HTLV-1 transactivator induces interleukin-2 receptor expression through an NF-κB-like factor

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Like other viruses that infect primate cells, the human T lymphotropic virus-I (HTLV-I) stimulates production of some host cell proteins. In particular, HTLV-I infected T cells synthesize interleukin-2 receptor α (IL-2Rα) chain, which is probably induced through the mediation of the *tat*-I gene product of the virus[1–5]. Activated T cells contain a transcription factor called NF-κB[6], which stimulates the expression of human immunodeficiency virus-1 (HIV-1) by binding to an 11-base-pair enhancer sequence called κB. We have now found evidence that a similar transcription factor is involved in the induction of IL-2Rα expression by *tat*-I. We have identified a sequence upstream of IL-2Rα which is the same as the κB site at 9 of 11 base pairs, competes for binding to the κB sequence, and serves as a *tat*-I responsive element when multiple copies are inserted upstream of a heterologous promoter. The *tat*-I product also induces κB and the IL-2Rα κB binding activity in transfected Jurkat T lymphoid leukaemia cells. Both HTLV-I and HIV-1 thus interact with NF-κB-like transcription factors which might normally regulate expression of a growth factor receptor gene.

Stimulation of T cells leads to the expression of NF-κB, which recognizes an 11-base-pair (bp) DNA sequence twice repeated in the HIV enhancer[6]; however, normal T-cell specific targets of NF-κB were unknown. Because the upstream regulatory region of IL-2Rα contains a site similar to κB (Fig. 1*b*), we analysed its potential role in IL-2Rα activation. To determine whether the IL-2Rα site was recognized by NF-κB, we performed electrophoretic mobility shift assays using a κB probe. Double-stranded oligonucleotide fragments containing the IL-2Rα κB site (Fig. 2*a*, lanes 4, 5) and the immunoglobulin κB site (Fig. 2*a*, lanes 2, 3)competed equally and specifically for binding, in contrast to an unrelated IL-2 site. Using a radiolabelled probe containing the IL-2Rα κB sequence, an inducible complex was identified (Fig. 2*b*, lane 2) which also competed with the HIV κB site (Fig. 2*b*, lanes 3, 4) or the IL-2*Rα* κB site (Fig. 2*b*, lanes 5, 6) fragments. Binding to the κB-like site in the native IL-2Rα enhancer was confirmed using a probe from the upstream region (Fig. 2*c*, lane 2). HIV or IL-2Rα κB fragments competed with the κB-like site for formation of the inducible complex (data not shown). When a similar probe modified at four base pairs in the IL-2Rα κB site (Fig. 1*a*) was used, this specific inducible complex did not form (Fig. 2*c*, lane 3). Finally, transfection of *tat*-I into Jurkat cells resulted in induction of both κB- (Fig. 2*d*, lane 2) and IL-2Rα κB- (Fig. 2*d*, lane 4) binding proteins.

The role of this κB-like site in IL-2Rα gene expression was determined with a bacterial plasmid containing the upstream region of IL-2Rα (−479 to +106) linked to the CAT gene (Fig. 1*a*). Expression of this plasmid was compared to the mutant plasmid by transient transfection. CAT activation in analogous plasmids has been shown previously to correlate with increased messenger RNA levels[4,7]. CAT activity in cells incubated with 12-O-tetradecanoyl phorbol 13-acetate (TPA) was induced by four- to sixfold (Fig. 3*a*). Comparable stimulation was seen in the mutant plasmid, suggesting that the IL-2Rα κB site is not responsive to TPA.

The mechanism of *tat*-I activation was examined by cotransfection of IL-2*Rα*-CAT with a plasmid that could express *tat*-I protein. IL-2Rα-CAT activity increased sixfold in the presence of the *tat*-I plasmid; however, no *tat*-I stimulation of the mutant plasmid was observed (Fig. 3*b*). The function of the

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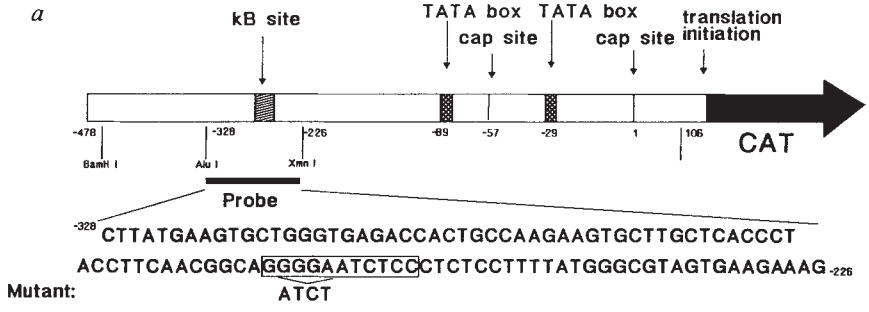

b

Gene	Sequence
HIV(6), mouse Ig kappa(16), SV40(24,25) CMV(26)	GGGGACTTTCC
beta 2-microglobulin(17), HIV(6), CMV(26)	AGGGACTTTCC
Human Ig kappa(27)	GGGGGATTTCC
Class I MHC (H2TF1)(14,15)	GGGGAATCCCC
IL-2R alpha	GGGGAATCTCC
Consensus:	G/A GGG AA/GC A/T TT/CC CC

Fig. 1 *a*, Schematic representation of the IL-2 receptor α chain upstream sequence. Four cap sites and associated TATA boxes have been defined, but only two are major transcriptional start sites; TATA boxes associated with these are indicated. The most downstream cap site is identified as +1. The probe from IL-2Rα used for binding gel analysis is shown. Bases altered in the mutant plasmid are indicated below the wild type sequence. *b*, Sequences of κB-related sites and their associated genes.

Methods. A plasmid containing a 1,352-bp *Pst*I fragment of IL-2Rα upstream region[3], kindly provided by Dr J. Depper, was digested with *Eco*RI and *Pst*I, incubated with T4 polymerase, and a 585-bp insert was isolated. This insert was ligated to p106-CAT[18], provided by Dr M. Gilman, previously digested with *Sma*I and calf intestinal phosphatase (CIP), and correct orientation determined by digestion with *Bam*HI. The mutant plasmid with the indicated base pair changes was derived by site-directed mutagenesis as previously described[6].

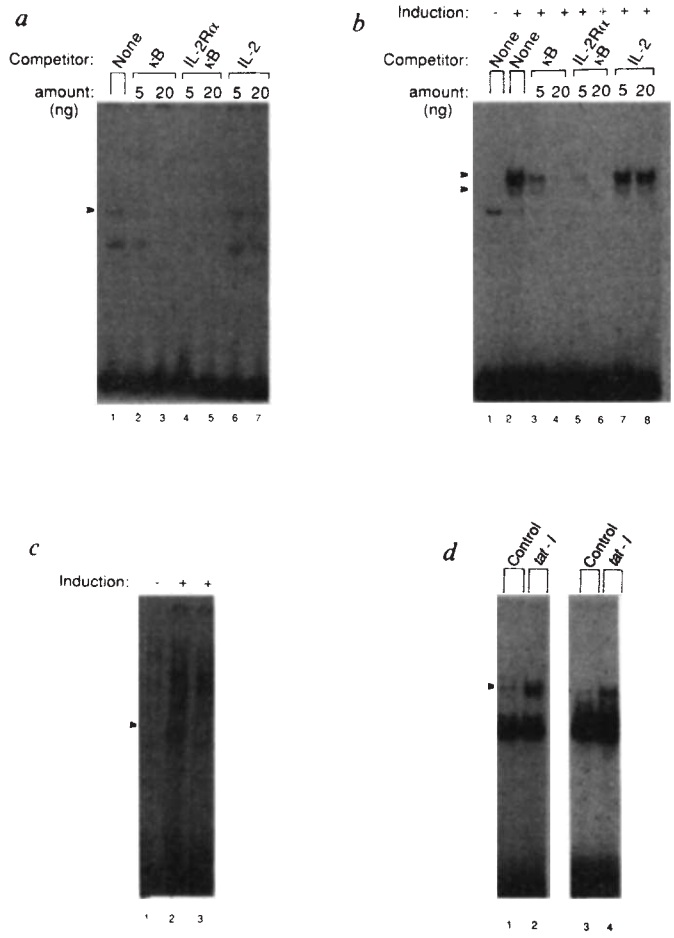

Fig. 2 Induction and specificity of factor binding to the κB-like site in Jurkat cells by phorbol esters and *tat*-I. Analysis of binding-site specificity and competition studies using electrophoretic mobility-shift assay with radiolabelled probe containing *a*, κB sites from HIV enhancer; *b*, IL-2Rα κB sites (double-stranded oligonucleotide probe); *c*, IL-2Rα probe, wild type (lanes 1, 2) or mutant (lane 3; fig. 1*a*). Nuclear extracts (8 μg) from unstimulated (−) or induced (+) Jurkat cells (40 nM TPA treatment for 4 h at 37 °C) were incubated with the relevant radiolabelled DNA probe alone or in the presence of indicated amounts of unlabelled competitors, double-stranded oligonucleotide containing a κB site, IL-2Rα κB site or a control (unrelated) fragment of the same size from the IL-2 promoter (B site)[19]. *d*, Extracts (25 μg) prepared from Jurkat cells transfected with HTLV-I-tat or pGEM-2, which resembles the vector containing the *tat*-I gene, were incubated with radiolabelled double-stranded oligonucleotide probe from κB sites of the HIV enhancer (lanes 1, 2) or IL-2Rα (lanes 3, 4) as above. Binding of the specific inducible complexes (see arrows) using the IL-2Rα probe (*c*), κB or IL-2Rα κB (*d*) also competed specifically with both κB-like sites. All samples contained poly dIdC (1 μg).

Methods. Nuclear extracts were prepared according to the method of Dignam *et al.*[20], and the electrophoretic mobility shift assay performed as previously described[6]. Radiolabelled κB site probe from HIV enhancer was prepared from a 93-bp *Hae*III fragment from the HIV enhancer[6]. A double-stranded oligonucleotide probe for the IL-2Rα κB site was derived from sequence −267 to −252 of the IL-2Rα receptor upstream region (CAGGGGAATCTCCCTC). The double-stranded oligonucleotide probe for the κB site was used as competitor and as radiolabelled probe in *d*. Its sequence was derived from the HIV enhancer, GATCAGGGACTTTCCGCTGGGGACTTTCC. IL-2Rα probe (Fig. 1) was prepared by isolation of a *Bam*HI to *Xmn*I fragment from IL-2Rα-CAT, digestion with *Alu*I, treatment with CIP, phenol and chloroform extraction, ethanol precipitation, and T4 polynucleotide kinase labelling. A mutant IL-2Rα probe was prepared as above using the mutant κB site IL-2Rα-CAT plasmid. HTLV-*tat*-I plasmid[21] was kindly provided by Dr Kuan-Teh Jeang. Jurkat cells (10^7) were transfected with the relevant HTLV-I-tat or control plasmid (20 μg) using DEAE-dextran[6], and nuclear extracts were prepared after 48 h.

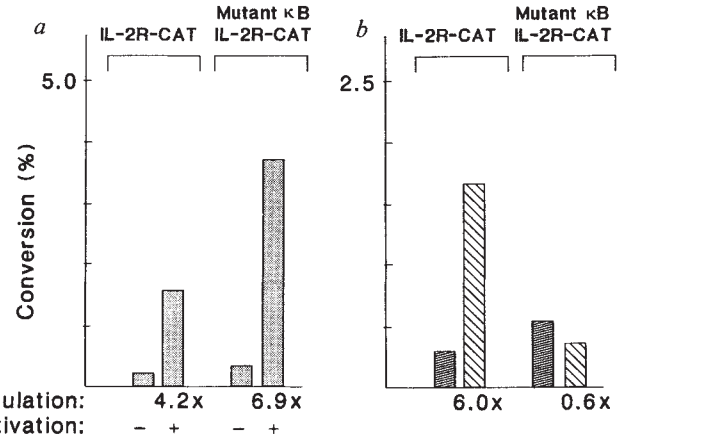

Fig. 3 Mutant IL-2Rα-CAT expression is stimulated by phorbol esters but not by the *tat*-I gene product. *a*, IL-2Rα-CAT or mutant IL-2Rα-CAT plasmid (20 μg) was transfected into Jurkat cells (10^7) using DEAE-dextran. After 16–20 h, cells were left untreated (−) or incubated with 40 nM TPA (+) (Sigma, St Louis, Mo) for the last 20 h of culture before harvest at 44 h. *b*, IL-2Rα-CAT or mutant plasmid (20 μg) was cotransfected as above with a HTLV-*tat*-I or a control (HTLV-1-tat deletion mutant) plasmid (5 μg). CAT activity was determined 44 h later.

Methods. Cells (10^7) were transfected and maintained as previously described[6]. The HTLV-*tat*-I deletion mutant control was prepared by digestion with *Acc*I and *Cla*I, isolation of linear fragment, and ligation with T4 DNA ligase, removing a 126-bp fragment near the 5′ end of the *tat*-I coding sequence. Cell extracts were prepared, protein concentration assayed, and transfection efficiencies standardized as described[1], or using cotransfection with an expression plasmid (5 μg) containing an IL-3 cDNA, measuring IL-3 activity as previously described[22]. CAT activity was determined according to standard methods[6,23]. Degree of conversion was determined by removing spots containing either unreacted ^{14}C-chloramphenicol or acetylated forms and measuring the amount of radioactivity in a liquid scintillation counter. Mutant plasmid was derived by site-directed mutagenesis as described in Fig. 1. Standard deviations for each CAT assay were less than 10%, and results are representative of at least six independent transfections.

IL-2Rα κB site in the absence of the other potential IL-2Rα regulatory sites was analysed using plasmids containing four copies of the different κB sites linked to the simian virus 40 (SV40) promoter and CAT gene. Whereas both *tat*-I and TPA stimulated CAT activity six- or eightfold in the κB plasmid, only *tat*-I activated expression of IL-2Rα κB site plasmid (Fig. 4). Because these plasmids are otherwise identical except for two base pairs in the enhancer, one or both of these bases probably confers TPA inducibility. No stimulation by *tat*-I or TPA was seen in a plasmid with a mutation in the IL-2Rα κB sites, showing that *tat*-I stimulation is dependent on recognition of the IL-2Rα κB site (Fig. 4*b*).

Our analysis shows that the IL-2α κB site is required for *tat*-I induction but not for TPA stimulation. The mechanism of stimulation by *tat*-I is unknown, but it probably induces an NF-κB-like factor rather than recognizing the site directly. There is little evidence that *tat*-I protein binds to a specific sequence of DNA. In fact, *tat*-I may activate more than one transcription factor

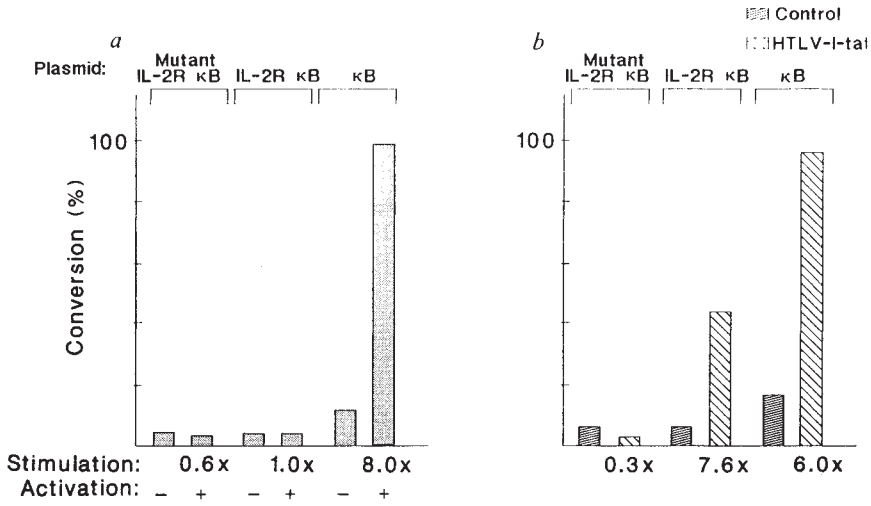

Fig. 4 Stimulation of plasmid containing IL-2Rα κB sites linked to a heterologous promoter by phorbol esters and the *tat*-I gene product. *a*, Jurkat cells were transfected with modified pSP-CAT plasmids, provided by Dr R. Sen, which contains the SV40 promoter linked to CAT. The plasmid was modified by inserting four copies of a mutant IL-2Rα κB, IL-2Rα κB, or κB site, upstream of the SV40 promoter. Cells were untreated (−) or activated with 40 nM TPA (+) as in Fig. 3. *b*, Mutant IL-2Rα κB, IL-2Rα κB or κB plasmids (20 μg) (see above) were transfected with the HTLV-*tat*-I plasmid (5 μg) or control plasmid (HTLV-*tat*-I deletion mutant) (5 μg) and maintained at 37 °C for 44 h, after which CAT activity was determined.

Methods. pSP-CAT was prepared by the isolation of *Bgl*II-*Xba*I fragment of pA10CAT2 containing the SV40 promoter linked to CAT, and ligation to a plasmid backbone prepared from SP-64 digested with *Bam*HI and *Xba*I. Four-copy insert plasmids were prepared by ligation of the following synthetic double-stranded oligonucleotide fragment to pSP-CAT digested with *Sac*I, *Sma*I and CIP: mutant IL-2Rα κB, GCTCAATCTCCAGAGCTCAATCTCCTCGAGCTCAATCTCCACTGCTCAATCTCCTCGA; IL-2Rα κB, GGGGAATCTCCAGAGGGGAATCTCCTCGAGGGGAATCTCCACTGGGGAATCTCCTCGA; κB, GGGGACTTTCCAGAGGGGACTTTCCTCGAGGGGACTTTCCACTGGGGACTTTCCTCGA. The anti-sense strands of each were complementary with an overhanging AGCT at the 3′ end, creating a *Sac*I-compatible end. Maxam-Gilbert sequencing confirmed the presence of the relevant insert in each plasmid. Transfection, normalization and determination of CAT activity are detailed in Fig. 3.

because its target site in the HTLV-I LTR[8–10] is unrelated to the IL-2Rα κB site.

The *tat*-I product activates some viral enhancers containing κB such as SV40[11] but not others, for example the complete HIV enhancer[12]. It is not clear whether this specificity results from the interaction of a single gene product with κB-like sites, or many. Singh *et al.* have reported the isolation of a complementary DNA encoding a polypeptide which recognizes two different κB-like sequences[13], the H2TF1 site of class I MHC genes[14,15] and immunoglobulin κB sequences[6,16]. The existence of this single copy gene, however, does not exclude the possibility that other proteins may recognize these sites. Our inducible complex co-migrates with NF-κB and competes in equimolar amounts for binding to the HIV κB sequence, but, unlike κB, the IL-2Rα κB-like site does not respond to TPA, raising the possibility of multiple κB recognition proteins. Alternatively, transcriptional activity of a single protein could be regulated by subtle differences in DNA sequence.

Analysis of cellular genes reveals that κB-like sites are associated with cell surface molecules (refs 14–17, Fig. 1*b*). It is possible that the NF-κB system evolved to control the synthesis of surface glycoproteins relevant to cellular activation and proliferation, but confirming this will require analysis of more genes. This regulatory site is also found frequently in primate viruses. Although the role of NF-κB in retroviral replication and leukaemogenesis is not completely understood, HTLV-I and HIV-1 use this class of transcription factors in different ways. Our data suggest that an NF-κB-like factor is a target of HTLV-I infection, activated by the *tat*-I product. In the case of T cells infected with HIV, NF-κB is not a target of viral transactivation but a cellular activator of HIV gene expression. In uninfected cells, it may regulate expression of a growth factor receptor, IL-2Rα.

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