JOURNAL OF VIROLOGY, Apr. 1989, p. 1578–1586
0022-538X/89/041578-09$02.00/0

Vol. 63, No. 4

Stimulation of the Human Immunodeficiency Virus Type 1 Enhancer by the Human T-Cell Leukemia Virus Type I *tax* Gene Product Involves the Action of Inducible Cellular Proteins

E. BÖHNLEIN,[1]† M. SIEKEVITZ,[2] D. W. BALLARD,[1] J. W. LOWENTHAL,[1] L. RIMSKY,[1] H. BOGERD,[1] J. HOFFMAN,[1] Y. WANO,[1]‡ B. R. FRANZA,[3] AND W. C. GREENE[1]*

Departments of Medicine and Microbiology-Immunology, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710[1]; Mount Sinai Medical Center, New York, New York 10029[2]; and Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724[3]

Received 16 August 1988/Accepted 9 December 1988

The human immunodeficiency virus type 1 (HIV-1) preferentially infects CD4$^+$ T lymphocytes and may exist as a latent provirus within these cells for extended periods. The transition to a productive retroviral infection results in T-cell death and clinically may lead to the acquired immune deficiency syndrome. Accelerated production of infectious HIV-1 virions appears to be closely linked to a heightened state of T-cell activation. The transactivator (Tax) protein of the type I human T-cell leukemia virus (HTLV-I) can produce such an activated T-cell phenotype and augments activity of the HIV-1 long terminal repeat. One Tax-responsive region within the HIV-1 long terminal repeat has been mapped to a locus composed of two 10-base-pair direct repeats sharing homology with the binding site for the eucaryotic transcription factor NF-κB (GGGACTTTCC). Tax-expressing Jurkat T cells contain one or more inducible cellular proteins that specifically associate with the HIV-1 enhancer at these binding sites. Microscale DNA affinity precipitation assays identified a Tax-inducible 86-kilodalton protein, HIVEN86A, as one of these HIV-1 enhancer-binding factors. The interaction of HIVEN86A, and presumably other cellular proteins, with the HIV-1 enhancer appears functionally important as oligonucleotides corresponding to this enhancer were sufficient to inpart Tax inducibility to an unresponsive heterologous promoter. These findings suggest that the Tax-inducible cellular protein HIVEN86A plays an important role in the transcriptional activation of the HIV-1 enhancer. These specific protein-DNA interactions may also be important for the transition of HIV-1 from a latent to a productive mode of infection. Furthermore, these findings highlight an intriguing biological interplay between HTLV-1 and HIV-1 through a cellular transcriptional pathway that is normally involved in T-cell activation and growth.

Two distinct pathogenic human retroviruses displaying tropism for CD4$^+$ T cells have now been identified (53). The type I human T-cell leukemia virus (HTLV-I) (38, 39) transforms CD4$^+$ T cells (16) and has been etiologically linked with adult T-cell leukemia (53). In addition to its structural genes (*gag*, *pol*, *env*), HTLV-I contains a pX region that encodes at least two *trans*-acting regulatory proteins, Tax and Rex (14, 53). The 40-kilodalton (kDa) Tax protein (*tat*-I, p40^x, x-lor) transactivates the HTLV-I long terminal repeat (LTR) (12, 21, 35, 41, 44, 46), thereby increasing viral gene expression, while the 27-kDa Rex protein appears importantly involved in the production of structural viral proteins (19, 21). Interestingly, the HTLV-I Tax protein also activates the expression of certain cellular genes (2, 5, 20, 25, 26, 28, 43, 47) including those encoding interleukin-2 (IL-2) (18) and the alpha subunit of the IL-2 receptor (IL-2Rα) (18). The deregulated expression of these two cellular genes may mediate a period of autocrine T-cell growth (18, 28) perhaps contributing to the transformation of T cells mediated by HTLV-I (16).

Human immunodeficiency virus type 1 (HIV-1) also infects CD4$^+$ cells, but unlike HTLV-I, this virus produces profound cytopathic effects within this lymphoid cell population (53, 57). HIV-1 has been identified as the cause of the acquired immune deficiency syndrome (3, 13). HIV-1 also encodes several regulatory gene products including *tat*, *rev*, and *nef* (114, 53). The concerted action of these viral proteins serves to regulate HIV-1 replication and perhaps the course of HIV-1 infection (6, 36, 53). Early after HIV-1 infection, the virus appears to enter a latent or chronically persistent state preceding the development of clinical disease. Similarly, in vitro culture conditions have been defined which result in the eclipse of HIV-1 gene expression (9, 57). In sharp contrast, high levels of viral gene expression are detectable during the lytic phase of infection (7, 53). The augmented production of infectious virions characteristic of this lytic phase leads to the further dissemination of the retrovirus within the CD4$^+$ T-cell population, resulting in the progressive depletion of this critical T-lymphocyte subset (57). Emerging evidence suggests that the state of T-cell activation plays an important role in regulating the state of HIV-1 replication. In this regard, the HIV-1 LTR contains *cis*-acting sequences that are activated by various T-cell mitogens (4, 8, 23, 24, 42, 48, 49, 51) and cytokines such as tumor necrosis factor-α (J. W. Lowenthal, D. W. Ballard, E. Böhnlein, and W. C. Greene, Proc. Natl. Acad. Sci. USA, in press). Furthermore, certain cellular factors (4, 10, 11, 15, 22, 24, 33, 54, 55) and *trans*-acting proteins derived from various DNA viruses (17, 31, 32) have been shown to activate the HIV-1 LTR. Previously, we have demonstrated that the Tax protein of HTLV-I is capable of activating the HIV-1 LTR (48). Interestingly, the HIV-1 LTR contains a directly re-

* Corresponding author.
† Present address: Sandoz, Forchungsinstitut, A-1235 Vienna, Austria.
‡ Present address: Fukui Medical School, Matsuoka, Yoshida-gun, Fukui 910-11, Japan.

peated enhancer element (33, 42, 48, 51) which shares striking homology with the NF-κB-binding sites (45) present in the κ light-chain gene enhancer and a related sequence present in the inducible IL-2Rα promoter (4). Several studies have documented the important role played by this enhancer element in mitogen- and Tax-mediated activation of the HIV-1 LTR (4, 23, 24, 33, 42, 48). In the present study, we explored the biochemical basis for Tax activation of the HIV-1 LTR using Jurkat T-cell lines that constitutively express the 40-kDa Tax protein (52). We demonstrated that Tax induces the expression of at least one cellular protein, HIVEN86A, which in turn specifically binds to the HIV-1 enhancer and presumably contributes to the activation of this transcription unit. These data indicate that the mechanism of Tax induction of HIV-1 is indirect and involves the requisite activation of one or more inducible *trans*-acting cellular factors.

MATERIALS AND METHODS

Plasmid construction. The nested series of *Bal* 31 deletion mutants of the HIV-1 LTR used in these studies has been previously described (48). All oligonucleotides were prepared on a DNA synthesizer (model 380; Applied Biosystems) and purified as previously described (4). Gel-purified oligonucleotides were annealed in 50 mM NaCl–10 mM Tris hydrochloride (pH 7.5)–0.2 mM EDTA and subsequently phosphorylated with T4 polynucleotide kinase (27). The oligonucleotides GATCCAGGGACTTTCC (HIV-R5′), GATCCGGGGACTTTCC (HIV-R3′), and GATCAGGGA CTTTCCGCTGGGGACTTTCCA (HIV-DR) were cloned into the unique *Bam*HI restriction site (−105) (30) in the herpes simplex virus thymidine kinase (TK) promoter (29). Copy number and orientation of the oligonucleotide inserts were determined by DNA sequencing (Sequenase; U.S. Biochemical Corp., Cleveland, Ohio). The pcTAX and pcREX expression plasmids encoding the 40-kDa Tax protein and 27-kDa Rex protein, respectively, have been previously described (40).

Cell culture and transfection assays. All cell lines were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. Jurkat T-cell lines stably expressing sense or antisense *tax* cDNAs were established by electroporation (52). Briefly, a functional HTLV-I pX cDNA encoding the Tax and Rex proteins (14) was inserted downstream of the homologous HTLV-I LTR in either the coding (sense, SpXHF/82-C) or noncoding (antisense, SpXHF/82-NC) orientation (52). These recombinant plasmids also contained the neomycin resistance gene placed under the transcriptional control of the simian virus 40 early region to permit G418 antibiotic selection of stably transfected Jurkat cells (50). Several individual G418-resistant cells were used to establish clonal populations of Tax-expressing Jurkat cells. A complete description of these Jurkat cell lines is provided by Wano et al. (52). The *tax* and anti-*tax* cell lines were transiently transfected with various *cat* reporter plasmids by using DEAE-dextran as previously described (26).

Nuclear extract preparation. Large-scale cell cultures were grown in roller bottles, and cells were harvested at a density of 2×10^5 to 5×10^5 cells per ml. Cell pellets were frozen in liquid nitrogen after a single wash with phosphate-buffered saline. The cells were subsequently thawed on ice in buffer 1 (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.3 mM sucrose, 0.5 mM phenylmethyl sulfonyl fluoride, 0.1 mM EGTA). The sedimented cells (10 min, 1,500 × *g* in a Sorvall RT6000 centrifuge) were resuspended in buffer 1 and homogenized with a loose-fitting Dounce homogenizer (15 strokes). The suspensions were transferred to an Eppendorf tube, and the nuclei were collected during a 40-s centrifugation. The supernatants were discarded, and the resuspended nuclei were then extracted at 4°C with mild agitation in buffer 2 (20 mM HEPES [pH 7.9], 25% glycerol, 0.3 M KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 0.1 mM EGTA; 0.3 ml/10^8 cells). High-molecular-weight debris was removed by centrifugation, and the supernatant was dialyzed for >3 h against at least 100 volumes of buffer 3 (20 mM HEPES [pH 7.9], 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol). Insoluble material was again sedimented in an Eppendorf centrifuge (5 min), and the cleared supernatants were frozen in liquid nitrogen (50-μl aliquots). For certain extracts, normal Jurkat cells were treated with phytohemagglutinin (1 μg/ml) and phorbol 12-myristate 13-acetate (PMA) (50 ng/ml) for 5 h prior to processing (4). The protein concentration of each extract was determined by the Bradford colorimetric assay (Bio-Rad Laboratories, Richmond, Calif.).

DNA binding studies. Routinely, 10 μg of nuclear extract was incubated at room temperature with radiolabeled DNA in a 20-μl final volume of buffer containing 50 mM KCl, 10 mM $MgCl_2$, 50 mM HEPES (pH 7.9), 1 mM EDTA, 1 mM dithiothreitol, 1 mg of bovine serum albumin per ml, 30% glycerol, and 1 μg of poly(dI-dC). Binding reactions were analyzed on 5% nondenaturing polyacrylamide gels as previously described (4, 26). Microscale DNA affinity precipitation assays employing [^{35}S]methionine-radiolabeled Tax cell extracts and biotinylated derivatives of the wild-type or mutated HIV-1 enhancer were also performed as previously described (4, 11).

RESULTS

Activation of HIV-1 LTR in Tax-expressing Jurkat T cells involves the directly repeated κB enhancer element. We have previously reported that the HTLV-I *tax* gene product activates the HIV-1 enhancer in transient cotransfection assays with a *tax* cDNA expression plasmid (48). However, owing to the relatively low transfection efficiency, crude nuclear extracts prepared from such transiently transfected Jurkat T cells proved unsuitable for the analysis of protein-DNA interactions involving the HIV-1 enhancer element. To circumvent this problem, we utilized experimental and control Jurkat T-cell lines stably transfected with HTLV-I pX cDNA positioned in either the coding or the noncoding orientation, respectively (52).

Several lines of evidence indicated that cells stably transfected with the sense pX cDNA expressed a functional Tax protein (52). The sense *tax* lines but not antisense *tax* or parental Jurkat cell lines supported transactivation of the HTLV-I LTR linked to the *cat* reporter gene (Table 1). Furthermore, the sense but not antisense *tax* cell lines constitutively expressed Tax-inducible cellular genes including IL-2Rα, IL-2, and granulocyte-macrophage colony-stimulating factor (52).

The Jurkat *tax*-9 and Jurkat anti-*tax*-10 cell lines were next transfected with *cat* expression plasmids containing a series of 5′ deletions within the HIV-1 LTR (48). Transfection of the full-length HIV-1 LTR construct (−671) resulted in a two- to threefold induction in the Jurkat *tax* cells compared with the control Jurkat anti-*tax* cells (Fig. 1A). Deletion of

TABLE 1. Transactivation of HTLV-I LTR[a]

Cell line	% Transacetylation
Jurkat	1.9
Jurkat anti-*tax*-2	1.6
Jurkat anti-*tax*-10	1.5
Jurkat *tax*-9	12.2
Jurkat *tax*-19	18.0

[a] HTLV-I LTR-*cat* plasmid DNA (47) (5 μg) was transfected into parental Jurkat cells, Jurkat anti-*tax*-2 or -10 cells, and Jurkat-*tax*-9 and -19 cells with DEAE-dextran (26, 47). CAT assays were performed as previously described (34). Percent transacetylation shown represents levels of conversion occurring after 60 min at 37°C. Similar results were obtained in four independent experiments.

the LTR to nucleotide −278 resulted in an absolute increase in chloramphenicol acetyltransferase (CAT) activity; however, the fold increase induced by Tax remained essentially unchanged. Greater absolute levels of CAT activity as well as increased fold induction were observed following further recession of the LTR to nucleotide −176. The highest levels of CAT activity and Tax induction were obtained with the deletion mutants terminated 5′ at nucleotides −117 and −103. Deletion to nucleotide −65, which removes both of the direct repeats (transcriptional enhancer) and two of the three Sp1-binding sites (22), resulted in a sharp decline in basal and Tax-induced CAT activity. This pattern of induction in the Jurkat *tax*-9 cell line closely paralleled results obtained in the previous transient *tax* cotransfection experiments (48) and was consistent with an involvement of the HIV-1 enhancer (κB motifs) in the induced response.

Since the SpXHF/82-C expression vector which had been used to establish the Jurkat *tax* lines (52) encodes both the 40-kDa Tax and 27-kDa Rex proteins of HTLV-I, we performed additional experiments to investigate whether the Tax protein represented the biologically active species mediating induction of the HIV-1 LTR. Jurkat T cells were transfected with the −117 HIV-1 LTR-*cat* expression vector in conjunction with the pcTAX or pcREX plasmid. As previously described (40), the pcTAX vector encodes the

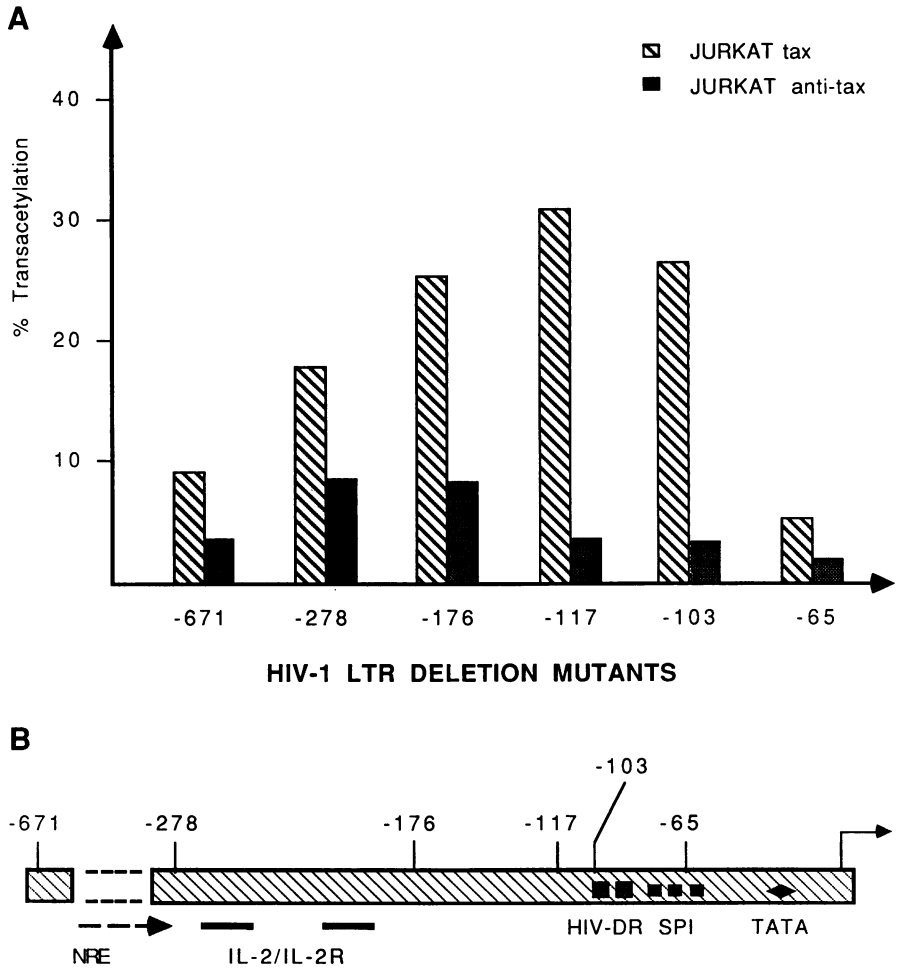

FIG. 1. Transcriptional activation of 5′-deleted forms of the HIV-1 LTR in Jurkat T cells stably expressing sense or antisense *tax* cDNA. (A) The Jurkat *tax*-9 and Jurkat anti-*tax*-10 cell lines were transfected with a series of 5′ HIV-1 LTR deletion mutants linked to the *cat* reporter gene (48). The 5′ endpoint of each deletion mutant is indicated. CAT expression was measured 48 h later as previously described (26, 34). Data presented correspond to the mean levels of transacetylation obtained in three independent transfection experiments. (B) Schematic of the structure of the HIV-1 LTR. The locations of the TATA box, Sp1 motifs, enhancer (direct repeats), regions of homology shared with the IL-2 and IL-2Rα genes, and negative regulatory element (NRE) (48) are shown. The 5′ endpoint of each HIV-1 LTR deletion mutant studied is also indicated.

TABLE 2. Activation of the −117 HIV-1 LTR is mediated by the HTLV-I Tax gene product[a]

Indicator plasmid	Effector plasmid	Inducer	% Transacetylation
−117 HIV-1 LTR-*cat*			0.45
−117 HIV-1 LTR-*cat*		PMA	2.90
−117 HIV-1 LTR-*cat*	pcTAX		9.50
−117 HIV-1 LTR-*cat*	pcTAX	PMA	14.40
−117 HIV-1 LTR-*cat*	pcREX		0.40
−117 HIV-1 LTR-*cat*	pcREX	PMA	2.00

[a] The pcTAX and pcREX expression plasmids (40) were cotransfected with the −117 HIV-LTR deletion mutant (48) into Jurkat T cells. PMA (50 ng/ml) was added 24 h after transfection. CAT assays were performed 24 h later as previously described (34). The CAT activities presented as percent transacetylation were obtained in assays performed for 60 min at 37°C.

Tax but not Rex protein, while the pcREX vector directs the synthesis of Rex but not Tax. Cotransfection of the pcTAX plasmid led to marked activation of the −117 HIV-1 LTR-*cat* plasmid that was further augmented by stimulation of the cultures with PMA (Table 2). In contrast, no stimulation of the −117 HIV-1 LTR was obtained in the presence of the pcREX expression plasmid. These results confirm that the Tax but not the Rex protein is responsible for activation of the HIV-1 LTR. Furthermore, these various findings indicate that the isogenically matched Jurkat *tax* and Jurkat anti-*tax* cells represent a well-suited experimental system for the study of putative Tax-inducible *trans*-acting factors that interact with the *cis* regulatory elements present in the HIV-1 LTR.

The κB elements of the HIV-1 enhancer confer Tax inducibility upon a heterologous promoter. To examine whether the NF-κB-binding sites in the HIV-1 enhancer alone are capable of mediating Tax induction, we prepared a series of chimeric expression vectors containing the upstream or downstream enhancer motifs as well as the complete reiterated enhancer. The oligonucleotides were inserted as one or two copies in various orientations upstream of the −105 deletion mutant of the Tax-unresponsive herpes simplex virus TK promoter linked to the *cat* reporter gene (30). Table 3 shows the relative CAT activities (percent transacetylation) obtained following the transfection of these recombinant TK-*cat* expression plasmids into both the Jurkat *tax* and anti-*tax* cell lines. All constructs containing the HIV-1 direct repeats proved inducible in the Jurkat *tax*-9 cell line but not in the Jurkat anti-*tax*-10 cell line, suggesting that the observed induction of this heterologous promoter is dependent on the action of a functional Tax protein. Furthermore, single κB elements corresponding to either the upstream (HIV-R5′) or the downstream (HIV-R3′) repeat were sufficient to confer Tax inducibility to the TK promoter. Activation by Tax occurred independently of insert orientation, a property consistent with the enhancerlike activity of these elements. PMA stimulation of Jurkat cells transiently transfected with these plasmids yielded similar stimulatory effects (4). Greater levels of Tax- or PMA-induced CAT activity were generally obtained with recombinant plasmids containing two tandem copies of the inserted oligonucleotides. Similar results have been reported for the κ light-chain enhancer-derived oligonucleotides linked to a c-*fos*–*cat* transcription unit (37).

Tax protein induces cellular protein(s) that specifically interacts with the HIV-1 enhancer. Nuclear extracts from Jurkat *tax* cell lines were subsequently prepared to study the interaction of inducible cellular factors with the HIV-1

TABLE 3. HIV-1 oligonucleotides are sufficient to confer Tax inducibility on the herpes simplex virus TK promoter[a]

Plasmid	Insert	Orientation	CAT activity		Fold induction
			Jurkat *tax*-9	Jurkat anti-*tax*-10	
DR 3	HIV-1 direct	→→	66.8	7.9	8.5
DR 6	repeat	→	26.8	6.5	4.1
DR 7		←	11.0	0.8	13.8
UN 21	HIV-1 5′	←←	28.1	1.3	21.9
UN 26	repeat	→→	8.9	1.1	8.0
UN 85		←	15.4	4.4	3.5
UN 87		→	6.8	2.1	3.2
DN 12	HIV-1 3′	←←	28.9	4.3	6.7
DN 80	repeat	→	11.3	3.0	3.8
DN 312		←	17.7	4.0	4.4
TK-*cat*	None		3.4	3.0	1.2

[a] The various recombinant TK-*cat* plasmids are indicated in the left column. Sequences corresponding to the HIV-DR, HIV-R5′, and HIV-R3′ inserts are given in the legend to Fig. 2. Insert copy number and orientation (determined by DNA sequencing) are shown by arrows in column 3. The mean CAT activities (percent transacetylation) obtained after DEAE-dextran transfection (26) of the Jurkat *tax*-9 and Jurkat anti-*tax*-10 cell lines (52) are presented in column 4. Fold induction of CAT activity in the *tax* versus anti-*tax* cell lines is shown in the last column.

enhancer. Initially, oligonucleotides containing both direct repeats (HIV-DR) were used as radiolabeled probes in gel retardation assays. Nuclear extracts from the Jurkat *tax*-9 cell line mediated the formation of two discrete protein-DNA complexes with this HIV-DR probe (Fig. 2, lane 4). Negligible amounts of these complexes were detected with extracts from the Jurkat anti-*tax*-10 cell line (lane 3), suggesting that these nucleoprotein complexes involved Tax-inducible cellular DNA-binding activities. Nuclear extracts from phytohemagglutinin- and PMA-induced (lane 2) but not uninduced (lane 1) Jurkat cells yielded two similarly retarded complexes, raising the possibility that the Tax-induced interactions involved a host factor(s) rather than Tax itself. Competition experiments confirmed the specific nature of these induced protein-DNA interactions. Preincubation with a 100-fold molar excess of unlabeled oligonucleotides corresponding to either one (HIV-R5′, HIV-R3′; Fig. 2, lanes 5 and 6) or both (HIV-DR, lane 10) κB sequence motifs blocked the formation of the Tax-induced protein-DNA interactions (Fig. 2, lane 4). Oligonucleotides mutated in only one of the two κB elements (HIV-DR-M1, HIV-DR-M2) also efficiently competed for the binding of this nuclear factor(s) (Fig. 2, lanes 7 and 8). In contrast, the addition of oligonucleotides containing mutations within both κB motifs (HIV-DR-DM, GGG→CTC) failed to interfere with the formation of these protein-DNA complexes (Fig. 2, lane 9). Pretreatment with an IL-2Rα promoter-derived oligonucleotide containing a sequence homologous to the κB motif (GATCCGGCAGGGGAATCTCCCT) also inhibited the formation of these specific complexes (Fig. 2, lane 11). In contrast, a mutated form of the IL-2Rα oligonucleotide (GGG→CTC) (Fig. 2, lane 12) or sequences from the coding region of the β-lactamase gene (*amp*) (Fig. 2, lane 13) did not compete for complex formation.

To further study the nature of the two complexes, the κB-like 16-base-pair oligonucleotides containing the upstream or downstream κB element, which were sufficient to confer Tax inducibility upon the −105 TK promoter (30),

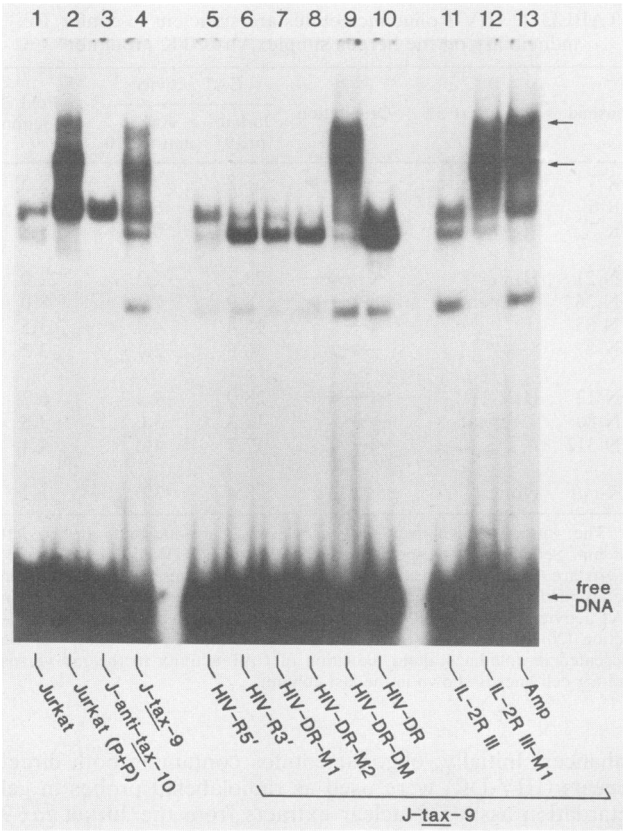

FIG. 2. Sequence-specific interactions of Tax-induced nuclear proteins with the κB motifs of the HIV-1 enhancer. Lanes 1 through 4 show control gel retardation assays performed with the radiolabeled HIV-DR oligonucleotide and the indicated nuclear extracts. A 5-h stimulation of Jurkat (J) cells with PMA and phytohemagglutinin is indicated (P + P). Preincubation with a 100-fold molar excess of unlabeled HIV-R5′ (lane 5), HIV-R3′ (lane 6), HIV-DR-M1 (lane 7), HIV-DR-M2 (lane 8), or HIV-DR (lane 10) oligonucleotides blocked the formation of the specific complexes with Jurkat *tax*-9 extracts and radiolabeled HIV-DR probe (indicated by arrows). The IL-2Rα-derived IL-2R III oligonucleotide (4), which contains a related κB element, also competed for the binding of these nuclear factors (lane 11). In contrast, addition of an HIV-DR oligonucleotide containing mutations in both κB elements (HIV-DR-DM, lane 9) or a mutated version of the IL-2R III oligonucleotide lacking an intact κB site (IL-2R III-M1, lane 12) failed to compete. Size-matched control oligonucleotides from the β-lactamase gene similarly did not block complex formation (lane 13). Additional nonspecific DNA-protein complexes were also detected that were not competed for by unlabeled homologous oligonucleotides (see lane 4 versus lane 10).

HIV-DR	gatcAGGGACTTTCCGCTGGGGACTTTCCa
HIV-DR-M1	gatcAc t c ACTTTCCGCTGGGGACTTTCCa
HIV-DR-M2	gatcAGGGACTTTCCGCTGc t c ACTTTCCa
HIV-DR-DM	Ac t c ACTTTCCGCTGc t c ACTTTCC
HIV-R5′	gatccAGGGACTTTCC
HIV-R3′	g a t c cGGGGACTTTCC

The wild-type or mutated version of each κB motif is underlined. Capital letters indicate authentic HIV-1 LTR-derived nucleotides. Mutations within the underlined κB motifs or linker sequences added at the ends for cloning purposes are indicated by lower-case letters.

were used as radiolabeled substrates in gel retardation assays. Both the radiolabeled HIV-R5′ and HIV-R3′ oligonucleotides formed a single specific complex with nuclear protein(s) isolated from Jurkat *tax*-9 cells or mitogen-induced Jurkat cells (Fig. 3, lanes 2, 4, 6, and 8). A size-matched oligonucleotide corresponding to the IL-2Rα κB element and its homologous flanking sequences (IL-2RNF) yielded a similarly retarded complex (Fig. 3, lanes 10 and 12). Competition experiments revealed that both the upstream and downstream HIV-1 κB oligonucleotides efficiently competed for the binding of the *trans*-acting factor(s) to the counterpart probe (data not shown). Only trace amounts of these Tax- and mitogen-inducible factors were detected in nuclear extracts from Jurkat anti-*tax* cells or uninduced normal Jurkat T cells (Fig. 3, lanes 1, 3, 5, 7, 9, and 11). Together, these results suggest that the same inducible *trans*-acting factors(s) binds to the upstream (HIV-R5′) and downstream (HIV-R3′) motifs of the HIV-1 enhancer in vitro.

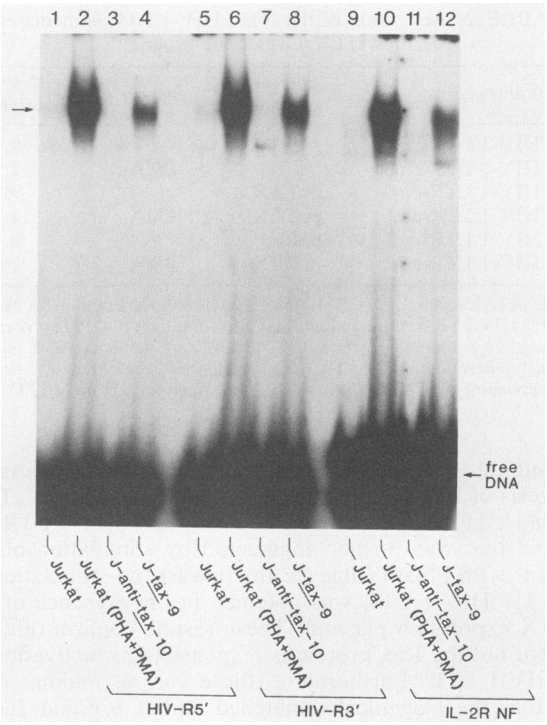

FIG. 3. The κB sequence is sufficient for the binding of Tax- and mitogen-induced cellular protein(s). Radiolabeled HIV-R5′ (lanes 1 to 4), HIV-R3′ (lanes 5 to 8), and IL-2RNF (GATCCGGCACGGGAATCTCCCT, lanes 9 to 12) oligonucleotides were incubated with nuclear extracts prepared from the indicated Jurkat (J) T cells. The specific protein-DNA complex is indicated by an arrow on the left, while free DNA is indicated by the arrow on the right. PHA, Phytohemagglutinin.

An 86-kDa cellular protein is induced by Tax and binds to the κB elements of the HIV-1 enhancer. To identify Tax-induced cellular proteins interacting with the HIV-1 enhancer, we performed microscale DNA affinity precipitation assays (4, 10, 11). These studies revealed the interaction of multiple isoforms of the inducible 86-kDa cellular protein (HIVEN86A) with the HIV-DR probe in the Jurkat *tax*-9 extracts (Fig. 4B). In contrast, much smaller amounts of this protein were recovered from nucleoprotein precipitations with extracts from the Jurkat anti-*tax*-10 cells (Fig. 4). As previously described (11), oligonucleotides mutated in the κB sequence motif failed to bind HIVEN86A (data not shown). Interestingly, a 72-kDa heat shock protein (HS72K) with nonspecific DNA-binding activity also appeared to be selectively induced in the Tax-expressing Jurkat cell line. A

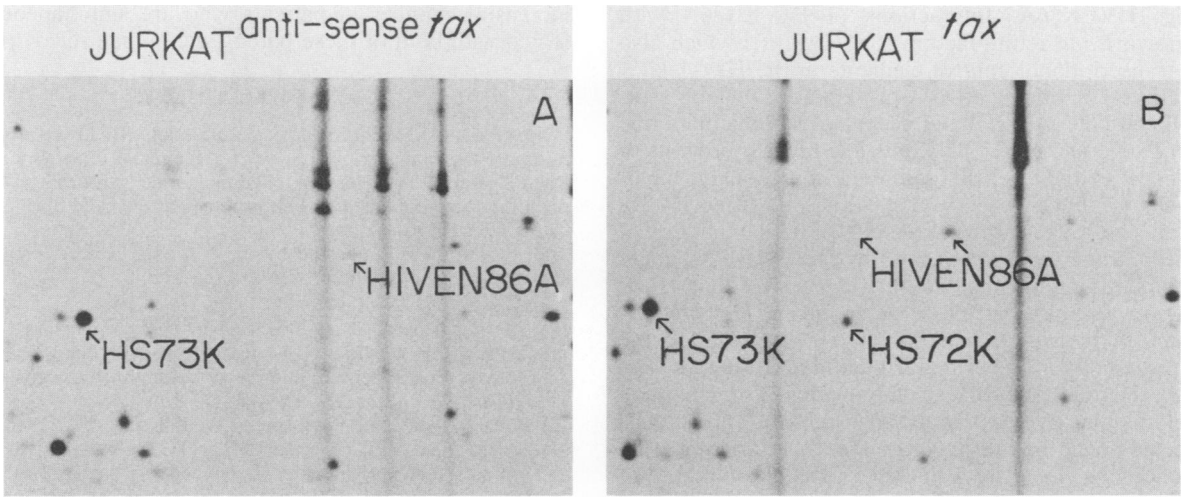

FIG. 4. HIVEN86A cellular protein is induced in Tax-expressing Jurkat cells. The microscale DNA affinity precipitation assays were performed as previously described (4, 10, 11). Biotinylated HIV-DR oligonucleotides were used to specifically precipitate [^{35}S]methionine-radiolabeled nuclear proteins from Jurkat anti-*tax*-10 (A) or Jurkat *tax*-9 (B) cells. The different isoelectric forms of HIVEN86A in the two-dimensional gel are indicated by arrows. The routinely precipitated heat shock protein (HS73K) as well as the apparently Tax-inducible heat shock protein (HS72K) are also identified.

second sequence-nonspecific heat shock protein (HS73K) was detected in both the Jurkat *tax* and anti-*tax* extracts, thus serving as an internal control for the efficiency of protein recovery. These results demonstrated that HIVEN86A represents one Tax-inducible cellular protein that specifically binds to the κB motifs present in the HIV-1 LTR.

DISCUSSION

Jurkat T-cell lines stably expressing sense and antisense HTLV-I-derived pX cDNAs encoding both the Tax and Rex proteins (14, 52) were used to study the molecular basis for transcriptional activation of the HIV-1 enhancer repeats by the Tax protein. The differential activation of the HTLV-I LTR-*cat* plasmid after transfection in the sense but not antisense cell lines confirmed the production of biologically active Tax protein in these cells. Transient transfection of deleted forms of the HIV-1 LTR into the Jurkat *tax* and anti-*tax* cell lines revealed selective activation of this heterologous viral regulatory region in the sense Jurkat *tax* cell lines, confirming previous cotransfection experiments (48). Studies with expression vectors uniquely encoding only the Tax or Rex retroviral protein demonstrated that the Tax gene product was responsible for activation of the HIV-1 LTR. The relative responses of LTR deletion mutants implicated the HIV-1 enhancer (direct repeat) as a critical *cis* element involved in the Tax-induced response. Furthermore, HIV-1-derived oligonucleotides corresponding to either one or both of the κB sequence motifs of the HIV-1 enhancer proved sufficient to confer Tax inducibility upon a heterologous promoter.

Nuclear extracts prepared from the Tax-expressing Jurkat cells were found to mediate the formation of two specific protein-DNA complexes with the complete HIV-1 enhancer. Interestingly, nuclear extracts from mitogen-induced Jurkat cells also yielded two similarly retarded protein-DNA complexes with this radiolabeled probe. Single complexes were detected with probes corresponding to each of the individual κB elements (HIV-R5′, HIV-R3′). In contrast, these specific protein-DNA complexes were virtually undetectable when the same substrates were incubated with nuclear proteins prepared from either uninduced Jurkat cells or the control Jurkat anti-*tax* cells, underscoring the Tax inducibility of the involved cellular factors.

Radiolabeled oligonucleotides derived from the IL-2Rα gene, which contains a functional and related κB-like element (2, 4), formed similar DNA-protein complexes with extracts from Tax- or mitogen-induced Jurkat cells. These results provided further support for the involvement of the κB sequence motifs in the activation of the HIV-1 LTR. Competition experiments directly confirmed the involvement of the κB sequence in the formation of these specific complexes. Unlabeled oligonucleotides spanning either the upstream or downstream κB elements, as well as HIV-DR derivatives that had been mutated in one of the reiterated motifs, competed effectively for the specific binding of host cell factors. However, the introduction of mutations at both of the HIV κB sequences rendered these DNA molecules ineffective as competitors. Studies with the shorter oligonucleotides (HIV-R5′, HIV-R3′) revealed effective binding to each κB sequence. In contrast to previous reports (15, 54), competition experiments with these probes revealed that the two κB elements were functionally equivalent and that each motif appeared to bind the same cellular factors(s) in vitro. Additionally, the transient transfection experiments employing the chimeric TK-*cat* expression plasmids indicated the functional importance of these reiterated sequences in vivo.

Microscale DNA affinity precipitation assays were performed to identify Tax-induced cellular proteins that specifically interacted with the HIV enhancer. These studies revealed an 86-kDa protein in Tax-expressing cells that was indistinguishable in size and isoelectric point heterogeneity from the previously described HIVEN86A polypeptide (11). HIVEN86A expression is also induced by T-cell mitogens and has been previously shown to bind to the κB motifs present in the HIV-1 LTR and the IL-2Rα promoter (4, 11). Recent results indicate that Tax activation of the IL-2Rα gene also involves the induction of cellular *trans*-acting factors interacting with the κB-like element (2, 25, 43)

including HIVEN86A. Interactions of HIVEN86A with other known *trans*-acting factors (Sp1, NF-κB) which also bind to transcriptional control elements in the HIV-1 LTR are quite likely. Utilizing a DNA-protein cross-linking technique, Ballard et al. (2) have recently demonstrated the binding of several distinct Tax-inducible nuclear proteins to the κB motif of the IL-2Rα promoter. Interestingly, major protein-DNA adducts in the 80- to 90- kDa and 50- to 55-kDa molecular size ranges were detected. A similar pattern of inducible proteins appears to bind to the HIV-1 enhancer (D. W. Ballard, unpublished data). It is possible that the 80 to 90-kDa cross-linked species corresponds to HIVEN86A, while the 50- to 55-kDa species may be identical to the NF-κB protein recently purified from human Burkett's lymphoma B cells (24). Microscale DNA affinity precipitation assays employing lysates from PMA-induced Jurkat cells radiolabeled under long-term (4 h) rather than short-term (30 min) conditions have revealed the specific binding of HIVEN86A as well as 71-, 65-, and 51-kDa proteins to the κB motifs of the HIV-1 enhancer and IL-2Rα promoter (10; Ballard, unpublished data). Wu and colleagues (55) reported the purification of a 63-kDa polypeptide which specifically interacts with the κB motifs in the HIV-1 LTR. These findings suggest that HIVEN86A represents one of multiple polypeptides that specifically recognize the HIV-1 enhancer. The interactions between these various *trans*-acting factors as well as their functional contribution to the HIV-1 expression remain to be resolved.

The ability of HIV-1 to utilize various inducible host cell transcription factors, including HIVEN86A and NF-κB, provides a potential explanation for the elevated levels of viral replication observed in activated T cells. Although HIV-1 enhancer activity is not solely restricted to T lymphocytes, the HIVEN86A protein and other *trans*-acting factors may be more abundantly expressed in T cells than in other cell types. In this regard, it will be of interest to study HIVEN86A expression in monocytes, as these cells uniquely appear to serve as a major cellular reservoir for latent or persistent forms of HIV-1.

In summary, Tax activation of the HIV-1 LTR involves inducible cellular transcription factors, including HIVEN86A, which specifically interact with the κB motifs present in the HIV-1 enhancer. Recently, Zack and colleagues (56) have reported transcriptional activation of the HIV provirus following HTLV-I infection of peripheral blood T cells. We suggest that this result reflects HTLV-I-mediated induction of specific cellular *trans*-acting factors, including HIVEN86A and NF-κB. Interestingly, both HTLV-I and HIV-1 interface with the same cellular transcriptional factors through convergent pathways. The Tax gene product of HTLV-I induces a κB-specific host cell transcription factor(s) (2, 25, 43) that then contributes to the activation of κB-dependent transcription units including the cellular genes encoding IL-2Rα and IL-2 (2, 26, 47, 52; B. Hoy, unpublished data). The deregulated expression of these cellular genes may induce an unrestricted period of proliferation of these virally infected cells which could be involved in leukemic transformation. Intriguingly, HIV-1 is uniquely adapted to respond to this same set of inducible cellular *trans*-acting factors owing to the presence of κB enhancer elements in the retroviral LTR. The interaction of HIVEN86A and additional cellular factors with the regulatory elements located in the LTR augments HIV-1 retroviral gene expression. This unusual molecular interplay between HTLV-I and HIV-1 may be of clinical importance in the increasing number of patients who are simultaneously infected with both of these pathogenic human retroviruses.

ACKNOWLEDGMENTS

We thank M. Malim for critical comments, B. Kissell for preparation of the manuscript, J. Ross for technical assistance, and R. Randall and K. Theisen for oligonucleotide synthesis. The high-resolution two-dimensional gels were run at the Cold Spring Harbor Quest laboratory.

B. R. Franza is supported by the National Cancer Institute and the American Foundation for AIDS research.

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