Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7070–7074, August 1992
Biochemistry

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

(DNA-binding protein/transcription factor)

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Communicated by Frederick C. Robbins, April 21, 1992 (received for review February 13, 1992)

ABSTRACT **HTLV-I Tax protein activates transcription from three 21-base-pair (bp) repeat sequences in the viral enhancer. The HTLV-I 21-bp repeat contains a TGACGT motif that is homologous to the cAMP-responsive element (CRE) and crucial for tax transactivation. Tax exhibits marginal affinity for DNA but rather interacts with cellular CRE-binding proteins to enhance their affinity for the HTLV-I 21-bp repeats. Using the HTLV-I 21-bp repeat and Jurkat T-lymphocyte nuclear extract in a gel electrophoretic mobility-shift assay, we previously detected three protein–DNA complexes that are specific for the CRE in the 21-bp repeat (complexes I, II, and IV). Complexes I and II but not IV interacted with Tax. We now show that complexes I, II, and IV are composed of CREB (CRE binding protein) homodimer, CREB/ATF-1 (activating transcription factor 1) heterodimer, and ATF-1 homodimer, respectively. Tax stabilizes complexes I and II via a direct interaction with the CREB moiety. In the absence of DNA, CREB and Tax continue to form a complex that can be immunoprecipitated by a Tax-specific antibody. These results suggest that one mechanism by which Tax activates transcription may be mediated through the direct interaction with CREB homodimer and/or CREB/ATF-1 heterodimer to stabilize their assembly on the Tax-responsive CRE motifs in the HTLV-I enhancer.**

The 3′ region of the HTLV-I proviral DNA encodes a 40-kDa nuclear protein, Tax, which stimulates transcription from three 21-base-pair (bp) repeat sequences in the viral enhancer (1–7) and from another cis-regulatory sequence, the NF-κB binding site (for reviews, see refs. 8 and 9). Purified Tax does not bind the HTLV-I 21-bp repeats (10). The fact that Tax activates transcription from two distinct cis elements also supports the notion that Tax does not directly interact with DNA. Saturation mutagenesis of the HTLV-I 21-bp repeat indicates that a sequence motif, TGACGT, is crucial for Tax activation (4, 10). The TGACGT motif, which appears in the enhancer/promoter region of numerous cellular and viral genes (11–13), has been termed the cAMP-responsive element (CRE) due to its role in mediating cAMP response. Several DNA-binding proteins of the bZIP family (containing basic and leucine zipper domains) that interact specifically with CRE have been cloned (14–18). The prototypic CRE-binding protein, CREB, is a 43-kDa protein that dimerizes via the leucine zipper domain in its carboxyl terminus and has been shown to mediate cAMP induction of gene expression (14, 15, 19). That the CRE motif mediates Tax transactivation implies a link between Tax and the CREB-like cellular transcription factors. Using a gel electrophoretic mobility-shift assay, we previously identified three specific protein–21-bp repeat complexes (I, II, and IV) with HeLa and Jurkat nuclear extracts (20). Proteins in complexes I, II, and IV contact the 21-bp repeats at the CRE-like motifs as evidenced by the abrogation of all three protein–DNA complexes by mutations in the CRE (10, 20). Tax bound to complexes I and II directly (20). Upon binding by Tax, the stability of complexes I and II became greatly enhanced. In this paper, we show that the interaction between Tax and complexes I and II is due to a direct interaction of Tax with CREB. Complex I is composed of CREB homodimer, and complex II derives from a heterodimer formed between CREB and a distinct DNA-binding protein, ATF-1 (activating transcription factor 1), which shows a high degree of sequence similarity to CREB (16, 17). The role of ATF-1 in modulating CREB function and Tax transactivation is discussed.

MATERIALS AND METHODS

Expression and Purification of CREB and ATF-1. The cDNA for CREB (15) was cloned at the *Nde* I/*Bam*HI sites of the pET-11a (21) expression plasmid, and that for ATF-1 (16) at the *Nco* I/*Bam*HI sites of pET-11d. Both cDNAs encode authentic proteins expressed from the bacteriophage T7 promoter. The transformed cells [*Escherichia coli* BL21(DE3)] containing the pET expression plasmids were grown and induced as described (21). The CREB or ATF-1 expressed after induction with isopropyl β-D-thiogalactopyranoside represents ≈10% of total cellular proteins as judged by Coomassie staining after SDS/PAGE. Both proteins remain soluble and can be readily purified on a DNA-cellulose column. The purified CREB and ATF-1 were identified by Coomassie staining, immunoblot analysis, and gel shift assays.

***In Vitro* Phosphorylation of CREB and ATF-1.** The method of Hurst *et al.* (22) was followed with minor modifications. ATF-1 or CREB (≈1 μg in 3 μl) purified by DNA-cellulose chromatography was mixed with 1 μl (5 units) of protein kinase A catalytic subunit (Sigma) and 1 μl of the 5× kinase buffer (0.25 M KH_2PO_4/K_2HPO_4, pH 7.2/50 mM $MgCl_2$/15 μM ATP) and incubated at 37°C for 15 min. The phosphorylated proteins were diluted 60-fold and used directly for gel shift assay.

RESULTS

The Cellular Factors That Bind Tax and the HTLV-I 21-bp Repeats Contain CREB. Using a 70-bp *Xho* I–*Nco* I DNA fragment containing two copies of the HTLV-I 21-bp repeat in gel shift assays with nuclear extracts prepared from Jurkat

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Abbreviations: HTLV-I, human T-cell lymphotropic virus type I; CRE, cAMP-responsive element.

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and HeLa cells, we previously identified three specific protein–DNA complexes (I, II, and IV; ref. 20) as shown in Fig. 1*A*, lanes 1 and 3. All three protein–DNA complexes were abrogated by mutations in the CRE, indicating that the proteins responsible for formation of complexes I, II, and IV contact the 21-bp repeats at the CRE motifs (10, 20). Upon addition of $TaxH_6$, a recombinant Tax containing six histidine residues at the carboxyl terminus, two effects were observed (20). First, complexes I and II were converted to I_X and II_X (Fig. 1*A*, lanes 2 and 4). Second, the levels of complexes I and II, and to a lesser extent, IV, were enhanced (compare lanes 1 and 2, and lanes 3 and 4). The presence of Tax in complexes I_X and II_X was shown by the supershift of these complexes by a Tax-specific antibody (20). Under the same experimental conditions, no Tax-induced mobility shift was observed for complex IV. When a 30-bp *Xho* I–*Bgl* II DNA fragment (10) containing the promoter-distal 21-bp repeat was used in the assay, bands similar to I, II, and IV were also observed (data not shown), suggesting that complexes I, II, and IV represent binding of three distinct cellular factors at one of the two CREs in the 70-bp *Xho* I–*Nco* I DNA fragment. Complexes formed with the *Xho* I–*Bgl* II DNA fragment could not be shifted by Tax (see *Discussion*). Two bands migrating at positions lower than complex III were also detected when HeLa nuclear extract was used (Fig. 1*A*, lanes 3 and 4). They appeared to be related to complex IV as shown below.

Since proteins responsible for complexes I, II, and IV bind to the CRE motifs in the HTLV-I 21-bp repeats, they most likely are composed of members of the CREB/ATF family of proteins. Indeed, CREB can activate transcription from the HTLV-I 21-bp repeats (23). Consistent with these results, transactivation by Tax has also been shown to require a functional cAMP regulatory pathway (25–28). To determine the relations between CREB and complexes I, II, and IV, a rabbit antibody prepared against a peptide corresponding to the phosphorylation domain of CREB (24) was added to the DNA-binding reaction mixtures. Bands I and II were shifted by the CREB antibody whereas band IV appeared unaffected (Fig. 1*B*, lane 2). The same results were obtained with HeLa nuclear extract (data not shown). Immunoblot analysis of Jurkat and HeLa nuclear extracts (Fig. 1*C*) showed that the CREB antibody recognized primarily one protein species. Thus, the mobility shifts of complexes I and II caused by the CREB antibody are most likely due to the presence of CREB in these complexes and suggest a direct link between Tax and CREB.

Direct Interaction Between Tax and CREB. To test the role of CREB in the formation of complexes I and II, we compared the mobility-shift profile of purified CREB (Fig. 2*A*, lane 3) with that of Jurkat nuclear extract (lane 2). As indicated, CREB bound the HTLV-I 21-bp repeats and yielded a protein–DNA complex (lane 3) that migrated at a position similar if not identical to that of complex I (lane 2). Addition of Tax to the reaction mixture containing CREB resulted in a mobility shift and a substantial increase in the level of the CREB–21-bp repeats complexes (compare lanes 3 and 4), suggesting that CREB and Tax interact directly.

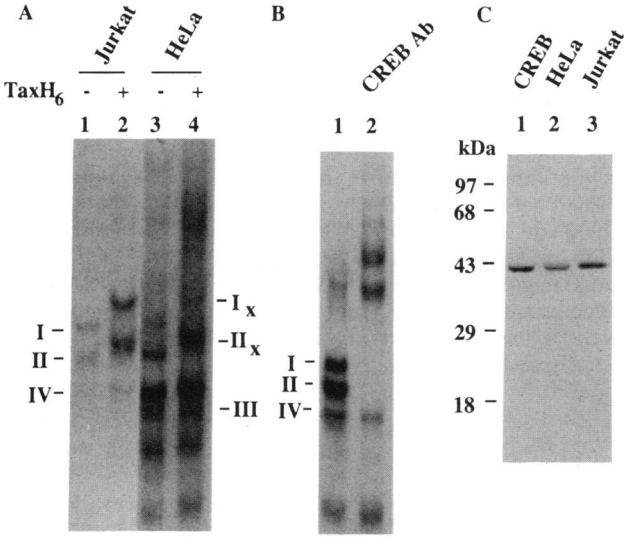


FIG. 1. Identification of CREB as a target of Tax. (*A*) Gel shift assay was carried out as described (20) with a ^{32}P-labeled DNA fragment containing two copies of the HTLV-I 21-bp repeats, 1 μl (protein concentration, 5 mg/ml) of Jurkat (lanes 1 and 2) or HeLa (lanes 3 and 4) cell nuclear extract, and 0.5 μg of poly(dI-dC). Nuclear extracts were prepared as described (20). The Jurkat (T-cell line) nuclear extract was partially purified on a DNA-cellulose column (23). Protein–21-bp repeats complexes are denoted as I–IV, and the ones containing Tax as I_X and II_X. To observe the mobility changes of complexes I and II in the presence of Tax, electrophoresis was carried out until the xylene cyanol tracking dye migrated 12 cm in a 4% polyacrylamide gel and the unbound DNA migrated out of the gel. Lanes 2 and 4 contained ≈0.1 μg of purified $TaxH_6$ [recombinant Tax with six histidine residues at the carboxyl terminus (20)]. (*B*) Gel shift assay was performed as in *A* with Jurkat nuclear extract without (lane 1) or with (lane 2) 0.5 μl of CREB antibody (24) prepared against the peptide (AESEDSQESVDSVTDSQKRREILSRRPSYRKIL) corresponding to the phosphorylation domain of CREB. (*C*) Western blot with the CREB antibody. Lanes 1, 2, and 3 contain 0.1 μg of purified CREB or 20 μg of partially purified HeLa or Jurkat nuclear extract, respectively.

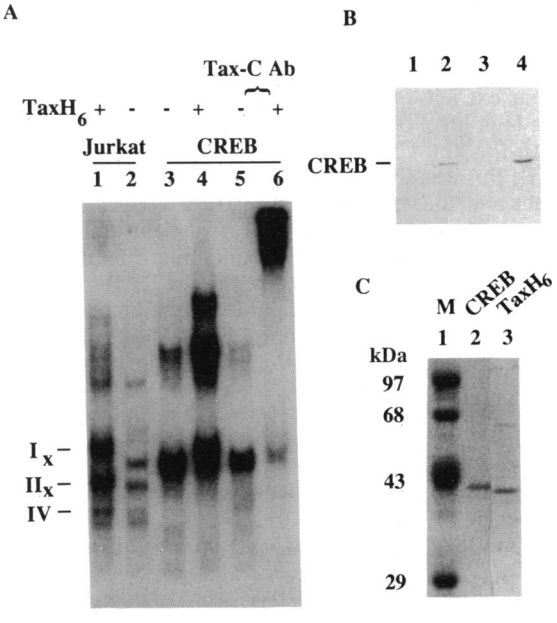


FIG. 2. Direct interaction between CREB and Tax. (*A*) Gel shift assay with Jurkat nuclear extract (lanes 1 and 2) or purified CREB (lanes 3–6). This CREB corresponds to CREB-341 (341 amino acids; ref. 15). Lanes 1, 4, and 6 contained 0.1 μg of $TaxH_6$, and lanes 5 and 6 contained 0.5 μl of Tax-C antibody [generated against the carboxyl-terminal portion of Tax (29)]. The bands in lane 1 are denoted I_X and II_X due to the presence of Tax in these complexes. The two bands migrating slightly lower than I_X and II_X in lane 2 correspond to complex I and II. (*B*) Coimmunoprecipitation of the CREB/Tax complex by Tax-C antibody. Purified CREB (0.2 μg) was incubated with 1 μl of Tax-C antibody (lanes 1 and 2), β-galactosidase antibody (lane 3), or CREB antibody (lane 4) in the presence (lanes 2 and 3) or absence (lanes 1 and 4) of 0.1 μg of $TaxH_6$ at 4°C for 1 hr. Protein A-Sepharose (2.5 mg) was then added and incubation continued for 1 hr. The precipitated proteins were separated by SDS/10% PAGE, transferred to Immobilon membrane (Millipore), and reacted with the antibody to CREB. (*C*) Purity of CREB and $TaxH_6$. CREB (lane 2) and $TaxH_6$ (lane 3) were analyzed by SDS/10% PAGE followed by staining with Coomassie blue. Lane 1, protein size markers (M).

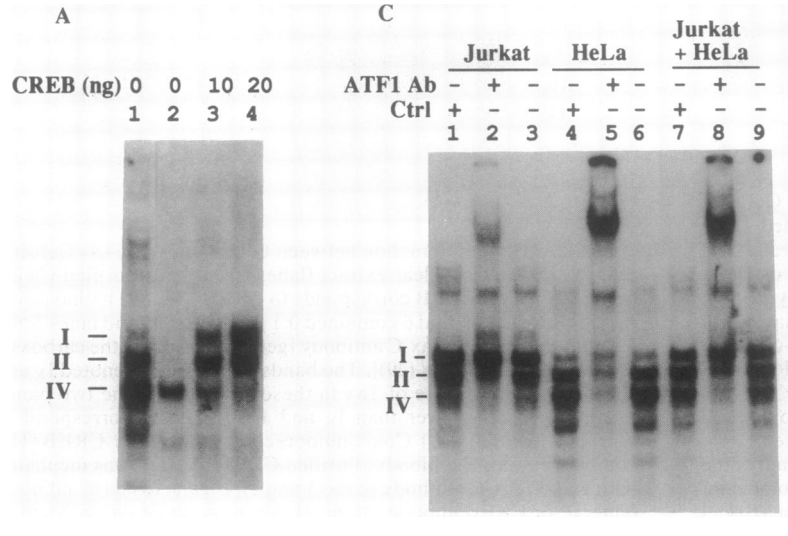
Complexes migrating at positions higher than complex I were observed in reactions with nuclear extracts or purified CREB (lanes 1–4). These complexes appeared only when higher amounts of CREB or extract were used and/or when their binding to DNA became enhanced by Tax. Their formation is most likely due to binding of protein factors to both CRE sites in the *Xho* I–*Nco* I fragment, which contains two copies of the HTLV-I 21-bp repeat.

The direct interaction between CREB and Tax was further demonstrated as follows. First, an antibody (Tax-C antibody) that recognizes the carboxyl-terminal region of Tax (29) was added to the binding reaction mixtures and shown to induce a supershift of the CREB–Tax complex (Fig. 2*A*, lane 6). CREB alone in the absence of Tax did not react with the Tax-C antibody (lane 5). Second, purified CREB (Fig. 2*C*, lane 2) and Tax (lane 3) when incubated together can be coimmunoprecipitated with the Tax-C antibody (Fig. 2*B*, lane 2; compare with CREB precipitated by the CREB antibody in lane 4) but not by a control antibody (lane 3), as detected by the immunoblot probed with CREB antibody. In the absence of Tax, CREB could not be precipitated by the Tax-C antibody (Fig. 2*B*, lane 1). From these results, we conclude that Tax interacts with complexes I and II via a direct binding to CREB and that the Tax–CREB interaction can occur in the absence of DNA. These data also indicate that CREB alone is sufficient for complex I formation.

CREB Forms a Complex with a Distinct CRE-Binding Factor, ATF-1. Complex I but not complex II could be reconstituted with CREB alone (Fig. 2*A*). Although complex II contains CREB (Fig. 1*B*), it appears to be distinct from CREB. The relationships of complexes I, II, and IV were revealed by the following experiments. Because the HeLa nuclear extract contained a low level of complex I (CREB) and a high level of complex IV (Fig. 1*A*, lane 3), we treated it with the anti-CREB antibody to deplete the endogenous CREB. Purified CREB was then added back to the depleted extract and the reconstituted mixture was analyzed by gel shift assay. The depleted HeLa nuclear extract yielded complex IV but not the CREB-containing complexes I and II (Fig. 3*A*, lane 2). Addition of purified CREB to this extract (lanes 3 and 4) resulted in a quantitative reduction in complex IV and the appearance of complexes I and II. These results indicate that complexes I, II, and IV can be reconstituted with CREB and a distinct protein factor responsible for complex IV formation and that complex II most likely derives from a complex formed between these two proteins.

ATF-1 [also called ATF-43 (22) or TREB36 (17)], a distinct member of the CREB/ATF family that shows a high degree of sequence similarity to CREB in the bZIP domains (Fig. 3*B*), forms a heterodimer with CREB (30). We investigated the relations between ATF-1 and complex IV by adding an antibody specific for the carboxyl-terminal region of ATF-1 (22) to the gel shift assay. Complexes II and IV but not complex I in Jurkat nuclear extract (Fig. 3*C*, lanes 1–3), HeLa nuclear extract (lanes 4–6), and a 1:1 mixture of Jurkat and HeLa nuclear extracts (lanes 7–9) were specifically shifted by the ATF-1 antibody (lanes 2, 5, and 8) but not by a control antibody (lanes 1, 4, and 7). Thus, the supershift of complexes II and IV, but not I, by the ATF-1 antibody is due to the presence of ATF-1 in both complexes. The complexes migrating at positions lower than complex IV, observed primarily in HeLa nuclear extract (Fig. 1*A*, lanes 3 and 4; Fig. 3*C*, lanes 4 and 6), were also shifted by the ATF-1 antibody (Fig. 3*C*, lanes 5 and 8), suggesting that they were derived from ATF-1.

Reconstitution of Complexes I, II, and IV with Purified CREB and ATF-1. That complexes II and IV contain ATF-1 was further demonstrated by reconstitution of all three complexes with *Escherichia coli*-synthesized CREB and ATF-1. A major ATF-1/HTLV-I 21-bp repeats complex (Fig. 4*B*, arrow) could be formed with purified ATF-1 (the faster-migrating band was most likely derived from the proteolytic degradation of ATF-1). Further, the ATF-1 protein–21-bp repeats complex could be supershifted by the ATF-1 antibody used in Fig. 3*C* (Fig. 4*B*) as anticipated. When bacterially produced CREB and ATF-1 were mixed and analyzed in the gel shift assay along with the ^{32}P-labeled 21-bp repeats (Fig. 4*C*, lane 1), a new protein–DNA complex (marked C/A) appeared between the CREB and ATF-1 complexes. This new complex apparently derived from the heterodimer formed between CREB and ATF-1 (compare with CREB in lane 2 and ATF-1 in lane 3). We observed that the bacterial ATF-1 protein–21-bp repeats complex (marked A in lanes 1 and 3) migrated substantially faster than complex IV (lane 5). Since complex IV reacted with the ATF-1 antibody and is likely to be composed of ATF-1 alone, the difference in



B

CREB bZIP

KREVRLMKNREAARECRRKKKEYVKCLENRVAVLENQNKTLIEELKALKDLYCHKSD
*** ********************************** ***** **
KREIRLMKNREAARECRRKKKEYVKCLENRVAVLENQNKTLIEELKTLKDLYSNKSV

ATF-1 bZIP

FIG. 3. Heterodimer formation between CREB and ATF-1. (*A*) Gel shift assay with HeLa nuclear extract (lane 1) or the same extract depleted of endogenous CREB by a CREB antibody (lanes 2–4). To deplete endogenous CREB, 10 μl of HeLa nuclear extract was incubated for 1.5 hr with 1 μl of CREB antibody and 2 mg of protein A-Sepharose beads. After a brief centrifugation to pellet the beads, 1 μl of the supernatant was used in the gel shift assay in the absence of CREB (lane 2) or with 10 ng (lane 3) or 20 ng (lane 4) of purified CREB. (*B*) Sequence comparison of the CREB and ATF-1 bZIP domains. Asterisks indicate amino acid sequence identity; dots denote differences. (*C*) Gel shift assay with Jurkat nuclear extract (lanes 1–3), HeLa nuclear extract (lanes 4–6), or a 1:1 mixture of Jurkat and HeLa nuclear extracts (lanes 7–9). The ATF-1 antibody was generated against the carboxyl-terminal peptide of ATF-1 (22). Ctrl, control preimmune serum. Complexes II and IV, and the complexes migrating at positions lower than complex IV (ATF-1-related) were specifically shifted by the ATF-1 antibody (lanes 2, 5, and 8) but not by the control antibody (lanes 1, 4, and 7) when compared with the complexes detected with extracts alone (lanes 3, 6, and 9).

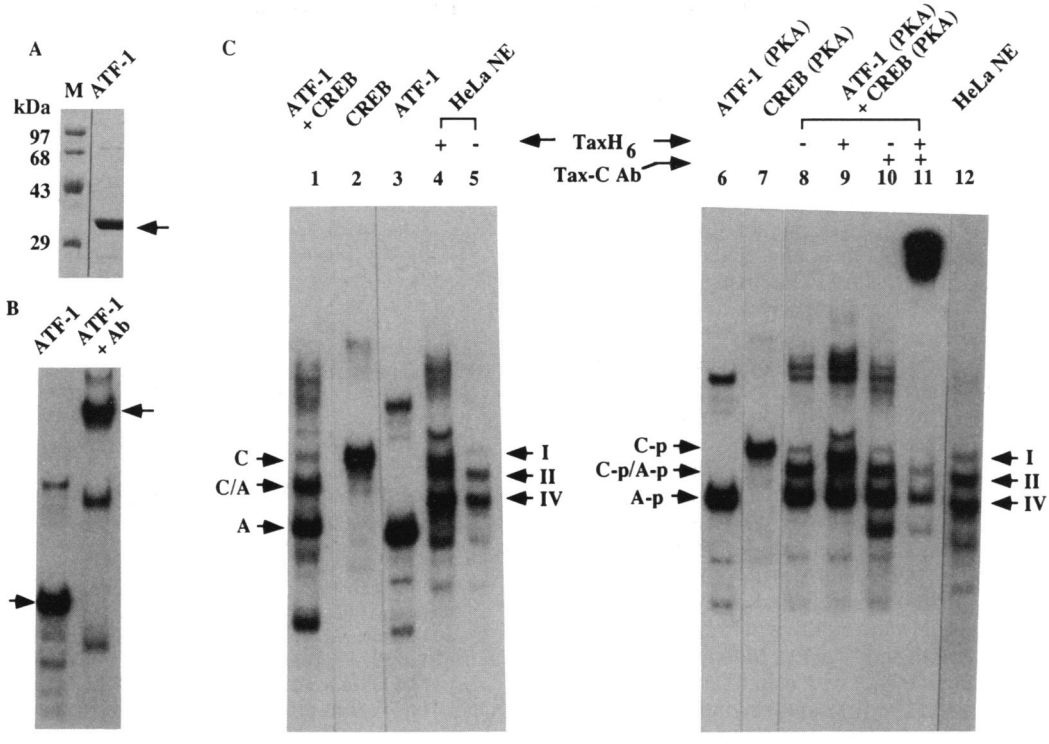


FIG. 4. Reconstitution of complexes I, II, and IV with *E. coli*-synthesized CREB and ATF-1. (*A*) *E. coli*-synthesized ATF-1 examined by SDS/10% PAGE followed by staining with Coomassie blue. Lane M, protein size markers. (*B*) Gel shift assay with *E. coli*-synthesized ATF-1 or ATF-1 and 0.25 μl of anti-ATF-1 carboxyl-terminal antibody (Ab). Assay condition and the radiolabeled DNA were the same as in Fig. 1, with 0.1 μg of ATF-1. The major ATF-1–DNA complex is marked with an arrow. The faster-migrating minor bands in both lanes most likely derived from breakdown products of ATF-1. The slower-migrating band represents ATF-1 binding to both 21-bp repeats present in the DNA fragment. (*C*) Gel shift assay with HeLa nuclear extract (HeLa NE), *E. coli*-produced ATF-1 and CREB, or ATF-1 and CREB phosphorylated *in vitro* (≈10 ng of protein each) by protein kinase A (PKA). Letters on the sides of the panel denote the protein components in the protein–DNA complexes: C, C/A, and A refer to CREB homodimer, CREB/ATF-1 heterodimer, and ATF-1 homodimer; C-p, C-p/A-p, and A-p refer to their phosphorylated forms. $TaxH_6$ (0.1 μg in 3 μl) was added to lanes 4, 9, and 11. Lanes 10 and 11 contained 0.5 μl of the Tax-C antibody. No Tax-C antibody super-shift was observed when $TaxH_6$ was excluded from the binding reaction (compare lanes 10 and 11). All the lanes in *C* were from the same autoradiograph but were regrouped for clarity.

electrophoretic mobilities between complex A and complex IV suggests structural differences between the bacterially produced ATF-1 and the ATF-1 from HeLa and Jurkat cells. The structural difference between these two forms of ATF-1 is probably due to the absence of phosphorylation of the *E. coli*-synthesized protein. When *E. coli* ATF-1 was phosphorylated *in vitro* by protein kinase A and used in the gel electrophoretic mobility-shift assay, a complex (marked A-p) migrating at a position close to complex IV was detected (Fig. 4*C*, compare lanes 6 with 12). Similarly, the protein kinase A-phosphorylated CREB also produced a slightly slower migrating CREB–21-bp repeats complex (marked C-p in lane 7). The alteration in mobility after *in vitro* phosphorylation observed for CREB, however, was not as pronounced as for ATF-1. Further, although protein kinase A-phosphorylated ATF-1–DNA complex (A-p) migrated to a position close to complex IV, a subtle difference in electrophoretic mobilities of A-p and IV remained. We think this is due to differences in the degree and/or sites of phosphorylation between the ATF-1 phosphorylated *in vitro* and the ATF-1 produced in HeLa and Jurkat cells. When the phosphorylated forms of CREB and ATF-1 were mixed, a new complex representing their heterodimer (C-p/A-p) was again observed (lane 8). As expected, $TaxH_6$ altered the mobilities of the CREB homodimer and the CREB/ATF-1 heterodimer (lane 9) as was observed for complexes I and II (lane 4). That the Tax-induced mobility changes in C-p and C-p/A-p were due to the physical interaction between Tax and CREB and CREB/ATF-1 complexes was confirmed by the supershift of the Tax/C-p and Tax/C-p/A-p complexes by the Tax-C antibody (lane 11). Considering the results shown in Figs. 3 and 4, we conclude that complexes I, II, and IV consist of CREB homodimer, CREB/ATF-1 heterodimer, and ATF-1 homodimer, respectively.

DISCUSSION

In this paper, we show that the HTLV-I transactivator, Tax, directly interacts with CREB. This work extends our previous results in which CRE-specific protein–DNA complexes were shown to be stabilized by a direct interaction with Tax (20). We now show that these complexes, I, II, and IV, contain CREB homodimer, CREB/ATF-1 heterodimer, and ATF-1 homodimer, respectively. The basis for Tax to interact with complexes I and II lies exclusively in the CREB moiety. This interaction most likely brings about the transactivation of the HTLV-I long terminal repeat. This notion is supported by the fact that mutations abolishing Tax effect are all localized in the CRE of the 21-bp repeats (10) and the observation that Tax effect was substantially diminished in a cell line lacking protein kinase A (28). In addition, when CREB and Tax were expressed after cotransfection, a synergistic effect on transactivation of the HTLV-I 21-bp repeats was observed (23). Our finding that CREB forms a heterodimer with ATF-1 is consistent with similar results reported by Hurst *et al.* (30). As both CREB homodimer and CREB/ATF-1 heterodimer interact similarly with Tax and bind the HTLV-I 21-bp repeats, their relative contribution to Tax transactivation is difficult to assess. It appears that ATF-1 can modulate CREB activity by competing for binding

to the 21-bp repeats and by heterodimer formation with CREB. Interestingly, when nuclear extracts from several human cell lines (Jurkat, MT4, Namalwa, JEG-3, and HeLa) were tested for the levels of complexes I, II, and IV, the CREB (complex I) level was found to be the highest in T cells (data not shown). The lower level of complex I activity (caused by CREB homodimer) in HeLa compared with Jurkat cells is due to a relative lack of CREB expression and an overabundance of ATF-1. The higher levels of CREB homodimer observed in T-cell lines can be explained by the higher molar ratio of CREB to ATF-1 and suggest a possible molecular basis for the T-lymphotropism of HTLV-I gene expression and pathology.

While two copies of the HTLV-I 21-bp repeat can confer a high level of Tax responsiveness, the first copy of the 21-bp repeat (the promoter-proximal copy), in combination with other transcription factor binding sites, can exert a similar effect (7, 31). It is therefore possible that CREB/Tax binding to the first copy of the 21-bp repeat constitutes the first step in Tax transactivation. Elevated Tax responsiveness then depends on contributions from transcription factor(s) bound to adjacent sequences. Consistent with this hypothesis, CREB has a higher affinity for the first copy of the 21-bp repeat and Tax interacts preferentially with this CREB–21-bp repeat complex (unpublished work). Marriott *et al.* (32) reported that Tax could interact with a 36-kDa protein bound to the sequence between the first and second copies of the 21-bp repeat in the HTLV-I long terminal repeat (32). The identity of the 36-kDa protein, however, remains unknown. A study in which Tax was fused with the yeast transcription factor GAL4 indicated that the GAL4–Tax fusion protein could transactivate via the GAL4 binding site (33). It is possible that GAL4–Tax may recruit CREB to the DNA and thus bring about transcriptional activation.

What is the biochemical nature of Tax–CREB interaction that enhances CREB binding to DNA? It is possible that via protein–protein interaction Tax may induce a conformational change in CREB to stabilize its interaction with DNA. Alternatively, when it is complexed with CREB, Tax may contact DNA sequences flanking the CRE motif in the 21-bp repeats to facilitate the assembly of the protein–DNA complexes. If the function of Tax is to assist in the assembly of CREB on the HTLV-I enhancer, then the mechanism of Tax transactivation would appear to be in contrast to those of herpes simplex virus VP16 and adenovirus E1A, proteins that activate transcription by providing acidic transactivation domains after interacting with sequence-specific DNA-binding proteins (for review, see ref. 34). The identification of CREB as one of the key cellular targets of Tax should facilitate the elucidation of the mechanism of Tax action and the etiology of adult T-cell leukemia.

We thank Dr. B. Cullen (Duke University) for the Tax-C antibody, Dr. J. Habener (Harvard Medical School) for the anti-CREB peptide antibody, Dr. M. Montminy (Salk Institute) for the purified CREB protein, Dr. M. Green (University of Massachusetts) for the ATF-1 cDNA clone, and Dr. H. Hurst (Imperial Cancer Research Fund) for the ATF-1 carboxyl-terminal peptide antibody. This work was supported by grants from the National Institutes of Health (R01 CA48709) and the Council for Tobacco Research (1728A).

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