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Direct evidence for translational regulation by leader RNA and Tat protein of human immunodeficiency virus type 1

(control of protein synthesis/double-stranded RNA-dependent protein kinase/RNA secondary structure)

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ABSTRACT **Translational effects of the RNA leader and Tat protein of human immunodeficiency virus type 1 (HIV-1) were investigated in rabbit reticulocyte lysate. Hybrid RNA species with natural or mutated HIV-1 leader fused to human interferon-γ mRNA were produced *in vitro* from recombinant plasmids. HIV-1 leader RNA was found to inhibit translation through two mechanisms. A 3-fold trans-inhibition of translation was demonstrated by mixing hybrid HIV-1 leader RNA with indicator interferon mRNA. By comparison, HIV-1 leader caused a 50-fold cis-inhibition in lysate in which two trans-inhibitory factors, double-stranded RNA-dependent protein kinase and (2′-5′)oligoadenylate synthetase, were suppressed. In contrast, purified HIV-1 Tat protein produced in *Escherichia coli* enhanced by 4-fold translation from HIV-1 leader–interferon mRNA but not from interferon mRNA lacking HIV sequences or from total poly(A)$^+$ RNA. Translation of mRNA containing either a single base substitution in the loop of the "trans-acting responsive" sequence (TAR) or an alternative stem–loop in TAR was nevertheless stimulated by Tat. The enhancement of translation by Tat was largely due to relief of cis-inhibition, since the effect was found even in lysate in which double-stranded RNA-dependent protein kinase was inhibited with 2-aminopurine. These results suggest that translation is an important level of control in the replication cycle of HIV-1.**

Regulation of human immunodeficiency virus (HIV) replication is a complex process involving both transcriptional and posttranscriptional events (reviewed in ref. 1). Control at the level of translation is suggested from several studies on HIV-1 leader RNA (2–4) and Tat protein (5–11). The RNA leader of HIV-1 contains two adjacent stem–loop structures and is present in all HIV-1 mRNA species (12, 13). The double-stranded portion of the leader has the potential to inhibit protein synthesis either in trans or in cis. In trans, the double-stranded regions of HIV-1 RNA activate double-stranded RNA (dsRNA)-dependent protein kinase (3, 4) and to a lesser extent (2′-5′)oligoadenylate (2-5A) synthetase (3). The kinase indirectly inhibits protein synthesis initiation by phosphorylating initiation factor eIF2α whereas 2-5A synthetase is part of an RNA degradation pathway (reviewed in ref. 14). RNA containing the first stem–loop of the HIV-1 leader both activated dsRNA-dependent protein kinase and inhibited protein synthesis in trans in a cell-free system (4). Furthermore, minimal mutations in the stem of the trans-acting responsive sequence (TAR) that disrupted secondary structure also relieved trans-inhibition, whereas a second compensatory mutation restored both the base pairing of the stem and its capacity for trans-inhibition (4). In cis, secondary structure near the 5′ terminus of mRNA can also reduce translational efficiency by impairing scanning by the 40S ribosomal subunit (reviewed in ref. 15). In addition, it was shown that the 5′ cap of HIV mRNA was relatively inaccessible for binding to protein synthesis initiation factor eIF-4B (2). Therefore, a cis effect of HIV-1 leader RNA on translation seemed likely.

Another potential translation control for HIV involves the trans-activator protein, Tat (16–18), an essential factor for HIV replication (19, 20). Tat enhances gene expression through the TAR element (21) in HIV leader RNA (22). The levels at which Tat and TAR interact to promote viral replication remain controversial. Trans-activation has been alternatively reported to increase mRNA accumulation through enhanced transcriptional initiation and elongation, to increase the efficiency of translation of TAR$^+$ mRNA, or to stimulate both transcription and translation (reviewed in ref. 23). Direct demonstration of either effect in cell-free systems supplemented with highly purified Tat has not been reported.

We have investigated translational effects of HIV leader RNA and Tat protein in rabbit reticulocyte lysate. HIV-1 leader RNA reduced translation through contributions from both trans and cis mechanisms. By attenuating translation the RNA leader could suppress virus replication and contribute to the maintenance of the virus-carrier state. In contrast, purified Tat protein specifically enhanced translation of mRNA containing HIV leader RNA.

MATERIALS AND METHODS

Plasmids. An 843-base-pair (bp) cDNA for human interferon (IFN-γ) including the complete 498-bp coding sequence, 60 bp of the 5′ untranslated region, and 285 bp of 3′ untranslated sequence was subcloned from plasmid pRJ22 (a gift from C. W. Dieffenbach, Uniformed Services University of the Health Sciences; ref. 24) into the *Bam*HI site of pSP64 (Promega; ref. 25) resulting in plasmid 1 (Fig. 1). To construct plasmid 3 (Fig. 1), a *Sal* I- and *Eco*RI-cut human IFN-γ cDNA fragment (873 bp) from plasmid 1 was subcloned in pSP6HIV+1 (a gift of D. Capon, Genentech, ref. 12) (containing HIV-1 nucleotides +1 to +231) cut by *Xho* I (at +231) and *Eco*RI. To synthesize plasmid 2 (Fig. 1), plasmid 3 was digested with *Hin*dIII (at +77) and *Xba* I (between HIV and IFN sequences) and ligated after the cut restriction sites were filled in. Two complementary oligonucleotides of the HIV-1 leader sequence from +232 to +289 were made by automated DNA synthesizer, annealed, and subcloned at +231 (*Xho* I site) of plasmid 3 to make plasmid 4 (Fig. 1). Three additional

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Abbreviations: dsRNA, double-stranded RNA; 2-5A, (2′-5′)oligoadenylate or $p_x[(5')A(2')p]_nA$ ($x = 2$ or 3, $n \geq 2$); HIV, human immunodeficiency virus; IFN, interferon; TAR, trans-acting responsive sequence; p_3IAA, ppp(5′)I(2′)p(5′)A(2′)p(5′)A; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase.

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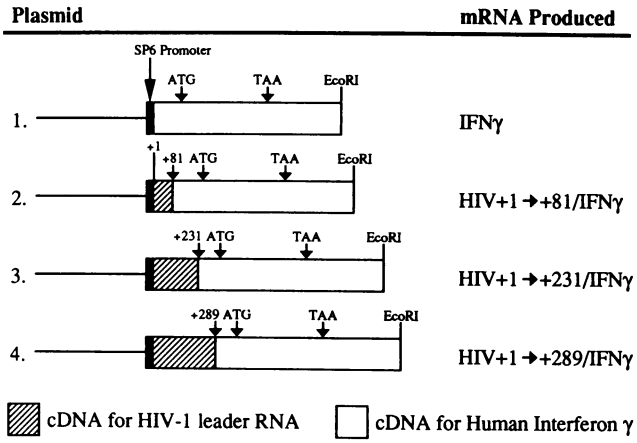

FIG. 1. Construction of plasmids and synthesis of mRNA (see *Materials and Methods*).

plasmids were constructed from plasmid 3: (*i*) pSP6-HIV+231(+52G)/IFN contained an A→G single base change at position 52 in the stem of TAR; (*ii*) pSP6-HIV+231(+31T,+52G)/IFN contained an additional base change, G→T, at position 31 in the TAR loop (as in the L3 mutant in ref. 26); and (*iii*) pSP6HIV+231(+52G,3'C1)/IFN contained a substitution on the 3' side of TAR inducing an alternative secondary structure (mutant 3'C1 in ref. 22). These mutant TARs were introduced into plasmid 3 as *Bgl* II–*Hin*dIII (+19 to +82) fragments. All plasmid constructions were confirmed by DNA sequencing.

Synthesis and Purification of mRNA. Plasmids were linearized with *Eco*RI (downstream of the IFN cDNA) and used as templates for *in vitro* transcription. Template DNA (2 μg) was incubated with transcription reaction buffer (Promega) that contained 1 mM ATP, CTP, and UTP; 0.1 mM GTP; 1 mM m^7GpppG (except where indicated); 20 μCi of [α-^{32}P]-UTP (1000 Ci/mmol; 1 Ci = 37 GBq); 15% (vol/vol) dimethyl sulfoxide; 0.2 M dithiothreitol, and 30 units of SP6 RNA polymerase (Promega). After 1 hr of incubation at 40°C, template DNA was digested with RQ1 DNase (Promega) and the RNA was extracted with phenol and then with chloroform, ethanol-precipitated, and resuspended in water. Two volumes of formamide-gel sample buffer was then added. All of the mRNA species were band-purified from sequencing gels to remove aberrant transcripts capable of forming dsRNA (27). The RNA solutions were heated at 70°C for 1 min and electrophoresed in 5% polyacrylamide/7 M urea gels (30 × 40 × 0.05 cm) for 3 hr at 45 W. The full-length transcripts were localized by autoradiography and were excised and eluted from the gel by a modification of the method of Skehel and Hay (28). The gel fragments were incubated in 0.5 M ammonium acetate/1.0 mM magnesium acetate/1% sodium dodecyl sulfate (SDS) at 30°C for 12–14 hr. The eluted RNA was filtered (Bio-Rad Poly-Prep columns), extracted with phenol and then with chloroform, and precipitated in ethanol. Finally the RNA pellets were washed in 70% ethanol and resuspended in 5 mM Hepes, pH 7.5/50 mM NaCl/0.05 mM EDTA.

Translation in Reticulocyte Lysate. The gel-purified RNAs were translated in rabbit reticulocyte lysate (micrococcal nuclease-treated, Promega). The RNA and lysate (10 μl) were used with or without (see text) preincubation at 30°C for 10 min prior to adding amino acids mixture minus methionine (Promega) and 30 μCi of L-[^{35}S]methionine (1106 Ci/mmol, Amersham). Translation was at 30°C for 1 hr and samples were run in SDS/12% polyacrylamide gels. Gels were treated with EN^{3}HANCE (NEN) prior to autoradiography. Autoradiograms were scanned with a densitometer (Hoefer, model GS 300) and relative peak areas were determined with a data processor [Shimadzu (Kyoto), model C-RIB].

Inhibition of dsRNA-Dependent Protein Kinase and 2-5A-Dependent RNase. 2-5A-dependent RNase was removed from the lysate with 2-5A-cellulose at 1 μM as described (29). In brief, the 2-5A-cellulose was incubated with the lysate for 1 hr on ice and the 2-5A-dependent RNase/2-5A-cellulose complex was removed by centrifugation at 1250 × *g* for 8 min at 2–4°C. The supernatant was removed and found to be lacking in 2-5A-dependent RNase as determined by a highly sensitive assay (30), whereas the RNase was detected in the untreated lysate (data not shown). Specific inhibitors of the dsRNA-dependent protein kinase were 2-aminopurine (10 mM) or poly(I)·poly(C) (25 μg/ml) (31), and ppp(5')I(2')p(5')-A(2')p(5')A (p_3IAA, 1.0 μM) (32) was used to inhibit 2-5A-dependent RNase. The inhibitors were added to the lysate prior to incubation at 30°C.

Analysis of HIV-1 Tat Protein. The 72-amino acid form of Tat from HIV-1 (LAV strain) was produced in *Escherichia coli* HB101. The purified Tat protein or lysozyme were introduced into cBENNCAT cells (33) by scrape-loading (34). Activation of the integrated HIV-1 long terminal repeat (LTR)–chloramphenicol acetyltransferase (CAT) gene was monitored by assay of [^{14}C]chloramphenicol acetylation in cell lysates. Either Tat supplemented with RNasin (Promega) at 1.8 units/μl and diluted in 10 mM 2-mercaptoethanol (in water) or a similarly prepared mock solution lacking Tat was preincubated with the RNA for 1 hr on ice prior to incubation with the reticulocyte lysate for 1 hr at 30°C.

RESULTS

Translation of IFN mRNA Is Inhibited in the Presence of HIV-1 Leader RNA. This study was designed to probe mechanisms by which HIV-1 leader RNA and Tat regulate translational efficiency. Protein synthesis in a reticulocyte lysate was measured by translating mRNA for human IFN-γ, a convenient indicator protein (22). IFN mRNA and hybrid mRNA species in which the first 81, 231, or 289 nucleotides of HIV leader RNA were fused to IFN mRNA were synthesized *in vitro* from recombinant plasmid DNA (Fig. 1) and were then band-purified through sequencing gels. Translation reactions whose products are shown in Fig. 2 were carried out after a 10-min preincubation at 30°C to allow activation of the trans-inhibitory enzymes, dsRNA-dependent protein kinase and 2-5A synthetase. The ability of HIV-1 leader RNA to inhibit protein synthesis in trans was determined by mixing HIV leader–IFN mRNA hybrids with the indicator IFN mRNA lacking HIV sequences (Fig. 2*A*). In the absence of the hybrid mRNAs, increasing the amount of the IFN mRNA resulted in a proportional increase in translation (Fig. 2*A*, compare lanes 1 and 2), whereas the opposite was true after addition of mRNA containing 81, 231, or 289 nucleotides of HIV-1 leader (lanes 3–5). The contribution to protein synthesis from the HIV leader–IFN mRNAs is negligible under these conditions (as described later). The same level of inhibition was obtained with all three HIV leader–IFN mRNAs even though the +81 hybrid is capable of forming only the first stem–loop of the leader whereas the +231 and +289 leaders form both stem–loops (12). Therefore, the second stem–loop and downstream sequences did not enhance the trans-inhibition of translation. The concentration of HIV-1 leader RNA required to produce a maximal inhibition of translation was determined by mixing dilutions of the +289 HIV leader–IFN mRNA with a constant level of the indicator IFN mRNA. Results showed that the greatest inhibition, about 3-fold, was obtained with the hybrid RNA at 0.52 μg/ml (Fig. 2*B*, lane 6).

Preparation of a Reticulocyte Lysate Deficient for the 2-5A and Protein Kinase Systems. To determine the cis effect of

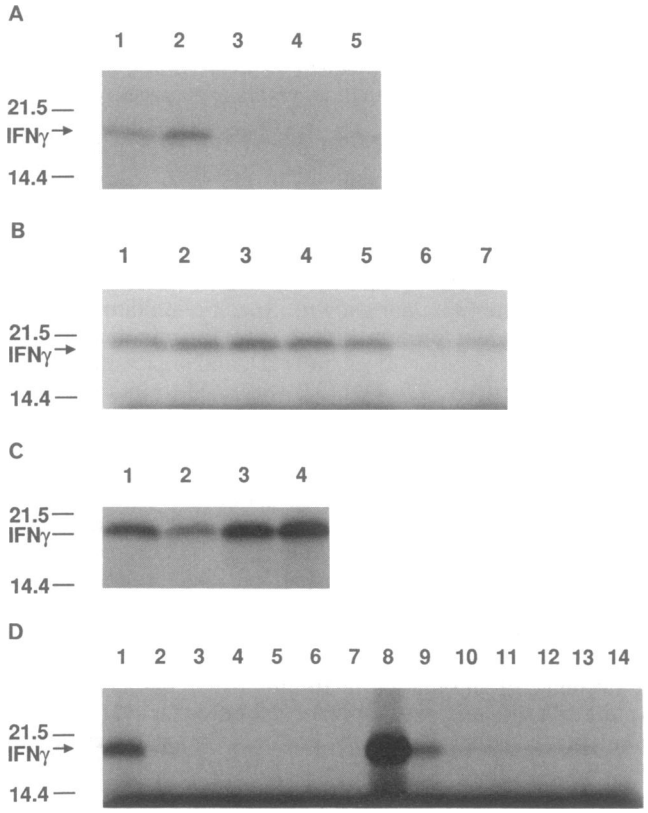

FIG. 2. HIV-1 leader RNA inhibits translation through both trans and cis mechanisms. (*A*) IFN mRNA at 2.6 μg/ml (lanes 1, 3–5) or 5.2 μg/ml (lane 2) was translated without (lanes 1 and 2) or with 2.6 μg/ml of +81 HIV leader–IFN mRNA (lane 3), +231 HIV leader–IFN mRNA (lane 4), or +289 HIV leader–IFN mRNA (lane 5). (*B*) IFN mRNA (2.6 μg/ml) was translated in the absence (lane 1) or presence (lanes 2–7) of +289 HIV leader–IFN mRNA at 26 ng/ml (lane 2), 52 ng/ml (lane 3), 130 ng/ml (lane 4), 260 ng/ml (lane 5), 520 ng/ml (lane 6), or 2.6 μg/ml (lane 7). (*C*) Translation of IFN mRNA (2.6 μg/ml) was in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of +289 HIV leader–IFN mRNA at 0.52 μg/ml. Lanes 3 and 4 were from lysate treated with 2-5A-cellulose (1 μM) and poly(I)·poly(C) (25 μg/ml). (*D*) The mRNAs (20 μg/ml) were translated in control lysate (lanes 1–7) or in the presence of high levels (25 μg/ml) of poly(I)·poly(C) in 2-5A-cellulose-treated lysate (lanes 8–14). Translation was with IFN mRNA (lanes 1 and 8), +81 HIV leader–IFN mRNA (lanes 2 and 9), +231 HIV leader–IFN mRNA (lanes 3 and 10), +289 HIV leader–IFN mRNA (lanes 4 and 11), +231(+52G) HIV leader–IFN mRNA (lanes 5 and 12), +231(+31T,+52G) HIV leader–IFN mRNA (lanes 6 and 13), and +231(+52G,3′C1) HIV leader–IFN mRNA (lanes 7 and 14). Translation was after a 10-min preincubation at 30°C. Positions of the molecular mass markers (kDa) and IFN (arrow) are indicated. Densitometer scanning resulted in the following relative peak areas. *A* lanes 1–5: 39, 83, 21, 16, and 20. *B* lanes 1–7: 91, 106, 121, 112, 87, 34, and 45. *C* lanes 1–4: 143, 92, 216, and 273. *D* lanes 1–14: 172, 8, 1, 1, 2, 4, 2, 491, 86, 16, 9, 8, 12, and 9.

HIV-1 leader RNA on translation, it was necessary to remove or inactivate the enzymes responsible for the trans-inhibition. 2-5A-dependent RNase was therefore depleted from the lysate by incubation with the highly specific affinity resin 2-5A-cellulose (29). A sensitive assay (30) demonstrated that the 2-5A-dependent RNase in the lysate was depleted from low to undetectable levels after removal of the RNase/2-5A-cellulose complex (data not shown). The dsRNA-dependent protein kinase, on the other hand, was inhibited by addition of a high level of poly(I)·poly(C). Paradoxically, low and high concentrations of dsRNA activate and inhibit the dsRNA-dependent protein kinase, respectively (31). The concentration of poly(I)·poly(C) used, 25 μg/ml, prevents activation of the kinase (data not shown). Mixing the +289 HIV leader–IFN mRNA with the indicator IFN mRNA failed to inhibit translation under these conditions (Fig. 2*C*, compare lanes 1 and 2 with lanes 3 and 4). Translation in general was improved in the trans-inhibitor-deficient lysate (Fig. 2*C*, compare lanes 1 and 3). We have found a similar stimulation by depleting lysates of 2-5A synthetase and the kinase with poly(I)·poly(C)-cellulose (data not shown). Presumably, reticulocyte lysate contains basal levels of these enzymes that limit the extent of translation. The predominant inhibitor is the kinase, since inhibition of the 2-5A system alone had little or no effect on translation of HIV leader–IFN mRNA (data not shown). There was, however, a low level of 2-5A-dependent RNase in the reticulocyte lysate.

HIV-1 Leader RNA Is Inhibitory to Translation in Cis. In the control lysate, the overall effect of HIV-1 leader RNA on translation was determined to be much greater than could be accounted for by the 3-fold trans effect (Fig. 2*D*, lanes 1–7). Translation of the hybrid HIV leader–IFN mRNA species (lanes 2–7) was reduced at least 20-fold compared with the control IFN mRNA (lane 1). The cis effect was also measured in the trans-inhibitor-deficient lysate in which 2-5A-dependent RNase was removed and dsRNA-dependent protein kinase was inhibited (Fig. 2*D*, lanes 8–14). Comparison of translation of the various mRNAs shows that HIV-1 leader sequences exert a potent inhibitory effect in cis. The effect increased with the length of the leader RNA, so that the +81, +231, and +289 HIV-1 leader segments caused inhibitions of about 6-, 31-, and 55-fold, respectively (Fig. 2*D*, lanes 9–11). Similar results were obtained with a lysate in which the kinase and RNase were inhibited with 10 mM 2-aminopurine (31) and 1.0 μM p_3IAA (32), respectively (data not shown). RNAs with point mutations in the first stem (lanes 5 and 12) or in the TAR loop (lanes 6 and 13) and a mutant that may result in an alternative stem–loop structure for TAR (lanes 7 and 14) were translated to a similar extent as the parent +231 HIV leader–IFN mRNA (lanes 3 and 10).

Tat Stimulates Translation of HIV Leader–IFN mRNA. HIV-1 Tat protein was added to determine whether it could partially relieve the inhibition caused by HIV-1 leader RNA. Tat produced in *E. coli* and purified by ion-exchange and reverse-phase chromatography appeared homogeneous as determined by silver staining after SDS/polyacrylamide gel electrophoresis (data not shown). To determine whether the recombinant Tat protein was biologically active, we introduced it by scrape-loading (34) into a cell line containing an HIV-1 LTR–CAT gene (cBENNCAT) (33). Tat induced expression of the integrated HIV-1 LTR–CAT gene, whereas control protein (lysozyme) in the same amounts showed no significant effect (data not shown).

Preincubation of RNA with Tat enhanced the translation of the +81, +231, and +289 HIV leader–IFN mRNAs about 2-fold (Fig. 3*A*, lanes 2–7; lane 1 was without added RNA). In contrast, Tat failed to stimulate translation of mRNAs lacking HIV leader sequences (Fig. 3*A* , lanes 10–13). There was no enhancement in response to Tat in the translation of either IFN mRNA (lanes 10 and 11) or total poly(A)$^+$ RNA (lanes 12 and 13). In fact, a slight inhibition in the translation of IFN mRNA was seen (lanes 10 and 11). Therefore, the stimulation of translation by Tat is not a general phenomenon, but rather it is specific for mRNAs that contain HIV-1 leader. The stimulatory effect of Tat on translation of HIV leader–IFN mRNAs cannot have been due to RNA degradation events in the leader that relieved inhibition, because there was no detectable translation of the capless +289 HIV leader–IFN mRNA without or with Tat (Fig. 3*A*, lanes 8 and 9).

Interestingly, Tat also enhanced translation of mRNA with the mutation in the loop of TAR (Fig. 3*B*, lanes 1 and 2) and to a lesser extent the mutant capable of forming an alternative stem–loop in TAR (lanes 3 and 4), thus differentiating effects

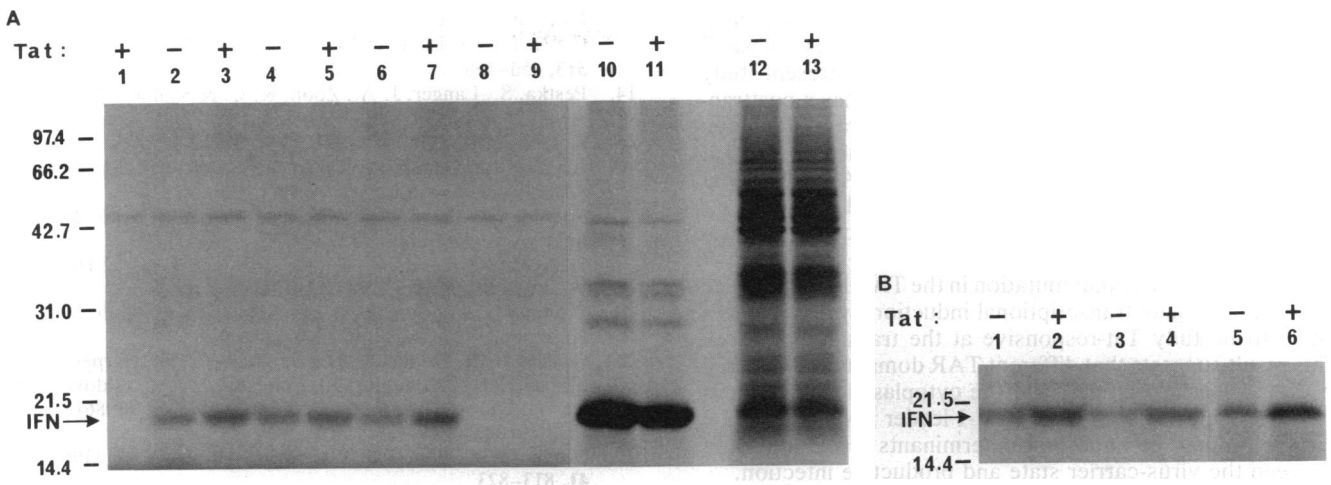

FIG. 3. Tat enhances translation from HIV leader–IFN mRNA. (*A*) Translation was from +81 HIV leader–IFN mRNA (lanes 2 and 3), +231 HIV leader–IFN mRNA (lanes 4 and 5), +289 HIV leader–IFN mRNA (lanes 6–9), IFN mRNA (lanes 10 and 11), and total poly(A)$^+$ RNA from mouse L cells (lanes 12 and 13; 78 μg/ml, a gift of H. Jacobsen, Deutsches Krebsforschungszentrum, Heidelberg). Lane 1 was without added RNA. The *in vitro* synthesized, gel-purified RNA species (28 μg/ml) were either capped (lanes 2–7, 10, and 11) or not capped (lanes 8 and 9). The autoradiograms were produced by exposing film for 24 hr (lanes 1–9) or 11 hr (lanes 10–13). (*B*) Translation was from +231(+31T,+52G) HIV leader–IFN mRNA (lanes 1 and 2) or +231(+52G,3'C1) HIV leader–IFN mRNA (lanes 3 and 4) at 29 μg/ml or from +81 HIV leader–IFN mRNA at 19 μg/ml in the presence of 10 mM 2-aminopurine with a 10-min, 30°C preincubation (lanes 5 and 6). Autoradiograms were exposed for 36 hr (lanes 1–4) or 6 hr (lanes 5 and 6). The presence or absence of Tat (200 ng; 12.5 μg/ml) and the positions of the molecular mass markers (kDa) and IFN (arrow) are indicated. Densitometer scanning resulted in the following relative peak areas. *A*, lanes 1–11: 0, 23, 49, 25, 40, 25, 49, 0, 0, 368, and 296. *B* lanes 1–6: 24, 62, 25, 42, 34, and 74.

of Tat on transcription and translation. Tat was primarily relieving cis-inhibition, because it stimulated translation of the +81 HIV leader–IFN mRNA even in lysate in which the dsRNA-dependent protein kinase was inhibited with 10 mM 2-aminopurine (Fig. 3*B*, lanes 5 and 6). From comparison of the results shown in Figs. 2 and 3, it is apparent that the absence of a 10-min preincubation step in the latter increased translational efficiency and resulted in synthesis of similar levels of IFN from the +81, +231 and +289 hybrid mRNAs (Fig. 3*A*, lanes 2–7).

A dose–response curve for translational stimulation by Tat was generated with the +81 HIV leader–IFN mRNA (Fig. 4). Translation was enhanced up to 4.4-fold in response to added Tat (Fig. 4). A half-maximal response was obtained with 100 ng of Tat, which represented a molar ratio of Tat to RNA of 6.8.

DISCUSSION

Our findings indicate that HIV-1 leader RNA inhibits translation in cis as well as in trans. The trans effect required sequences present in the first 81 nucleotides of the HIV-1 leader with no further inhibition from downstream sequences to nucleotide +289. In contrast, cis-inhibition is most efficient from the complete 289-nucleotide HIV leader, producing a 15-fold-greater reduction in translation rates than seen for the trans-inhibition. When studying the effect of the HIV leader on translation, therefore, it is important to include the complete leader sequence. The observed cis-inhibition is most likely caused by the extensive RNA structure in the HIV-1 leader, which may impair scanning by the 40S ribosome subunit (15) or decrease accessibility of the cap structure (2). On the other hand, the trans effect is largely due to activation of the dsRNA-dependent protein kinase (3, 4). The recent report (35) of downregulation of dsRNA-dependent protein kinase by Tat or HIV infection, however, suggests that HIV has evolved a mechanism for circumventing the kinase. Although the RNA used here and in previous studies (3, 4) was extensively purified, one cannot completely rule out the possibility that the trans effect is due in part to contaminating dsRNA.

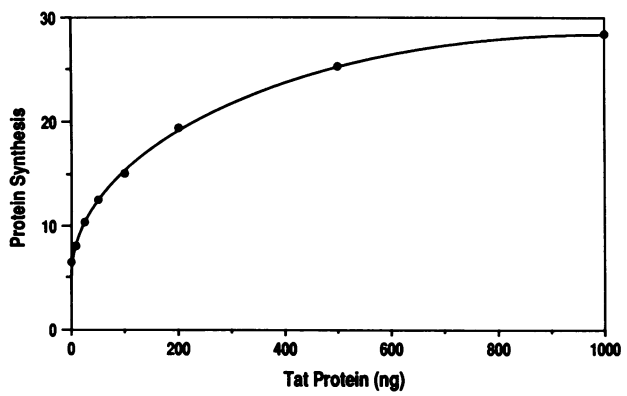

FIG. 4. Dose–response curve for translational trans-activation by Tat. Results were obtained by densitometer scanning from an autoradiogram. IFN was synthesized from the +81 HIV leader–IFN mRNA preincubated with various amounts of Tat.

The enhancement of translation by Tat provides a pathway by which the virus could overcome the inhibitory effect of the RNA leader. The level of translational stimulation by Tat that we obtained was consistent with the 5-fold effect estimated from studies involving intact cells (as reviewed in ref. 23). Although indirect immunofluorescence experiments localized Tat in the nucleolar regions of nuclei (10), the minuscule amounts of Tat needed for full function in the cytoplasm may not be experimentally demonstrable. The mechanism for the stimulation is unknown but may involve melting of secondary structure found at the 5' end of the HIV leader. By promoting, directly or indirectly, unwinding and ribosome scanning of the leader RNA, Tat could overcome both trans- and cis-inhibition of translation. For instance, unwinding could be a consequence of Tat binding to the TAR directly (36) or through a Tat–cellular protein–TAR complex (37). The effect of Tat reported here however, is, mediated primarily through a partial relief of cis-inhibition, since the stimulation occurred even in lysate in which the kinase was inhibited (Fig. 3*B*, lanes 5 and 6).

These findings are consistent with previous observations of a translational component to trans-activation by Tat (5-11).

Several studies have also demonstrated that the predominant effect of Tat is to enhance transcription of genes whose 5′ leaders contain TAR (reviewed in ref. 23). Our present study offers a direct demonstration that Tat action has a posttranscriptional component. In the viral life cycle, trans-activation of transcription would be complemented by the effect of Tat on translation. Although the features of TAR RNA that permit transcriptional trans-activation are well defined (e.g., refs. 11, 12, and 26), the sequence and structural requirements for the translational effect await further studies. However, mRNA with a point mutation in the TAR loop sequence known to abrogate transcriptional induction by Tat (26) was found to be fully Tat-responsive at the translational level. This result suggests that different TAR domains are involved in the nuclear as compared to the cytoplasmic activities of Tat. The opposing actions of HIV-1 leader RNA and Tat on translation may be important determinants in the transition between the virus-carrier state and productive infection.

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