Modulation of Tax and PKA-mediated expression of HTLV-I promoter via cAMP response element binding and modulator proteins CREB and CREM

Josef Bodor[a], William Walker[a], Erik Flemington[b], Anna-Lena Spetz[b], Joel F. Habener[a,*]

[a]*Laboratory of Molecular Endocrinology, Massachusetts General Hospital (WEL320), Howard Hughes Medical Institute, Boston, MA 02114, USA*
[b]*Division of Tumor Virology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02114, USA*

Received 26 September 1995; revised version received 25 October 1995

Abstract **Nuclear proteins of the human peripheral blood T lymphocytes that bind to the CREs located within three 21-bp repeat enhancers of the HTLV-I promoter belong to the CREB/CREM family of bZIP transcription factors. It has been shown previously that Tax enhances transactivation of these CREs by direct interactions with the bZIP domain of the transcription factors to stabilize DNA-binding. We show that CREB and CREM bind all three CRE sequences of the HTLV-I promoter which are important determinants in Tax-elicited transactivation as well as PKA-mediated activation of the HTLV-I promoter. Tax and PKA activate transcription from a HTLV-I-LTR CAT reporter plasmid transfected to NIH 3T3 cells, and CREM attenuates the activation. In the context of a GAL4 CREB fusion protein in which the DNA-binding bZIP domain of CREB is replaced by GAL4 binding domain, a single amino acid substitution of serine-133, phosphorylated by PKA and critical for the transactivation function of CREB, attenuates both Tax and PKA-mediated transcriptional responses. These observations suggest that Tax enhances CREB-mediated transactivation of the HTLV-I promoter by a mechanism apart from, and/or in addition to, the reported stabilization of DNA-binding by interaction with the bZIP domain of CREB.**

Key words: Genetics; Protein kinase A; cAMP element binding protein; Promoter region; Trans-activation; Regulatory sequence

1. Introduction

The lymphotropic and neurotropic pathogenic action of the HTLV-I virus is well established in HTLV-I-associated diseases, including adult T cell leukemia [1,2], inflammatory arthropathy resembling rheumatoid arthritis [3,4], and the neurological disorder tropical spastic paraperesis [5]. The chronic nature of these diseases indicates a tight transcriptional regulation of HTLV-I expression. The HTLV-I promoter contains three imperfect repeats of 21 nucleotides with asymmetric CREs that govern the response of the promoter to the transactivator protein Tax and to varying cAMP levels [6–8]. The critical role of CREs in Tax-mediated transactivation of the HTLV-I promoter has been proven by saturation mutagenesis of the HTLV-I promoter [6,9]. Tax is the viral gene product responsible for the autoregulation of the HTLV-I promoter and has been shown not to bind directly to target DNA [10,11]. Instead, Tax is believed to indirectly interact with CREs of the HTLV-I 21-bp repeats by forming protein-protein complexes with transcription factors [12,13]. It has been reported that Tax stabilizes the binding of CREB and other CRE-binding proteins to the CREs specifically in the 21-bp repeats of the HTLV-I promoter by interactions with the basic region/leucine zipper domain involved in DNA-binding [14–16]. Here we report evidence that Tax stimulates CREB-mediated transcription of the HTLV-I LTR by a mechanism in addition to the stabilization of DNA-binding.

2. Materials and methods

2.1. Plasmid constructs

For the preparation of the (3 × GAL4) HTLV-I LTR CAT reporter used in these studies, site-directed mutagenesis, using oligonucleotides corresponding to the 17mer GAL4 DNA-binding recognition sequences UAS_G [17], was performed to substitute all three 21-bp repeats of the HTLV-I LTR CAT reporter (CR-CAT)[18]. The sequences and the orientations of the inserted oligonucleotides were confirmed by dideoxy-chain termination sequencing. The GAL4CREB and Ser-133 to Ala mutant expression vector $GAL4CREB^{mutS133A}$ were prepared by PCR and site-directed mutagenesis [19,20]. Plasmid constructs encoding human full-length CREB [19] and CREM (CREM$\tau\alpha$) [21] were cloned into bacterial expression vector pRSET (Invitrogen, San Diego, CA) using standard procedures [22], and bacterial lysates were prepared as described [23]. The eukaryotic expression vectors for Tax and the catalytic subunit of PKA were kindly provided by Dr. M. Nerenberg [24] and Dr. R. Maurer [25], respectively.

2.2. Cell lines and transient transfections

NIH3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf bovine serum and antibiotics to approximately 25% confluence in 100-mm-diameter culture dishes (Gibco BRL, Gaithersburg, MD). The cells were transfected with 2 μg of reporter and 1 μg of activator and transactivator DNA expression constructs as indicated. Transient transfections were performed using the calcium phosphate precipitation method [26]. Harvesting of cells, preparation of cell extracts, CAT assays and quantification on the PhosphoImager (Betascope) were performed as described [27].

2.3. Isolation of peripheral blood T cells

Buffy coat leukocyte were obtained from healthy adult blood donors, and peripheral blood mononuclear cells were isolated by using Ficoll-Hypaque (Pharmacia, Piscataway, NJ). Monocytes were depleted by adherence to plastic, 2 × 45′ in RPMI (Gibco BRL, Gaithersburg, MD) at 37°C. Recovered cells were depleted of B cells by using magnetic cell sorting (MACS, Miltenyi Biotec, Sunnyvale, CA). Staining with anti-CD19-microbeads and separations on MACS columns were performed according to the manufacturer's instructions, except that the buffer used was RPMI with 1.0% bovine serum albumin. Cell surface staining

*Corresponding author. Fax: (1) (617) 726-6954.

Abbreviations: bZIP, basic region/leucine zipper; CRE, cAMP response elements; CREB, CRE binding protein; CREM, CRE modulator protein; EMSA, electromobility shift assay; HTLV-I, human T cell leukemia virus; PKA, protein kinase A.

SSDI 0014-5793(95)01299-0

and flow cytometry was performed as described [28].The T cell-enriched populations of cells typically contained less than 4% $CD20^+$B cells, less than 3% $CD14^+$ monocytes, and 15–23% $CD16^+$ natural killer cells, as revealed by flow cytometry (data not shown).

2.4. Nuclear extracts and electromobility shift assays (EMSA)

Nuclear extracts were prepared by modified method of Dignam [29]. Binding reactions were performed in a 20 μl reaction volume containing 20 mM HEPES, 1 mM $MgCl_2$, 50 mM KCl, 12% glycerol, 0.1 mM EDTA, 0.5 mM PMSF (phenylmethylsulfonic acid), 0.5 mM DTT (dithiothreitol), 1 μg of nuclear protein and 1 μg poly{d(A-T)} (Boehringer-Mannheim, Germany) as an unspecific competitor. ^{32}P-Labeled oligonucleotide and, where indicated, unlabeled competitor oligonucleotides in excess were added and incubated for 30 min at room temperature. Samples were run on a 4% polyacrylamide gel in 0.5 × TBE at 150 V for 2 h. The dried gels were exposed for autoradiography overnight.

2.5. Oligonucleotides

Oligonucleotides were annealed and labeled with [α-^{32}P]dATP (New England Nuclear, Billerica, MA). Oligonucleotides with 5′ overhang were labeled with Klenow polymerase (Boehringer-Mannheim, Germany). The sequences of the oligonucleotides used are shown in Table 1.

2.6. Immunochemical identification of nuclear factors (antibody EMSA)

Nuclear extracts, antibodies, and oligonucleotides were incubated at room temperature for 30 min. The sera used were rabbit polyclonal R1090 anti-CREB antiserum raised against the P-box peptide consisting of amino acids 92–124 of CREB [19] and preimmune rabbit serum.

2.7. DNA-protein binding and UV-crosslinking

Short, photoreactive BrdU-substituted 1H21CRE^c oligonucleotide duplexes were prepared as described [30]. For in situ DNA–protein crosslinking, polyacrylamide slabs from EMSA were placed on a 300 nm UV light source (Fotodyne; 7000 mW/cm^2) and irradiated for 20 min. DNA–protein complexes were excised and analyzed by discontinuous SDS/7.5% PAGE under reducing conditions.

3. Results

The palindromic CRE sequence (TGACGTCA) exemplified by COL8 [19] is composed of two functional CGTCA units essential for cAMP response [31] which overlap in opposite DNA strands, forming a symmetrical CRE (Table 1). In contrast, CREs in the 21-bp repeats of HTLV-I (H21CRE) fall into the second category of low affinity sites represented by asymmetric CREs (Table 1). Asymmetric CREs contain only one CGTCA unit in the opposite antisense orientation and an imperfect CGTCA unit in the sense orientation. Although sequences adjacent to CRE elements are important for the binding of proteins, it is generally believed that the presence of at least one CGTCA unit, and not the presence of the palindromic

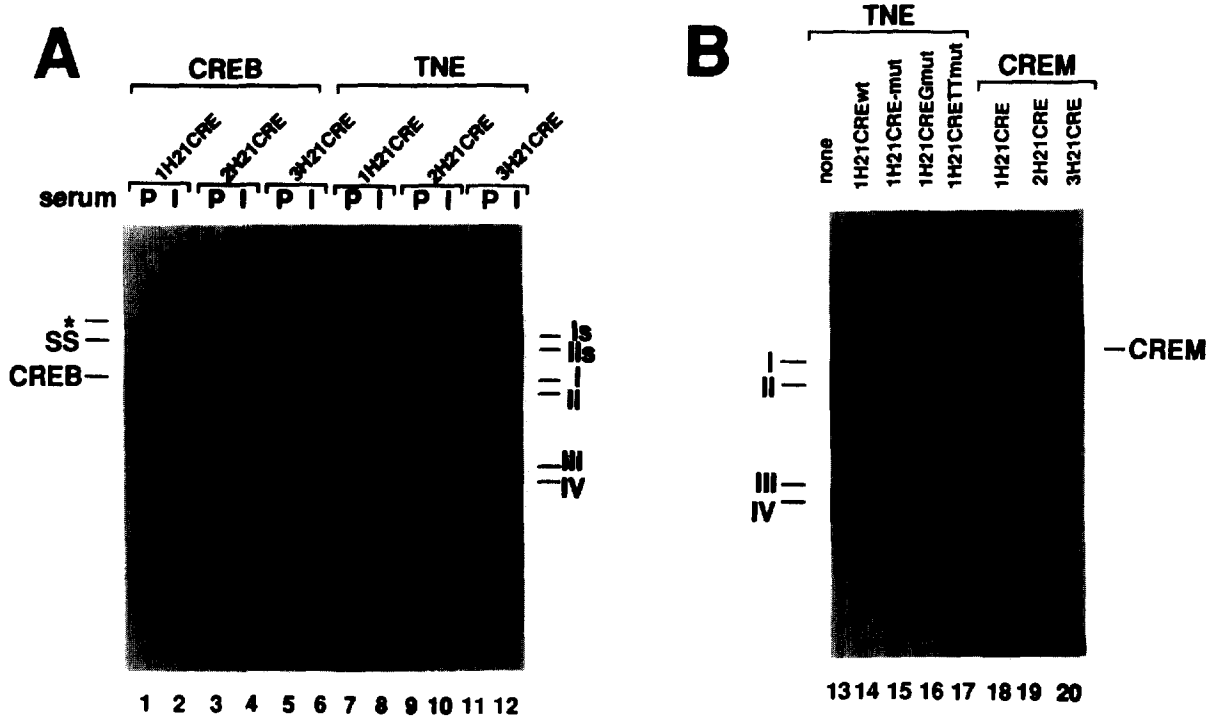

Fig. 1. EMSAs with oligonucleotides containing CRE sites in the 21-bp repeats of the HTLV-I promoter. (A) Binding of bacterially expressed recombinant CREB and proteins in extracts of nuclei prepared from peripheral blood T cells (TNE) to ^{32}P-labeled oligonucleotides corresponding to 21-bp repeats of HTLV-I LTR (lanes 1–12). The EMSA compares the binding of CREB and T cell nuclear proteins to wild type HTLV-I 21-bp repeats 1H21CRE, 2H21CRE, 3H21CRE (Table 1). The effects of incubating CREB or nuclear extracts with either rabbit preimmune serum (P), or immune R1090 CREB antiserum (I) raised against the CREB 'P-box' peptide in the EMSA binding reactions are shown. 'CREB' indicates the prevalent complex formed between bacterially expressed CREB protein and DNA. 'SS' indicates complexes that are supershifted with the CREB R1090 antiserum, * denotes a non-specific complex detected with various intensity also with the preimmune rabbit serum. Of the four complexes (I, II, III and IV) detected in the EMSA of peripheral blood T cell nuclear proteins with preimmune rabbit serum (P), incubation with R1090 CREB antiserum (I) supershifted complexes I and II to the complexes denoted as Is and IIs and eliminated complex III. (B) Cross-competition of binding for EMSA complexes I, II, III and IV in T cell nuclear extracts (TNE) with the 1H21CRE ^{32}P-radioactively labeled probe 1H21CRE (lanes 13–17) indicate that competition with a 100-fold excess of unlabeled probe 1H21CREwt competes all four complexes while the mutated probes 1H21CRE-mut, 1H21CREGmut and 1H21CRETTmut compete only complexes III and IV. Bacterially expressed recombinant CREM binds effectively to the ^{32}P-labeled oligonucleotides corresponding to the three 21-bp repeats of HTLV-I LTR (lanes 18–20).

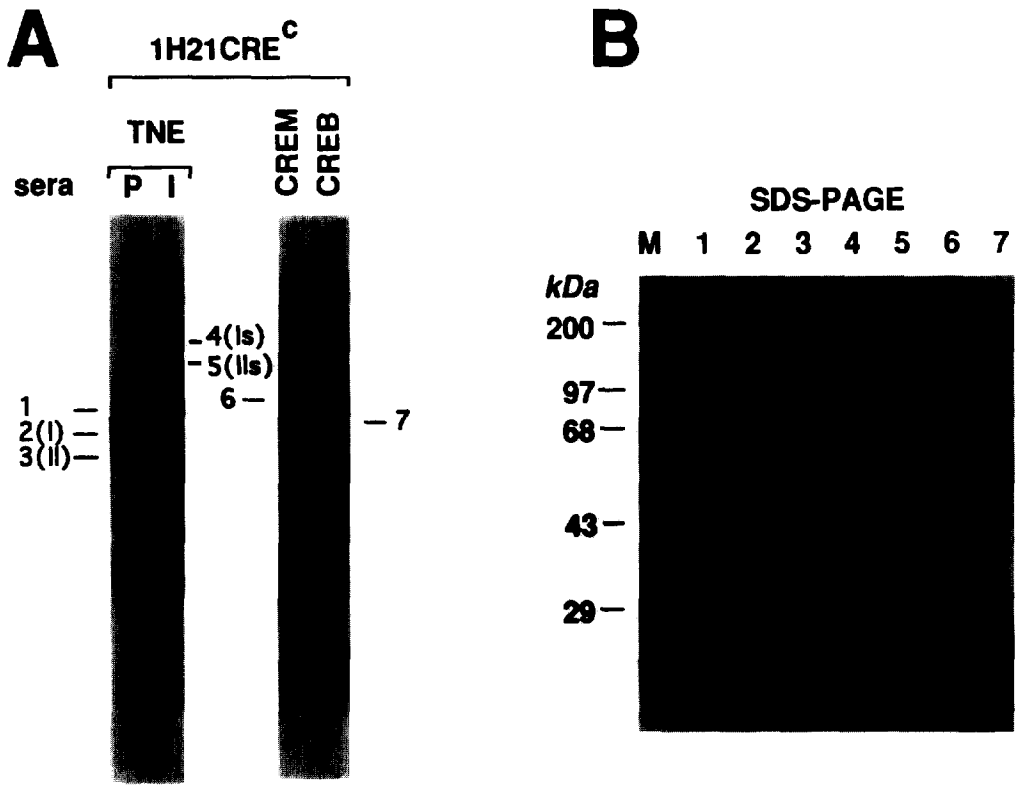

Fig. 2. In situ UV crosslinking of peripheral blood T cell nuclear proteins to the Br-dUTP-substituted 1H21CRE^c probe. (A) Preparative EMSA gel with resting T cell nuclear extract (TNE) and bacterially expressed recombinant CREM and CREB proteins. EMSA of nuclear extract from human peripheral blood T cells (TNE) with the 1H21CRE^c probe is shown in the presence of preimmune rabbit sera (P) or R1090 antibody (I). Band 1 depicts DNA–protein complex bound outside CRE of HTLV-I 21-bp repeat. Bands 4 and 5 are supershifted bands 2 and 3 of DNA–protein complexes binding via CRE of HTLV-I 21-bp repeat. Complexes in bands 2, 3, 4 and 5 observed with BrdU substituted 1H21CRE^c probe correspond to complexes I, II, Is and IIs observed with the HTLV-I 21 bp repeat probes respectively. Bands 6 and 7 depict DNA-protein complexes with full-length CREM and full-length CREB. The UV-irradiated wet gel was exposed to autoradiography film for 1 h at 4°C and then the complexes in bands 1–7 were excised and fractionated by SDS-PAGE. (B) Autoradiogram of the analytical SDS-PAGE gel showing the proteins detected in EMSA complexes 1 to 7 shown in panel A. The positions of molecular markers are shown along the left margin.

Table 1
Coding strand sequences of CREs in 21-bp repeats of HTLV-I LTR oligonucleotides used in the DNA-binding studies

Oligonucleotide	Coding sequence
1H21CRE	5′-AAGGCTC TGACGTCT CCCCCC-3′
2H21CRE	5′-TAGGCCC TGACGTGT CCCCCT-3′
3H21CRE	5′-CAGGCGT TGACGACA ACCCCT-3′
1H21CREc	5′-AAGGCTC TGACGTCT CCCCCCGGAGGGCAG-3′
1H21CRE-mut	5′-AAGGCTC CAACGTCT CCCCCC-3′
1H21CREGmut	5′-AAGGCTC TGAGGTCT CCCCCC-3′
1H21CRETTmut	5′-AAGGCTC TGATTTCT CCCCCC-3′
COL8	5′-CGGC TGACGTCA TCAAGCTA-3′
Sense unit	CGTCA
CRE consensus	TGACGTCA
Antisense unit	ACTGC

All of the oligonucleotides have 5′ GATC overhangs. The wild type HTLV-I 21-bp repeats oligonucleotides 1H21CRE, 2H21CRE, 3H21CRE comprise sequences of the HTLV-I LTR from nucleotide −251 to −231 (1H21CRE), −203 to −184 (2H21CRE), and −103 to −82 (3H21CRE) relative to the transcription start site (+1). The mutated bases in the distal 21-bp repeat oligonucleotides (1H21CRE-mut, 1H21CREGmut, 1H21CRETTmut) are underlined. 1H21CRE^c was used for the preparation of Br-dUTP-substituted probe for the in situ UV crosslinking.

sequence itself, confers the binding of CREB homodimers to DNA [32]. To directly compare the affinities of CREs in the HTLV-I 21-bp repeats, these sequences were incubated with bacterially expressed CREB protein in EMSA (Fig. 1A).

Next, we determined whether bacterially expressed recombinant CREB can be recognized in EMSA by an antiserum (R1090) directed against a peptide from the P-box of CREB. In the presence of R1090 we observed a supershift of the more intense band, indicating that CREB bound to the CREs of the 21-bp repeats can be specifically recognized by an antiserum to a CREB peptide (Fig. 1A, lanes 1–6). Recombinant CREM also binds to all three of the HTLV-I CREs (Fig. 1B, lanes 18–20).

To identify T-cell nuclear proteins that bind to the CRE elements of the HTLV-I 21-bp repeats, we investigated the binding of proteins in nuclear extracts prepared from human peripheral blood T cells (Fig. 1A, lanes 7–12). Incubation with the 21-bp probes (1H21CRE, 2H21CRE, 3H21CRE) yielded four distinct complexes, denoted I, II, III and IV (Fig. 1A). Two complexes (III and IV) could be explained by DNA–protein interactions involving sequences outside of the CRE element as they were competed with an excess of oligonucleotides containing mutated CRE recognition sequences (1H21CRE-mut,

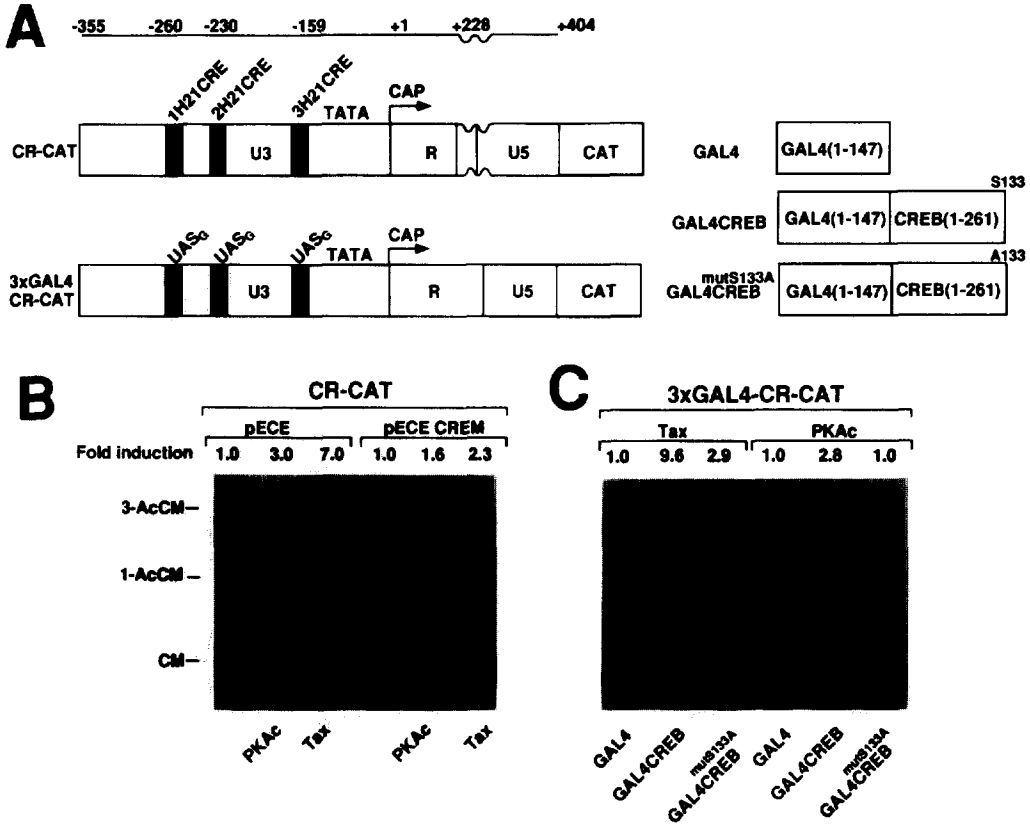

Fig. 3. CREB and CREM modulate Tax and PKA response of HTLV-I LTR. (A) Schematic representation of constructs encoding HTLV-I LTR reporters (CR-CAT) where 21-bp repeats are replaced with GAL4 DNA binding sites UAS_G (3 × GAL4-CR-CAT) and GAL4-CREB fusion proteins GAL4CREB and GAL4CREB mutant substituted by Ala on Ser-133 ($GAL4CREB^{mutS133A}$). (B) Overexpression of CREM downregulates PKA_c and Tax response of CR-CAT. The fold stimulations in the presence of Tax of PKA_c are shown as indicated. (C) Mutation of Ser-133 of CREB alone can modulate the Tax and PKA response in GAL4CREB-mediated 3 × GAL4-CR-CAT transactivation. CAT activities were normalized relative to the basal activity of the 3 × GAL4-CR-CAT reporter in the presence of GAL4 DNA binding domain only. Although the figure represents the results of a single experiment, similar Tax and PKA_c activation was observed in four independent experiments.

1H21CREGmut, 1H21CRETTmut) (Fig. 1B, lanes 13–17, and Table 1). The remaining two bands (I and II) contained DNA–protein complexes that were not competed by oligonucleotides with mutated CRE recognition sequences, indicating that protein in complexes I and II are bound via the CRE element. This observation suggested that the proteins in complexes I and II bind the 21-bp repeats via the CRE element similar to that of CREB. To address this question we determined whether T cell nuclear proteins present in complexes I and II could be recognized by antiserum R1090 that is specific for the recognition of CREB and CREM. Indeed, both complexes I and II, but not complexes III and IV, were supershifted by the antiserum to give new complexes Is and IIs with 21-bp repeats tested. Notably, complex III was disrupted by antiserum R1090 on all three 21-bp repeats tested (Fig. 1A, lanes 8, 10, and 12). These findings indicated the presence of CREB and/or CREM isoforms bound to the CREs in the complexes I and II (Fig. 1B).

To further elucidate the nature of the proteins in complex I and II, a specific BrdU-substituted probe for DNA-protein crosslinking by UV light was prepared. Incubation with this probe (1H21CRE^c) based on the sequence of the distal 21-bp repeat of HTLV-I LTR, including the flanking sequences (Table 1), yielded *several* complexes, three of which were further pursued in our analysis (bands 1 to 5, Fig. 2A). Band 1 depicts a DNA–protein complex bound outside of the core CRE of 1H21CRE^c probe. Bands 2 and 3 observed with BrdU substituted 1H21CRE^c probe (Fig. 2) correspond to complexes I and II recognized by the HTLV-I 21 bp repeat probes (Fig. 1). Similarly bands 4 and 5 (Fig. 2) supershifted in the presence of R1090 antiserum correspond to complexes Is and IIs (Fig. 1), suggesting that CREB and/or CREM isoforms are members of these complexes. For comparison, bacterially expressed full-length CREM and CREB were incubated with the oligonucleotide probe (Fig. 2A, bands 6 and 7). DNA-binding proteins were crosslinked to the labeled BrdU-substituted 1H21CRE^c probe using UV light. DNA–protein adducts (1–7) (Fig. 2A) were excised and the complexes were resolved by SDS-gel electrophoresis (Fig. 2B, SDS-PAGE). The migration of complexes excised from bands 2 and 3 (Fig. 2B, lanes 2 and 3) were similar to that of the bacterially expressed recombinant CREM and CREB isoforms in lanes 6 and 7, respectively. Furthermore, complexes excised from bands 2 and 4, as well as 3 and 5, displayed almost identical patterns, supporting the conclusion that complexes Is and IIs are supershifted by the antiserum R1090, respectively. These data indicate that certain of the proteins in nuclear extracts of human resting T cells that bind

to the 21-bp repeats of HTLV-I via the CREs are CREB/CREM proteins. Not all of the proteins binding to the 21-bp repeats comigrate with CREB/CREM isoforms, because some proteins bind outside of the CRE. For example, the complex in band 1 (Fig. 2A) migrated in a clearly different range of molecular weight (about 100 kDa) (Fig. 2B).

To elucidate whether the binding of CREB to the CREs is functionally significant, we carried out transient transcriptional transactivation assays in transfected fibroblast NIH 3T3 cells, using a HTLV-I LTR-CAT reporter plasmid (CR-CAT) and expression plasmids encoding either Tax or the catalytic subunit of protein kinase A (PKA_c). Both Tax and PKA_c activated the CR-CAT reporter (Fig. 3B). Further, the activation was inhibited by cotransfection of an expression plasmid encoding the cAMP response element modulator CREM (Fig. 3B). Because the mechanism of action of Tax-mediated stimulation of transcription is reported to be a stabilization of the binding of CREB and other bZIP proteins to the CRE through specific interaction with the bZIP domain [13–16], and because of the presence of multiple endogenous CRE-binding proteins, we used the 'domain-swap' approach in which the DNA-binding domain of CREB is removed and substituted for by the DNA-binding domain of the yeast transactivator GAL4, GAL4(1–147). In addition, this approach obviates undesired interactions with the multiple endogenous CRE-binding proteins, including isoforms of CREB and CREM. GAL4 is not known to bind to DNA control elements in animal cells [20]. Correspondingly, all three 21-bp repeats in the wild type HTLV-I-LTR-CAT reporter (CR-CAT) were substituted with three GAL4 DNA binding sites (3 × GAL4-CR-CAT) (Fig. 3A). In addition, recent observations indicate that GAL4 DNA-binding domains dimerize in a manner analogous to bZIP domains, although this dimerization is not facilitated by Tax [14]. Thus Tax is not expected to enhance transcription by stabilizing the binding of GAL4CREB fusionproteins. Overexpression of the GAL4(1–147) DNA-binding domain itself did not activate 3 × GAL4 CR-CAT activity in the presence of Tax in NIH 3T3 cells (Fig. 3C). However, overexpression of the GAL4CREB(1–261) fusion protein (GAL4CREB) and Tax (Fig. 3A) resulted in a 9.6-fold activation, showing that in the context of the 3 × GAL4-CR-CAT reporter, the Tax response could be at least partially reconstituted with GAL4CREB fusion protein. The effect of PKA_c on CREB-mediated activation of HTLV-I transcription was approximately 3-fold above the basal level (GAL4).

To determine whether the Tax and the PKA response mediated by GAL4CREB is dependent upon specific phosphorylation of serine-133 phosphorylated by the cAMP-dependent protein kinase A, we used a GAL4CREB mutant with serine-133 altered to alanine GAL4CREBmut^{S133A}. Substitution of serine-133 to alanine diminished the Tax response by 3-fold (Fig. 3B). In accordance with earlier observations, substitution of serine-133 by alanine also abrogated the PKA response. These results directly implicate serine-133 of CREB in the Tax response, even in the absence of the bZIP domain of CREB which is lacking in the GAL4CREB. This finding further suggests that the reported failure of Tax to elevate cellular cAMP levels [8] does not necessarily exclude Tax from the utilization of the same target molecule (CREB) via signaling pathway(s) apart from PKA-mediated phosphorylation of the serine-133 of CREB.

4. Discussion

Our DNA-binding studies performed with nuclear extracts prepared from human peripheral blood T lymphocytes confirm that proteins of the CREB/CREM family of transcription factors bind to the CREs of the HTLV-I promoter. The role of CREB in regulation of the activity of the HTLV-I promoter was further studied using GAL4CREB fusion proteins and an HTLV-I promoter construct in which the 21-bp repeats were replaced by GAL4 DNA-binding sites. In this context, both Tax and PKA stimulate GAL4CREB-mediated transactivation of gene transcription in the absence of the CREB bZIP domain. This transactivation was significantly reduced when serine-133 of CREB was substituted by alanine. Phosphorylation of serine-133 by PKA, or other protein kinases, is known to be critical for the generation of its transactivational activity [19,33,34], probably by enhancement of its interactions with the CREB-binding protein (CBP), a novel cAMP-responsive protein that couples enhancer binding proteins to the basal RNA polymerase complex [35–37]. Our observations indicate that Tax stimulates CREB-mediated transactivation of the CREs in the HTLV-I 21-bp repeats by a mechanism in addition to the stabilization of bZIP-specific binding to the DNA, and suggest that the mechanism may be to enhance interactions of CREB with CBP. Our data are consistent with the idea that phosphorylated CREB is a target for CBP, and that Tax may also enhance the recruitment and/or stabilization of the binding of phosphoCREB to CBP. Our findings are also consistent with the reported observations that sequences located amino-proximal to the binding domain of CREB are required for the Tax-mediated stimulation of DNA-binding [38] and that Tax directly binds to CBP [39].

Acknowledgements: This work was supported by a grant from National Institute of Health DK25532 (J.F.H.). We thank to H. Hermann, and J. Lin, for technical assistance and T. Budde for secretarial help. J.F.H. is an investigator with the Howard Hughes Medical Institute.

References

[1] Gallo, R.C., Kalyanaraman, V.S., Sarngadharan, M.G., Sliska, A., Vonderheid, E.C., Maeda, M., Nahao, Y., Yamada, K., Ito, Y., Gutensohn, N., Murphy, S., Bunn, P.A., Catovsky, D., Greaves, M.F., Balyney, D.W., Blattner, W., Jarrett, W.F.H., zur Hausen, H., Seligmann, M., Brouet, J.C., Haynes, B.F., Jegasothy, B.V., Jaffe, E., Cossman, J., Broder, S., Fisher, R.I., Golde, D.W. and Robert-Guroff, M. (1983) Cancer Res. 43, 3892–3899.
[2] Franchini, G., Wong-Staal, F. and Gallo, R. (1984) Proc. Natl. Acad. Sci. USA 81, 6207–6211.
[3] Iwakura, Y., Tosu, M., Yoshida, E., Takiguchi, M., Sato, K., Kitajima, I., Nishioka, K., Yamamoto, K., Takeda, T., Hatanaka, M., Yamamoto, H. and Seikiguchi, T. (1991) Science 253, 1026–1028.
[4] Sato, K., Maruyama, I., Maruyama, Y., Kitajima, J., Nakajima, Y., Higaki, M., Yamamoto, K., Myiasaka, N., Osame, M. and Nishioka, K. (1991) Arthritis and Rheumatism 34, 714–721.
[5] Gessain, A.F., Barin, F., Vernant, J.C., Gout, O., Maurs, L., Calender, A. and de The, G. (1985) Lancet ii, 407–410.
[6] Jeang, K.T., Boros, I., Brady, J., Radonovich, M. and Khoury, G. (1988) J. Virol. 62, 4499–4509.
[7] Nakamura, M., Niki, M., Ohtani, K. and Sugamura, K. (1989) Nucleic Acids Res. 13, 5207–5221.
[8] Poteat, H.T., Kadison, P., McGuire, K., Park, L., Park, L.E., Sodroski, J. and Haseltine, W.A. (1989) J. Virol. 63, 1604–1611.
[9] Niki, M., Ohtani, K., Nakamura, M. and Sugamura, K. (1992) J. Virol. 66, 4348–4357.

[10] Chen, I.S.Y., Slamon, D.J., Rosenblatt, J.D., Shah, N.P., Quan, S.G. and Wachsman, W. (1985) Science 229, 54–58.
[11] Sodroski, J., Rosen, C., Goh, W.C. and Haseltine, W. (1985) Science 228, 1430–1434.
[12] Zhao, L.J. and Giam, C.Z. (1991) Proc. Natl. Acad. Sci. USA 88, 11445–11449.
[13] Zhao, L.J. and Giam, C.Z. (1992) Proc. Natl. Acad. Sci. USA 89, 7070–7074.
[14] Wagner, S. and Green, M.R. (1993) Science 262, 395–399.
[15] Baranger, A.M., Palmer, R.C., Hamm, M.K., Giebler, H.A., Brauweiler, A., Nyborg, J.K. and Schepartz, A. (1995) Nature 376, 606–608.
[16] Perini, G., Wagner, S. and Green, M.R. (1995) Nature 376, 602 605.
[17] Webster, N.J., Green, S., Jin, J.R. and Chambon, P.R. (1988) Cell 54, 145–161.
[18] Josephs, S., Wong-Staal, F., Mazari, V., Gallo, R.C., Sodroski, J., Trus, M., Perkins, D., Patarca, R. and Haseltine, W. (1984) Virology 139, 340–345.
[19] Hoeffler, J.P., Meyer, T.E., Yun, Y., Jameson, J.L. and Habener, J.F. (1988) Science 242, 1430–1433.
[20] Sadowski, J., Ma, J., Triezenberg, S. and Ptashne, M. (1988) Nature 335, 563–564.
[21] Meyer, T.E. and Habener, J.F. (1992) Nucleic Acids Res. 20, 6106.
[22] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY.
[23] Studier, F.W., Rosenberg, A.H. and Dunn, J.J. (1990) Methods Enzymol. 185, 61–89.
[24] Nerenberg, M., Hinrichs, S.H., Reynolds, R.K., Khoury, G., and Jay, G. (1987) Science 237, 1324–1329.
[25] Maurer, R.A. (1982) J. Biol. Chem. 264, 6870–6873.
[26] Graham, F.L. and van der Eb, J. (1973) Virology 52, 456–461.
[27] Phillippe, J., Drucker, D.J., Knepel, W., Jepeal, L., Misulovin, Z. and Habener, J.F. (1988) Mol. Cell. Biol. 8, 4877–4888.
[28] Brooks, E.G., Schmalstieg, F.C., Wirt, D.P., Rosenblatt, H.M., Adkins, L.T., Lookingbill, D.P., Rudloff, H.E., Rakusan, T.A. and Goldman, A.S. (1990) J. Clin. Invest. 86, 1623–1631.
[29] Dignam, J.D., Lebowitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1475–1489.
[30] Molitor, J.A., Walker, W.H., Doerre, S., Ballard, D.W. and Green, W.C. (1990) Proc. Natl. Acad. Sci. USA 87, 10028–10032.
[31] Fink, J.S., Verhave, M., Kasper, S., Tsukada, T., Mandel, G. and Goodman, R.H. (1988) Proc. Natl. Acad. Sci. USA 85, 6662–6666.
[32] Nichols, M., Weih, F., Schmid, W., DeVack, C., Kowenz-Leutz, E., Luckow, B., Boshart, M. and Schutz, G. (1992) EMBO, J. 9, 3337–3346.
[33] Gonzales, G. and Montminy, M.R. (1989) Cell 59, 675–680.
[34] Sheng, M., Thompson, M.A. and Greenberg, M.E. (1991) Science 252, 1427–1430.
[35] Chrivia, J.C., Kwok, R.P.S., Lamb, N., Hagiwara, M. and Montminy, R.M. (1993) Nature 365, 855–859.
[36] Arany, Z., Newsome, D., Oldread, E., Livingston, D.M. and Eckner, R. (1995) Nature 374, 81–84.
[37] Lundblad, J.R., Kwok, R.P.S., Laurance, M.E., Harter, M.L., and Goodman, R.H. (1995) Nature 374, 85–88.
[38] Brauweiler, A., Garl, P., Franklin, A.A., Giebler, H.A., and Nyborg, J.K. (1995) J. Biol. Chem. 270, 12814–12822.
[39] Kwok, R.P.S., Laurance, M.E., Lundblad, J.R. and Goodman R.H. (1995) submitted.