HIV-1, HTLV-I and the Interleukin-2 Receptor: Insights into Transcriptional Control

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A. Introduction

T-cell proliferation is regulated by the induced expression of the cellular genes encoding the T-cell growth factor interleukin 2 (IL-2) and the alpha subunit of the high-affinity IL-2 receptor (IL-2Rα, p55, Tac) [1]. Infection of $CD4^+$ T-lymphocytes by the type I human T cell leukemia virus (HTLV-I), the etiologic agent of adult T cell leukemia (ATL) [2], leads to the deregulation of IL-2Rα gene expression. Virtually all ATL cell lines thus far examined have been found to constitutively display a large number of high- and low-affinity receptors for IL-2. This response appears to involve an action of the 40-kDa transactivator gene product (*tax*, tat-1, $p40^x$) encoded within the pX region of this retrovirus [3, 4]. Transient cotransfection assays have revealed that *tax* markedly augments the activity of the IL-2Rα promoter and partially activates the IL-2 promoter in Jurkat T cells [5–9]. In addition to this effect on these cellular genes, *tax* is also capable of activating the transcriptional enhancer located within the long terminal repeat (LTR) of type 1 human immunodeficiency virus (HIV-1) [10]. In contrast to HTLV-I, infection of $CD4^+$ T cells with HIV-1 leads to cell death and is clinically associated with the acquired immune deficiency syndrome (AIDS) [2]. Like *tax*, various T-cell mitogens including phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) activate both the IL-2Rα promoter and the LTR of HIV-1 [10, 11]. Sequence analysis of the IL-2Rα promoter has revealed the presence of a 12 base pair (bp) element (GGGGAATCTCCC) [11] that shares significant homology with the NF-κB binding site present as a duplicated element in the HIV-1 enhancer [12] and as a single motif in the immunoglobulin κ chain gene enhancer [13]. These findings raised the possibility that a common regulatory intermediate may bind to these conserved sequences and play a role in the induced expression of these cellular and viral genes. We now describe transient transfection studies with 5′ deletion and site-specific mutants of the IL-2Rα promoter, establishing that the κB motif is importantly involved in both *tax* and mitogen inducibility. Furthermore, using gel retardation [14, 15] and DNA footprinting [16] assays, we demonstrate that both *tax* and T-cell mitogens induce the expression of DNA-binding protein(s) that specifically bind to this 12-bp IL-2Rα promoter sequence as well as to the transcriptional enhancer of HIV-1 [17]. Microscale DNA-affinity precipitation assays [18] performed with biotinylated oligonucleotides derived from the IL-2Rα promoter have permitted identification of one of these proteins as an 86-kDa cellular factor termed HIVEN86A [11, 18–20]. Taken together, these results suggest that the inducible cellular protein, HIVEN86A, plays a central role in the transcriptional regulation of both the IL-2Rα gene and the HIV-1 LTR me-

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diated by either T-cell mitogens or the *tax* protein of HTLV-1.

B. Materials and Methods

I. Cell Culture and Transfection Assays

All cell lines were grown in RPMI-1640 media supplemented with 10% fetal calf serum. Jurkat cell lines stably expressing sense or anti-sense *tax* cDNA were established by electroporation of the respective expression plasmids and subsequent selection for G418 antibiotic resistance [19]. Transient transfection experiments using DEAE dextran were performed as previously described [8]. The nested series of 5′ deletion mutants of the IL-2Rα promoter linked to the chloramphenicol acetyltransferase (CAT) reporter gene was prepared as previously described [9].

II. DNA-Protein Binding Assays

Nuclear extracts were prepared from large-scale cultures of cells grown at 2–5×10^5 cells/ml. The cell pellets were routinely frozen in liquid nitrogen after a single wash in 1 × PBS. The cells were thawed on ice in 1 ml buffer I (10 m*M* HEPES of pH 7.9, 1.5 m*M* $MgCl_2$, 10 m*M* KCl, 0.5 m*M* DTT, 0.3 m*M* sucrose, 0.5 m*M* PMSF, 0.1 m*M* EGTA). The cells were then repelleted, homogenized (15 strokes with a loose-fitting Dounce homogenizer), and the nuclei were collected in an Eppendorf centrifuge. The nuclei were then extracted at 4° C with mild agitation in buffer II (20 m*M* HEPES of pH 7.9, 25% glycerol, 0.3 *M* KCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* EDTA, 0.5 m*M* PMSF, 0.5 m*M* DTT, 0.1 m*M* EGTA; 0.3 ml/10^8 cells). Debris was removed by centrifugation, and the supernatant was dialyzed for over 3 h against at least 100 volumes of buffer III (20 m*M* HEPES of pH 7.9, 20% glycerol, 100 m*M* KCl, 0.2 m*M* EDTA, 0.5 m*M* PMSF, 0.5 m*M* DTT). Insoluble material was pelleted (5 min) and 50-µl aliquots were frozen in liquid nitrogen. Protein concentration was determined using the Bio-Rad colorimetric assay. Routinely, 2–10 µg nuclear extract was incubated with radiolabeled DNA at room temperature in the presence of 50 m*M* KCl, 10 m*M* $MgCl_2$, 50 m*M* HEPES of pH 7.9, 1 m*M* EDTA, 1 m*M* DTT, 1 mg/ml BSA, 30% glycerol, and lug poly(d[I-C]) (20 µl final volume). Reaction mixtures were then analyzed by electrophoresis in low ionic strength polyacrylamide gels as previously described [11]. For the in situ DNA footprinting assays, the binding mixture was increased five fold, and the DNA-protein complexes were subsequently separated on 5% nondenaturing polyacrylamide gels. These gels were then exposed to 1,10 phenanthroline copper for 7–12 min, and the samples were purified as described by Ballard et al. [20]. The microscale DNA-affinity precipitation assays were performed as previously reported [11, 18].

C. Results and Discussion

I. Mapping of Functional *cis*-Acting Elements Involved in the IL-2Rα Promoter Activation

Transient transfection of various IL-2Rα promoter deletion mutants revealed that upstream sequences located between nucleotides −281 and −248 were required for mitogen (PMA, 50 ng/ml) induction (Fig. 1). Similarly, an overlapping promoter segment was shown to be essential for activation of the IL-2Rα promoter by the HTLV-I derived *tax* gene product (Fig. 1). Identical results were obtained with transient cotransfection of *tax* cDNA expression plasmids and with transfection of Jurkat cells stably expressing the *tax* protein [19, 20]. In contrast, transfection of the IL-2Rα promoter-CAT plasmids in uninduced Jurkat T-cells or control Jurkat cell lines expressing an anti-sense *tax* cDNA produced only basal levels of CAT activity. As noted above, a κB-like binding site is present between nucleotides −267 and −256 of the IL-2Rα promoter. Site-di-

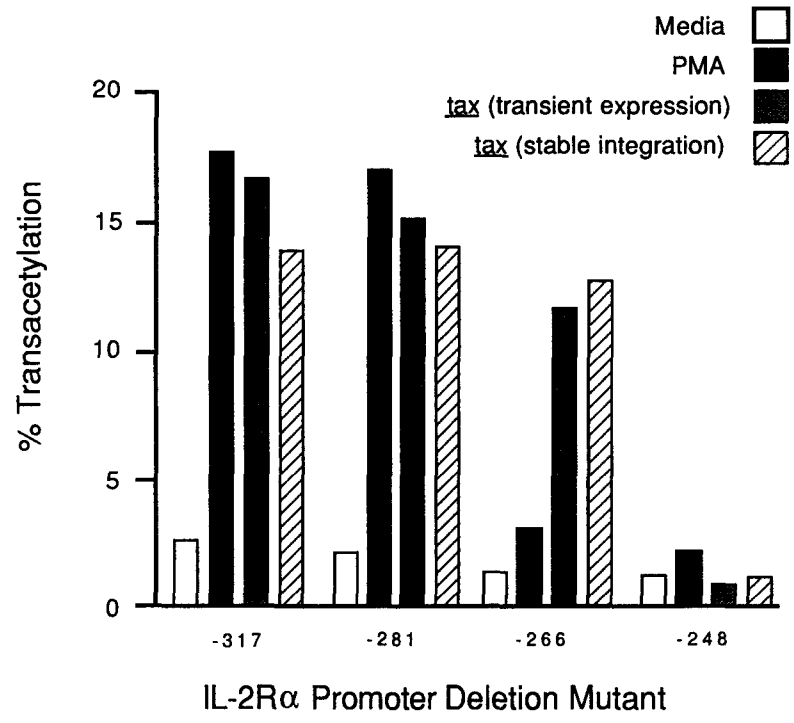


Fig. 1. PMA and *tax* stimulation of 5′ IL-2Rα deletion mutants linked to the CAT indicator gene. IL-2Rα-CAT deletion mutants [9], terminating 5′ at the nucleotide position indicated, were transfected into Jurkat T cells with DEAE dextran as previously described [9]. CAT activity, expressed as percent transacetylation, was measured after 48 h culture [22]. Transcriptional activity (measured by CAT production) obtained with uninduced Jurkat cell extracts (*open bars*) was similar to the level present in anti-*tax* Jurkat cells (data not shown). Stimulation induced by PMA (50 ng/ml) is shown in the *closed bars*, effects of cotransfection of *tax* cDNA in the *dotted bars*, and results in the stable *tax* cell line are shown in the *hatched bars*

rected deletion of this κB element from the active −317 IL-2Rα promoter construct produced a marked decrease in mitogen and *tax* inducibility in Jurkat T cells (data not shown), suggesting that this motif was importantly required for promoter activation. To test whether the κB element was sufficient to support *tax* and mitogen activation of a heterologous promoter, 47-bp or 18-bp oligonucleotides containing this sequence [11] were linked to the thymidine kinase (TK) promoter [21] upstream of the CAT reporter gene. Transfection of these modified TK CAT plasmids into Jurkat cells revealed marked inducibility by either *tax* or T-cell mitogens (Table 1). These oligonucleotides were effective in either orientation, and amplified effects were observed when the binding site was reiterated. Control plasmids containing various mutations in the κB site (IL-2R III M1, IL-2R VII M1, [20]) failed to confer mitogen or *tax* inducibility to the TK promoter (Table 1), further implicating the κB sequence in IL-2Rα promoter induction. Together, these results suggest that the κB-like sequences are both necessary and sufficient for IL-2Rα promoter activation by either *tax* or mitogens. However, these two inducers appear to differ with respect to the requirement for additional sequences flanking the κB element for maximal transcriptional activation (see Fig. 1; [7, 9]).

II. Inducible Nuclear Proteins Bind to the κB Element of the IL-2Rα Promoter

To study the potential interactions of cellular proteins with the κB element of the IL-2Rα promoter, oligonucleotides that conferred *tax* and mitogen inducibility upon the TK promoter were analyzed in gel retardation assays. Nuclear extracts prepared from Jurkat cells induced with

Table 1. IL-2Rα promoter oligonucleotides containing the κB element are sufficient to confer PMA and *tax* inducibility to the TK promoter

Insert	Orientation	J-*tax*-9	J-anti-*tax*-10	Jurkat + PMA
None		1.5	1.3	1.4
IL-2R III	→	10.8	1.1	6.6
	←	16.2	1.4	7.3
	←→	51.8	1.4	29.5
IL-2R III M1	→	2.1	1.7	1.8
	←	1.4	1.1	1.4
	→→	1.8	1.0	1.6
IL-2R VII	→	14.3	1.3	7.2
	←	10.7	1.1	6.3
	→→	28.8	1.5	20.5
	←←	34.2	1.4	22.7
IL-2R VII M1	→	1.7	0.9	1.5
	→→	1.2	1.1	1.5
	←←	1.5	1.0	1.2

Double-stranded oligonucleotides (IL-2R III, −291 to −245; IL-2R III M1, GGG to CTC substitution at −266 to −264 within the κB element; IL-2R VII, −272 to −255; IL-2R VII M1, four substitutions in κB element, see [20]) were inserted upstream of the TK promoter (nucleotide −105) linked to the CAT reporter gene. Arrows denote orientation of the inserts, and copy number is indicated by the number of arrows. Transfections were performed as described in Fig. 1. CAT conversion values for the parental TK CAT construct without an insert are shown in the first line.

PMA (50 ng/ml) and PHA (1 μg/ml) for 5 h yielded two specific DNA-protein complexes with a radiolabeled 47-bp oligonucleotide designated IL-2R III (−291 to −245; Fig. 2A, lane 3). In contrast, nuclear extracts prepared from unstimulated cells failed to mediate detectable complex formation. Analysis of the Jurkat cell lines stably expressing the *tax* gene product also yielded two similarly retarded complexes (Fig. 2B, lanes 3, 4). In constrast, these complexes were not detected with extracts prepared from two independent Jurkat anti-*tax* cell lines (Fig. 2, lanes 1, 2). Competition studies using oligonucleotides mutated in the κB site did not inhibit complex formation while wild-type unlabeled DNA probes competed effectively (data not shown). These findings indicated that the observed DNA-protein complexes were not only sequence specific, but also that the κB element was involved in protein binding. In situ DNA-footprinting experiments confirmed protein contacts over the κB region using nuclear extracts from either *tax*- or mitogen-induced Jurkat cells (Fig. 3). Furthermore, the sequences protected in DNA footprinting experiments with PMA- and *tax*-activated Jurkat nuclear extracts proved virtually identical.

To characterize the protein(s) interacting with this site within the IL-2Rα promoter, microscale DNA-affinity precipitation assays [18] were performed. Consistent with the gel retardation profiles, an 86-kDa cellular protein, HIVEN86A, was detected with the biotinylated IL-2R III probe in extracts obtained from either PHA + PMA induced Jurkat T cells or the *tax*-producing Jurkat cell lines. In contrast, this protein was not detectable in extracts from Jurkat anti-*tax* or unstimulated Jurkat cells (Fig. 4). Binding of this same inducible

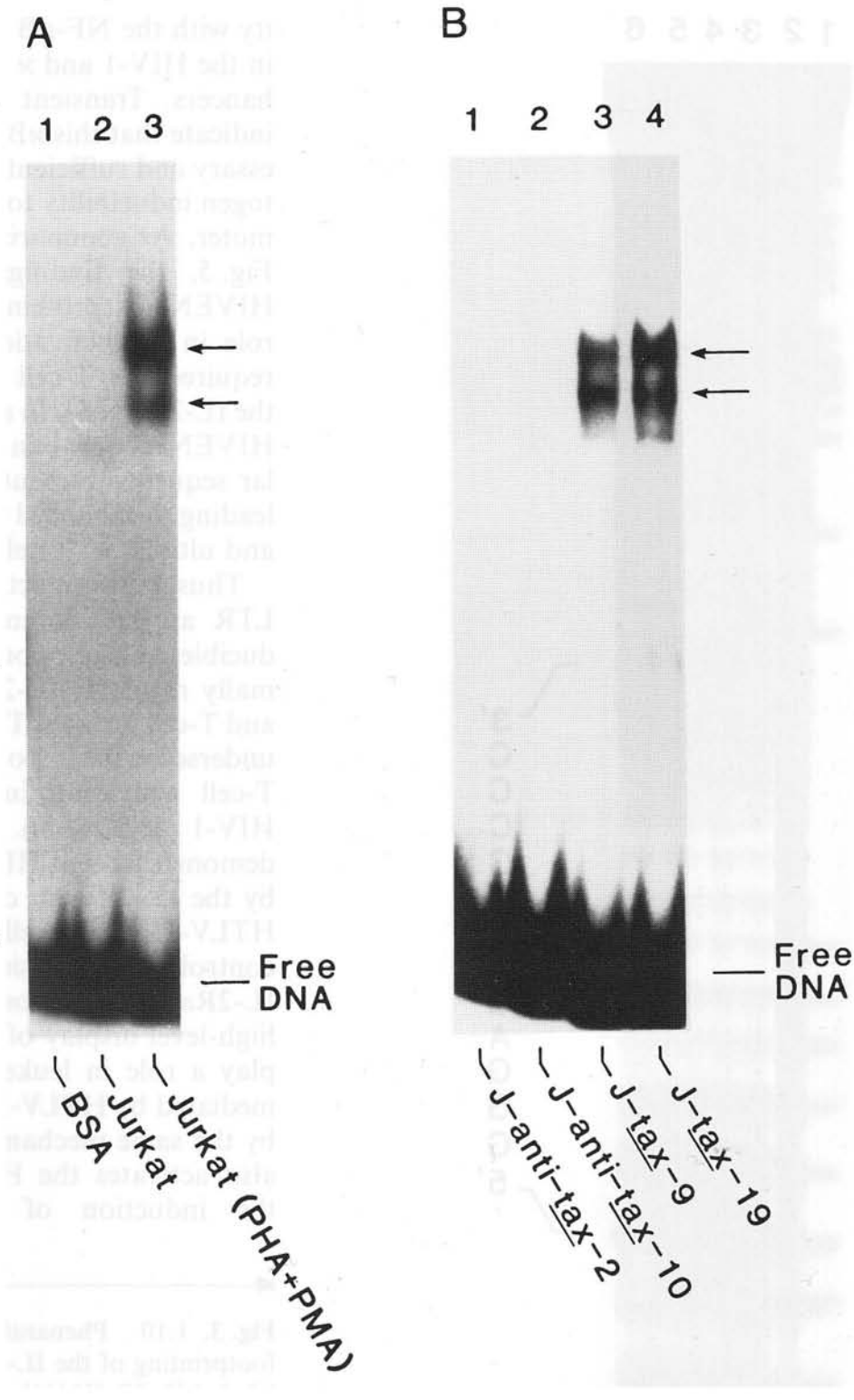
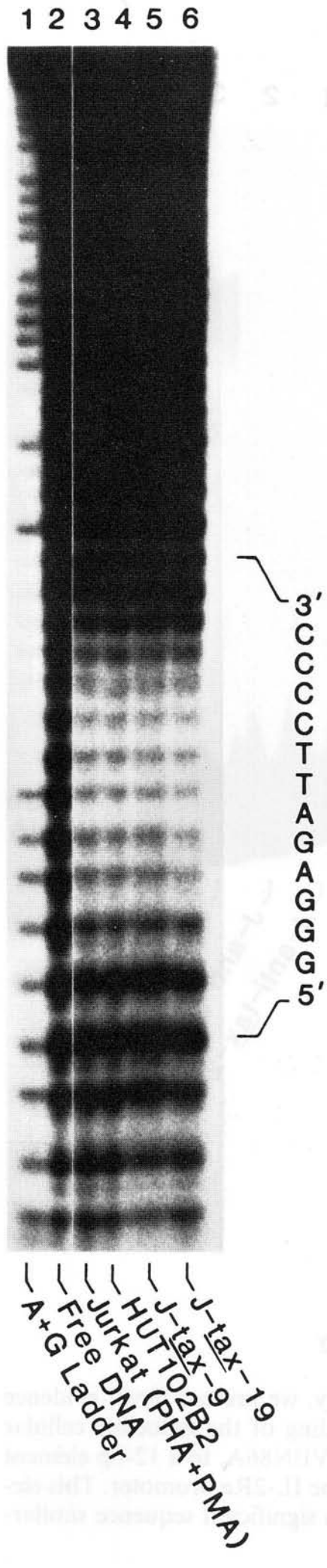


Fig. 2A, B. Induction of nuclear proteins that bind to the IL-2Rα promoter. **A** Mitogen induction. The 47-bp ^{32}P-radiolabeled IL-2R III [11] oligonucleotide was incubated with bovine serum albumin (*lane 1*), nuclear extracts from uninduced Jurkat T cells (*lane 2*), or nuclear extracts from Jurkat T cells activated with PHA (1 μg/ml) and PMA (50 ng/ml) for 5 h (*lane 3*). **B** *tax* induction. Radiolabeled IL-2R III was incubated with extracts from control anti-sense *tax* cell lines (*lanes 1, 2*) or sense Jurkat *tax* cell lines (*lanes 3, 4*). In both panels, the migration of free DNA and the formation of DNA-protein complexes are indicated by *arrows*

protein to the HIV-1 enhancer has been previously reported [11, 18]. Together, these results indicate that mitogens and *tax* share the capacity to induce the same nuclear protein which in turn interacts with the κB elements present in the IL-2Rα promoter and the HIV-1 enhancer.

D. Summary

In this study, we present direct evidence for the binding of the inducible cellular protein, HIVEN86A, to a 12-bp element present in the IL-2Rα promoter. This element shares significant sequence similar-



ity with the NF-κB binding sites present in the HIV-1 and κ immunoglobulin enhancers. Transient transfection studies indicate that this κB element is both necessary and sufficient to confer *tax* or mitogen inducibility to a heterologous promoter. As summarized schematically in Fig. 5, the findings suggest that the HIVEN86A protein may play a central role in the activation of cellular genes required for T-cell growth, specifically the IL-2Rα gene. In addition, the induced HIVEN86A protein also binds to a similar sequence present in the HIV-1 LTR leading to enhanced viral gene expression and ultimately T-cell death.

Thus, mitogen activation of the HIV-1 LTR appears to involve the same inducible transcription factor(s) that normally regulates IL-2Rα gene expression and T-cell growth. These findings further underscore the importance of the state of T-cell activation in the regulation of HIV-1 replication. Our results also demonstrate that HIVEN86A is induced by the *tax* protein of HTLV-I. Thus, in HTLV-I infected cells, normally the tight control of the transient expression of the IL-2Rα gene is lost. The constitutive high-level display of IL-2 receptors may play a role in leukemic transformation mediated by HTLV-I (ATL). Apparently by the same mechanism, the *tax* protein also activates the HIV-1 LTR through the induction of HIVEN86A. This

Fig. 3. 1.10 Phenanthroline-copper DNA footprinting of the IL-2Rα promoter. Radiolabeled IL-2R III [11] was incubated with nuclear extracts prepared from induced Jurkat cells (PHA + PMA), two different Jurkat *tax* lines, as well as the HTLV-I infected HUT102B2 T-cell line. The DNA-protein complexes were electrophoretically separated from free DNA on a 5% polyacrylamide gel and subjected to partial chemical digestion for 10 min. Reaction products including free DNA (*lane 2*) were processed as described [20] and subsequently analyzed on a 10% sequencing gel. An A + G ladder (*lane 1*) was prepared and loaded for sequence determination thus permitting identification of the protected area

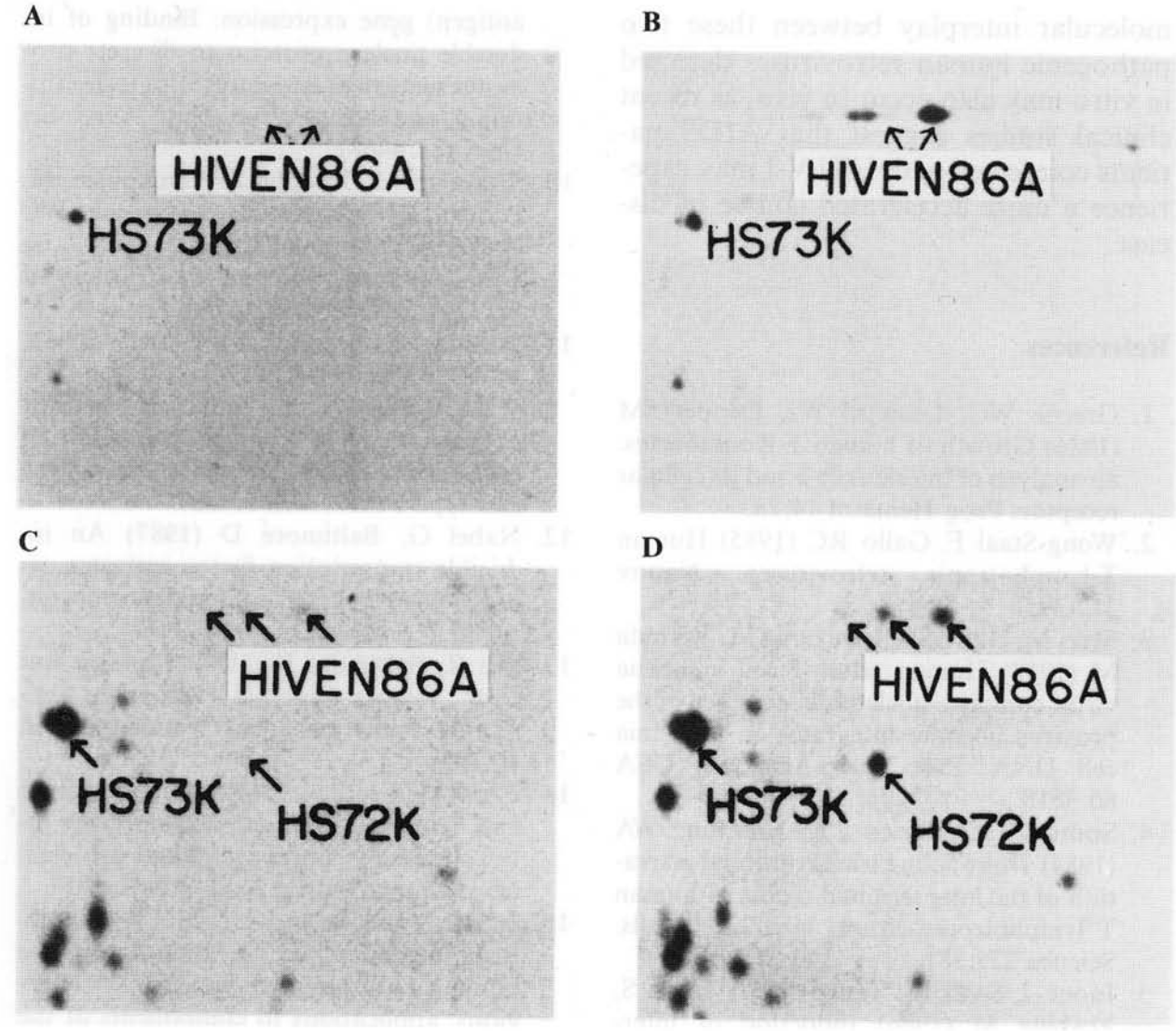


Fig. 4A–D. Microscale DNA-affinity precipitation assays. ^{35}S-Labeled cellular extracts prepared from uninduced Jurkat cells **(A)**, Mitogen-induced Jurkat cells (PMA 50 ng/ml, PHA 1 μg/ml; **B**), an anti-*tax* cell line **(C)**, and Jurkat *tax* line **(D)** were precipitated with a biotinylated IL-2R III DNA probe as previously described [16]. A 73-kDa heat-shock protein (HS73K) is coprecipitated in all assays independent of the biotinylated probe used (data not shown). A second heat-shock protein, HS72K, appears to be induced by *tax* but binds DNA in a non-sequence-specific manner

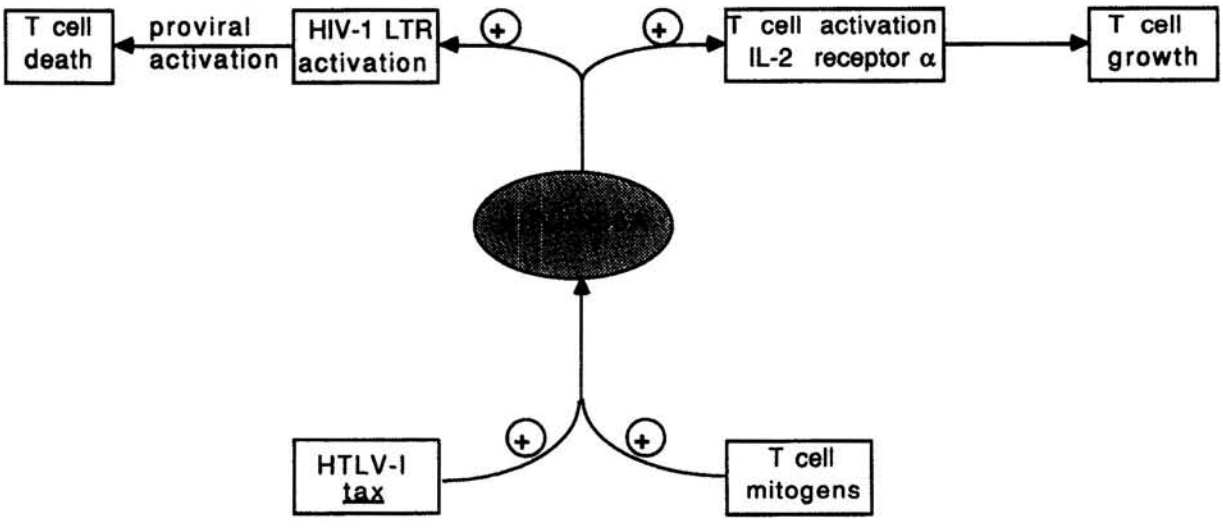


Fig. 5. Working model for the role of HIVEN86A in mitogen and *tax* activation of T-cell growth and HIV-1 replication

molecular interplay between these two pathogenic human retroviruses detected in vitro may also occur in vivo, as recent clinical studies suggest that AIDS patients coinfected with HTLV-I may experience a more accelerated course of disease.

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