JOURNAL OF VIROLOGY, Sept. 1995, p. 5806–5811 Vol. 69, No. 9
0022-538X/95/$04.00+0


# The Amino Terminus of Tax Is Required for Interaction with the Cyclic AMP Response Element Binding Protein

ITAMAR GOREN,[1]† OLIVER J. SEMMES,[2] KUAN-TEH JEANG,[2] AND KARIN MOELLING[3]*

*Max-Planck-Institut fuer Molekulare Genetik, D-14195 Berlin (Dahlem), Federal Republic of Germany[1]; Institut fuer Medizinische Virologie, Universitaet Zuerich, CH-8028 Zuerich, Switzerland[3]; and Molecular Virology Section, Laboratory of Molecular Biology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892[2]*

Received 30 January 1995/Accepted 23 May 1995

**Tax of human T-cell leukemia virus type 1 was analyzed for interaction with the cyclic AMP response element binding protein (CREB) in vitro with and without Tax response element DNA. Mutations in the carboxy terminus of Tax (L296G and L320G) did not affect binding to CREB and led to supershifts. In contrast, mutants with changes in the amino-terminal cysteine-rich region lost the ability to bind to CREB. The S10A mutant protein bound moderately. Thus, the amino terminus of Tax is essential for Tax-CREB interaction.**

Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia (23, 41) and tropical spastic paraparesis HTLV-1 associated myelopathy (13). It codes for a 40-kDa Tax protein (Fig. 1A) which is a potent transactivator of the HTLV-1 long terminal repeat (LTR), as well as other viral and cellular genes (8, 18, 20, 26, 31, 35, 38). Tax functions by binding indirectly to DNA, forming multiprotein complexes with host cell transcription factors to prompt RNA synthesis (4, 9, 10, 14, 17, 19, 37). Three tandem 21-bp repeats, the Tax response elements (TxREs), are located in the U3 region of the HTLV-1 LTR (30) (Fig. 1C). A cyclic AMP response element-like sequence, TGACG, is located in the core of each repeat and is crucial for transcriptional activation by the activating transcription factor (42) and the cyclic AMP response element binding protein (CREB) shown in Fig. 1B (4, 17, 42, 43). The G- and/or C-rich sequences flanking the cyclic AMP response element motif are required for the formation of a stable Tax-CREB-DNA ternary complex (22) and necessary for Tax transactivation in vivo (12).

Mutational analyses of Tax have indicated that Tax transactivation of viral and cellular promoters is mediated through distinct functional domains (28, 33). The extreme amino terminus of Tax (amino acids 1 to 13) has been shown to be important for promoter selectivity between the HTLV-1 and HTLV-2 LTRs (6). A zinc finger-like structure located in the amino terminus has been implicated in localization of Tax in the nucleus (34), as well as for the overall protein folding (27).

In this study, we found that the interaction of Tax and CREB requires the integrity of the amino terminus of Tax in vitro.

**Tax-CREB-DNA interaction analyzed in gel electrophoretic mobility shift assays.** The human CREB CDNA (a kind gift of K. Lee) contained in a 1.02-kb fragment with blunted 5′ *Eco*RI and staggered 3′ *Bam*HI sites was inserted into T7 vector pET3CH6 (25), which contained the sequence for six histidines upstream of the cloning site (15) through blunted *Nco*I and staggered *Bam*HI sites. Histidine-tagged CREB (Fig. 1B) was expressed in *Escherichia coli* BL-21 DE3 as previously described (36) and purified with Ni-NTA agarose (24) (Fig. 2A).

Amino-terminal mutant Tax proteins were cloned by PCR into p5TaxH6 (graciously supplied by C.-Z. Giam) via *Hin*dIII-*Mlu*I sites. The carboxy-terminal mutant proteins were cloned into p5TaxH6 by using *Cla*I-*Psp*AI sites. A Tax(His)$_6$ wild-type protein and Tax mutant proteins were expressed and purified essentially as previously described (42) (Fig. 2A). A glutathione *S*-transferase (GST)–Tax fusion protein was constructed by ligating the Tax wild-type coding sequence into pGEX2TNX via *Nco*I-*Bam*HI sites and expressed and purified as previously described (32). A 143-bp DNA fragment (−213 to −71; Fig. 1C) was amplified by PCR with plasmid pURI (35) as the template and labelled with [$\gamma$-$^{32}$P]ATP. The recombinant proteins (500 pg) were incubated in DNA binding buffer (10 mM Tris-HCl [pH 7.6], 50 mM NaCl, 2.5 mM MgCl, 2 mM spermidine, 2 mM dithiothreitol, 10 $\mu$M $Zn_2SO_4$, 10% glycerol, 3 $\mu$g of bovine serum albumin) in the presence of poly(dI-dC) (0.8 $\mu$g) in 20 $\mu$l for 30 min at room temperature. DNA-protein complexes were separated by electrophoresis through 5% polyacrylamide gels (29:1 acrylamide/bisacrylamide ratio) in TGE buffer (25 mM Tris, 192 mM glycine, 1 mM EDTA, pH 8.5) at 4°C. Complexes were visualized with a Phosphorimager (Molecular Dynamics).

As shown in Fig. 2B, two complexes arose, C1 and C2, consisting of one and two dimers of CREB, respectively, and DNA. In the presence of wild-type Tax, C1 and C2 were shifted, creating two more-slowly migrating protein-DNA complexes, C1* and C2*. This indicates the presence of Tax in stable multiprotein-DNA complexes. The three zinc finger mutant proteins (C36S, R42G, and C49S) and GST-Tax increased the amount of CREB bound to DNA in this assay, perhaps by improving CREB dimerization, yet they failed to interact stably with the CREB-DNA complex and did not lead to the formation of more-slowly migrating protein-DNA complexes. Carboxy-terminal mutant proteins L296G and L320G gave rise to results similar to those obtained with wild-type Tax, indicating that this region is not involved in complex formation. Amino-terminal mutant protein S10A behaved similarly to the wild type, except that the intensity of the complex was reduced, especially that of C2. GST-Tax, in contrast to Tax(His)$_6$, did not lead to the formation of the slowly migrating complex, thereby resembling the three zinc finger mutants proteins. Quantitative evaluation of DNA bound in complexes C1 and

* Corresponding author. Mailing address: Institut fuer Medizinische Virologie, Universitaet Zuerich, Gloriastrasse 30, CH-8028 Zuerich, Switzerland.

† Present address: Hadassah Medical School, Hebrew University, 91010 Jerusalem, Israel.

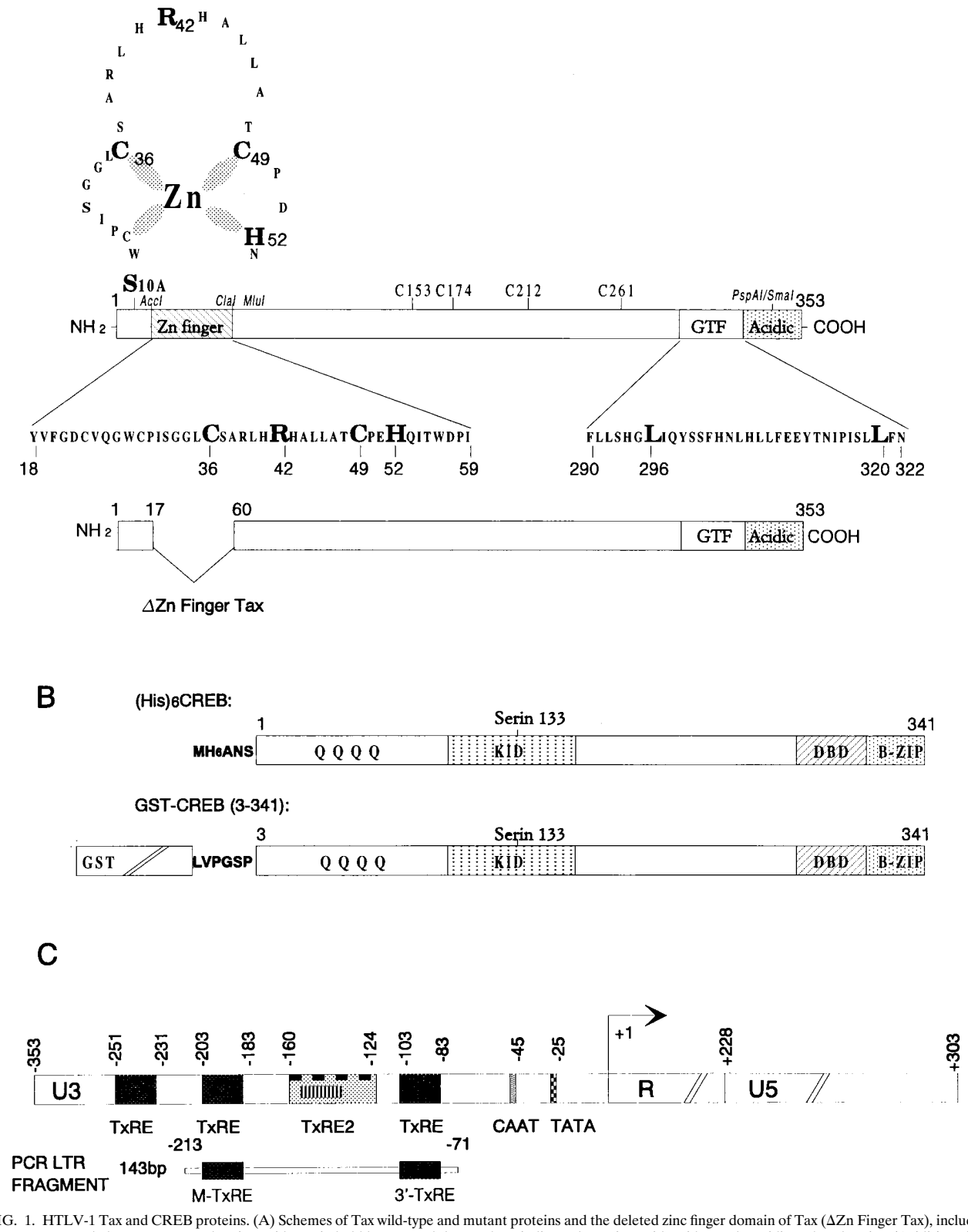


FIG. 1. HTLV-1 Tax and CREB proteins. (A) Schemes of Tax wild-type and mutant proteins and the deleted zinc finger domain of Tax (ΔZn Finger Tax), including important structural elements. Mutated amino acids are in bold letters, and the numbers indicate the amino acid residues. The acidic carboxyl region (Acidic) and the proposed region for interaction with general transcription factors (GTF) are shown as well. Some restriction sites used for cloning are indicated. (B) Histidine-tagged CREB [$(His)_6$CREB] and GST-CREB fusion proteins. Glutamine-rich domain Q, the kinase-induced domain (KID), the DNA binding domain (DBD), and the basic region with the leucine zipper (B-ZIP) are indicated. The histidine-tagged protein and the fusion protein contain additional amino acid residues as indicated by the one-letter symbols upstream of the CREB sequence. (C) HTLV-1 LTR promoter with the three 21-bp TxREs, a related TxRE2, and CAAT and TATA boxes. The arrow at nucleotide +1 indicates the start of mRNA synthesis, R indicates the redundant region, and U5 and U3 are unique regions of the LTR. The PCR-amplified promoter fragment used in this assay is indicated below. It contains the middle TxRE (M-TxRE) and the proximal TxRE (3′-TxRE) of the promoter.

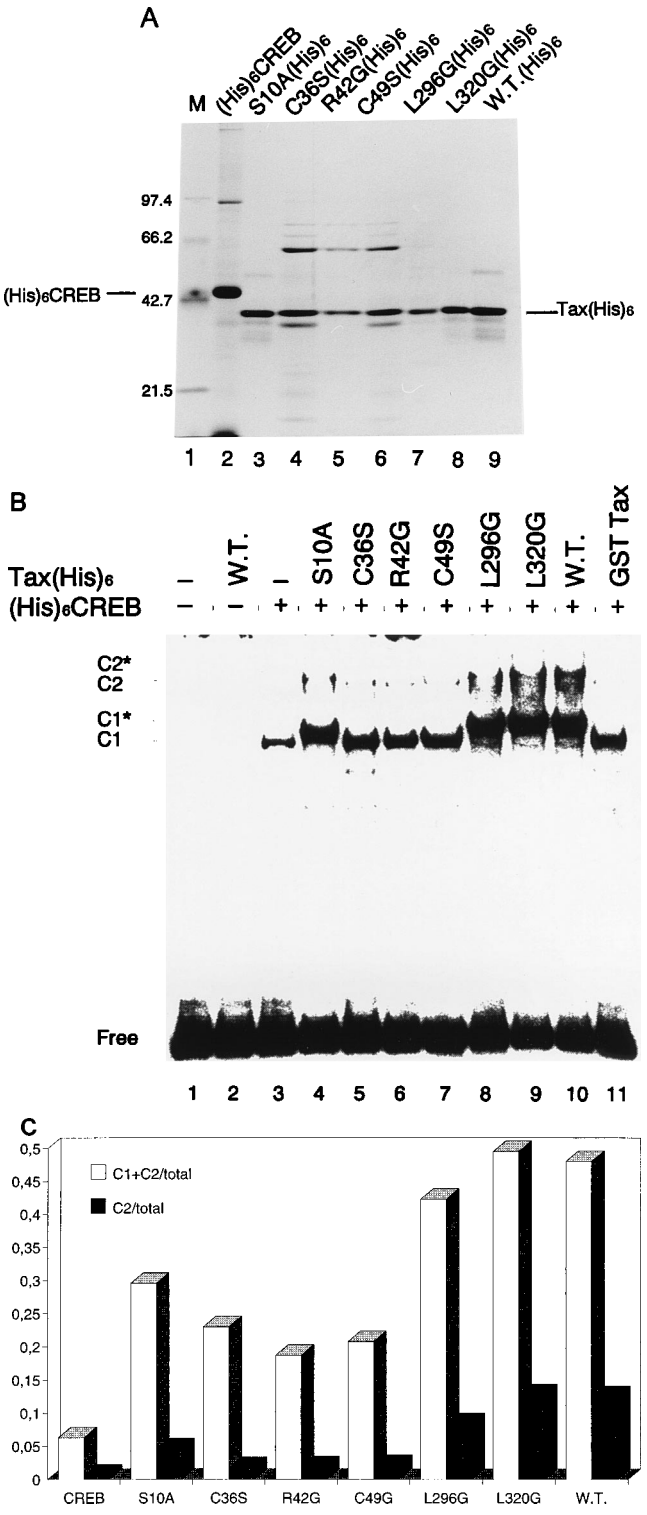


FIG. 2. Effect of Tax and Tax mutant proteins on CREB binding activity to the HTLV-1 LTR promoter fragment. (A) Proteins used in the DNA binding reaction were analyzed by SDS–10% PAGE and visualized by silver staining (5). Lanes: 1, molecular weight standards (sizes are indicated to the left in kilodaltons); 2, $(His)_6$CREB; 3 to 9 wild-type and mutant Tax$(His)_6$ proteins as indicated above the lanes. The amount of protein was estimated by comparison with a standard by using silver staining (data not shown). (B) Electrophoretic mobility shift assay using the $^{32}$P-labelled LTR DNA fragment (Fig. 1C) and purified wild-type (W.T.) and mutant $(His)_6$CREB (20 ng) and Tax$(His)_6$ (100 ng) proteins as described in text. C1 represents one TxRE occupied by a CREB dimer. C2 represents two TxREs both occupied by CREB dimers (i.e., a fully occupied promoter fragment). Shifted complexes are represented by asterisks (C1* and C2*). (C) DNA-protein complexes measured with a Phosphorimager and ratio of DNA bound in C1 plus C2 to total DNA (□) and ratio of two TxREs occupied by CREB dimers (C2) to total DNA (■).

C2 was achieved by Phosphorimager analysis (Fig. 2C). The ratio of DNA bound in C1 plus C2 (white column) or C2 (black column) versus the total DNA input was lowest for the cysteine mutants. It was reduced five- to sevenfold in C2.

**Filter binding analysis of Tax-CREB-DNA interaction.** Tax-CREB-DNA interaction was further characterized by filter binding assays. A synthetic oligodeoxynucleotide corresponding to the middle TxRE 21-bp motif, spanning nucleotides −203 to −183 of the HTLV-1 LTR, was synthesized and extended by five additional oligonucleotides, end labelled with [$\gamma$-$^{32}$P]ATP, annealed, and treated as previously described (3). Various amounts of recombinant $(His)_6$CREB protein were incubated with 100 pg of labelled DNA in DNA binding buffer in a final volume of 100 μl for 30 min at room temperature essentially as previously described (21). Where indicated, poly(dI-dC) was added as a nonspecific competitor alone or together with 100 ng of Tax$(His)_6$ wild-type or mutant protein (Fig. 3). Addition of increasing amounts of CREB gave rise to a saturation curve well fitted to the two-step protein-DNA binding model previously suggested for $(CREB)_2$-TxRE interaction (2). Addition of 1 μg of poly(dI-dC) abolished this binding activity almost completely. Addition of 100 ng of wild-type Tax protein, however, restored the binding activity of CREB. In addition to what was previously described (2) and the shift assay results (Fig. 2B), Tax was found to increase the binding activity of CREB to the 21-bp repeat in this assay, especially at lower concentrations of CREB (50 ng). The molecular ratio of CREB to Tax was about 2.5:1. These results support the notion that Tax increases the specific affinity of CREB dimers for the 21-bp repeat DNA. Carboxy-terminal mutant Tax proteins L320G and L296G were almost as efficient in restoring this CREB-DNA binding as the wild-type protein. In contrast, the C36S mutant protein had no influence on CREB-TxRE complex formation and the binding activity of CREB remained as low as with the addition of poly(dI-dC) alone. Amino-terminal mutant protein S10A restored only some of the CREB-binding activity to the TxRE DNA, making it similar to what was obtained in the DNA shift assay.

**Direct interaction between Tax and CREB.** The interaction of wild-type and mutant Tax proteins with CREB was further analyzed in the absence of DNA in vitro. CREB cDNA present in a 1-kb fragment (amino acids 3 to 341) was cloned into pETGST via *Nco*I-*Bam*HI restriction sites, expressed in *E. coli* B1-21 DE3 (36), and purified as previously described (32). Wild-type and mutant Tax proteins were cloned into vector pGNE [a pGEM3 derivative (Promega Co.) which contains β-globin leader sequences upstream and a $(dA)_{30}$ sequence downstream of the cloning site (15)] via *Nco*I-*Bam*HI sites. Deletion of the zinc finger domain of Tax (amino acids 18 to 54) was generated by religating previously mung bean nuclease-blunted, *Acc*I-digested, Klenow-blunted, *Cla*I-digested plasmid pGTaxNE. An *Eco*RI-linearized plasmid (1 μg) was used for in vitro transcription with T7 RNA polymerase (Promega Co.), and 1/10 of the transcribed RNA was translated in rabbit reticulocyte lysate in the presence of [$^{35}$S]methionine and resolved by sodium dodecyl sulfate (SDS)–10% polyacrylamide gel electrophoresis (PAGE) (Fig. 4A). Equal amounts of labelled protein adjusted to 10 μl with nonprogrammed reticulocyte lysate were incubated with 1 μg of bacterially

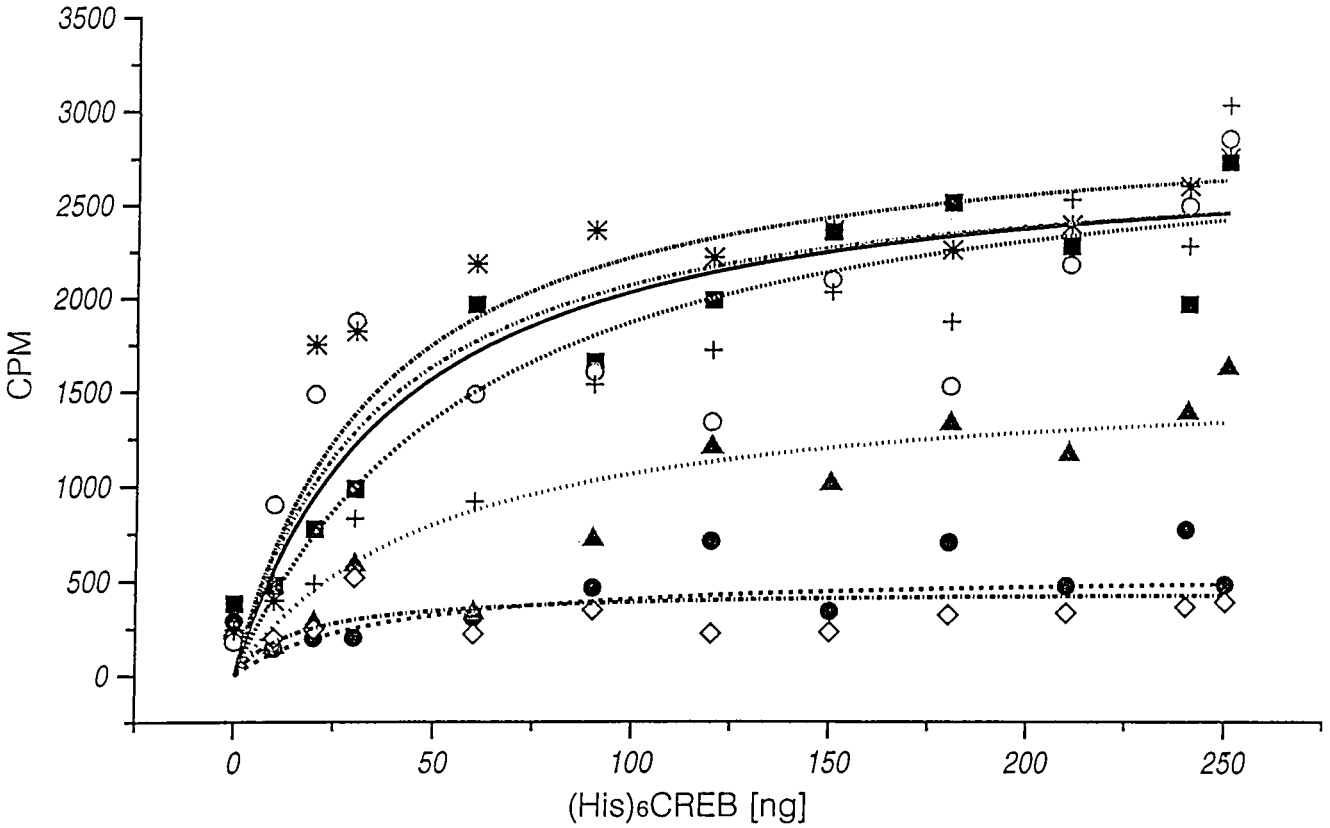


FIG. 3. Filter binding assay results. A double-stranded DNA oligonucleotide containing the middle TxRE was radiolabelled and treated with increasing concentrations of $(His)_6$CREB protein. The amount of the filter-bound CREB-TxRE complex as a function of the CREB concentration was measured. Nonspecific DNA competitor poly(dI-dC) (1 μg) alone or supplemented with 100 ng of wild-type or mutant Tax$(His)_6$ protein was added to all reactions except a control reaction containing CREB only. Poly(dI-dC) abolished the binding of CREB to DNA, which was restored by wild-type Tax and some of the mutant proteins. Symbols: ■, CREB; ●, CREB plus poly(dI-dC); ▲, CREB plus poly(dI-dC) plus Tax S10A; ◇, CREB plus poly(dI-dC) plus Tax C36S; ○, CREB plus poly(dI-dC) plus Tax L296G; +, CREB plus poly(dI-dC) plus Tax L320G; ❋, CREB plus poly(dI-dC) plus wild-type Tax.

expressed GST-CREB immobilized on 15 μl of glutathione-Sepharose beads and treated as previously described (40). GST-CREB-bound proteins were resolved by SDS–PAGE (Fig. 4B). Mutant proteins with changes in the cysteine- and histidine-rich region, including a mutant protein with a deleted zinc finger, did not bind. Carboxy-terminal mutant proteins L296G and L320G allowed binding somewhat similar to that allowed by the wild type. Figure 4C shows the GST-CREB proteins used for binding and visualized by Coomassie blue staining of the gel shown in Fig. 4B. Quantitative analysis of the binding reaction was obtained with a Phosphorimager. The amount of wild-type Tax bound to GST-CREB was arbitrarily set to 1, and the other reactions were quantified accordingly. The result shows that the mutant proteins with changes in the zinc finger region exhibited 5- to 10-fold reduced binding efficiencies (Fig. 4D). The two carboxy-terminal mutants bound about half as efficiently as the wild type. In a control experiment, Tax was found to bind exclusively to the $NH_2$-deleted GST–TATA-binding protein (TBP) (168-339) fusion protein [GST-TBP(C); Fig. 4E, section II, lane 4], in agreement with what was previously reported (7), and not to the COOH-deleted GST–TBP(1-163) fusion protein [GST–TBP(N); lane 3] or GST alone (lane 2).

We found that mutant proteins with three single amino acid substitutions located in the zinc finger domain of Tax and the GST-Tax fusion protein were unable to interact with CREB and HTLV-1 LTR DNA. Mobility shift assays showed, however, that the amount of protein-DNA complexes was slightly increased over that obtained with CREB alone and no decrease in the mobility of the protein-DNA complex was obtained. Moreover, in filter binding assays, we observed no enhancement of CREB binding to the 21-bp repeat DNA mediated by the C36S cysteine substitution mutant protein, although the binding activity of CREB was enhanced by wild-type or mutant Tax protein. This supports the notion that the induction of CREB binding to TxRE DNA mediated by Tax depends, to some extent, on stable interaction of Tax with the CREB-DNA complex.

Mutant Tax protein S10A, with a mutation adjacent to the cysteine-rich region, was still able to interact with the CREB-DNA complex to a moderate level and also reduced the mobility of this complex in the shift assay. This suggests that the sequence adjacent to the zinc finger domain is also important for the interaction of Tax with CREB on the HTLV-1 LTR promoter, as was recently demonstrated by Adya and Giam (1).

The finding that the COOH-terminal mutant Tax proteins, although they do not activate the HTLV-1 LTR promoter in vivo (28), can still bind to the CREB 21-bp repeat complex and enhance the binding of CREB to the HTLV-1 LTR DNA in a manner comparable to that of the wild-type protein in vitro points to the role of this domain in interaction with other factors, such as the general transcription factors. These mutant proteins were unable to inhibit (squelch) the activity of a GAL4-

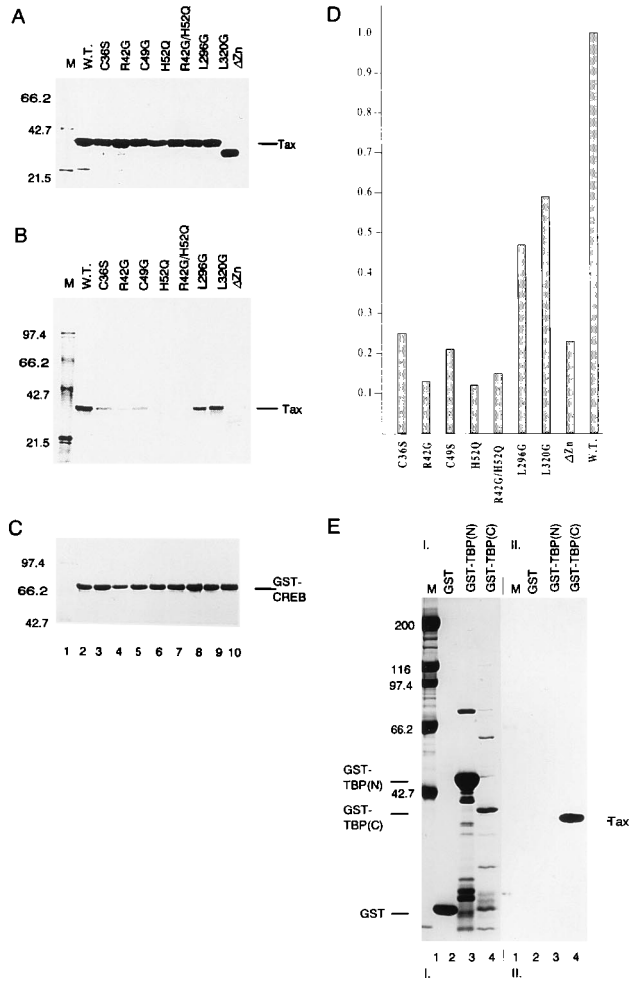


FIG. 4. In vitro association of wild-type (W.T.) and mutant Tax proteins with CREB. (A) In vitro-translated, [$^{35}$S]methionine-labelled wild-type and mutant Tax proteins were subjected to SDS–10% PAGE, and radioactivity was measured with a Phosphorimager. (B) Equal amounts of in vitro-translated [$^{35}$S]methionine-labelled wild-type Tax and mutant Tax were incubated with GST-CREB (1 μg) immobilized on glutathione-agarose beads, washed extensively, and analyzed by SDS–10% PAGE. Radiolabelled wild-type and mutant Tax proteins bound to GST-CREB were measured with a Phosphorimager. (C) Coomassie blue staining of the same gel as in panel B showing GST-CREB fusion protein in each reaction. (D) Binding of mutant Tax to GST-CREB relative to the binding of the wild-type protein (set as 1) plotted by quantitation of the results (shown in panel B) obtained with a Phosphorimager. (E) In vitro association of Tax with recombinant GST protein (lane 2), GST-TBP(N) (amino terminus) fusion protein (lane 3), or GST-TBP(C) (carboxy terminus) fusion protein (lane 4) (a kind gift of T. Kouzarides [16]). [$^{35}$S]methionine-labelled Tax was incubated with immobilized GST-TBP fusion proteins and treated as described above for Tax-CREB association. Protein complexes were analyzed by SDS–10% PAGE and visualized by Coomassie blue staining (I). The gel was then dried and exposed for autoradiography (II). Lanes M contained molecular weight markers (sizes are indicated in kilodaltons at the left).

Tax fusion protein in cotransfection assays and hence lack the ability to interact with transcription cofactors (29). The existence of a transactivation domain located in the COOH terminus of Tax which can be replaced by the heterologous activation domain of herpes simplex virus VP16 has been suggested on the basis of functional activation assays with GAL4-Tax (11, 39). Therefore, interaction of Tax with CREB, which requires the intact $NH_2$-terminal domain of Tax and induces CREB binding, is a prerequisite but is probably not sufficient to account for activation (29).

We are grateful to K. Lee, C.-Z. Giam, and T. Kouzarides for the generous supply of CREB, Tax(His)$_6$, and the GST-TBP expression plasmids, respectively. I.G. is indebted to H. Schuster for the use of laboratory facilities.

This work was supported by the Deutsche Forschungsgemeinschaft and the Max Planck Society.

## REFERENCES

1. **Adya, N., and C.-Z. Giam.** 1995. Distinct regions in human T-cell lymphotropic virus type I Tax mediate interactions with activator protein CREB and basal transcription factors. J. Virol. **69:**1834–1841.
2. **Anderson, M. G., and W. S. Dynan.** 1994. Quantitative studies of the effect of HTLV-I Tax protein on CREB protein-DNA binding. Nucleic Acids Res. **22:**3194–3201.
3. **Beimling, P., and K. Moelling.** 1990. Tax-independent binding of multiple cellular factors to the Tax response element DNA of HTLV-I. Oncogene **5:**361–368.
4. **Beimling, P., and K. Moelling.** 1992. Direct interaction of CREB protein with 21 bp Tax-response elements of HTLV-I LTR. Oncogene **7:**257–258.
5. **Blum, H., H. Beier, and H. J. Gross.** 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis **8:**93–99.
6. **Cann, A. J., J. D. Rosenblatt, W. Wachsman, and I. S. Y. Chen.** 1989. In vitro mutagenesis of the human T-cell leukemia virus types I and II *tax* genes. J. Virol. **63:**1474–1479.
7. **Caron, C., R. Rousset, C. Beraud, V. Monocllin, J. M. Egly, and P. Jalinot.** 1993. Functional and biochemical interaction of the HTLV-I Tax 1 transactivator wih TBP. EMBO J. **12:**4269–4278.
8. **Fujii, M., P. Sassone-Corsi, and I. M. Verma.** 1988. c-*fos* promoter transactivation by the tax$_1$ protein of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA **85:**8526–8530.
9. **Fujii, M., H. Tsuchiya, T. Chuhjo, T. Akizawa, and M. Seiki.** 1992. Interaction of HTLV-I Tax1 with p67-$^{SRF}$ causes the aberrant induction of cellular immediate early genes through CArG boxes. Genes Dev. **6:**2066–2076.
10. **Fujii, M., H. Tsuchiya, T. Chuhjo, T. Minamino, K. Miyamoto, and M. Seiki.** 1994. Serum response factor has functional roles both in indirect binding to the CArG Box and in the transcriptional activation function of human T-cell leukemia virus type I Tax. J. Virol. **68:**7275–7283.
11. **Fujii, M., H. Tsuchiya, and M. Seiki.** 1991. HTLV-I Tax has distinct but overlapping domains for transcriptional activation and for enhancer specificity. Oncogene **6:**2349–2352.
12. **Fujisawa, J.-I., M. Toita, and M. Yoshida.** 1989. A unique enhancer element for the *trans* activator (p40$^{tax}$) of human T-cell leukemia virus type I that is distinct from cyclic AMP- and 12-*O*-tetradecanoylphorbol-13-acetate-responsive elements. J. Virol. **63:**3234–3239.
13. **Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, A. Calender, and G. de The.** 1985. Antibodies to the human T lymphotropic virus type 1 in patients with tropical spastic paraparesis. Lancet **ii:**407–410.
14. **Giam, C.-Z., and Y.-L. Xu.** 1989. HTLV-I *Tax* gene product activates transcription via pre-existing cellular factors and cAMP responsive element. J. Biol. Chem. **264:**15236–15241.
15. **Goren, I., and S. Volkmann.** 1992. Unpublished data.
16. **Hagemeier, C., S. Walker, R. Caswell, T. Kouzarides, and J. Sinclair.** 1992. The human cytomegalovirus 80-kilodalton but not the 72-kilodalton immediate-early protein transactivates heterologous promoters in a TATA box-dependent mechanism and interacts directly with TFIID. J. Virol. **66:**4452–4456.
17. **Jeang, K.-T., I. Boros, J. Brady, M. Radonovich, and G. Khoury.** 1988. Characterization of cellular factors that interact with the human T-cell leukemia virus type I p40$^{x}$-responsive 21-base-pair sequence. J. Virol. **62:**4499–4509.
18. **Leung, K., and G. J. Nabel.** 1988. HTLV-I transactivator induces interleukin-2 receptor expression through an NF-κB like factor. Nature (London) **333:**776–778.
19. **Mariott, S. J., I. Boros, J. F. Duvall, and J. N. Brady.** 1989. Indirect binding of human T-cell leukemia virus type I tax$_1$ to a responsive element in the viral long terminal repeat. Mol. Cell. Biol. **9:**4152–4160.
20. **Miyatake, S., M. Seiki, R. D. Malefijit, T. Heike, J. Fujisawa, Y. Takebe, J. Nishida, J. Shlomai, T. Yokota, M. Yoshida, K. Arai, and N. Arai.** 1988. Activation of T cell-derived lymphokine in T cells and fibroblasts: effect of human T cell leukemia virus type I p40$^{x}$ protein and bovine papilloma virus encoded E2 protein. Nucleic Acids Res. **16:**6547–6567.
21. **Müller, G., B. Strack, J. Dannull, B. S. Sproat, A. Surovoy, G. Jung, and K. Moelling.** 1994. Amino acid requirements of the nucleocapsid protein of HIV-1 for increasing catalytic activity of a Ki-ras ribozyme *in vitro*. J. Mol. Biol. **242:**422–429.
22. **Paca-Uccaralertkun, S., L.-J. Zhao, N. Adya, J. V. Cross, B. R. Cullen, I. M. Boros, and C.-Z. Giam.** 1994. In vitro selection of DNA elements highly responsive to the human T-cell lymphotropic virus type I transcriptional activator Tax. Mol. Cell. Biol. **14:**456–462.
23. **Poiesz, B. F., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1980. Detection and isolation of type C retrovirus particles from fresh

cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA **77:**7415–7419.
24. **Qiagen Inc.** 1992. The QIAexpressionist: the high level expression & protein purification system. Qiagen Inc., Hilden, Germany.
25. **Rosenberg, A. H., B. N. Lade, D. S. Chui, S. W. Lin, J. Dunn, and F. W. Studier.** 1987. Vector for selective expression of cloned DNAs by T7 RNA polymerase. Gene **56:**125–135.
26. **Seiki, M., S. Hattori, Y. Hirayama, and M. Yoshida.** 1983. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. Proc. Natl. Acad. Sci. USA **79:**3618–3622.
27. **Semmes, O. J., and K.-T. Jeang.** 1992. HTLV-I Tax is a zinc-binding protein: role of zinc in tax structure and function. Virology **188:**754–764.
28. **Semmes, O. J., and K.-T. Jeang.** 1992. Mutational analysis of human T-cell leukemia virus type I Tax: regions necessary for function determined with 47 mutant proteins. J. Virol. **66:**7183–7192.
29. **Semmes, O. J., and K.-T. Jeang.** 1995. Definition of a minimal activation domain in human T-cell leukemia virus type I Tax. J. Virol. **69:**1827–1833.
30. **Shimotohno, K., M. Takano, T. Teruuchi, and M. Miwa.** 1986. Requirement of multiple copies of a 21-nucleotide sequence in the U3 regions of human T-cell leukemia virus type I and type II long terminal repeats for trans-acting activation of transcription. Proc. Natl. Acad. Sci. USA **83:**8112–8116.
31. **Siekevitz, M., S. F. Josephs, M. Dukovitch, N. Pfeffer, F. Wong-Staal, and W. C. Greene.** 1987. Activation of the HIV-1 LTR by T-cell mitogens and the trans-activator protein of HTLV-I. Science **238:**1575–1578.
32. **Smith, D. B., and K. S. Johnson.** 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene **67:**31–40.
33. **Smith, M. R., and W. C. Greene.** 1990. Identification of HTLV-I *tax trans*-activator mutants exhibiting novel transcriptional phenotypes. Genes Dev. **4:**1875–1885.
34. **Smith, M. R., and W. C. Greene.** 1992. Characterization of a novel nuclear localization signal in the HTLV-I Tax transactivator protein. Virology **187:** 316–320.
35. **Sodroski, J., C. Rosen, W.-C. Goh, and W. Haseltine.** 1985. A transcriptional activator protein encoded by the x-*lor* region of the human T-cell leukemia virus. Science **228:**1430–1434.
36. **Studier, F. W., and B. A. Moffatt.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of clonal genes. J. Mol. Biol. **189:**113–130.
37. **Tanimura, A., H. Teshima, J.-I. Fujisawa, and M. Yoshida.** 1993. A new regulatory element that augments the Tax-dependent enhancer of human T-cell leukemia virus type 1 and cloning of cDNAs encoding its binding proteins. J. Virol. **67:**5375–5382.
38. **Tsuchiya, H., M. Fujii, T. Niki, M. Tokuhara, M. Matsui, and M. Seiki.** 1993. Human T-cell leukemia virus type 1 Tax activates transcription of the human *fra-1* gene through multiple *cis* elements responsive to transmembrane signals. J. Virol. **67:**7001–7007.
39. **Tsuchiya, H., M. Fujii, Y. Tanaka, H. Tozawa, and M. Seiki.** 1994. Two distinct regions from a functional activation domain of the HTLV-I transactivator Tax1. Oncogene **9:**337–340.
40. **Xu, X., C. Prorock, H. Ishikawa, E. Maldonado, Y. Ito, and C. Gélinas.** 1993. Functional interaction of the v-Rel and c-Rel oncoproteins with the TATA-binding protein and association with transcription factor IIB. Mol. Cell. Biol. **13:**6733–6741.
41. **Yoshida, M., I. Miyoshi, and Y. Hinuma.** 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc. Natl. Acad. Sci. USA **79:**2031–2035.
42. **Zhao, L.-J., and C.-Z. Giam.** 1991. Interaction of the human T-cell lymphotrophic virus type I (HTLV-I) transcriptional activator Tax with cellular factors that bind specifically to the 21-base-pair repeats in the HTLV-I enhancer. Proc. Natl. Acad. Sci. USA **88:**11445–11449.
43. **Zhao, L.-J., and C.-Z. Giam.** 1992. Human T-cell lymphotropic virus type I (HTLV-I) transcriptional activator, Tax, enhances CREB binding to HTLV-I 21-base-pair repeats by protein-protein interaction. Proc. Natl. Acad. Sci. USA **89:**7070–7074.