TAR overcome the translational block and obviate the need for Tat (Braddock et al. 1990), indicating that translational inhibition might arise from the binding of a cellular protein to the loop of TAR. Although it is not clear whether the TAR loop-binding protein responsible for transcriptional activation in mammalian nuclei is the same protein as that responsible for translational repression in Xenopus, the relative absence of translational repression in mammalian systems (Rice and Matthews 1988) indicates that a fundamental property or subcellular distribution of the TAR loop-binding proteins must differ significantly between mammalian cells and the Xenopus oocyte. Nonetheless, the finding that this RNA modification step is blocked by a known inhibitor of transcription elongation (Braddock et al. 1991) indicates that some processes that occur in this system may also be important for transcriptional control by Tat in mammalian cells.

To better define the host cell proteins that assist Tat function, we have characterized two nuclear proteins that bind specifically to TAR RNA. One of these is a large heteromeric complex containing a 185-kD protein that directly contacts sequences in the loop of TAR. The second factor binds specifically to the TAR bulge, in a region directly overlapping the binding site for Tat. Analysis of the effects of these proteins on binding and transcription in the presence of Tat indicates that they are nuclear RNA-binding transcription factors required for the trans-activation process. The implications of these findings for the mechanism of transcriptional activation by Tat are discussed.

Results

Nuclear factors TRP-1 and TRP-2 bind independently to the upper portion of the TAR RNA hairpin

Preliminary experiments with gel mobility-shift experiments revealed that a large number of nuclear proteins in HeLa cell extracts can bind to the full-length TAR RNA hairpin (+1 to +80), consistent with previous reports (Gatignol et al. 1989, 1991; Gaynor et al. 1989); however, none of the most abundant of these complexes was found to interact specifically with the loop of TAR. To simplify the identification of relevant TAR-binding proteins, RNA fragments corresponding to the upper half of the hairpin (+17 to +43) were used. These shorter RNAs contain the minimal domain of TAR that is necessary, as well as sufficient, for significant transcriptional activation by Tat in vivo (Selby et al. 1989). The short TAR RNA fragments formed a single predominant ribonucleoprotein complex with HeLa nuclear proteins that was stable in the presence of high levels of total HeLa RNA as competitor (Fig. 1).

To better define the host cell proteins that assist Tat function, we have characterized two nuclear proteins that bind specifically to TAR RNA. One of these is a large heteromeric complex containing a 185-kD protein that directly contacts sequences in the loop of TAR. The second factor binds specifically to the TAR bulge, in a region directly overlapping the binding site for Tat. Analysis of the effects of these proteins on binding and transcription in the presence of Tat indicates that they are nuclear RNA-binding transcription factors required for the trans-activation process. The implications of these findings for the mechanism of transcriptional activation by Tat are discussed.

Figure 1. Biochemical separation of two HeLa nuclear TAR RNA-binding proteins, TRP-1 and TRP-2. (A) Resolution of TRP-1 and TRP-2 by FPLC-Mono Q column chromatography. Fractions were analyzed by mobility-shift experiments after incubation of TAR RNA fragments with 1 µg of each Mono Q fraction in the presence of HeLa competitor RNA (30 µg/ml). Lane IN represents the input fraction to the column [a heparin–agarose 0.2 M KCl step fraction]. The specific TRP-1 and TRP-2 complexes are indicated, and a nonspecific protein that generates a more rapidly migrating complex is also marked. (B) Competition between TRP-1 and TRP-2 for binding to TAR RNA. [Lane 1] TRP-1 alone, Mono Q-purified (0.9 µg); [lanes 2 and 9] TRP-2 alone (1.5 µg); [lanes 3–5] 1.5 µg of TRP-2 plus 0.3, 0.9, and 1.8 µg of TRP-1, respectively; [lanes 6–8] 0.9 µg of TRP-1 with 0.5, 1.5, and 3.0 µg of TRP-2, respectively.
complex was purified sequentially on phosphocellulose and heparin–agarose (HA) resins, and was found to split into two successive step fractions (0.2 and 0.4 M KCl) upon elution from the HA column. A more complete separation of these two RNA-binding protein complexes was obtained by chromatography of the HA 0.2 M KCl step fraction on an FPLC-Mono Q column (Fig. 1A). What appeared initially to be a single TAR RNA-binding species [IN input to the column] was found to resolve into early- and late-eluting protein activities, designated TRP-1 and TRP-2, respectively. Both TRP-1 and TRP-2 protein fractions bound specifically to TAR sequences in that they formed a stable complex with wild-type TAR sense RNA but not with anti-sense RNA transcripts (not shown). A distinct RNA-binding activity that eluted between the TRP-1 and TRP-2 factors [labeled nonspecific] could not discriminate between sense and anti-sense TAR RNAs and was not characterized further. The two specific TAR RNA-binding complexes could be resolved by extended electrophoresis, where TRP-1 remained as a single band and TRP-2 activity was resolved as a doublet, with the upper band migrating close to the position of TRP-1. The upper and lower bands of TRP-2 might arise from different multimerization states of the protein, or the upper band might be a complex between TRP-2 and a second, distinct protein. As indicated below, both the upper and lower forms of TRP-2 bind to TAR RNA with identical specificity and are distinct from TRP-1.

The mixing of the TRP-1 and TRP-2 proteins resulted in competition for binding to TAR, without the formation of a larger intermediary supershifted complex, which indicates that the binding of these two proteins to TAR RNA is mutually exclusive (Fig. 1B). Under these conditions, TRP-1 behaved as a more efficient competitor for TAR than TRP-2 when equivalent RNA-binding activities were assayed (Fig. 1B and other data not shown). Thus, it appears that these two cellular proteins cannot bind simultaneously to TAR RNA.

**TRP-1 and TRP-2 contact TAR loop and bulge sequences**

To define the specific residues in TAR that are contacted by these cellular proteins, base substitution mutants in TAR were tested for binding in vitro. Mutant RNAs containing either multiple base substitutions in the loop (e.g., +30/+33, +29/+34), or a single point mutation converting C → A at position +29, were not bound efficiently by TRP-1 (Fig. 2). These three TAR loop mutants have been shown to reduce trans-activation by Tat in vivo to levels of 2–11% of the wild-type promoter (Berkhout and Jeang 1989; Selby et al. 1989). In addition, single-base changes converting C → U at positions 31 or 33 in the loop reduced binding of TRP-1 by a factor of two- to threefold in vitro (data not shown), consistent with a report that substitution of all three of the guanine residues in the loop reduces Tat activity by 69% in vivo (Dingwall et al. 1990). Proper folding of the TAR RNA hairpin was also important, as mutations that disrupt base-pairing in the stem of the hairpin (mutants +14/+18 and +39/43) reduced binding of TRP-1 by a factor of five- to sevenfold, whereas a mutation at the base of the stem (+1/+5; Selby et al. 1989) had no effect (data not shown). Thus, the binding of TRP-1 to these RNAs correlates well with the TAR structural and loop sequence requirements for trans-activation by Tat in vivo.

In contrast, the binding of the TRP-2 protein was not affected by base changes in the loop, including substitution of all six loop residues (mutant +29/+34; Fig. 2), indicating that it recognizes a different region of the RNA. Binding of TRP-2 was reduced by a point mutation converting residue U22 in the bulge to A (mutant +22, (Fig. 1B), or by a triple-site mutation converting the bulge sequence from UCU to AGA (mutant +22/+24, not shown). Therefore, TRP-1 binds to the loop, whereas TRP-2 binds to the bulge. TRP-1 must recognize the loop without forming additional contacts with the bulge, as its binding was not affected by either of the two bulge mutations (Fig. 2; other data not shown). Because the specific contact sites for TRP-1 and TRP-2 do not overlap directly, the observed competition between these proteins for binding to TAR (Fig. 1B) may result from indirect occlusion of the bulge or loop sequences, or from an overlap in the regions of TAR that are important for nonspecific protein–RNA interactions.

**Efficient binding of TRP-1 to the loop requires the bulge of TAR**

To test whether the bulge was completely dispensable for the binding of TRP-1 to TAR, we analyzed two additional mutants in which the bulge was deleted or was moved to the 3' side of the stem. In contrast with the results obtained using base substitution mutants in the bulge, TRP-1 could not efficiently recognize either of these two new mutant TAR RNAs (Fig. 3). These findings suggest that TRP-1 may interact nonspecifically with the bulge, or its binding may require a particular conformation of the RNA that is imparted by the bulge. The importance of the bulge for the binding of TRP-1 was illustrated further by the fact that TRP-1 could not recognize a mutant RNA in which the bulge was retained intact, but the distance between the bulge and the loop was increased by three additional GC base pairs in the upper stem of the hairpin (Fig. 3). Thus, binding of TRP-1 required that specific loop residues be presented in the context of an RNA hairpin that contains a properly positioned bulge, although the sequence of the bulge was not important for binding. In addition, TRP-2 was unable to bind efficiently to these mutants (Fig. 3), indicating that it might form additional specific contacts with TAR RNA outside of the bulge.

Although the TAR bulge–loop extension mutant was not recognized by the cellular RNA-binding proteins, it was bound by Tat (see below). To determine the extent to which Tat can activate transcription in vivo in the absence of the two cellular TRP proteins, the TAR bulge–loop extension mutant was subcloned into a chloramphenicol acetyltransferase (CAT) expression vector and tested by transient expression assays in HeLa cells.
The TAR RNA-binding proteins TRP-1 and TRP-2 contact different regions of TAR RNA. Wild-type and mutant HIV-1 TAR RNAs were incubated with 1 μg of Mono Q-purified TRP-1 or TRP-2 in reactions containing either 60 μg (first and third lanes in each panel) or 20 μg of competitor HeLa RNA (second and fourth lanes in each panel), as described in Materials and methods. Reactions containing wild-type TAR RNA are shown in the first two lanes of each panel, and the remaining lanes contain the specific TAR loop or bulge mutant RNA that is indicated alongside each panel. The +30/+33 TAR loop mutation was tested in the context of a large (80 nucleotides) RNA fragment and is compared with the corresponding 80-nucleotide wild-type TAR RNA; all of the other mutants shown here and in Fig. 3 were assayed as short TAR RNA fragments (+17 to +43), and binding to each mutant was compared with the corresponding short wild-type TAR RNA fragment.

The mutant promoter was reduced in its ability to be trans-activated by Tat (8–10% of the wild-type HIV-1-TAR promoter), either when assayed in the context of the shorter TAR RNA or in the context of the full-length (+1 to +80) HIV-1-TAR promoter (data not shown). These data indicate further that Tat is only marginally functional in vivo in the absence of cellular TAR RNA-binding factors.

Molecular sizes of TRP-1 and TRP-2

A number of cellular TAR RNA-binding proteins ranging from 48 to 105 kD have been described previously (Gagnon et al. 1989, 1991; Gaynor et al. 1989), including a 68-kD protein shown by UV cross-linking experiments to bind the loop specifically (Marciniak et al. 1990b). To determine whether the TRP-1 and TRP-2 proteins are similar to any of these factors, we examined the size of the TRP proteins using different RNA-labeling techniques. A single large protein of 185 kD was detected in the TRP-1 protein fraction by UV cross-linking experiments carried out with labeled minimal TAR RNA fragments. This 185-kD protein was also detected when the gel-shifted TRP-1 complex was excised and eluted from the gel, and analyzed by SDS-PAGE and autoradiography (Fig. 4A). Interestingly, we found that the eluted 185-kD protein could be detected by its association with labeled TAR RNA regardless of whether or not the shifted complex was treated with UV light. Similar findings have been reported for unrelated cellular RNA-binding proteins (e.g., Malter 1989) and most likely reflect a very strong association between the protein and its RNA target. We were unable to detect TRP-1 activity by transferring the protein to nitrocellulose, renaturing and incubating with labeled TAR RNA (Northwestern blot), indicating that the 185-kD protein cannot be readily

Downloaded from genesdev.cshlp.org on June 24, 2017 - Published by Cold Spring Harbor Laboratory Press
70 kD) that were detected with the wild-type TAR RNA and were bound only inefficiently by the +22/+24 TAR bulge substitution mutant (Fig. 4B). Thus, TRP-1 and TRP-2 are large nuclear proteins and are distinct in size from TAR-binding proteins that have been described previously. Unlike the p185 component of TRP-1, each of the TRP-2 proteins appeared to be capable of binding independently to TAR RNA.

TRP-1 is a cell type-specific heteromeric protein complex

The inability of p185 to be detected by Northwestern blotting experiments indicated that it might bind to TAR RNA as a complex with distinct factors. This possibility was confirmed by glycerol gradient sedimentation (Fig. 5A). The input TRP-1 activity to the gradient (IN) was abolished by sedimentation, but could be restored by mixing the p185-containing fraction with a different fraction from the gradient (lanes 4–6), neither of which was capable of binding to TAR alone (lanes 2,3). The cofactor activity required for binding was sensitive to heat and N-ethylmaleimide, indicating that at least one other protein must interact with p185 to restore specific binding to the loop.

Transcriptional induction by Tat has been shown to be cell or species specific, or both, in that it functions poorly in Drosophila or yeast (Jeang et al. 1988) as well as in certain rodent and hamster cell lines (e.g., NIH-3T3 cells and CHO cells). To learn whether this restriction might be due to different levels in the TAR RNA-binding proteins, we fractionated nuclear extracts from 3T3 cells and analyzed TRP activity. These extracts contained a protein that binds to the bulge, similar to TRP-2 isolated from HeLa cells, but lacked significant levels of TRP-1, the protein that binds to the loop (Fig. 5B). Nuclear extracts from yeast (Saccharomyces cerevisiae) lacked both TRP-1 and TRP-2, and no specific complexes were formed with TAR RNA. However, a cofactor activity that could complement binding of p185 to the loop was present in yeast extracts (Fig. 5C). Therefore, the p185 component of the TRP-1 heteromeric complex seems to vary in a cell-specific (or species-specific) manner, and its absence could down-regulate Tat activity in some cells.

TRP-1 and TRP-2 factors compete with Tat for binding to TAR RNA in vitro

As mentioned above, some models have postulated a role for a cellular TAR loop-binding protein in stabilizing the binding of Tat to the bulge of TAR RNA (for review, see Cullen 1990). To examine this possibility, we expressed two different Tat proteins in bacteria: [1] full-length Tat(1–86), and [2] Tat(1–67)CP, which contains the first exon of Tat (the minimal region required for transcriptional activation in vivo and binding to RNA in vitro) joined to the RNA-binding domain of the MS2 bacteriophage coat protein gene (Selby and Peterlin 1990). Both

![Figure 3](#) Binding of TRP-1 and TRP-2 requires appropriate spacing between the loop and bulge residues of TAR RNA. Reactions contained the wild-type or mutant RNAs indicated above each lane, and the amounts of protein and competitor RNA are as described in the legend to Fig. 2. The Δ bulge and bulge-loop extension mutant RNA probes contain a band that migrates above the double-stranded RNA probe, which could be removed by heating and renaturing of the RNA; however, this treatment did not affect the binding of TRP-1 or TRP-2 (data not shown).
TAR RNA-binding transcription factors

Tat proteins can activate the HIV-1 long terminal repeat (LTR) promoter in vivo, and the Tat[1–67] protein also appears to be capable of inducing the expression of a chimeric promoter in which TAR sequences are replaced by the MS2 coat protein RNA-binding site (pHIVSACAT; Selby and Peterlin 1990). As shown in Figure 6, the bacterial Tat[1–67] protein (expressed in bacteria and purified by fractionation over HA resin) bound specifically to TAR in that it could recognize both wild-type TAR RNA and the +29 point mutant in the loop but did not bind to the +22 point mutation in the bulge. In addition, Tat bound to the TAR bulge-loop extension mutant (which introduces three GC base pairs in the upper stem) as efficiently as to the wild-type TAR RNA (Fig. 6). As summarized in Materials and methods, the conditions used here to monitor binding of Tat to TAR RNA were less stringent than those used to assay the nuclear TAR RNA-binding proteins; we were unable to detect the Tat–TAR complex using our standard conditions.

Mixing experiments were carried out to determine whether either of the TRP factors can bind to TAR in conjunction with Tat. At moderate levels of TRP-1, both the Tat and TRP-1 complexes were detected (Fig. 6A, lane 5). As TRP-1 levels were increased, the Tat–TAR complex disappeared, concomitant with a slight decrease in the mobility of the TRP-1 complex. The decreased mobility was not the result of the formation of a ternary complex in which TRP-1 and Tat are bound to the loop and bulge, respectively, because a similar complex could also be observed using a TAR bulge mutant to which Tat cannot bind (+22; data not shown). High levels of Tat protein competed very inefficiently for the binding of TRP-1 (Fig. 6A, lanes 9–12). As expected, TRP-2 also competed for the binding of Tat to the bulge of TAR (Fig. 6B). In the converse experiment, we were unable to compete TRP-2 by addition of high levels of Tat, and low levels of Tat actually seemed to stabilize the binding of TRP-2 (data not shown). At approximately equivalent levels of RNA-binding activities, TRP-2 appeared to be a more efficient competitor of Tat than TRP-1 (cf. lanes 6 and 16). The competition observed between TRP-1 and Tat could result from conformational changes in the structure of TAR that occur with binding of TRP-1, sterical inhibition, or nonspecific interactions between TRP-1 and the TAR bulge. Partially purified Tat[1–86] protein behaved identically to Tat[1–67] protein in RNA-binding specificity and ability to be competed by the TRP proteins (data not shown).

Upon further purification of the Tat[1–86] protein by gradient elution from an FPLC–Mono S resin, its RNA-binding activity was significantly diminished, possibly as a result of some oxidation of the protein or inactivation of the more purified Tat protein by the HeLa competitor RNA present in the binding reaction. As a consequence, this highly purified preparation of Tat[1–86] protein (Fig. 7A) was unable to compete for the binding of either TRP-1 or TRP-2 to TAR. In addition, low levels of the Tat protein (≤50 ng) did not affect the affinity or mobility of the TRP-1 complex (data not shown). As had been observed with the partially purified Tat protein, however, we found that addition of the Mono S-purified Tat[1–86] protein enhanced the binding of TRP-2 to TAR and promoted the formation of the more slowly migrating TRP-2U complex (Fig. 7B). As shown below, this more purified Tat[1–86] protein was fully capable of activating TAR-dependent transcription in vitro.

Transcriptional activation by Tat in vitro requires TAR and the nuclear TAR RNA-binding proteins

To assess the role of the cellular TAR RNA-binding proteins, we carried out in vitro transcription experiments in the presence and absence of Tat. Consistent with previous reports, transcription from the HIV-1 LTR was relatively insensitive to mutations in TAR (e.g., mutant +30/+33 in the loop or mutant +24/+27 in the bulge and upper stem) when assayed in the absence of Tat. Addition of the bacterially expressed Tat proteins, either Tat[1–67] or the full-length Tat[1–86] protein, activated transcription from the wild-type HIV-1–TAR promoter and did not affect transcription from the +30/+33 or +24/+27 TAR mutant promoters (Fig. 8). We consistently observed greater transcriptional activity...
with the Tat[1–86] preparation, which might be the result of its higher state of purity. Tat did not affect transcription from the human cytomegalovirus (HCMV) immediate-early (IE) promoter [the slight decrease in HCMV–IE transcription seen with the Tat[1–67]CP fraction might be nonspecific inhibition, owing to increased salt concentrations]. As also shown in Figure 8, the Tat proteins activated transcription from the wild-type HIV-2 promoter and did not affect transcription from an HIV-2 3’-deletion mutant that lacks TAR sequences downstream of +34 [HIV-2 ΔTAR]. In addition, neither protein affected transcription from pHIVSRCAT, in which HIV-1 sequences downstream of +1 are substituted with sequences specifying the RNA-binding site of the MS2 coat protein. Therefore, trans-activation by Tat in vitro is absolutely dependent on the presence of a functional TAR element.

To evaluate the effects of the cellular TAR RNA-binding proteins on transcriptional induction by Tat, it was first necessary to inhibit the activity of the endogeneous TRP-1 and TRP-2 proteins present in the nuclear extract. This was accomplished by incubation with a mass excess of synthetic wild-type TAR RNA to compete for binding of the cellular TRP factors. In the experiment shown in Figure 9, the Tat response was diminished in the presence of synthetic TAR transcripts and addition of TRP-1 enhanced transcription from the wild-type HIV-1 and HIV-2 promoters in the presence of Tat, whereas transcription of TAR mutant promoters was either unaffected or slightly diminished in the presence of the Tat or TRP-1 fractions, or both. We could not restore Tat-dependent transcription under these conditions by the addition of TRP-2 alone (i.e., without additional TRP-1), although we did observe a slight stimulation of Tat-mediated transcription with the TRP-2 protein fraction in the absence of competitor RNA (Fig. 9). These findings suggest that TRP-1 and TRP-2 function as RNA-binding transcription factors in the presence of Tat.

**Discussion**

In this study we have identified two distinct nuclear RNA-binding proteins that bind specifically to the minimal HIV-1 Tat trans-activation domain, located in the upper portion of the TAR RNA hairpin. Analysis of base substitution mutants in TAR revealed that TRP-1 specifically recognizes the loop, whereas TRP-2 contacts the bulge, including the same residue (U23) that is critical for the binding of Tat to TAR in vitro [Dingwall et al. 1989; Roy et al. 1990a; Weeks et al. 1990]. Although TRP-1 interacts specifically with the loop and its binding was unaffected by base substitutions in the bulge, it could not recognize TAR mutants in which the bulge was deleted or in which the distance between the bulge and the loop was increased by the addition of 3 bp in the upper stem. Thus, the TRP-1 complex might form nonspecific contacts with the bulge. Alternatively, as biophysical studies have shown that RNA bulges are important determinants of local structure and can provide a distinctive bend or kink to the RNA [Bhattacharyya et al. 1990], the bulge may confer a distinctive conformation to TAR that is necessary for the binding of TRP-1. Although the precise number of residues in the bulge varies among different HIV isolates, a corresponding alteration in the length of the upper stem conserves the distance between individual loop and bulge residues [Berkhout and Jeang 1989]. Our findings indicate that the conserved structure could be important for trans-activation in vivo as well as the specific binding of the nuclear TRP factors in vitro, although it is not as important for the Tat–TAR interaction [Fig. 6].

RNA-labeling experiments indicate that TRP-1 activity derives from a heteromeric protein complex containing a 185-kD RNA-binding protein, whereas TRP-2 act-
TAR RNA-binding transcription factors

Figure 6. (A) TRP-1 competes for binding of Tat to TAR RNA in vitro. Binding of Tat(1-67)CP (250 ng, where indicated) was analyzed using the GC bulge-loop extension mutant (lane 1), the +29 TAR loop mutant (lane 2), the +22 bulge mutant (lanes 3,13), or the wild-type TAR RNA (lanes 4-12,14-17) (Lanes 5-7) 250 ng of Tat plus 0.3, 0.6, or 1.8 µg of Mono Q-purified TRP-1, respectively; (lanes 8,9) 0.6 µg of TRP-1 without added Tat, (lanes 10-12) the effect of adding 50, 100, and 300 ng of Tat, respectively, to reactions containing 0.6 µg of TRP-1. (B) TRP-2 competes for binding of Tat to TAR RNA in vitro. (Lanes 15-17) The effect of adding 0.4, 0.8, or 2.4 µg of TRP-2 to reactions containing 250 ng of Tat (1-67)CP.

Figure 7. (A) SDS-PAGE analysis of Mono S-purified Tat(1-86) protein. Proteins were visualized by silver staining. Tat(1-86) protein is indicated with an arrow; [M] molecular size standards. (B) Mono S-purified Tat(1-86) enhances binding of TRP-2 in vitro. TRP-2 binding was monitored by gel mobility-shift assay, using the conditions described in Fig. 6. The first lane contains 1.8 µg of TRP-2 alone; the remaining lanes contain TRP-2 plus 10, 20, or 40 ng of Mono S-purified Tat(1-86), respectively. Arrows indicate the upper (U) and lower (L) forms of the TRP-2 complex. Additional bands are nonspecific RNA-binding proteins present in the TRP-2 preparation.

Activity derives from a family of 110- to 70-kD proteins. The relatively large sizes of these two TRP activities distinguish them from other cellular TAR RNA-binding proteins that have been described previously, including p68, a protein shown by UV cross-linking experiments to bind to the loop of TAR (Marciniak et al. 1990a,b). To date, we have not detected specific binding of a 68-kD protein to the loop of TAR, nor does the 185-kD TRP-1 protein appear to be processed or degraded to a 68-kD species; therefore, it will be important to compare the TAR RNA-binding specificity and affinity of the TRP-1 and p68 proteins directly in solution-binding experiments. A large heteromeric TAR loop-binding protein complex that appears to be identical to TRP-1 has been characterized recently by Gaynor and colleagues (Wu et al. 1991). The observation that TAR loop-binding activity requires the joint action of two distinct proteins introduces an additional level of complexity to the potential regulation of TRP-1 (and TAR) activity in the cell. We find that the p185 component of TRP-1 is absent (or present at relatively low levels) in NIH-3T3 cells and in yeast; thus, its absence could contribute to the low level of Tat activity in some cells. In contrast, the cofactor that stabilizes the binding of p185 to the loop appears to be a ubiquitously distributed activity. The absence of the 185-kD protein cannot account for the inactivity of Tat in all nonresponsive cell types, however, as our preliminary studies indicate that a factor similar to TRP-1 is present in nuclear extracts from CHO cells (C.T. Sheline, unpubl.).

Given the restricted expression of TRP-1 activity, it is interesting that TAR loop sequences have a dramatic effect on post-transcriptional regulation by Tat in Xenopus oocytes (Braddock et al. 1990). In this system, the TAR RNA hairpin inhibits HIV-1 mRNA translation in the cytoplasm, an effect that is abrogated by point mutants in the loop of TAR. Thus, if TRP-1 is the translational repressor in Xenopus, it may shuttle between the nucleus to the cytoplasm. Alternatively, translational repression might be carried out by a unique Xenopus TAR loop-binding protein that is localized in the cytoplasm of the oocyte.

To begin to understand the functional role of the nuclear TRP-1 and TRP-2 RNA-binding proteins, first we examined the effects of these factors on the binding of Tat to TAR RNA in vitro. The specific Tat–TAR interaction was detected only under relatively low-stringency...
Figure 8. TAR-dependent trans-activation of the HIV-1 and HIV-2 promoters by Tat in vitro. The transcriptional activity of 30 ng of bacterially expressed Tat[1–86] protein [the Mono S-purified protein shown in Fig. 7A] or 150 ng of Tat[1–67]CP protein [partially purified by fractionation on heparin–agarose resin] was evaluated with the addition to a Jurkat nuclear extract [Materials and methods]. Transcription was monitored by runoff assay using the following linear promoter templates: HIV-1 wild-type promoter [pLTR/CAT], or the +30/+33 or +24/+27 TAR mutants, each linearized with NcoI to generate a 630-nucleotide transcript; the HCMV promoter, linearized with AvaI to generate a 550-nucleotide transcript; and the wild-type HIV-2 promoter, pHIV-2/CAT or HIV-2 ΔTAR [a 3' deletion mutant to position +34], each linearized with NcoI to generate 724- and 584-nucleotide runoff transcripts, respectively.

binding conditions, and Tat was readily displaced from the RNA by the addition of either the TRP-1 or TRP-2 proteins. The displacement of Tat by TRP-1 may be the result of physical changes in the conformation of TAR RNA that occur with binding, or steric problems, whereas the competition between TRP-2 and Tat is likely to be more direct because both proteins contact the same residue in the bulge. We did not observe any indication that either of the cellular proteins functions by stabilizing the binding of Tat to the bulge. The only effect that was reproducibly observed in these experiments involved a stabilization by Tat of the binding of TRP-2 to TAR. Further experiments are required to determine whether this is a specific effect of Tat and whether the ability of Tat to enhance binding of TRP-2 to the bulge contributes to TAR-dependent transcriptional regulation. Nevertheless, this effect may be significant because a model in which Tat must bind directly to TAR to function might predict that TRP-2 should behave as a repressor [by virtue of competition with Tat], whereas the in vitro transcription experiments indicate that both the TRP-1 and TRP-2 proteins activate TAR-dependent transcription from the HIV-1 promoter in the presence of Tat.

The ability of TRP-1 to bind specifically to the loop of TAR and promote TAR-dependent transcription in the presence of Tat indicates that it will play a key role in trans-activation. In this regard, TRP-1 may act in a manner similar to the bacterial NusA protein, a transcription elongation factor that appears to become stably associated with RNA polymerase only when the λN antiterminator protein has recognized the nut boxB RNA hairpin [Batik et al. 1987]. The N protein bound to nut has been proposed to interact with the RNA polymerase-bound NusA factor through looping of the intervening mRNA [Whalen and Das 1990]. By analogy, TRP-1 might become dissociated from the RNA and attach to the RNA polymerase II elongation complex in a step promoted by the binding

Figure 9. TRP-1 and TRP-2 fractions assist Tat and TAR-dependent transcription in vitro. Reactions were carried out as described for Fig. 8, with the exception that the reactions in the first two panels contained 100 and 80 ng, respectively, of synthetic wild-type TAR RNA as competitor. Where indicated, reactions were supplemented with 30 ng of Mono S-purified Tat[1–86] or 1.8 μg of Mono Q-purified TRP-1 or TRP-2 fractions, or both. Promoter templates were as described in the legend to Fig. 8.
of TRP-2 or Tat to TAR RNA. Dissociation from the RNA could be required to permit either Tat or the cellular RNA-binding proteins to interact with the RNA polymerase II transcription elongation complex.

In considering specific models for trans-activation, a key question is whether Tat needs to bind directly to TAR RNA to activate transcription through the TAR element. Tat binds to TAR through a short arginine-rich basic domain, which is homologous to a motif found in the AN antiterminator protein, as well as ribosomal proteins that are thought to interact non- or semispecifically with RNA [Lazinski et al. 1989]. Although several reports have established a correlation between the ability of wild-type or mutant Tat proteins to bind to TAR RNA in vitro and activate the HIV-1 promoter in vivo, some of these studies are inherently difficult to interpret because the basic domain is also necessary for proper localization of the Tat protein in the nucleus/nucleolus. Moreover, it has been reported that the Tat basic domain can be substituted by the basic segment of the functionally unrelated Rev protein, or by other unrelated basic peptides that seem to require only one centralized arginine residue [Calnan et al. 1991]. In support of the hypothesis that binding of Tat to TAR is required for TAR-dependent transcription, Marciniak et al. [1990a] have shown that a peptide containing the Tat basic domain could weakly suppress Tat trans-activation in vitro, whereas an altered peptide that could not bind to TAR had no effect on transcriptional activity. However, high levels of these peptides might also interfere with the binding of TRP-1, as Tat and TRP-1 compete for binding to TAR in vitro (Fig. 6).

In support of a model in which Tat functions without binding directly to TAR RNA, a previous study found that a short Tat peptide [Tat 1–47], which lacks the basic domain, was as efficient as the wild-type Tat protein at activating HIV-1 transcription in a cell-free system [Jeyapaul et al. 1990]. These results indicate that specific binding of Tat to TAR RNA might not be essential for transcriptional activation through TAR. Similarly, a Tat protein lacking the basic region [Tat 1–48] was capable of activating translation of TAR-containing transcripts upon injection into the nucleus of Xenopus oocytes [Braddock et al. 1989]. These findings, taken together, suggest that at least some TAR-dependent transcriptional and post-transcriptional regulation can occur without direct binding of Tat to TAR RNA. It remains to be determined how Tat is recruited to the promoter by the TAR element under these conditions. Our preliminary experiments indicate that a mutant Tat protein containing a short in-frame deletion of the basic domain is relatively inactive in vitro [C. Sheline, unpubl.]; however, we have also noted that purification of the Tat[1–86] protein used in this study resulted in a considerable loss of RNA-binding activity without a corresponding decrease in transcriptional activity. The Tat- and TAR-dependent in vitro transcription system will be extremely useful in identifying the domains of Tat that are most critical for trans-activation and understanding how these regions of the protein affect the binding of Tat or the nuclear TRP factors to TAR RNA. At present, our findings suggest that TRP-1 plays a direct role in transcription, rather than an indirect role such as stabilizing the binding of Tat to TAR RNA. TRP-2 might assist transcription indirectly, for example, by displacing TRP-1 from TAR, or it might also act directly in the reaction.

In addition to its postulated role in transcription, the TRP-2 protein could also affect the post-transcriptional fate of TAR RNA. Recently, Sharmeen et al. [1991] have demonstrated that a nuclear enzyme, the "ds RNA unwindase," induces a covalent modification of TAR RNA (conversion of adenosine-26 in the upper stem to inosine) upon conjuction of Tat and TAR into Xenopus oocytes. In the absence of Tat, the TAR RNA appeared to be protected from modification by its association with nuclear RNA-binding proteins. A triple-base substitution in the bulge of TAR was sufficient to remove protection and expose the RNA to the ds RNA unwindase in the absence of Tat [Sharmeen et al. 1991]. Because we have shown that a comparable mutation prevents binding of TRP-2 but not TRP-1 (Fig. 2), it appears that a protein similar to TRP-2 protects TAR from modification by the unwindase in the nucleus of the oocyte. Because the unwindase is a ubiquitously distributed enzyme, TRP-2 might carry out a similar role in mammalian cells and this could, for example, serve to protect the integrity of viral RNA transcripts that are destined for packaging into virions.

In conclusion, our experiments indicate that two nuclear proteins bind to TAR and facilitate transcriptional regulation by Tat. These factors may therefore represent a new class of proteins that affect transcription through specific binding to RNA. It is intriguing to speculate that these TRP proteins might also affect transcription of cellular genes, and that transcriptional control by nuclear sequence-specific RNA-binding proteins is not a process that is uniquely restricted to the regulation of HIV-1 gene expression.

Materials and methods

Plasmids used to analyze wild-type and mutant TAR RNAs

Plasmids used for the production of short HIV-1 TAR RNAs (+17 to +43) were derived by subcloning an oligonucleotide containing the wild-type HIV-1 TAR sequence 5'-TATCTGACGCTTGAGCTCTCGA-3', or the appropriate mutant TAR sequence, into the EcoRI and HindIII sites of pGEM1. The identity of the mutant plasmids was confirmed by dideoxynucleotide sequencing. RNA was prepared from HindIII-digested DNA templates using T7 RNA polymerase (Promega), and labeled RNA transcripts were recovered after removal of free nucleotides with a P-10 microspin column and precipitation with 0.3 m sodium acetate and ethanol. The +30/ +33 loop mutant RNA was analyzed within the context of the full-length TAR RNA probe (+1 to +80) by digestion of a pGEM1 derivative of construct pLTR/CAT +30/+33 [Jones et al. 1988] with HindIII and transcription with T7 RNA polymerase, and was compared with an equivalent RNA transcript from a HindIII-digested pGEM-1 derivative of HIV-1 pLTR/CAT.

To test the efficiency of the TAR bulge-loop extension mu-
Sheline et al.

tant, HeLa SL-3 cells were transfected with 10 μg of the wild-type pHIV-1-CAT plasmid or the TAR bulge-loop extension mutant in the presence of 1 μg of pSV-Tat (Kao et al. 1987) or carrier DNA, using the DEAE/dextran/chloroquine protocol. CAT activity was determined for each of these in duplicate by a nonchromatographic assay (Sleigh 1986), which was normalized for reaction time and protein concentration.

TRP-1 and TRP-2 Protein Isolation

Nuclear extracts from HeLa SL-3 spinner cells [2 x 10^10 cells] were desalted using an 800-ml P-10 column, and passed through a 40-ml phosphocellulose column in TGED buffer [50 mM Tris-HCl (pH 8.2), 1 mM EDTA, 10% glycerol, 0.1 mM PMSE, 1 mM DTT] containing 100 mM KCl. The flowthrough fraction, which contained all of the specific TAR-binding activity, was then applied to a 35-ml HA column equilibrated in TM buffer [50 mM Tris-HCl (pH 7.9), 12.5 mM MgCl_2, 1 mM EDTA, 20% glycerol, 0.1 mM PMSE, 1 mM DTT] containing 0.1 mM KCl. The column was washed with five volumes of TM 0.1 M, and successive step elutions were obtained using TM 0.2 M and TM 0.4 M buffers.

The protein peaks were pooled, dialyzed against TGED buffer [50 mM Tris-HCl (pH 8.2), 1 mM EDTA, 10% glycerol, 1 mM DTT, 0.1 mM PMSE] containing 0.075 M KCl, and applied to an FPLC-Mono Q HR 5/5 column (Pharmacia). The protein was eluted with a salt gradient from 0.075 to 0.6 M KCl, and the resultant TRP-1 and TRP-2 fractions were tested directly for RNA-binding activity using gel mobility-shift experiments. To determine the levels of TRP proteins in murine NIH-3T3 cells, a nuclear extract from 3 x 10^8 cells was analyzed by the same procedure, using columns calibrated to one-tenth scale. For RNA-binding reactions 15 μl total) contained α-32P-labeled HIV-1 TAR RNA [10^5 cpm/reaction, 2 x 10^8 cpm/μg] and 1 μg of Mono Q-purified TRP-1 or TRP-2 in RNA-binding buffer [final concentration: 10 mM Tris-HCl (pH 7.9), 2 mM MgCl_2, 0.1 mM EDTA, 4% glycerol, and 50 mM KCl] with different amounts of total HeLa RNA as competitor, as indicated in each figure legend. The electroeluter was run at 120,000g for 30 min. The supernatant was dialyzed twice against HEMGN buffer containing 0.5 mM guanidine-HCl, then stepwise against HEMGN buffer containing 0.15 M guanidine-HCl, followed by a short centrifugation to remove any insoluble proteins. A portion of the lysate (2 ml) was then loaded onto a 2-ml HA column, washed extensively with TM 0.25 mM/10 mM DTT, and eluted with TM 0.5 mM/10 mM DTT. The Tat(1-86) protein used for transcription was then dialyzed against HEMGN buffer [lacking MgCl_2 and NP-40] and purified further by gradient elution from an FPLC-Mono S resin. This second purification step reduced the RNA-binding activity of the preparation approximately twofold but did not alter the transcriptional activity of the Tat protein. RNA mobility-shift reactions containing Tat [Fig. 6] were performed following the conditions described by Roy et al. (1990a), except that binding reactions were incubated at 25°C and contained 1 x 10^6 cpm of total HeLa RNA as competitor.

In vitro transcription assays

In vitro transcription reactions were prepared as described by Marciniak et al. (1990a), with the following modifications: (1) The preincubation step was omitted, (2) 70 μg of desalted Jurkat

SDS as the exterior well buffer. The electroeluter was run at 175 V at room temperature for 2 hr. The labeled material was collected from the cathode membrane trap, concentrated to 30 μl under vacuum, and heated for 10 min at 95°C in SDS-sample loading buffer before 6% SDS-PAGE. RNA blot analysis was performed on TRP-2 as described for the Southwestern DNA-blotting protocol [Waterman and Jones 1990], with the following exceptions: (1) All renaturation and washing steps were carried out in RNA-binding buffer [10 mM Tris-HCl (pH 7.9), 2 mM MgCl_2, 0.1 mM EDTA, 4% glycerol, and 50 mM KCl]; and (2) the denaturation step was carried out with RNA-binding buffer plus 6 M guanidine HCl. The prehybridization and hybridization buffers contained 0.02% BSA, 0.02% polyvinyl pyrrolidone (PVP), and 0.02% Ficoll in RNA-binding buffer, and the hybridization buffer contained 10 μg/ml of HeLa RNA and 50 μg/ml of poly[r] (r)-CNAs and 10° cpm/ml of the wild-type or +22/+24 mutant TAR RNA.

Preparation of HIV-1 Tat Protein

To express Tat in bacteria, the full-length open-reading frame of HIV-1 Tat was obtained by polymerase chain reaction (PCR) amplification from the vector pSVTat (Kao et al. 1987) as an Xhol–NotI fragment, and was inserted into a modified pGEMEX vector such that Tat was fused in-frame with the first 21 amino acids of the bacterial gene 10 protein. The Tat(1-67) gene (Selby and Peterlin 1990) was similarly isolated by PCR amplification and inserted into the modified pGEMEX vector as an Xhol–HindIII fragment. Expression of the Tat proteins was induced by incubating transformed pLysS cells [Studer et al. 1990] with 1 mM IPTG for 3 hr at 37°C. The cell pellet from a 400-ml culture was washed in PBS/MgCl_2 and resuspended in 5 ml of lysis buffer [50 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.1 mM KCl, 10 mM DTT] containing 0.1 mM PMSE, 2 mg/ml of benzamidine, 4 μg/ml of leupeptin, 10 μg/ml of aprontin and, 1 μg/ml of pepstatin A. The cells were lysed by two successive rounds of freezing and thawing, and the lysate was centrifuged for 20 min at 15,000 g at 2°C. The pellet was extracted with 8 ml of 4 M guanidine-HCl in HEMGN buffer [25 mM HEPEs (pH 7.6), 12.5 mM MgCl_2, 0.1 mM EDTA, 0.1% NP-40, 10% glycerol, 10 mM DTT, and protease inhibitors as listed above] at 4°C for 45 min on a rotator, and then diluted twofold with HEMGN buffer lacking guanidine-HCl. The pellet was removed by centrifugation at 120,000 g for 30 min. The supernatant was dialyzed twice against HEMGN buffer containing 0.5 mM guanidine-HCl, then stepwise against HEMGN buffer containing 0.15 M guanidine-HCl, followed by a short centrifugation to remove any insoluble proteins. RNA blotting protocol of the bacterial gene 10 protein. The Tat(1-67) gene was similarly isolated by PCR amplification and inserted into the modified pGEMEX vector as an Xhol–HindIII fragment. Expression of the Tat proteins was induced by incubating transformed pLysS cells [Studer et al. 1990] with 1 mM IPTG for 3 hr at 37°C. The cell pellet from a 400-ml culture was washed in PBS/MgCl_2 and resuspended in 5 ml of lysis buffer [50 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.1 mM KCl, 10 mM DTT] containing 0.1 mM PMSE, 2 mg/ml of benzamidine, 4 μg/ml of leupeptin, 10 μg/ml of aprontin and, 1 μg/ml of pepstatin A. The cells were lysed by two successive rounds of freezing and thawing, and the lysate was centrifuged for 20 min at 15,000 g at 2°C. The pellet was extracted with 8 ml of 4 M guanidine-HCl in HEMGN buffer [25 mM HEPEs (pH 7.6), 12.5 mM MgCl_2, 0.1 mM EDTA, 0.1% NP-40, 10% glycerol, 10 mM DTT, and protease inhibitors as listed above] at 4°C for 45 min on a rotator, and then diluted twofold with HEMGN buffer lacking guanidine-HCl. The pellet was removed by centrifugation at 120,000 g for 30 min. The supernatant was dialyzed twice against HEMGN buffer containing 0.5 mM guanidine-HCl, then stepwise against HEMGN buffer containing 0.15 M guanidine-HCl, followed by a short centrifugation to remove any insoluble proteins. A portion of the lysate (2 ml) was then loaded onto a 2-ml HA column, washed extensively with TM 0.25 mM/10 mM DTT, and eluted with TM 0.5 mM/10 mM DTT. The Tat(1-86) protein used for transcription was then dialyzed against HEMGN buffer (lacking MgCl_2 and NP-40) and purified further by gradient elution from an FPLC-Mono S resin. This second purification step reduced the RNA-binding activity of the preparation approximately twofold but did not alter the transcriptional activity of the Tat protein. RNA mobility-shift reactions containing Tat [Fig. 6] were performed following the conditions described by Roy et al. (1990a), except that binding reactions were incubated at 25°C and contained 5–10 μg/ml of total HeLa RNA as competitor.

In vitro transcription assays

In vitro transcription reactions were prepared as described by Marciniak et al. (1990a), with the following modifications: (1) The preincubation step was omitted, (2) 70 μg of desalted Jurkat

2518 GENES & DEVELOPMENT
nuclear extract was used per reaction. [3] Tat was added before the nuclear extract, and [4] reactions were carried out in a 50-μl final volume and contained 10 ng/μl of DNA template. Where indicated, reactions contained either 150 ng of Tat[1-67]CP protein or 30 ng of Tat[1-86] protein (expressed in bacteria and purified as described above), alone or in combination with 1.8 μg of TRP-1 or TRP-2 protein fractions. Half of each reaction was loaded on a sequencing gel and visualized by autoradiography after 3- to 8-hr exposures of the gel with an intensifying screen.

Acknowledgments

We thank B. Matjia Peterlin for the Tat[1-67]CP and pHiVSR-CAT plasmids and Marian Waterman for the Jurkat nuclear transcription extract. This study was funded by the National Institutes of Health and partially supported by a grant from the American Foundation for AIDS Research. C.T.S. is supported by a postdoctoral fellowship from the California Universitywide Biomedical Research Scholars Program. The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References


Two distinct nuclear transcription factors recognize loop and bulge residues of the HIV-1 TAR RNA hairpin.

C T Sheline, L H Milocco and K A Jones

Genes Dev. 1991 5: 2508-2520
Access the most recent version at doi:10.1101/gad.5.12b.2508

References
This article cites 39 articles, 18 of which can be accessed free at:
http://genesdev.cshlp.org/content/5/12b/2508.full.html#ref-list-1

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.

Work with our RNA experts to find biomarkers in exosomes.

To subscribe to Genes & Development go to:
http://genesdev.cshlp.org/subscriptions

Copyright © Cold Spring Harbor Laboratory Press