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A Human Chromosome 12-Associated 83-Kilodalton Cellular Protein Specifically Binds to the Loop Region of Human Immunodeficiency Virus Type 1 *trans*-Activation Response Element RNA

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***trans* activation of human immunodeficiency virus type 1 (HIV-1) involves the viral *trans*-activator protein (Tat) and a cellular factor(s) encoded on human chromosome 12 (HuChr12) that targets the *trans*-activation response element (TAR) in the viral long terminal repeat. Because nascent TAR RNA is predicted to form a secondary structure that specifically binds cellular proteins, we investigated the composition of the TAR RNA-protein complex for HuChr12-specific proteins. UV cross-linking of TAR RNA-nuclear protein complexes formed in vitro identified an 83-kDa protein in human cells and in a human-hamster hybrid cell containing only HuChr12. The 83-kDa TAR RNA-binding protein was absent in the parental hamster cells. TAR RNA mutations that inhibited binding of the 83-kDa protein in vitro also inhibited HuChr12-dependent Tat *trans* activation. These TAR mutations changed the native sequence or secondary structure of the TAR loop. The TAR RNA binding activity of the 83-kDa protein also correlated with a HuChr12-dependent increase in steady-state HIV-1 RNA expression during Tat *trans* activation. Our results suggest that either a species-specific 83-kDa TAR RNA loop-binding protein is directly encoded on HuChr12 or a HuChr12 protein(s) induces the expression of an 83-kDa TAR-binding protein in nonprimate cells.**

Human immunodeficiency virus type 1 (HIV-1) gene expression is controlled by multiple interactions of viral *trans*-activator proteins and cell factors with regulatory sequences located throughout the viral genome (for reviews, see references 4, 9, and 45). One of the major HIV-1 regulatory mechanisms requires the viral *trans*-activator protein Tat in combination with cellular factors to *trans* activate the viral long terminal repeat (LTR) and thereby increase overall viral RNA transcription (for a review, see reference 40). An important cellular component of this *trans*-activation mechanism is encoded on human chromosome 12 (HuChr12) (22, 24, 32). The HuChr12 mechanism and the viral Tat protein both target the *trans*-activation response element (TAR) (positions +1 to +80; +1 is the RNA transcription start) in the viral LTR (2, 3, 7, 13, 21, 37, 46). HIV-1 TAR RNA is predicted to form a stem-loop secondary structure which is the primary site of Tat- and cell factor-mediated *trans* activation (8, 14). The 3-nucleotide bulge region of TAR RNA (+23 to +25) binds Tat protein in vitro and is necessary for *trans* activation in vivo (13, 14, 37, 46). The native TAR RNA loop sequences (+31 to +34) are required for the HuChr12 support of *trans* activation (21).

In addition to the viral Tat protein, TAR RNA binds nuclear proteins in vitro (16–20). Certain TAR RNA-binding proteins are reported to bind specifically to the TAR RNA loop structure and increase LTR-directed transcription in vitro (27, 28, 39, 47). Additional TAR RNA-binding proteins specifically bind to the stem region (17, 35, 36). Mutational analyses of TAR RNA currently suggest, however, that the native bulge and loop nucleotide sequences are more critical than native stem nucleotide sequences in supporting Tat-directed *trans* activation (13–15, 21). Complementary stem mutations that maintain the predicted secondary structure of TAR RNA do not significantly affect Tat *trans* activation; bulge or loop mutations that maintain secondary structure do dramatically inhibit *trans* activation (14, 15), including the HuChr12-encoded mechanism (21). These results suggest that the HuChr12-encoded mechanism may provide a critical cellular factor in the protein-TAR RNA loop component of Tat-directed *trans* activation.

Although we have previously discovered that the native TAR RNA loop sequences (+31 to +34) are required for HuChr12 support of *trans* activation, the role of TAR RNA stem sequences and the native TAR RNA secondary structure in HuChr12-supported *trans* activation is not known. To more thoroughly define the TAR regions required for HuChr12-dependent *trans* activation, a Chinese hamster ovary (CHO) cell line (UCW56 [10]), a HuChr12-containing human-CHO hybrid cell line (HHW271), and a human T-cell line (CEM) were assayed for support of HIV-1 LTR *trans* activation in the presence of TAR mutations that maintain or disrupt the predicted TAR RNA secondary structure (Fig. 1A). HIV-1 Tat-directed *trans* activation of the HIV-1 LTR with a wild-type TAR (TAR-WT) confirmed our previous reports (21, 22) that the addition of a single copy of HuChr12 to the CHO genome (HHW271) greatly increased *trans* activation (Table 1). The stem mutation (TAR-ΔS), which disrupts the predicted TAR RNA secondary structure (Fig. 1A), and the loop mutation (TAR-ΔL), which retains the native secondary structure, both decreased *trans* activation in HHW271 and CEM to near-basal levels. In contrast, the complementary stem mutations (TAR-

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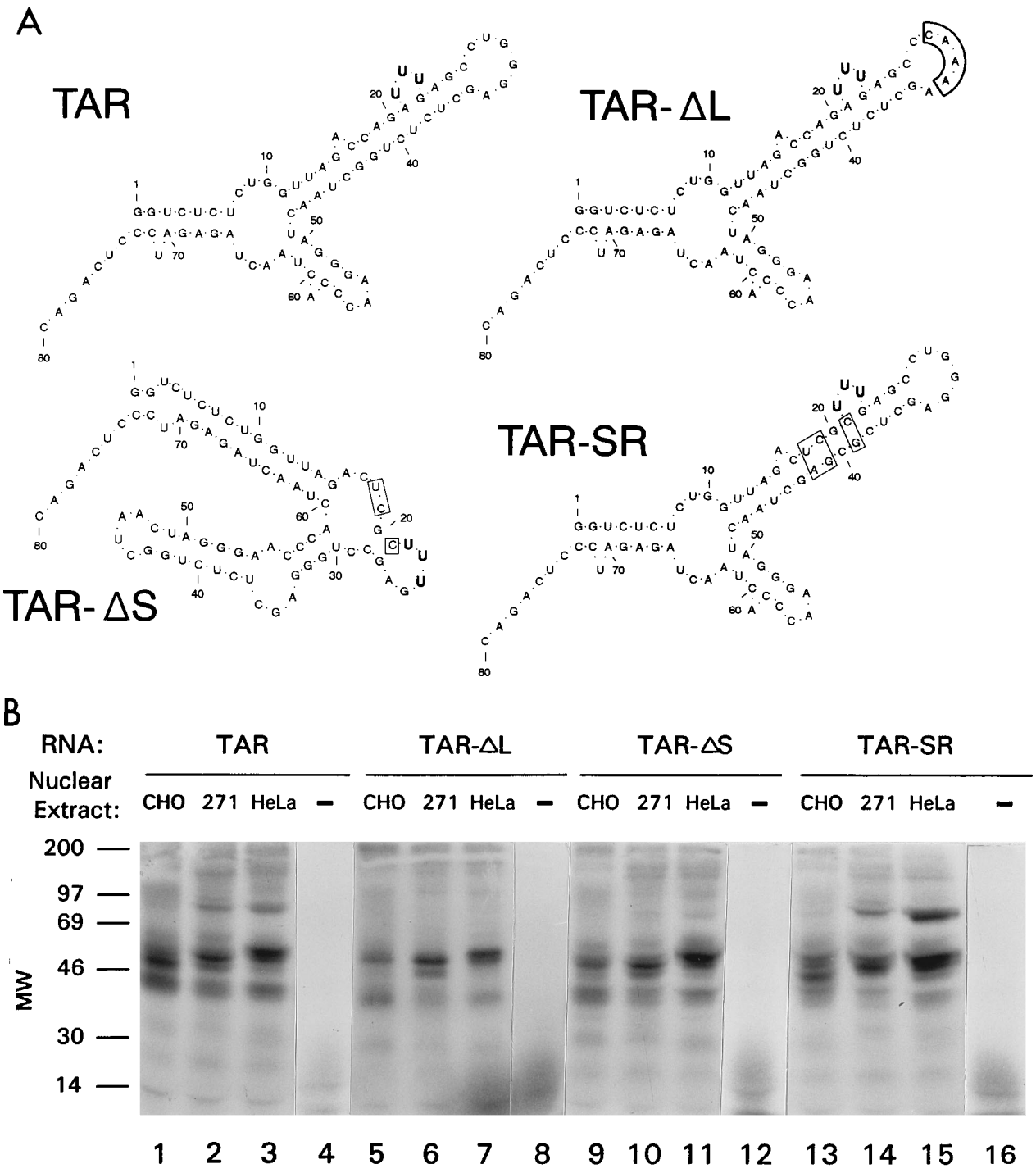

FIG. 1. UV cross-linking analysis of TAR RNA-binding proteins. (A) Secondary structure, predicted by the Zuker and Stiegler method (48), of in vitro-synthesized native TAR RNA ($\Delta G = -36.4$ kcal/mol) and RNAs with the loop mutation TAR-ΔL ($\Delta G = -36.4$ kcal/mol), the stem mutation TAR-ΔS ($\Delta G = -32.1$ kcal/mol), and the complementary stem mutation TAR-SR ($\Delta G = -37.7$ kcal/mol). TAR RNAs were synthesized as described in the text. Boldface nucleotides (+22 to +24) form the bulge region necessary for Tat binding (13, 14, 37, 46). Sequence mutations in TAR-ΔL, TAR-ΔS, and TAR-SR RNAs are outlined. (B) TAR RNA-binding proteins in nuclear extracts from CHO, HHW271 (271), and HeLa cells and no nuclear extract (−) were UV cross-linked to [α-^{32}P]RNA by using the indicated TAR RNAs as described in the text. MW, molecular weight (in thousands).

SR), which restore the native TAR RNA structure, supported 63 and 79% of the TAR-WT *trans*-activation levels in HHW 271 and CEM, respectively. For the human T-cell line, CEM, the level of *trans* activation in the presence of TAR mutations closely corresponds with those in studies using a nonlymphoid human cell line, HeLa (15, 47). The parallel levels of *trans* activation of HHW271 and CEM in the presence of TAR mutations strongly suggest that HuChr12-encoded factors are providing a vital cellular component in the TAR-dependent HIV-1 *trans*-activation process. In addition, HuChr12-encoded

TABLE 1. HuChr12 support of HIV-1 LTR *trans* activation in the presence of TAR mutations[a]

LTR	Fold increase in *trans* activation (% of wild-type value) in cell type[b]		
	CHO	HHW271	CEM
TAR-WT	2.0 (100)	19 (100)	34 (100)
TAR-ΔL	2.1 (105)	1.3 (7)	1.3 (5)
TAR-ΔS	1.8 (90)	2.1 (11)	1.8 (7)
TAR-SR	1.2 (60)	12 (63)	27 (79)

[a] Cells were transfected with an HIV-1 LTR-CAT plasmid or cotransfected with an LTR-CAT construct and a plasmid (pSV-*tat*) containing the HIV-1 *tat* gene under control of the simian virus 40 early promoter (5).

[b] Fold increase in CAT activity of the cotransfections (LTR-CAT + pSV-*tat*) compared with the CAT activity of the same LTR-CAT construct transfected without pSV-*tat*. CAT activity was quantitated in the linear range of the assay. Values in parentheses are *trans*-activation values of the mutant TAR constructs compared with the *trans*-activation value of the wild-type LTR (set at 100%) for each cell line. Values for individual cell lines are the averages for two or more experiments, with a variation between experiments of <20%.

support of *trans* activation appears to require the native TAR loop sequences in the context of a wild-type TAR RNA secondary structure.

To analyze the protein composition of the TAR RNA-protein complexes, a UV cross-linking procedure described by Marciniak et al. (28) was used to form covalent bonds between ^{32}P-labeled TAR RNA and proteins. Nuclear extracts were prepared from the CHO parental cell line (UCW56) (10), a human-UCW56 hybrid cell line containing a single copy of HuChr12 (HHW271) (22), and human cells by the procedure described by Dignam et al. (12). CHO nuclear extracts were included since these cells support very low levels of both HIV-1 virus production (22) and TAR loop-dependent *trans* activation (2, 21).

TAR RNA was synthesized in vitro from the TAR-WT or TAR mutants TAR-ΔL, TAR-ΔS, and TAR-SR as described previously by Wu et al. (47). TAR RNAs were synthesized by using their respective plasmid DNA templates that were linearized with *Hin*dIII at the +80 nucleotide in TAR and by using the Riboprobe System II with T7 RNA polymerase (Promega, Madison, Wis.), following the manufacturer's protocol. Radiolabeled TAR RNA was produced during the in vitro synthesis reaction by the addition of [α-^{32}P]CTP. TAR RNA was isolated from the reaction mixture by electrophoresis on an 8 M urea–8% polyacrylamide gel. The gel fragment containing TAR RNA was suspended in 0.5 M NH_4 acetate–1 mM EDTA–1% sodium dodecyl sulfate, placed in dialysis tubing, and electroeluted against 0.5× Tris-borate-EDTA at room temperature for 2 h at 80 V. The electroeluted TAR RNA was extracted with phenol-chloroform (1:1) twice and chloroform once, precipitated, and stored in ethanol until use. The predicted secondary structure of native TAR RNA is not altered with the loop mutations in TAR-ΔL (Fig. 1A). The stem mutation (TAR-ΔS; +18 to +21) produces a different stem-loop structure wherein the native loop and bulge sequences are present but in different secondary structures. The complementary stem mutations (TAR-SR; +18 to +21 and +39 to +42) restore the native stem-loop structure but with a different nucleotide sequence in the stem (Fig. 1A).

For UV cross-linking studies, nuclear extract (100 μg) and 2×10^6 cpm of [^{32}P]CTP-labeled TAR RNA were incubated in 125 μl of reaction A buffer (RxB) containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.9), 62 mM KCl, 2 mM $MgCl_2$, 150 μM dithiothreitol, 6% glycerol (vol/vol), and heparin sulfate (1 mg/ml) for 30 min at 4°C. The TAR RNA-protein interactions were then covalently cross-linked by using a 254-nm UV light source (model UVGL-25; UVP Inc., San Gabriel, Calif.) at a 4-cm distance for 10 min. RNase A was then added to a final concentration of 1 mg/ml for 30 min at 37°C. Proteins radiolabeled with covalently linked [^{32}P]CTP were electrophoresed on a 12% denaturing polyacrylamide gel. The gel was processed for fluorography with Enhance (Amersham, Arlington Heights, Ill.) by following the manufacturer's protocol. By this technique, nuclear extracts from CHO, HHW271, and HeLa were found to contain many of the same TAR RNA-binding proteins (Fig. 1B). Major TAR RNA-binding proteins were observed at 52 to 53 kDa, 48 kDa, and 42 kDa in all three cell types (Fig. 1B, lanes 1 to 3). No [^{32}P]CTP-labeled bands appeared when the UV cross-linking procedure was done in the absence of nuclear extract (Fig. 1B, lanes 4, 8, 12, and 16). An 83-kDa TAR RNA-binding protein present in HeLa and HHW271 cells (Fig. 1B, lanes 2 and 3) was absent in CHO extracts (Fig. 1B, lane 1). A minor 89-kDa protein observed with the HHW271 extract, which was absent in CHO and HeLa extracts, was closely associated with the major 83-kDa protein. The pattern of major TAR RNA-binding proteins observed in the presence of the loop mutation, TAR-ΔL, was similar to that for native TAR RNA except for the absence of the major 83-kDa protein (Fig. 1B, lanes 5 to 7). The stem mutation, TAR-ΔS, bound the same major proteins as native TAR except for an almost total reduction in the binding of the loop sequence-dependent 83-kDa protein (Fig. 1B, lanes 9 to 11). This result indicates that the predicted secondary structure of the loop sequence was also important in the interaction of the 83-kDa protein with TAR RNA; the other major TAR RNA-binding proteins (52 to 53 kDa, 48 kDa, and 42 kDa) were not noticeably sensitive to the loop and stem mutations. TAR-SR RNA, which had the additional stem mutations from +39 to +42, bound the same major proteins, including the 83-kDa protein, as TAR-WT (Fig. 1B, lanes 13 to 15). These results indicate that the proteins at 52 to 53 kDa, 48 kDa, and 42 kDa are binding to double-stranded RNA in a sequence-independent manner or are binding in a region of TAR unaffected by the mutations. In addition, it appears from their presence in all three cell lines tested that these proteins are ubiquitously expressed.

Partial purification of the nucleic acid-binding proteins of HHW271 and human cells by heparin-Sepharose chromatography was performed to further analyze their 83-kDa TAR-binding proteins. Nuclear extracts (11.2 mg of protein) were clarified by centrifugation (27,200 × *g* for 15 min), brought to final buffer concentrations of 20 mM HEPES (pH 7.9), 6% glycerol (vol/vol), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride (column buffer), and applied to a heparin-Sepharose column (1.77-cm^2 surface area, 2.5-cm height) equilibrated with the column buffer. Unbound proteins (flowthrough) were eluted with column buffer (flow rate, 6 ml/h). Bound proteins were eluted with a KCl linear gradient (12 ml; 0.05 to 0.5 M KCl) in column buffer. Fractions (840 μl) were collected, dialyzed against RxB, and assayed by UV cross-linking for identification of individual TAR RNA-binding proteins. The major TAR RNA-binding activity of nuclear proteins from CEM and HHW271 eluted at the same point in the KCl gradient (compare Fig. 2A and B) and corresponded to the major protein peak eluted during the KCl gradient (data not shown). The flowthrough protein fractions of CEM (Fig. 2A, lane 2) and HHW271 (not shown) were void of TAR RNA-binding activity. The nuclear proteins of CEM (Fig. 2A, lane 12) and HHW271 (not shown) which bound tightly to the column and eluted in the presence of high salt concentrations (0.5 M KCl)

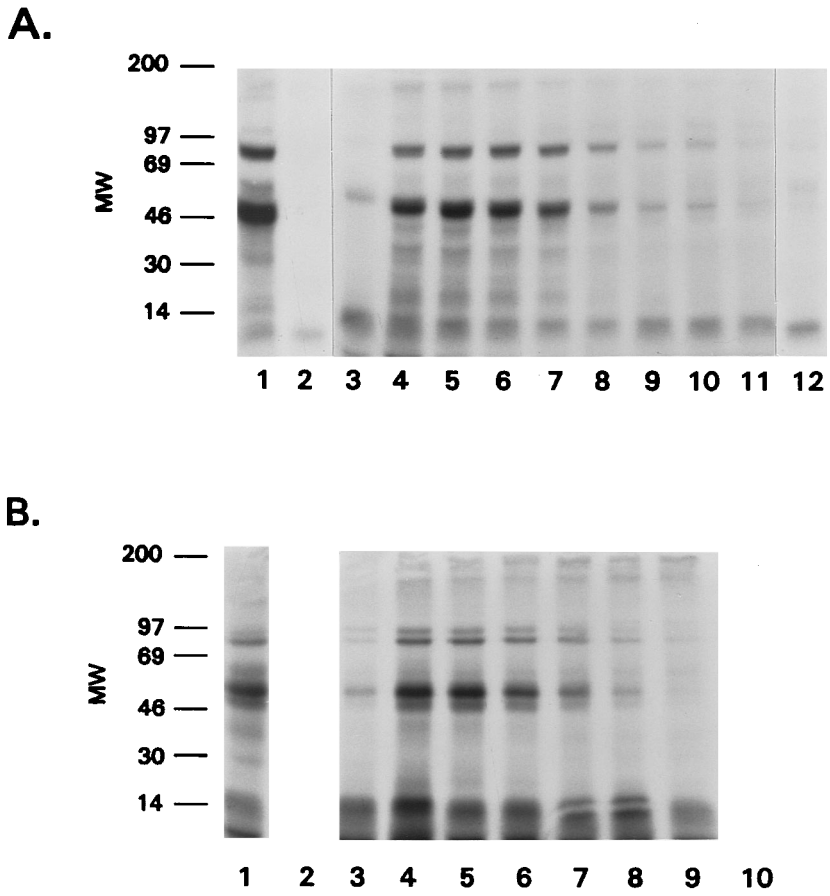

FIG. 2. Heparin-Sepharose fractionation of TAR RNA-binding proteins in nuclear extracts from CEM (A) and HHW271 (B). Bound and unbound proteins were eluted and fractions were analyzed as described in the text. (A) Lanes: 1, total nuclear extract; 2, flowthrough fraction; 3 through 11, KCl gradient fractions 25 through 33; 12, 0.5 M KCl fraction 39. (B) Lanes: 1, total nuclear extract; 2, flowthrough fraction (data not shown); 3 through 9, KCl gradient fractions 25 through 31; 10, 0.5 M KCl fraction 39 (data not shown). MW, molecular weight (in thousands).

did not contain significant levels of TAR RNA-binding activity. Many of the major TAR-binding proteins in the whole nuclear extracts were also observed in the fractions that contained the highest levels of TAR RNA-protein complexes. The TAR loop-specific 83-kDa protein eluted at the same point in the KCl gradient in CEM and HHW271 nuclear extracts (Fig. 2A and B, respectively). The minor 89-kDa protein in the unfractionated HHW271 nuclear extract coeluted with the 83-kDa protein, suggesting that these two proteins had similar ionic charges under these conditions. The 53- and 48-kDa TAR-binding proteins also appeared to coelute with the 83-kDa protein in the KCl gradient in both CEM and HHW271 extracts (Fig. 2A and B, respectively). The coelution of the TAR-binding 83-kDa and 53- to 48-kDa proteins in the KCl gradient may indicate that these proteins were part of a larger TAR-binding complex, although our data do not specifically address this issue. However, only the 83-kDa protein in these fractions was dependent on the native TAR loop sequences for binding (data not shown).

Although the addition of HuChr12 to the CHO cell genome generates a species-specific TAR loop-dependent *trans*-activation mechanism (2, 6, 21) that greatly increases Tat-directed HIV-1 gene expression (3, 22, 32), our previous studies did not address the question of whether the HuChr12 effect is due primarily to an increase in Tat-induced HIV RNA expression. To investigate the effect of HuChr12 on HIV-1 LTR-directed RNA expression, a semiquantitative reverse transcription PCR (RT-PCR) method was used to measure steady-state HIV-1 RNA levels.

First, to construct a clonal cell line that constitutively expresses HIV-1 Tat (HHW271-7), HHW271 was cotransfected with plasmid pSV-*tat* and with plasmid pSV-Neo (5), which contains the neomycin antibiotic resistance gene aminoglycoside phosphotransferase 3′ (*APH*), under transcriptional control of the simian virus 40 early promoter as previously described (21). Three days after transfection the medium was supplemented with the antibiotic Geneticin (300 μg/ml; Gibco Laboratories, Grand Island, N.Y.) for selection of cells expressing the *APH* phenotype. The neomycin-resistant HHW271 cells were plated at single-cell cloning density. Surviving single-cell clones were expanded and analyzed for Tat-directed *trans* activation of the HIV-1 LTR by using the chloramphenicol acetyltransferase (CAT) reporter gene assay as described previously (21). Expression of *tat* RNA in cells was monitored by RT and gene amplification by using the primers and RNA-based PCR technique described previously (23).

To assay for steady-state HIV-1 RNA and CAT enzymatic activity, 2×10^6 cells (CHO, HHW271, and HHW271-7) were plated for each transfection by the $CaPO_4$ technique as described previously (21). Cell cultures were cotransfected 24 h later with 10 μg of plasmid containing the HIV-1 LTR upstream of the CAT reporter gene (LTR-CAT) (5), with or

without 10 μg of pSV-*tat*. To control for transfection efficiency, all transfections contained 10 μg of a plasmid (pSV-βgal) (5) containing the β-galactosidase gene under control of the simian virus 40 early promoter. Sonicated salmon sperm DNA was added to transfections, when necessary, to normalize input DNA to 30 μg. Twenty-four hours posttransfection, cells were harvested by incubation in cold phosphate-buffered saline with 2 mM EDTA; one half of each cell culture was taken for analysis of CAT and β-galactosidase enzyme activities as described previously (5, 21). Equivalent amounts of each transfected culture, determined by protein concentration, were assayed for CAT and β-galactosidase activity. Quantitative measurements were taken in the linear range of the assays. The other half of each culture was extracted for total RNA in RNazol-Blue (Tel-Test, Friendsworth, Tex.) by following the manufacturer's protocol. Isolated RNA (0.5 μg) was reverse transcribed by using 10 U of avian myeloblastosis virus reverse transcriptase. PCR amplification of LTR-CAT cDNA was carried out for 20 cycles by using the reaction conditions described previously (31) and a sense-strand ^{32}P-labeled 5′ primer (GGGTCTCTCTGGTTAGA) (1) from +1 to +16 (+1 is the RNA transcription start site in the HIV-1 LTR) in HIV_{LAI} and an antisense 3′ primer in the CAT gene (+157 to +133) as described previously (25). Simultaneous PCR amplifications, without RT, were used to control for DNA contamination. A standard curve for quantitation of the amplified cDNAs from transfected cultures was generated by the parallel amplification of serial dilutions of the pLTR-CAT plasmid from 2.7×10^5 to 1.6×10^7 molecules. One-twentieth of each ^{32}P-labeled amplified product was electrophoresed on a 4% denaturing polyacrylamide gel and quantitated by using a PhosphorImager system (Molecular Dynamics, Sunnyvale, Calif.).

This RT-PCR analysis indicated that the level of LTR-CAT RNA expression in CHO cells was not increased in the presence of Tat (Fig. 3A). Similarly, the CAT protein level in CHO cells, measured by CAT enzyme activity, increased only 1.4-fold in the presence of Tat. In HHW271 cells, basal-level LTR-CAT RNA expression (minus Tat) was below the limit of detection and CAT protein activity was correspondingly 14-fold lower than in CHO cells. In the presence of Tat, HHW271 cells greatly increased levels of both LTR-CAT RNA (11-fold) and CAT protein (16-fold) over those in CHO cells. In the Tat-expressing HHW271-7 cells, LTR-CAT RNA and CAT protein levels were similarly elevated (22-fold and 23-fold, respectively) over those in CHO cells. The relatively equivalent Tat-induced increases in LTR-CAT RNA and CAT protein in HuChr12-containing cells suggest that HuChr12 is most likely increasing HIV-1 gene expression at the level of transcription.

Since HuChr12 appears to work in concert with Tat to increase steady-state HIV-1 RNA levels, we investigated the TAR RNA-binding properties of the HuChr12-related 83-kDa protein in the context of an in vivo-expressed Tat protein in HHW271-7. Analysis of the TAR RNA-protein complex by UV cross-linking suggests that Tat expression did not alter the interaction between the 83-kDa protein and TAR (Fig. 3B, lanes 1 and 2). The TAR RNA-binding characteristics of the 83-kDa protein from HHW271-7 remained TAR loop dependent, as shown by the absence of binding in the presence of the loop mutation (TAR-ΔL) (Fig. 3B, lanes 3 and 4), and stem sequence independent, as shown by the presence of binding in the presence of the complementary stem mutations (TAR-SR) (Fig. 3B, lanes 5 and 6). Other TAR RNA-binding proteins were unaffected by the expression of Tat with the exception of a 33-kDa protein in HHW271 that was absent in HHW271-7. The Tat-sensitive 33-kDa protein appeared to be TAR loop and stem sequence independent (Fig. 3C, lanes 3 and 5, respectively). Conversely, a 27-kDa TAR-binding protein, appearing only in the presence of Tat (HHW271-7), was not observed with TAR-ΔL RNA but was present with TAR-SR RNA (Fig. 3B, lanes 2, 4, and 6, respectively). These results suggest that the 33- and 27-kDa proteins are influenced by the presence of Tat, but how they relate functionally, if at all, to the HuChr12-associated 83-kDa protein is presently not known.

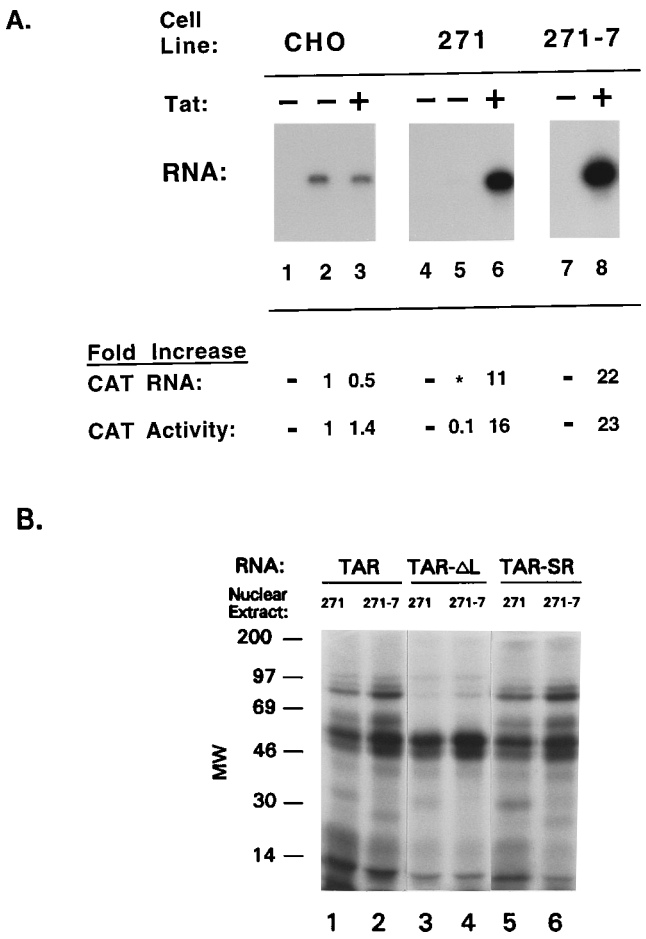

FIG. 3. In vivo steady-state HIV-1 LTR-CAT RNA and CAT protein levels (A) and in vitro TAR RNA-protein interactions (B) in the presence or absence of HuChr12 and HIV-1 Tat. (A) RT-PCR amplification of total RNA from CHO (lanes 2 and 3), HHW271 (lanes 5 and 6), and HHW271-7 (lane 8) cells expressing LTR-CAT in the absence or presence of Tat, respectively. Control RT-PCR amplifications, using the PCR primers for detection of LTR-CAT RNA, were from total RNA of CHO (lane 1), HHW271 (lane 4), and HHW271-7 (lane 7) cells transfected with pSV-βgal. *, no PCR product detected. (B) UV cross-linking of TAR-binding proteins from nuclear extracts of HHW271 (lanes 1, 3, and 5) and HHW271-7 (lanes 2, 4, and 6) with the indicated TAR RNAs. All conditions were as described in the text. MW, molecular weight (in thousands).

The goal of this study was to investigate, in vitro, the interactions of TAR RNA and host cell proteins in the context of the HuChr12-encoded mechanism that supports Tat-directed *trans* activation. Previously, we identified the TAR RNA loop and the HuChr12 mechanism as critical interdependent elements in Tat *trans* activation (21). The relatively equivalent HuChr12-dependent increases in steady-state levels of HIV-1 RNA and transcribed protein during Tat *trans* activation observed in this study indicate that a HuChr12-encoded factor(s) is functioning most likely at the level of RNA transcription. The HuChr12 factor does not appear to be a general activation

factor even in the presence of Tat; gene expression directed from the simian virus 40 early promoter was not increased in HHW271 or HHW271-7 cells compared with that in CHO cells (data not shown). Previously, we also discovered that the HuChr12 mechanism did not enhance human T-cell leukemia virus type 1 or type 2 LTR-directed gene expression in the presence of their own *trans*-activator genes, Tax_I and Tax_{II}, respectively, or in the presence of HIV-1 Tat (24). Further studies will be required to determine if the specific effect of HuChr12 factors on HIV-1 steady-state RNA is exerted through increased initiation, processivity, or transcript stability.

The minor differences between the individual TAR RNA-binding proteins of CHO and HHW271 cells suggest that the 83-kDa TAR loop-binding protein is encoded on, or regulated by, HuChr12. Furthermore, it would be predicted that the same 83-kDa protein, which is present in HeLa and CEM cells, is a major TAR RNA loop-binding protein in human cells. Previous reports have identified TAR RNA loop-binding proteins from human cells (28, 39, 47) that appear to have a size different from the 83 kDa we report. A 68-kDa protein (27) and a 185-kDa protein (39, 47) are loop sequence-dependent TAR RNA-binding proteins that enhance HIV-1 LTR-directed transcription in vitro and share some of the in vitro properties of the 83-kDa protein. A TAR RNA-binding protein doublet from a nuclear extract of the human T-cell line MT-4 (29), which appears to be in the same size range as the 83- to 89-kDa doublet we observed, also eluted from a heparin-Sepharose column at a similarly low KCl concentration. The loop-dependent characteristics of this protein doublet, however, were not reported (29). The 185-kDa TAR RNA-binding protein (39, 47) and our 83-kDa protein have the same loop-dependent binding specificities but appear to have dissimilar species-specific expression. The 83-kDa protein found in human cells and human-hamster hybrid cells containing HuChr12 was not present in CHO cells. In contrast, the 185-kDa protein found in human cells is reported to have a similar CHO homolog (39), which suggests that the expression of this TAR-binding protein is not dependent on the presence of HuChr12. The mixed results of differing molecular weights and similar, but not always identical, biochemical and in vitro properties of these RNA-binding proteins suggest that some of these proteins may be closely related genetically or functionally. The generation of molecular clones of these proteins will be necessary to determine the extent of homology, if any, between these similar TAR RNA-binding proteins.

The molecular mechanism that supports Tat *trans* activation through a pathway that is dependent on the TAR RNA loop primary sequence and secondary structure is not yet understood. The correlation of our in vivo *trans*-activation results with the in vitro binding of the 83-kDa protein to the TAR loop suggests that these observations have identified an important cellular component of Tat-dependent *trans* activation. In vitro binding studies report that Tat and cellular TAR RNA loop-dependent binding proteins may compete for access to their closely adjacent binding sites on the TAR RNA bulge and loop, respectively (39, 47). Our results suggest that the binding of the 83-kDa protein to TAR was unaffected in the presence of Tat produced in vivo. Although it is possible that the Tat protein synthesized in HHW271-7 cells lost activity during the nuclear extract isolation procedure, previous studies indicate that nuclear extracts prepared by this procedure (12) from HIV-1-infected cells do activate LTR-directed transcription in vitro (33, 34), presumably through Tat *trans* activation. The changes in TAR-binding proteins that did occur in the presence of Tat, i.e., the loss of a 33-kDa protein and the addition of a 27-kDa protein, do suggest that Tat expression in the HHW271 cells affected TAR RNA-binding proteins in our in vitro assay. A 38-kDa cellular protein that competes with Tat in TAR RNA binding assays and that recognizes the bulge and upper stem of TAR RNA has been described (6). The 33-kDa protein we observed and the 38-kDa protein may be closely related, since both proteins recognize TAR RNA independently of the loop sequences and their TAR-binding capacities are decreased in the presence of Tat. The function of these two proteins in HIV replication and normal cellular metabolism will require their purification for further study in vitro.

A recent report suggests that a species-specific component of HIV-1 *trans* activation involves the interaction of a cellular factor or protein complex with Tat and that this protein complex then binds specifically to TAR RNA to activate viral transcription (26). A similar species-specific mechanism that enhances Tat-TAR RNA interaction is proposed to be encoded on HuChr12 (2). The rather ubiquitous occurrence of Tat-binding proteins in yeast, hamster, and human cells (11, 30, 41, 44) and the observation that Tat effectively *trans* activates heterologous promoters in the absence of HuChr12 (21, 24) indicate that cellular mechanisms that support Tat activity per se are not species restricted. TAR-independent Tat *trans* activation is also reported for the HIV-1 LTR, in which the TAR region is replaced with an exogenous RNA sequence target for an RNA-binding protein-Tat chimera (3, 38, 42, 43). These observations indicate that the presentation of Tat at or near the transcription complex is vital for *trans* activation to occur and that there may be multiple mechanisms, both artificial and natural, that are capable of presenting Tat to an RNA-protein *trans*-activation complex. Our previous studies have shown the interdependency of HuChr12-encoded factors and native TAR loop sequences in HIV-1 and HIV-2 *trans* activation (21, 22, 24). The discovery of the 83-kDa TAR RNA loop-binding protein and its ability to bind TAR mutations in vitro that support *trans* activation in vivo provides evidence for a molecular component of the HuChr12 support of HIV *trans* activation. Further in vitro characterization and molecular cloning of the 83-kDa protein will be necessary to understand how a species-specific component in the TAR RNA-nuclear protein complex leads to the interdependent *trans*-activation mechanism involving HuChr12-encoded factors and the TAR RNA loop.

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