Differential Activation of Viral and Cellular Promoters by Human T-cell Lymphotropic Virus-I Tax, CRE-binding proteins, and CBP

by

Megan E. Laurance

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CERTIFICATE OF APPROVAL

This is to certify that the Ph.D. thesis of

Megan E. Laurance

has been approved

[redacted]
Professor in charge of thesis

[redacted]
Member

[redacted]
Member

[redacted]
Member

[redacted]
Associate Dean for Graduate Studies

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ABSTRACT

HTLV-1 Tax activates viral gene expression through elements in the HTLV-1 LTR that resemble the cellular cAMP-regulated enhancer (CRE). This activation, as well as the activation of selected cellular genes depends on interactions of Tax with cellular CRE-binding proteins. This thesis focuses on understanding the mechanism by which Tax utilizes transcription factors involved in the cAMP-responsive pathway to mediate this activation. Specifically, three CRE-binding proteins, the activator CREB and the inhibitors CREMα and CREM Δ(C-G), have been used to elucidate the mechanism by which Tax recruits DNA-binding proteins to viral and cellular enhancer elements. By utilizing naturally occurring basic/leucine zipper (bZIP) isoforms I demonstrate which of the highly conserved functional domains in these transcription factors are necessary and sufficient for activation of viral and cellular promoters. I use transient transfection studies to characterize Tax-mediated transactivation of viral and cellular CRE-containing genes in the presence and absence of PKA. Tax-mediated activation of the viral and cellular CREs by bZIP factors differs with respect to their dependence on protein kinase A (PKA)-phosphorylation. Furthermore, Tax converts the repressors CREMα and CREM Δ(C-G) into activators of gene expression. I use fluorescence polarization binding assays to examine the role of Tax in recruiting DNA-binding proteins to viral and cellular enhancer elements. These experiments demonstrate that Tax is able to enhance binding bZIP factors to the HTLV-I CRE-element, but has no effect on the binding of these factors to their consensus cellular CRE-element.

The varying requirement for phosphorylation in Tax-mediated activation of viral and cellular promoters suggests a potential role for Tax in recruiting the transcriptional co-activator CREB-binding-protein (CBP) to these enhancers. Using a biochemical approach, I demonstrate that while Tax has no effect on the affinity of CBP for a phosphorylated

bZIP:cellular CRE complex, Tax does indeed facilitate a high affinity interaction between CBP and a bZIP:viral CRE complex, and renders this interaction phosphorylation independent.

The differential requirement for PKA in recruitment of CBP suggests that Tax mediates activation of viral and cellular CREs through different domains of these conserved bZIP proteins. In order to test this model, I use a naturally occurring isoform of CREMα, CREM Δ(C-G), which contains a conserved bZIP domain, but no KID, in functional and biochemical assays. Indeed, results from transient transfection assays, and fluorescence polarization binding assays support a model in which the basic/leucine zipper domain is sufficient for activation by Tax and CBP in the context of the HTLV-I promoter. Conversely, a phosphorylated KID is required for activation of the cellular CRE by Tax and CBP.

The results from this thesis led to the conclusion that Tax activates the HTLV-1 CRE by enhancing the assembly of bZIP on viral DNA, by increasing the affinity of the transcriptional co-activator CBP for these CRE-binding proteins, and by binding to CBP directly. All of these actions occur independent of phosphorylation of bZIP factors by PKA. Conversely, Tax activates the cellular CRE by binding directly to CBP and, in this instance, requires phosphorylation of the KID by PKA.

INTRODUCTION

The human T-cell lymphotropic virus Type-I: a transforming retrovirus

Since the initial discovery of RNA and DNA tumor viruses researchers have learned that identification of cellular proteins critical to the progression of the viral life cycle (Figure 1) leads to an understanding of the host proteins which play key regulatory roles in the normal processes of the cell. In particular, research into the mechanism of transformation by such animal retroviruses as the rous sarcoma virus and murine leukemia virus has served to identify oncogenes such as v-src, and the role of their cellular proto-oncogene counterparts in various signal transduction pathways.

The search for related viruses in man led to the discovery of a human retrovirus, isolated from cultured T-lymphocytes of a patient in the United States with a mature T-cell malignancy (Poiesz *et al*, 1980 and Yoshida *et al*, 1982). This virus, now designated the human T-cell leukemia virus type-I, is the prototype for isolates of the same virus obtained from various parts of the world. Another member of this family, HTLV- type II, was isolated from the transformed T-lymphocytes of a patient with a T-cell variant of hairy cell leukemia (Kalyanaraman *et al*, 1982). While there are striking similarities between HTLV-type I and II, HTLV-II is not as well characterized as HTLV-I. Thus the focus of this thesis reflects the efforts to elucidate the regulatory mechanisms of HTLV-I.

The etiological link of this virus to leukemia was provided in 1980 upon investigation of a syndrome first described in Japan called adult T-cell leukemia (ATL) (Manns *et al*, 1991). Subsequent studies with nucleic acid probes from HTLV-I isolates confirmed that the virus causing ATL was identical to HTLV-I (Wong-Staal *et al*, 1985). Epidemiological results, studies of in vitro transformation in primary human T-cells (Gazzolo *et al*, 1987), and the comparison of the molecular properties of isolates from ATL patients led to the conclusion that HTLV-I is the primary cause of this cancer. Studies from

Gallo and colleagues (Wong-Staal *et al*, 1985), and Yoshida and co-workers (1982) led to the conclusions that 1) all ATL cells contain a HTLV-I provirus, 2) the provirus is clonal, indicating that infection was before the time of the initial transformation event, and 3) the integration sites vary from on patient to another. This variation in integration site suggested that transformation by HTLV-I probably occurs through a trans-mechanism, as opposed to long terminal repeat (LTR) activation of a nearby cellular gene, (i.e. insertional mutagenesis).

HTLV-I-associated diseases

The malignant cells in the peripheral blood of ATL patients are lymphocytes which stain with CD4 monoclonal antibodies and are of the same origin as cells found infiltrating the lymph nodes in these patients. They contain clonally integrated HTLV-I viral sequences (Murphy *et al*, 1988). Many cell types can be infected with HTLV-I including lymphoid, endothelial, fibroblasts, osteoid, muscle (Clapham *et al* 1983, Fann *et al*, 1992, Ho *et al*, 1984, Hoffman *et al*, 1992, and Kirchbaum-Stenger *et al*, 1987). However, HTLV-I has been shown to only immortalize T-cells. HTLV-I is required, but not sufficient to induce ATL. Indeed, less than 0.1% of infected individuals ever develop the disease, and the latency period can last as long as 20 years (Hinuma *et al*, 1982). Fresh isolates of peripheral leukemic cells frequently do not have detectable viral antigens. These lines of evidence suggest that an independent, second event is required beyond infection in order to induce ATL. As a consequence, viral products are not required for maintenance of the malignant phenotype.

Tropical spastic paraparesis (TSP), or HTLV-I associated myelopathy (HAM) is a slowly progressive myelopathy that is characterized by paraparesis and spasticity of the lower extremities (Molgaard *et al*, 1989). The incidence and prevalence of TSP/HAM correlates well with those geographic regions endemic for HTLV-I, particularly the Caribbean basin, Africa, Colombia, southern Japan, and the United States (Gessain *et al*,

1985). Established TSP/HAM cell lines are of T-cell lineage, with activated T-cell markers. Patients with TSP/HAM reveal high levels of circulating HTLV-I-specific cytotoxic T-lymphocytes which predominantly recognize a regulatory protein Tax, encoded by HTLV-I (Jacobson *et al*, 1990). Cytotoxic T-lymphocytes directed against HTLV-I Tax have been demonstrated in TSP/HAM patients and have been suggested to correlate with disease progression. These findings provided initial evidence that the presence of Tax may be related to productive viral infection or to viral pathogenesis and neurologic degeneration.

HTLV-I and II differ from other leukemia and sarcoma retroviruses in that they do not contain cell-derived oncogenes, nor do they require active viremia for transformation/ leukemogenesis. In addition, no common integration site of the provirus has been found in primary tumor cells (Seiki *et al*, 1984). This suggested a mechanism of leukemogenesis involving a trans-acting viral element. In this sense, transformation by HTLV-I appeared to be somewhat analogous to transformation by DNA tumor viruses which encode viral oncogenes that primarily function as nuclear trans-activators of viral gene expression, and can also induce transformation due to their effects on the expression of host cell genes. The constitutive expression of IL-2 receptors in HTLV-I positive ATL cells supports this model (Kronke *et al*, 1985, and Tendler *et al*, 1990). The IL-2 receptors of these virus-positive leukemic cells cannot be down-regulated, and are unusually abundant. T-cell lines immortalized by *in vitro* infection by HTLV-I were partially or completely independent of exogenous IL-2, and expressed high levels of IL-2 receptors (Inoue *et al*, 1986). The identification of a viral protein that mediates the biological effects of HTLV-I infection would provide a natural explanation for the ability of HTLV-I to transform cells *in vitro*, and for the absence of preferential integration sites in tumor cells.

Direct role of HTLV-I Tax in transformation

Evidence supporting a role for the regulatory protein Tax in leukemogenesis came from analysis of leukemic cells from ATL patients that contained defective HTLV-I proviral

genomes which had preferentially retained the 3' ORF encoding Tax (Yoshida *et al*, 1982). In vitro studies demonstrated that overexpression of Tax could immortalize primary T cells, although the cells remained IL-2 dependent (Grassman *et al*, 1989). When the HTLV-I regulatory protein coding sequences were integrated into a transformation-defective herpesvirus, it was able to transform T-cells of the same phenotype as found with HTLV-I (Grassman *et al*, 1989). Of the three proteins encoded by the pX region, the Tax gene is critical for this transformation since specific mutations of the Tax initiation codon eliminated the transformation potential of the herpesvirus-HTLV-I pX recombinant virus. Introduction of Tax into primary rat embryo fibroblasts immortalized cells and, in cooperation with the ras oncogene, transformed primary fibroblasts such that they were tumorigenic in nude mice (Pozzatti *et al*, 1992, Tanaka *et al*, 1990). Tax has been shown to induce neurofibromas and mesenchymal and fibroblastic tumors in transgenic mice (Hinrichs *et al*, 1987, and Nerenberg *et al*, 1987)

Activation of cellular genes by Tax

Tax has been shown to regulate the expression of several cellular genes (Table I). It has been hypothesized that Tax may immortalize and transform cells through the induction of cellular genes implicated in cell growth regulation, and the resultant loss of the normal transcriptional control of these genes in the infected cell. For example, it has been suggested that an autocrine loop involving Tax-induction of IL-2Rα and IL-2 expression may lead to the immortalization and transformation of HTLV-I-infected lymphocytes (Maruyama *et al*, 1987). Tax-mediated transcription of cellular genes appears to occur through several structurally unrelated cellular transcriptional activator proteins, including members of the ATF/CREB family; serum response factor; and the NF-kB family of transcription regulatory proteins.

Tax transactivates the c-fos, Krox-20 and Krox-24 genes through multiple cellular proteins, including the CREB/ATF family of transcription factors, and serum response factor (Alexandre *et al*, 1991, Alexandre *et al*, 1991, Fujii *et al*, 1988, and Fujii *et al*, 1992)

HTLV-I Tax has been shown to negatively regulate the host DNA repair enzyme DNA-polymerase β, which may contribute to chromosome mutations (Jeang *et al*, 1990). A possible mechanism of repression of the β-polymerase gene, a gene involved in DNA repair, has recently been elucidated. Unittenbogaard *et al*, (1994) hypothesized that Tax interacts with bHLH proteins to disrupt normal regulation of β-polymerase and promote entry into the cell cycle. Indeed, the putative "E-box" in the β-polymerase promoter conferred Tax repression to a heterologous promoter. Tax repressed gene expression through all 3 classes of bHLH proteins, and in all cases, repression by Tax was dependent upon the E-box (5'-CANNTG-3').

NF-κB regulation of cellular gene transcription displays several levels of complexity. At the DNA level several distinct κB-regulatory binding sites have been found in association with various cellular and viral genes. The proteins in the NF-κB complex consist of several distinct protein species. These include a 50 kDa DNA-binding subunit and an associated transactivation protein of approximately 65 kDa. Both subunits are related to the rel oncogene.

In unstimulated cells, NF-κB can be detected in the cytoplasm associated with an inhibitor protein (IκB) that complexes to NF-κB and inhibits DNA-binding activity. This complex can be disassociated by treatment with different protein kinases in vitro. This phosphorylation likely results in modification of the inhibitor IκB, although it may also affect phosphorylation of NF-κB, and it has been demonstrated that release from IκB may facilitate translocation into the nucleus.

Tax induction of NF-κB is an important mechanism of transcriptional activation of some cellular genes. Tax expression in virally infected and transfected cells induces IL-2Rα and IL-2 gene expression through induction of NF-κB proteins which bind to an 11

bp promoter element containing the NF-κB consensus sequence (Leung *et al*, 1988). Other cellular genes containing NF-κB domains that are stimulated by Tax include vimentin (a cytoskeletal growth-regulated gene, Lilienbaum *et al*, 1990), TNF-β (Paul *et al*, 1990), and murine GM-CSF (Schreck *et al*, 1990). It has been demonstrated that Tax-expressing cell lines show increased expression of the NF-κB DNA-binding proteins (p50, p55, p65, p75, p85, and p92, Arima *et al*, 1991 and Lacoste *et al*, 1991). There is indirect evidence that Tax activates NF-κB -dependent transcription by dissociating the NF-κB/ IκB complex (Ruben *et al*, 1989).

Tax-mediated activation of NF-κB may display a further level of complexity, in that the Tax protein has been detected in the extracellular growth medium of HTLV-I infected and transformed cells (Lindholm *et al*, 1990). Recombinant extracellular Tax protein introduced into cell culture media was taken up by cells and induced the nuclear accumulation of NF-κB DNA-binding proteins in pre-B lymphocytes (Marriott *et al*, 1991). The cellular uptake of Tax and the induction of NF-κB was associated with the stimulation of cellular genes such as IgK light chain and TNF-β, which contain NF-κB binding sites in their promoters.

Recent evidence suggests that Tax interacts with two subunits of the 20s proteasome (Rousset *et al*, 1996). In vivo, Tax strengthens a weak interaction between the NF-κB precursor p105 and the proteasome and may stimulate the processing of p105 to p50. Thus the mechanism of Tax induction of NF-κB is incompletely understood, but appears to involve stimulation of NF-κB gene expression, stimulation of NF-κB /IκB dissociation by a membrane signaling pathway, processing or modification of NF-κB proteins, and/or indirect interactions with the NF-κB /IκB complex.

Genomic structure of HTLV-I

Identification of HTLV-I Tax as the trans-acting viral element necessary for activation of viral transcripts began with identification of the genomic structure of HTLV-I.

The HTLV-I proviral genome has been molecularly cloned and the complete nucleotide sequence has been determined (Seiki *et al*, 1983, Josephs *et al*, 1984, and Shimotohmo *et al*, 1985). Similar to other retroviruses, open reading frames which code for the Gag, Pol, and Env proteins are present in the 5' portion of the viral genome (Figure 2). Interestingly, the HTLV-I genome accommodates one or more coding regions in addition to the usual viral replicative genes. These sequences, unique to the human retroviruses, are located at the 3' end of the region encoding the viral structural proteins. In HTLV-I this region, designated pX, contains four open reading frames encoding the regulatory proteins Rex, Rof, Tof, and Tax (Figure 2). A subdomain of this region, designated x-lor (for long open reading frame in pX) encoding the regulatory proteins Tax (40kDa) and Rex, was found to be highly conserved between HTLV-I and II, and a similar bovine leukemia virus (BLV) (Seiki *et al*, 1983). The high degree of x-lor conservation, and the retention and expression of the Tax gene in almost all transformed cells, including those containing single proviruses deleted in the entire *env* gene, suggested that Tax may be critical for transformation. In addition, Tax was unrelated to all previously known retrovirus-replicative genes. These data suggested that the Tax gene product may be the trans-acting viral element which mediates the transformation and replication activity of the virus.

Transactivation of the HTLV-I LTR by Tax

Multiple studies have shown that Tax induction of HTLV-I transcription is required for efficient synthesis of the viral mRNA (Cann *et al*, 1985, Chen *et al*, 1985, Felber *et al*, 1985, Fujisawa *et al*,1985, Seiki *et al*, 1986, and Sodroski *et al*, 1984). These studies demonstrated that the steady-state mRNA level, and consequently the enzymatic activity of an HTLV-I LTR-reporter construct was greatly enhanced in virus infected cells expressing Tax. This phenomenon of transacting transcriptional regulation (TAT) activation of an HTLV-I LTR-CAT gene was also observed in cells transfected with only the Tax gene. (Felber *et al*, 1985).

Further direct evidence of Tax transactivation of the viral promoter was provided by Giam *et al* (1986) who showed that bacterially derived Tax transactivates the HTLV-I LTR *in vivo*. Bacteria producing Tax were fused to mammalian cells carrying an integrated copy of a HTLV-I-chloramphenicol acetyltransferse gene (CAT) construct by PEG-mediated protoplast fusion. This resulted in a 10-20 fold activation of the promoter. The time course for increase in CAT enzymatic activity correlated with the increase in CAT mRNA levels as measured by quantitative S1 nuclease protection assay

While these studies demonstrated the necessity of Tax in activation of the viral LTR, and therefore for replication, the regulatory elements which mediated this function had yet to be identified.

Identification of HTLV-I Tax-responsive elements

Studies by Fujisawa *et al* (1986) identified the sequences in the HTLV-I LTR that were responsible for transactivation by Tax. These promoter deletion studies identified two 42-base pair repeats and one 21-base pair repeat that were essential for activation of the HTLV-I LTR by Tax. Insertion of 59 base pairs containing one of the 42-base pair direct repeats was sufficient to rescue an inactive enhancer-minus SV40 promoter in trans-activation by Tax. These sequences were active irrespective of site and orientation, characteristics commonly displayed by upstream transcriptional enhancer elements. This enhancer sequence was activated by Tax directly, or indirectly, since the LTR-SV40 construct was activated in cells expressing Tax or in cells co-transfected with Tax. At the time, most known transcriptional enhancers consisted of tandemly repeated sequences. Thus, a 42-base pair, or 21-base pair unit seemed likely to be an enhancer responsible for the transcriptional activation mediated by Tax.

Evidence supporting an essential role for these elements was provided by Rosen *et al* (1987, also see Figure 3) who used transient transfection studies to localize the regions within the HTLV-I LTR that are essential for promoter activity and trans-activation by Tax.

These studies led to the identification of the 21-base pair "Tax-responsive element" (TRE). Placement of a synthetic copy of the TRE 5' to the basal HTLV-I promoter rendered the promoter responsive to the Tax in Jurkat cells. These studies demonstrated that one TRE (promoter proximal) was sufficient to confer Tax-responsiveness. These studies also confirmed the orientation-independent manner of the TREs.

Brady *et al* (1987) chemically synthesized transcriptional regulatory sequences required for Tax transactivation and cloned them upstream of the basal HTLV-I LTR to monitor activation induced by Tax. Weak induction by one 21-base pair element increased when a multimer of 2-3 of these repeats were present. Again, orientation and position of these Tax responsive elements had no effect on transactivation. Quantitative S1 nuclease analysis demonstrated that activation occurred at the level of transcription.

These data led to the hypothesis that Tax may induce or modify a set of enhancer-binding proteins that recognize the enhancer sequence in the HTLV-I LTR, or that perhaps it was an enhancer binding protein itself. At this time, the Tax-responsive elements had no significant homology to known cellular transcriptional enhancers. While some transcriptional enhancers had demonstrated cell type or tissue specificity, the HTLV-I enhancer appeared to require its own viral protein, but was not cell type specific, as it enhanced activation of the viral LTR in non-lymphoid cells infected with HTLV-I.

The subsequent identification of the cellular cAMP-response element (CRE) led to the observation that these TRE-1 sequences were highly related to a common transcriptional enhancer element (Figure 4). The CRE was originally characterized in cellular genes through its ability to confer transcriptional responsiveness to cyclic AMP (Montminy *et al*, 1986, Short *et al*, 1986, Comb *et al*, 1986). Indeed, the HTLV-I LTR is inducible by cAMP via forskolin and a lipid soluble analog of cAMP, dibutyl cyclic AMP (Poteat *et al*, 1989). Additionally, the sequences required for cAMP induction are also required for Tax-responsiveness. Tax does not require elevated levels of cAMP for function, nor does Tax appear to alter intracellular levels of cAMP. Interestingly, increased levels of cAMP are

reported to inhibit expression of the IL-2 gene and several other genes required for growth in human T-cell populations. It has therefore been speculated that induction of Tax responsive genes (i.e. the HTLV-I LTR) by cyclic AMP may circumvent the normal repressive effects of this second messenger on T-cell growth.

Genomic footprinting by Brown *et al* (1996) has confirmed many of the findings from previous studies of Tax activation of the HTLV-I promoter. However, some notable differences were seen. HTLV-I genomic protein-binding patterns corresponded more closely to elements defined by transient transfection expression studies than to those mapped by *in vitro* protein binding studies. Results showed that the most predominant HTLV-I genomic footprints corresponded to the exact locations of the three 21-base pair Tax responsive elements as established by previous studies utilizing CAT assays and site directed mutagenesis (Brady *et al*, 1987, Giam *et al*, 1989, Marriott *et al*, 1989, Muchardt *et al*, 1992, and Shimotohno *et al*, 1986). In contrast to the genomic footprinting results for the viral HTLV-I LTR in human T-cells, the transgenic HTLV-I LTR in mouse fibroblasts, splenocytes, and thymocytes exhibited smaller footprints over the TREs, and a large number of additional footprints in the R region. These results indicate that the HTLV-I LTR may be regulated differently according to species or tissue type or when present as a transgene.

A second region weakly activated by Tax has been located between the second and most promoter proximal 21-base pair repeat. This sequence contains binding sites for several cellular factors, and appears to facilitate Tax transactivation through cooperation with the 21-base pair repeats (Figure 3, and Bousselut *et al*, 1990, Bousselut *et al*, 1992, and Gegonne *et al*, 1993).

Identification of cellular factors that bind the HTLV-I TREs

Initial promoter footprinting studies had demonstrated that similar DNA-protein interactions were observed in the absence or presence of Tax (Altman *et al*, 1988, and

Nyborg *et al*, 1988). It seemed unlikely, therefore that Tax bound to DNA directly or led to de novo synthesis of cellular factors that mediated Tax action. Thus it was hypothesized that Tax expression resulted in the post-translational modification of host cell transcription factors from an inactive to an active form (enhanced DNA binding, enhanced functional or transcriptional activity). Tax may additionally participate directly in the formation of an active transcription initiation complex. As the TGACG motif found common to each of the TREs was recognized as highly homologous to ATF-binding sites and CREs, several investigators asked whether cellular CRE-binding proteins might mediate Tax-inducible activation of the HTLV-I promoter.

Nyborg *et al* (1988) used DNase I footprinting to identify specific protein-DNA interactions in the HTLV-I LTR using both unfractionated nuclear extracts and fractions eluted from a heparin agarose column. These techniques were used to map the protein binding sites in the HTLV-I LTR and to identify cell-specific differences in those footprints. This study demonstrated that the imperfect 21-base pair repeats was occupied by a single factor, at the time designated HEF-1. HEF-1 binding activity was present in all cell lines tested, consistent with the observation that HTLV-I LTR is active and can be transactivated by Tax in a wide variety of cell lineages. At the time, HEF-1 was not known to be related to other cellular transcription factors. The purification of HEF-1 to near homogeneity revealed a complex of two polypeptides shown in later studies to contain a DNA-binding domain homologous to that of the CRE-binding protein (CREB).

Tan *et al* (1989), in order to identify nuclear factors that bind specifically to the HTLV-I TRE, used a gel-mobility shift assay to screen HeLa cell nuclear extract-derived chromatographic fractions. This screen identified TRE-binding proteins 1, 2, and 3 (TREB1, TREB2, TREB3). Mutations in the core CRE-like element of the TRE disrupted the formation of these three protein-DNA complexes *in vitro*. Affinity purified TREB-1 (35-43 kDa) activated transcription from the HTLV-1 promoter *in vitro*. In addition, gel-mobility shift assays demonstrated binding of TREB-1 to cellular CRE and ATF-binding

sites. The observation that TREB-1 and ATF had similar molecular masses suggested that TREB-1 and ATF were highly related proteins. These studies supported a model in which Tax activation of viral TREs was mediated by the CREB/ATF-1 family of transcription factors.

Further evidence that the TRE-binding proteins were members of the CREB family of transcription factors, was provided by Yoshimura *et al* (1990). These studies identified human cDNAs encoding proteins that bound specifically to the HTLV-I 21-bp enhancer. Sequence analysis of these clones showed that all three clones contained a leucine zipper structure and an adjacent basic amino acid DNA-binding domain. Interestingly, these proteins, later identified as C/EBP-1/ATF-2, ATF-1, and lambda BP-1 showed slightly different footprints on each of the 21-base pair repeats, indicating that the binding of each protein differed depending on the flanking sequences and core sequences of the TRE. While these studies clearly demonstrate a role for basic/leucine zipper factors in activation of the viral TREs, the mechanism of that action was unknown.

Direct effect of HTLV-I Tax on cellular TRE-binding proteins

In order to elucidate the mechanism by which Tax utilized these basic/leucine zipper proteins, the interactions among Tax, viral TREs and cellular CRE-binding proteins have been studied extensively. There have been several approaches to this end including *in vitro* gel mobility shift assays, co-immunoprecipitation of purified proteins, genetic yeast two-hybrid assays, and various in vivo transactivation assays.

Zhao *et al* (1992), using the HTLV-I 21-base pair repeat and Jurkat T-lymphocyte nuclear extract in a gel mobility shift assay, detected three protein-DNA complexes that were specific for the CRE in the 21-base pair repeat. Using antibodies directed at CRE-binding proteins, they showed that these complexes are composed of CREB homodimer, CREB/ATF-1 heterodimer, and ATF-1 homodimer. They further showed that Tax stabilized CREB-DNA complexes via a direct interaction with the CREB-monomer of these

dimerized partners. A direct Tax-CREB interaction was demonstrated in the absence of DNA via co-immunoprecipitation with a Tax specific antibody and purified proteins *in vitro*. These studies provided an initial model for Tax-mediated activation of the HTLV-I LTR in which Tax interacts directly with CREB homodimer and or CREB/ATF heterodimer to stabilize their assembly on the Tax -responsive CRE motifs in the HTLV-I enhancer.

Suzuki *et al* (1993) used gel mobility shift assays with a multimerized TRE-probe, purified TRE-binding proteins, and purified Tax. These studies confirmed the binding of Tax to CREB in the absence of DNA, and showed that the complex binds to the 21-base pair enhancer forming a stable Tax:CREB:TRE complex. They additionally demonstrated a similar complex comprised of Tax, CREM, and the TRE.

An interaction between CREM and Tax was confirmed in studies by Bodor *et al* (1995) in which T-cell nuclear extracts bound to HTLV-I TREs were supershifted with CREB and CREM specific antibodies in gel mobility shift assays. Purified CREB and CREMτ also produced shifted bands identical in size to those seen with the T-cell nuclear extract shifts.

Franklin *et al* (1993) identified ATF-2 and CREB as the principal T-cell proteins that bind the three TREs in vitro. Functional studies demonstrated that purified Tax protein augmented the level of RNA synthesis induced by ATF-2 and CREB in a cell-free transcriptional assay. Furthermore, Tax increased the binding of both the T-cell derived and recombinant forms of ATF-2 and CREB to teach of the 21-base pair repeats. Together, these data suggested that Tax transactivates HTLV-I gene expression by increasing the number of bound ATF-2 and CREB molecules at the viral promoter. Using truncated versions of CREB and ATF-2, they demonstrated that the amino acids located in or near the DNA binding and leucine zipper domains of CREB and ATF-2 were essential for the effect of Tax on DNA binding.

Studies by Wagner and Green (1993) suggested that Tax acts by increasing the *in vitro* DNA binding of multiple ATF proteins. Tax also stimulated DNA binding by other unrelated bZIP proteins, but did not affect DNA binding proteins that lack a bZIP domain. Crosslinking studies suggested that this increase in DNA binding occurred due to Tax-mediated dimerization of these bZIP factors in the absence of DNA. They proposed that this elevated concentration of the bZIP dimer in turn increases the on-rate of DNA binding. In support of their model, they demonstrated that Tax increases dimerization in the absence of DNA, and this dimerization requires only a minimal bZIP domain. Their model proposes that Tax binds preferentially to a bZIP dimer, thereby shifting the monomer-dimer equilibrium toward dimer formation. The resulting increased concentration of the bZIP homodimer would presumably account for the increased extent and rate of DNA binding.

Studies by Armstrong *et al* (1993) suggest that the effect of Tax on DNA binding activity of transcription factors extends beyond the CREB/ATF family. Gel-mobility shift assays were used to demonstrate enhanced binding of purified SRF to the AT-rich SRE. Tax also produced an increase in the binding activity of the Fos-Jun heterodimer to an AP-1 enhancer element in vitro. Tax also increased, albeit to a lesser extent, the binding of the NF-κB heterodimer p50/p65 to a kB recognition site from the HIV LTR. Tax also increased the amount of protein-DNA complex formed between purified Sp1 and a probe containing the Sp1 recognition site from the HTLV-I LTR.

While the results from these studies implicate a number of unrelated bZIP family members in Tax-mediated binding to the HTLV-I TREs, CREB, in particular, has been consistently identified as a target of Tax. In order to more closely examine the Tax:CREB:TRE interaction, Giam *et al* performed in vitro oligonucleotide selection studies in the presence and absence of Tax (Paca-Uccatalertkun *et al*, 1994). Two distinct, but related groups of sequences containing the CRE flanked by long runs of G and C residues in the 5' and 3' regions were preferentially recognized by the Tax-CREB complex. CREB alone bound only to CRE motifs (GNTGACG$^{T}/_{C}$) without the flanking G-C rich

sequences. The consensus Tax-CREB-selected binding site ($GGGGG^{T}/_{G}TGACG^{T}/_{C}{}^{A}/_{C}TA^{T}/_{C}CCCCC$) is highly homologous to the HTLV-I 21-base pair TRE. Gel mobility shift assays and transfection studies indicated that the G and C-rich sequences adjacent to the CRE motif are crucial for the formation of a stable Tax:CREB:DNA complex and Tax transactivation. DNase I footprinting analysis indicated that these flanking sequences were not in direct contact with the Tax-CREB complex. Thus, the long runs of G and C rich residues may affect DNA conformation in the vicinity of the CRE motif so that the structurally altered CRE could be recognized efficiently only by the Tax-CREB complex.

Evidence for a more selective interaction between Tax and bZIP factors was provided by Adya *et al* (1994). Using gel shift analysis, they found that, despite the extensive amino acid similarities shared between ATF-1 and CREB, Tax interacts only marginally with ATF-1. Amino acid sequence comparison between CREB and ATF-1, coupled with domain switching and targeted mutagenesis identify the Ala-Ala-Arg residues just N-terminal to the basic domain of CREB to be crucial for the Tax-CREB interaction. Specific amino acid substitutions in AAR of CREB weakened or abolished the Tax-CREB interaction. Reciprocal amino acid substitutions in ATF-1 conferred an ability to interact with Tax. Co-immunoprecipitation assays demonstrated a stable Tax-CREB complex, but were unable to precipitate a Tax-ATF-1 complex. These studies not only identified residues in the N-terminus of CREB necessary for interaction with Tax, but also lent some specificity to the interactions between Tax and the bZIP family of proteins.

Brauweiler *et al* (1995) performed equilibrium binding assays and determined dissociation kinetics to specifically study the interaction of CREB and ATF-2 with the 21-base pair repeat sequences in the presence and absence of Tax. These studies demonstrated that the three 21-base pair repeat TREs represent lower affinity CRE sites, and that Tax enhances the equilibrium binding affinity of both CREB and ATF-2 for the TREs, as well as to a consensus cellular CRE. However, only CREs flanked by the G-C rich sequences

of the TREs conferred Tax transactivation to a heterologous promoter *in vivo*. The addition of Tax to a relatively unstable CREB:TRE complex was highly specific, and relied on specific amino acid sequences in CREB, and well as the G-C-rich sequences flanking the viral CRE.

Yin *et al* (1995) used *in vitro* binding studies, and a "mammalian two-hybrid" assay to address the specificity of Tax interaction with members of the ATF/CREB family and other leucine zipper proteins. These studies supported previous data which suggested that the bZIP region of CREB and the N-terminus of Tax were critical for the Tax-CREB interaction. Interestingly, Tax was able to interact with CREB but not CRE-BP1 or Jun in a mammalian two-hybrid assay. These data did not support the model set forth by Green *et al*, who demonstrated a pleiotropic DNA-binding effect on many bZIP factors. In contrast, while Tax enhanced binding of CREB to all 3 21-base pair repeats, it did not effect binding to a consensus cellular CRE, as had been shown by Brauweiler *et al.*

Perini *et al* (1995) used chimeras of various unrelated bZIP and leucine zipper proteins in gel shift studies to demonstrate that the bZIP region was essential for Tax mediated enhancement of binding to a variety of enhancer elements *in vitro*. However, in apparent conflict with previous studies, this enhanced binding was not limited to Tax responsive elements of the HTLV-I LTR. While the results indicated that Tax-mediated DNA binding depends on the combination of core-binding element and its flanking sequences, the elements used in these studies were synthetic ATF and AP1 sites. For example, Tax appeared to increase the affinity of CREB, ATF1, GCN4 and a Fos-ZTA fusion protein for all ATF-1 and AP1 sites tested. Perini *et al* proposed that the pleiotropic effect of Tax on a variety of unrelated bZIP proteins in vitro may result in aberrant activation of cellular genes, in turn contributing to the oncogenic activity of Tax.

In vivo genetic studies to characterize the transactivation of the HTLV-I promoter were performed by Bantignies *et al* (1996) and Shnyreva *et al* (1996). Expression of a TRE-driven reporter gene was induced by co-expression of Tax and CREB. Analysis of

different CREB mutants with this system indicated that the bZIP domain of CREB was necessary to mediate transactivation by Tax. Using this yeast system to screen a cDNA library, Bantignies *et al* determined that CREB, CREM, or ATF-1 could cooperate with Tax to activate the viral TRE. Expression of ATF-2 in this system did not activate TRE-1 in the presence of Tax.

The data from these studies support two, somewhat conflicting models for Tax activation of viral TREs. Studies by Green *et al* (1994, 1996) and Nyborg *et al* (1996) suggest a pleiotropic effect of Tax. Their model suggests a rather non-selective effect of Tax in enhancing dimerization, and therefore DNA-binding of a variety of unrelated bZIP factors to both viral and cellular CREs. A model supported by Giam *et al*, and Gaynor *et al*, suggest that the mechanism of Tax action is more specific: Tax enhances DNA binding of particular members of the bZIP family, and only to CREs flanked by the G-C rich regions of the HTLV-I TREs.

Clearly, some discrepancy exists as to the specificity of Tax recruitment of various bZIP factors to viral and cellular DNA elements. Some of these discrepancies results from significant differences in DNA elements used, in the method employed to measure DNA binding, and the use of fusion proteins as a result of "domain swapping". A more sensitive and quantitative method is necessary to distinguish the specificity of Tax within the bZIP family of proteins, and to determine whether Tax affects the binding of these factors to cellular CREs in addition to the HTLV-I CRE.

It is clear that although Tax does not directly bind DNA, its actions are critically dependent upon the nature of the CRE. The mechanism of transactivation by Tax and CREB remained poorly understood. The inability to consistently demonstrate activation of cellular cAMP responsive genes, or recruitment of CREB to cellular CREs by Tax remained puzzling. Recent advances in our understanding of cAMP regulated gene expression opens up further possibilities as to the mechanism of Tax-mediated activation of cellular and viral promoters.

Functional domains of HTLV-I Tax

Secondary structure prediction and analysis of single and double point mutations in Tax has provided identification of additional functional domains in Tax (Figure 5). A series of double amino acid substitutions in the putative zinc finger domain of Tax (amino acids 17-48) suggests that this domain serves as a unique nuclear localization signal. Indeed, this region is sufficient to retarget β-galactosidase to the nuclei of expressing cells. (Smith *et al*, 1990). Direct evidence for coordination of zinc by this region has not be demonstrated, however. For example, recombinant Tax partially purified from E-coli has been reported to bind zinc chelating columns (Marriot *et al*, 1990). However, recombinant Tax immobilized on nitrocellulose does not bind zinc specifically under conditions where known metal-binding protein bind divalent metals (Smith and Greene, 1990).

Mutagenesis studies have demonstrated that distinct amino acid residues of Tax are required for transactivation of the CREB and NF-κB pathways. Mutation of a region located between amino acids 315 and 325 abrogated CREB-mediated Tax transactivation without affecting NF- κB mediated transactivation. Conversely, scattered mutations in Tax between amino acids 113 and 258 resulted in intact CREB but deficient NF- κB mediated transactivation. Tax transactivation likely occurs via distinct regions that targeted specific transcription factors (Smith and Greene, 1990).

Using Gal4 DNA binding domain:Tax fusions, several groups have demonstrated transcriptional activation of a reporter construct carrying GAL4-binding sites (Fujii *et al*, 1991, Fujisawa *et al*, 1991, Tsuchiya *et al*, 1994, and Semmes *et al*, 1995). These studies support the hypothesis that Tax carries a potent transcriptional activation domain. Indeed, Tax has been shown to form a stable complex with several members of the basal transcriptional machinery including TFIIA (Clemens *et al*, 1996), TAFII28 (Caron *et al*, 1996), and TBP (Caron *et al*, 1993).

Mutations in Tax confirmed that the amino terminus was required for interaction with CREB (Goren *et al*, 1995). Specifically, mutants with changes in the putative zinc finger lost the ability to bind a CREB:HTLV-I TRE complex in a filter binding assay. In contrast, C-terminal point mutations did not effect the Tax :CREB interaction. The C-terminus of Tax is not dispensable for this effect, however, as a C-terminal truncation mutation, as well as double-point mutations disrupt the ability of Tax to interact with GST-CREB. These C-terminal mutations were also disfunctional in a CREB:HTLV-I TRE biotin-streptavidin binding assay. An N-terminal mutation (3H-S), and a distinct C-terminal mutation (319LL-RS) independently displayed attenuated transactivation of the CREB/ATF HTLV-I LTR, while retaining nuclear localization, and the ability to transactivate the NF-κB -dependent HIV-LTR. These mutations support a model in which an intact N- and C-terminus are necessary for activation of the HTLV-I CREs.

Regulation of cellular cAMP responsive genes

The role of CREB in Tax-activation of the HTLV-I TREs, and the mechanism of activation of cellular CRE containing genes by Tax is incompletely understood. However, the regulation of the cAMP-response pathway in normal cellular processes has been studied extensively (Figure 6).

Cyclic AMP-regulated gene expression occurs through the cAMP-response element (CRE). The CRE binds several cellular transcription factors including CREB and CREMα, members of the basic/leucine zipper family of transcription factors (Meyer and Habener, 1993). These transcription factors are characterized by a leucine zipper domain which facilitates dimerization. Dimerization juxtaposes the basic regions of these proteins over cellular CREs, forming a bimolecular DNA binding domain. CREB binds to the CRE constitutively and is activated via phosphorylation by protein kinase A on a single serine residue within its transcriptional activation domain (Gonzalez *et al*, 1989). CREB contains, within this phosphorylation domain (or kinase inducible domain, KID) phosphorylation

sites for protein kinase A (PKA), protein kinase C, calcium/calmodulin-dependent protein kinase II, CaM kinase II and casein kinase II (Figure 7). CREMα is also phosphorylated by PKA on a single serine, but normally acts as a repressor of transcription from cAMP responsive genes (Foulkes *et al*, 1991).

The structurally related transcription factors CREB, CREM, and ATF-1 are key mediators of cAMP-regulated gene transcription. While the regulatory properties of ATF-1 remain poorly defined, isoforms of CREB and CREM have well-characterized functions as activators or repressors of transcription, respectively. In addition to the N-terminal phosphorylation domain (kinase inducible domain; KID), CREB and CREM genes both encode glutamine-rich transcriptional activation domains.

Alternative splicing and selective translational initiation of CREB and CREM transcripts generate a family of proteins ranging from full-length transcriptional activators to truncated transcriptional repressors (Laoide *et al*, 1993). Specifically, CREMα, which lacks the glutamine-rich domains found in activator isoforms such as CREB and CREMτ, has been shown to be a potent repressor of cAMP-activated transcription (Foulkes *et al*, 1991). Another repressor isoform of CREMα, CREM Δ(C-G) lacks both the glutamine-rich regions and the KID (Walker *et al*, 1994).

The most critical phosphorylation site in the KID appears to be the serine residue at position 133 which can be phosphorylated by PKA, CaM kinase II and IV. Mutation of this serine to alanine destroys the ability of CREB to function as a transcriptional activator (Gonzalez and Montminy, 1989). The role of phosphorylation by PKA in activation of CREB remains somewhat controversial. While phosphorylation of single serine residues has been documented to induce structural and functional changes in some enzymes, studies demonstrating similar structural changes in CREB have been inconsistent (Brindle *et al*, 1995, Richards *et al*, 1996). Other evidence suggests that CREB phosphorylation may primarily serve to allow interaction with another nuclear protein, CBP, that functions as a transcriptional co-activator (Chrivia *et al*, 1993, Kwok *et al*, 1994). Consistent with this

model, Arias *et al* (1994) have shown that microinjection of an antibody that disrupts the interaction of CBP with PKA phosphorylated CREB blocks activation of a CRE-reporter gene.

Role of CBP in cAMP-mediated gene activation

Our understanding of mechanism regulating transcription of cellular CREs has increased significantly with the cloning of the transcriptional co-activator CREB-binding protein (CBP). CBP was originally cloned by screening a human thyroid λgt11 library with recombinant CREB labeled at the PKA phosphorylation site with [γ-^{32}P]ATP (Chrivia *et al*, 1993). CBP was found to be located almost exclusively in the nucleus, as determined by indirect immunofluorescence, with minimal cytoplasmic staining. Comparison of the primary sequence of CBP to known proteins, as well as secondary structure predictions, identified several motifs in CBP including: four putative zinc-finger domains, a bromodomain, and a glutamine-rich domain. The unique cysteine-histidine rich regions are homologous to zinc-finger domains found in other proteins (Ponting *et al*, 1996). The bromodomain is conserved among several co-activator proteins including yeast GCN5 (Georgakopoulos, 1992), yeast Swi/Snf2 (Peterson, 1992), Drosophila brahma (Tamkun, 1992), and TAF250 (Hisatake, 1993). The presence of these conserved domains, in addition to the glutamine rich C-terminus (a motif common to many transcriptional activators, Mitchell and Tjian, 1989) strongly implicated CBP in mediating transcriptional activation.

CBP bound specifically to PKA phosphorylated CREB, as non-phosphorylated CREB and casein kinase II phosphorylated CREB were unable to bind to CBP immobilized on a membrane (Chrivia *et al*, 1993). In addition, CBP specific antibodies were able to co-imunoprecipitate PKA phosphorylated CREB, but not non-phosphorylated or casein kinase II phosphorylated CREB. A functional activation domain in CBP was defined in vivo using full-length CBP fused to the GAL4 DNA binding domain. This GAL4:CBP fusion

protein activated transcription of a multimerized GAL4-reporter construct over 10-fold. Addition of PKA increased that activation up to 45-fold.

In order to further characterize the phosphoCREB:CBP interaction, Kwok *et al* (1994), used fluorescence polarization binding assays to define the equilibrium binding parameters of this interaction. This method utilized a fluorescent oligonucleotide containing the somatostatin CRE. Upon binding of a CREB dimer to that element, the rotation of the oligonucleotide slows. The change of the rotational movement of the oligonucleotide translates into an increase in the polarization (or anisotropy) of light emitted from the fluorescent oligonucleotide. An increase in anisotropy upon binding of macromolecules to the specific DNA probe provides a quantitative measure of the increase in the rotational correlation time and hence the molecular volume of a fluorescent complex (Lundblad *et al*, 1996). This method has considerable advantages over other methods of examining protein:DNA and protein:protein interactions which are limited by their inability to provide equilibrium determinations of binding constants. In addition, the fluorescence anisotropy binding assay is sensitive to subtle changes in affinities of DNA:protein and protein:protein interactions, allowing close examination of the role of Tax in assembly of transcription complexes on both viral and cellular promoters. Using this method, CBP demonstrated a high affinity specifically for a phosphoCREB:CRE complex (Kd=220nM).

The importance of PKA phosphorylation in mediating an interaction with CBP was further demonstrated by Shih *et al* (1996). These studies utilized a variation of the genetic yeast two-hybrid assay to screen for mutations in CREB which interrupt its association with CBP. Interestingly, the majority of mutations which led to disruption of the CBP:CREB interaction were located in the phosphorylation domain of CREB, specifically within amino acids 130-133 (RRPS).

Kwok *et al* (1994) demonstrated that CREB, PKA, and CBP coordinately stimulate expression of a CRE reporter in a CBP-dose dependent manner. These studies further defined the activation domain in C-terminus of CBP by demonstrating a direct interaction

between the C-terminal zinc finger domain of CBP (a.a. 1680-1812) and a GST:TFIIB fusion protein. These results provided a possible mechanism for the co-activator function of CBP. Specifically, CBP provided a link between the upstream enhancer elements and promoter elements by bridging phosphorylated CREB to members of the basal transcriptional machinery.

Arias *et al* (1994) demonstrated the necessity for CBP in mediating cAMP dependent transcription by blocking CRE-lacZ reporter activity with a CBP specific antibody. Co-injection of CBP antibodies also blocked activation of an SRE-lacZ and a TPA-responsive lacZ reporter in NIH3T3 cells, implicating CBP in signalling pathways other than that mediated by cAMP.

While CBP binding to phosphorylated CREB is necessary for transcriptional activation, Sun *et al* (1995) have demonstrated that the phosphoCREB:CBP interaction is not sufficient for activation. For example, while a GAL4:CREB protein, in which amino acid 142 has been replaced by aspartic acid, is able to bind to CBP with the same affinity as PKA phosphorylated CREB, this complex is transcriptionally inactive.

Further evidence of the interaction of CBP with the basal transcriptional machinery was provided by Kee *et al* (1996). Analysis of HeLa nuclear extracts fractionated over a phosphocellulose resin demonstrated that CBP fractionates with RNA polymerase II. RNA polymerase II was also directly immunoprecipitated with a CBP antibody. A CREB specific antibody was able to co-precipitate CBP and RNA polymerase II from PKA treated HeLa cell extracts, but not from untreated extracts.

Rehfuss *et al* (1996), and Chrivia *et al* (1996) have further defined the regions in CBP which are required specifically for CREB-mediated transcription. Loss of function in transient transfection studies of CBP deletion mutants was used to identify regions required for CREB-mediated transcription. These studies determined that the N-terminal half of CBP is sufficient to support the cAMP-mediated transcriptional activation demonstrated originally by Kwok *et al* (1994). These studies also identified of a novel N-terminal

activation domain in CBP. Interestingly, the transcriptional activation of the N-terminal activation domain contained in a GAL:CBP 1-460 fusion protein is enhanced in the presence of PKA in PC12 cells. In addition, this N-terminal activation domain in CBP was shown to interact directly with TBP.

CBP is highly homologous to the adenovirus E1A-associated protein p300. The transcriptional activity of p300 and CBP is severely compromised by E1A. This effect requires the N-terminus of E1A and is mediated through the most C-terminal putative zinc finger domain in p300 and CBP. Specifically, E1A interferes with cAMP-dependent activation of cellular CREs (Eckner *et al*, 1995, and Lundblad *et al*, 1995).

While these studies highlight the role of CBP in the cAMP-signal transduction pathway, subsequent studies have demonstrated that an increasing number of transcription factors are able to form stable physical complexes with, and respond to the co-activating properties of CBP (Figure 8). For example, CBP can associate with c-jun, specifically with the JNK phosphorylated form of c-jun (Arias *et al*, 1994). A similar interaction was demonstrated with c-fos (Bannister *et al*, 1995). CBP also serves as a co-activator for c-Myb; an interaction that is compromised by E1A (Dai *et al* 1996).

The ligand-binding domains of the nuclear hormone receptors RAR and RXR, as well as ER, PR, TR, and GR, interact with the N-terminal region of CBP (Kamei *et al*, 1996, and Chakravarti *et al*, 1996). Neutralizing antisera against CBP block hormone-dependent transcriptional activation. Co-activators implicated in the hormone-dependent activation of nuclear receptors also interact with receptor-bound CBP. For example, co-expression of CBP with steroid receptor co-activator-1 (SRC-1) stimulates the activities of the estrogen and progesterone receptor in a co-operative manner (Smith *et al*, 1996). These data suggest that multiple interfaces within CBP may be simultaneously occupied by different transcription factors.

CBP also functions in the JAK/STAT mediated pathway of transcriptional activation. STAT1, STAT2 and p48 comprise a transcriptional complex utilized in

activation of a variety of interferon-α responsive genes. CBP binds to the N-terminal region of STAT2, and this co-activation is impeded by E1A (Bhattachary *et al*, 1996).

A role for CBP in regulation of transcription factors which are dedicated to controlling differentiation within specific cell lineages has recently been demonstrated. MyoD, together with MEF2, utilize CBP to activate transcription of muscle-specific genes. Injection of CBP antisera has been demonstrated to prevent myogenesis (Eckner *et al*, 1996 and Yuan *et al*, 1996).

In addition to providing a bridge between sequence specific transcription factors and the basal transcription machinery, CBP may function at the level of chromatin remodeling to activate transcription. Indeed, CBP interacts with P/CAF, a homologue of the yeast histone acetylase GCN5 (Yang *et al*, 1996). Acetylation of histones H3 and H4 in nucleosomes has been proposed to decrease the interaction between these proteins and DNA. Recent evidence, however (R. Brennan, personal communication), suggests that acetylation may disrupt histone:histone interactions. Specifically, histone 2A (H2A) provides a negatively charged binding pocket for the lysine tail of histone 4 (H4). Acetylation of the H4 lysine tail may disrupt a H2A:H4 interaction, opening up the nucleosome. The unwinding of chromatin resulting from remodeling of chromatin may allow previously masked promoter elements to be accessed by transcription factors. In further support of a role for CBP in this process, CBP has been shown to have intrinsic histone acetylase activity located in a domain between amino acids 1099-1877 (Bannister *et al*, 1996). The demonstration that P/CAF and CBP appear to have different substrate specificity in the nucleosome emphasizes the potential role for CBP in various promoter contexts. Specifically, signaling events which may disrupt the P/CAF:CBP interaction may have no effect on the intrinsic histone acetyl-transferase activity of CBP.

Specific Aims

While the results from previous research on Tax have provided some insight into the molecular mechanisms of activation of the HTLV-I LTR, several aspects of the current model for Tax-mediated activation remain unclear. The work presented in this thesis details the in vivo functional and in vitro biochemical effects of HTLV-I Tax on CRE-binding proteins. The purpose of this work is to elucidate the mechanism underlying Tax-mediated activation of viral and cellular cAMP-response elements. The specific aims of this project are to:

1. *Examine the transactivation of the HTLV-I and somatostatin promoters by Tax and basic/leucine zipper (bZIP) factors in vivo.* I will use HTLV-I LTR and somatostatin reporter constructs to functionally analyze Tax action in transient transfection studies. The somatostatin element was chosen because it contains a consensus cellular CRE sequence.
2. *Determine the effect of Tax on binding of bZIP proteins to HTLV-I and cellular CREs in vitro.* I will use fluorescence polarization binding assays to determine the equilibrium binding constants of bZIP proteins to viral and cellular CREs in the presence and absence of Tax.
3. *Determine the effect of Tax on recruitment of the co-activator CBP to bZIP:CRE complexes in vitro.* I will use fluorescence polarization binding assays to determine the affinity of CBP for bZIP:CRE complexes in the presence and absence of Tax. The effect of phosphorylation on the assembly of these complexes will also be determined.
4. *Determine the discrete functional domains of these bZIP factors necessary and sufficient to recruit the Tax:CBP complex to viral and cellular promoters.* Isoforms of CREMα which retain some, but not all of the functional domains of full length CREB will be used in functional and biochemical assays to determine which of these domains

are necessary to recruit the Tax:CBP complex to the HTLV-I TRE and the cellular CRE.

The studies in Chapter I represent a collaborative effort between Roland Kwok, Phyllis Goldman, Hsiu-Ming Shih, and myself. These studies were published as titled in the journal Nature (Kwok et al, 1996).

The studies in Chapter II represent data generated by myself. These studies were published as titled in the Journal of Biological Chemistry (Laurance et al, 1997).

Figure 1.

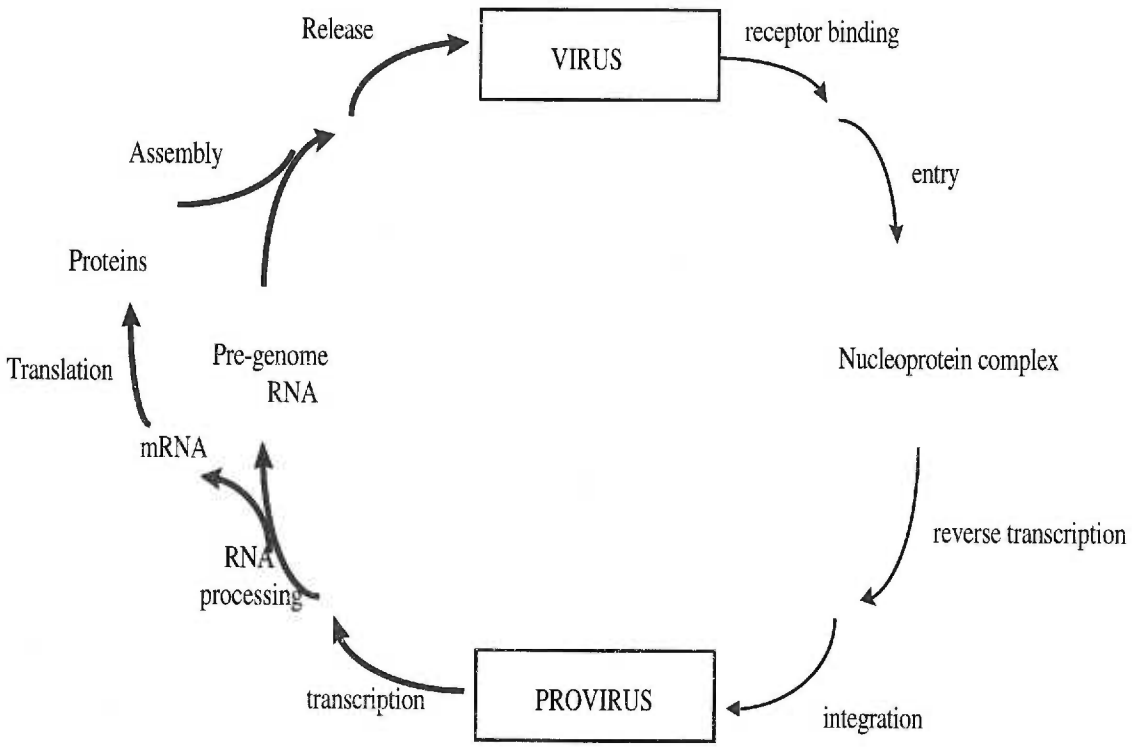

Release
VIRUS
receptor binding
Assembly
entry
Proteins
Translation
Pre-genome
RNA
Nucleoprotein complex
mRNA
reverse transcription
RNA
processing
transcription
PROVIRUS
integration

Figure 1. Schematic of the HTLV-I life cycle. The life cycle of the virus is marked initially by binding of viral envelope proteins to host cell surface proteins. The virus enters the cell via receptor-mediated endocytosis. Entry of the virus initiates the conversion of the enveloped particle into an enzymatically active nucleo-protein complex competent for reverse transcription and integration. Once the provirus is integrated, it is highly dependent on the host for 1) replication of the provirus, 2) transcription of the provirus by RNA polymerase II, 3) processing of viral RNA transcripts, and 4) translation of mRNAs by host polyribosomes.

Figure 2.

Genomic organization of HTLV-1

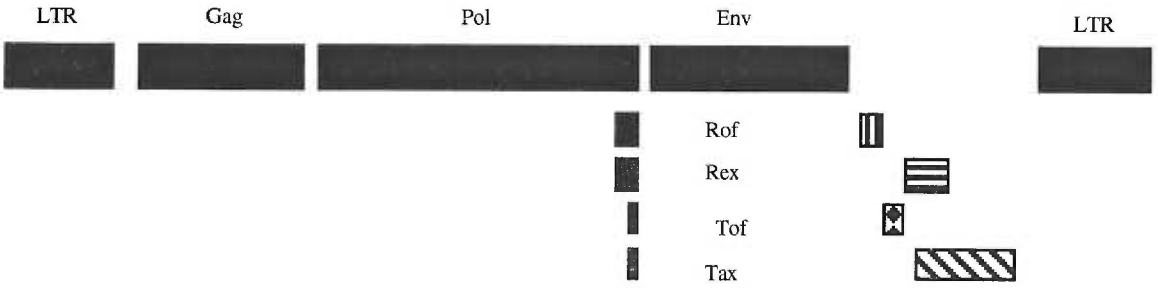


Figure 2. Genomic organization of HTLV-I. Genomic sequences that regulate the structural transformation of the viral genome during the life cycle are clustered near the ends of the RNA (long terminal repeats LTR). Between the regulatory regions of the LTRs are coding sequences for gag (encoding core proteins), env (encoding the envelope glycoproteins), and pol (encoding integrase, reverse transcriptase, and a protease). Coding sequences for the HTLV-I regulatory proteins Rof, Tof, Rex, and Tax are as marked.

Figure 3.

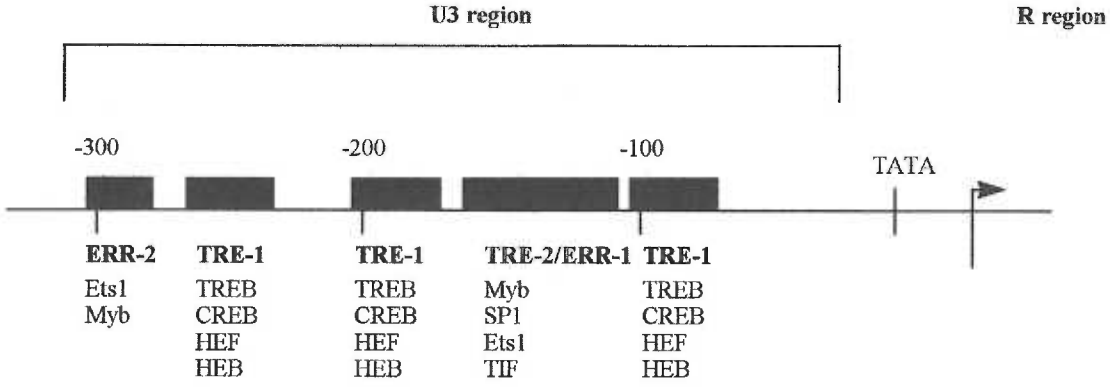

U3 region
R region
-300
-200
-100
TATA
ERR-2
Ets1
Myb
TRE-1
TREB
CREB
HEF
HEB
TRE-1
TREB
CREB
HEF
HEB
TRE-2/ERR-1
Myb
SP1
Ets1
TIF
TRE-1
TREB
CREB
HEF
HEB

Figure 3. Upstream regulatory elements of the HTLV-I LTR. Signals that modulate HTLV-I transcription are contained primarily in the U3 region of the LTR. ERR refers to the Ets-response regions 1 and 2. The three 21-base pair repeat Tax-responsive-elements (TREs) are highly homologous, and referred to as TRE-1. TRE-2/ERR-1 represents a composite Tax/Ets responsive site. Proteins known to bind to these sites are as noted.

Figure 4.

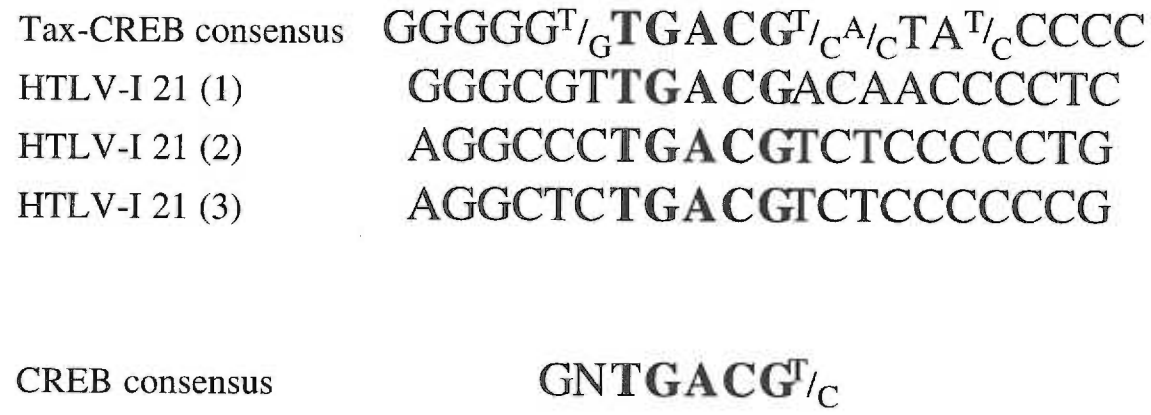

Tax-CREB consensus
GGGGG T/G TGACG T/C A/C TA T/C CCCC
HTLV-I 21 (1)
GGGCGTTGACGACAACCCCTC
HTLV-I 21 (2)
AGGCCCTGACGTCTCCCCCTG
HTLV-I 21 (3)
AGGCTCTGACGTCTCCCCCCG
CREB consensus
GNTGACG T/C

Figure 4. Tax-responsive elements in HTLV-I LTR. The consensus site selected by the Tax:CREB complex is compared to the known Tax-responsive elements of the HTLV-I LTR, with HTLV-I 21 (1) representing the promoter proximal TRE. These sequences are compared to the consensus cellular CRE-site (CREB consensus).

Figure 5.

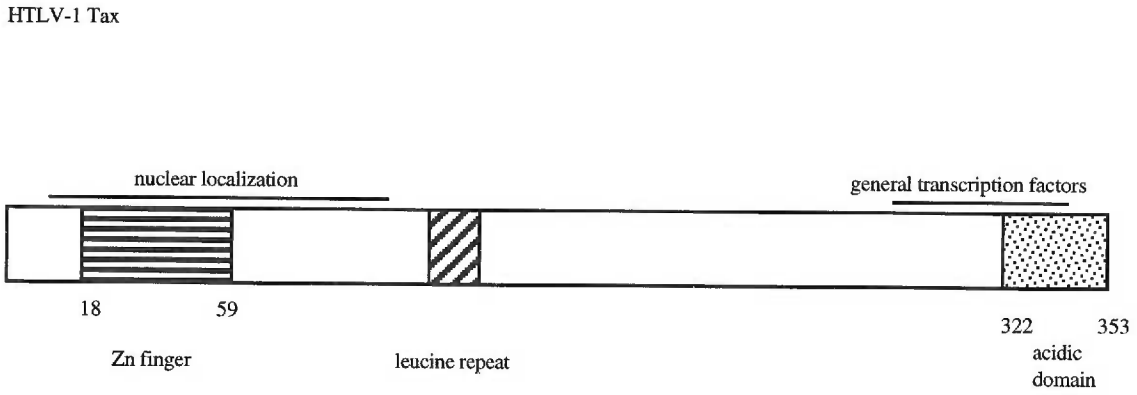

HTLV-1 Tax
nuclear localization
general transcription factors
18
59
322
353
Zn finger
leucine repeat
acidic
domain

Figure 5. Schematic representation of the functional domains in HTLV-I Tax. Primary structure prediction identifies a putative zinc-finger domain located in the N-terminus of Tax; a N-terminal leucine repeat; and an acidic C-terminal domain. Known regions of protein:protein interaction are noted.

Table I:

Effect of Tax on transcription of cellular genes

Activation of:	
IL-2	Greene et al, 1986, Ballard et al, 1988
Il-2R α	Inoue etal, 1987, Cross et al, 1987, Leung et al, 1988, Ruben et al, 1988
IL-3	Miyatake et al, 1988
IL-4	Miyatake et al, 1988
TNF-α	Paul et al, 1990
TNF-β	Paul et al, 1990
GM-CSF	Miyatake, et al, 1988
proenkephalin	Low et al, 1994
Vimentin	Lilienbaum et al, 1990
fos/jun	Fujii et al, 1988, Alexandre et al, 1991, Fujii et al, 1991, Fujii et al 1992
NF-κB/Rel	Arima et al, 1991, Lacoste et al, 1991
Egr/Krox	Alexandre et al 1991, Fujii et al 1992
c-myc	Duyao et al, 1992
Repression of :	
human β-polymerase	Jeang et al, 1990

Figure 6.

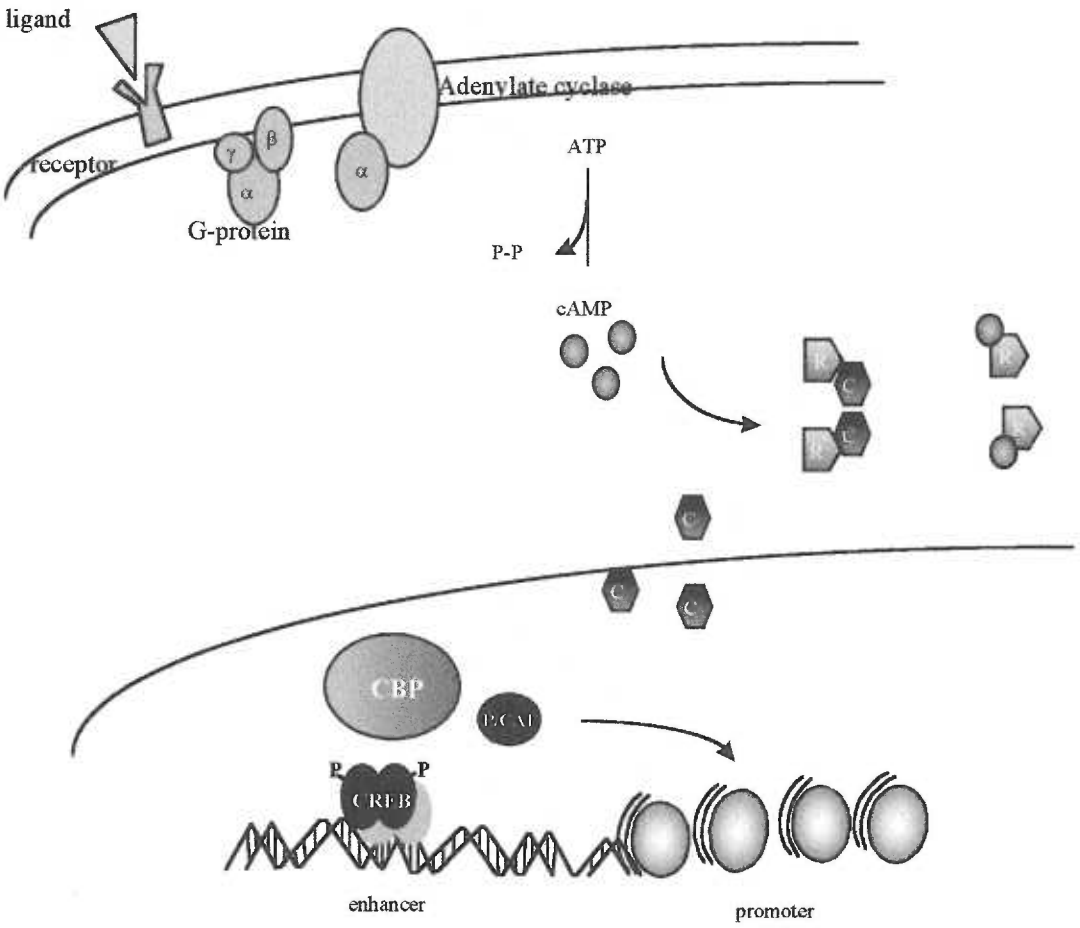

ligand
receptor
Adenylate cyclase
G-protein
ATP
P-P
cAMP
CBP
P
P
enhancer
promoter

Figure 6. Model for cAMP mediated gene activation. Upon binding of a hormone (ligand) to a G-protein coupled receptor, adenlyate cyclase is activated to produce cAMP from ATP. cAMP binds the regulatory subunit of PKA, freeing the catalytic subunit to traverse to the nucleus. Phosphorylation of CRE-bound CREB by activated PKA allows binding of the transcriptional co-activator CBP. CBP mediates transcriptional activation by linking the upstream enhancer elements (CRE) and their sequence specific binding proteins (CREB) to the basal transcriptional machinery bound to the promoter. CBP may also function to alter chromatin structure.

Figure 7.

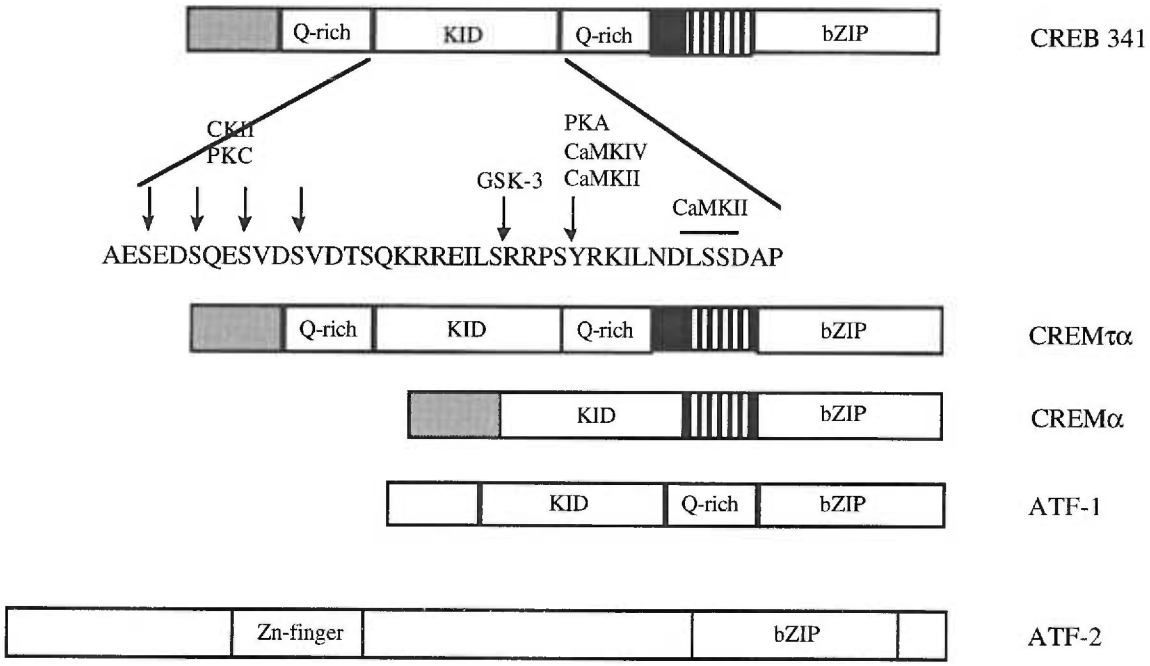

Q-rich
KID
Q-rich
bZIP
CREB 341
CKII
PKC
GSK-3
PKA
CaMKIV
CaMKII
CaMKII
AESEDSQESVDSVDTSQKRREILSRRPSYRKILNDLSSDAP
Q-rich
KID
Q-rich
bZIP
CREMτα
KID
bZIP
CREMα
KID
Q-rich
bZIP
ATF-1
Zn-finger
bZIP
ATF-2

Figure 7. Schematic representation of CRE-binding proteins. Functional domains conserved within this family include the glutamine-rich domains (Q-rich), the kinase inducible domain (KID), and the basic/leucine zipper dimerization/DNA-binding domain (bZIP).

Figure 8.

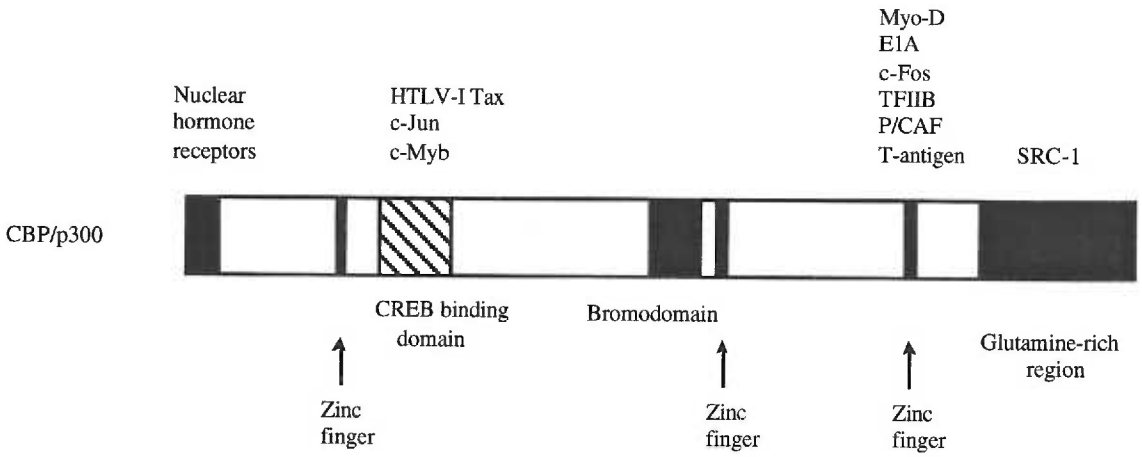

Myo-D
E1A
c-Fos
TFIIB
P/CAF
T-antigen
Nuclear
hormone
receptors
HTLV-I Tax
c-Jun
c-Myb
SRC-1
CBP/p300
CREB binding
domain
Bromodomain
Glutamine-rich
region
Zinc
finger
Zinc
finger
Zinc
finger

Figure 8. Functional domains of the CREB-binding protein (CBP). Primary structure comparison of CBP to other proteins has identified three cysteine-histidine rich putative zinc finger domains (CH-1, 2, 3), a bromodomain, and a C-terminal glutamine-rich domain. Areas mediating protein:protein interactions are indicated.

CHAPTER I

Control of cAMP-regulated enhancers by the viral transactivator Tax through CREB and the co-activator CBP

Roland P.S. Kwok, Megan E. Laurance, James R. Lundblad,
Phyllis S. Goldman, Hsiu-ming Shih, Laurie M. Connor*, Susan J. Marriott*
and Richard H. Goodman

Vollum Institute, Oregon Health Sciences University
Portland, Oregon 97201-3098

*Division of Molecular Virology, Baylor College of Medicine
Houston, Texas 77030

Address correspondence to:

Richard H. Goodman, M.D., Ph.D.
Vollum Institute, L474
Oregon Health Sciences University
3181 S. W. Sam Jackson Park Road
Portland, Oregon 97201-3098
Telephone: 503-494-5078
FAX: 503-494-4353

SUMMARY

The Tax protein of HTLV-1 activates expression of the HTLV-1 LTR through a DNA element that resembles the cellular cAMP-regulated enhancer (CRE) (Rosen *et al*, 1985, Paskalis *et al*, 1986). Tax contains a transcriptional activation domain (Fujii *et al*, 1991), but its ability to activate gene expression depends upon interactions with cellular CRE-binding proteins such as CREB. Whether Tax can activate the expression of cellular CRE-containing genes has been controversial. We present evidence that Tax can activate both the HTLV-1 and consensus cellular CREs, and propose that this activation may occur through mechanisms that are differentially dependent upon CREB phosphorylation. In the context of the viral CRE, Tax not only increases the binding of CREB but also recruits the transcriptional co-activator CBP (Chrivia *et al*, 1993, Kwok *et al*, 1994) in a manner that is independent of CREB phosphorylation. In contrast, association of Tax with the cellular CRE occurs through CBP which, in turn, is recruited only in the presence of phosphorylated CREB.

Although the participation of CRE-binding proteins such as CREB in Tax activation of the HTLV-1 LTR is well-established (Zhao *et al*, 1992, Franklin *et al*, 1993), the mechanisms underlying Tax activation of cellular CRE-containing genes are unclear. Several studies have shown that Tax function requires DNA sequences that are specific to the HTLV-1 promoter (Fujisawa *et al*, 1985, Felber *et al*, 1985, Paca-Uccaralertkun *et al*, 1994) . Additionally, cellular CRE-containing genes have generally been found to be unresponsive to Tax (Yin *et al*, 1995, Brauweiler *et al*, 1995) . Nonetheless, genetic analysis of Tax mutants indicates that cellular CRE-containing genes account for critical aspects of Tax-mediated cell transformation (Smith *et al*, 1991). To compare the ability of Tax to activate cellular and viral CREs, we constructed reporter genes containing either a single copy of the somatostatin CRE (Montminy *et al*, 1986) , as a prototype of a cellular CRE, or the entire HTLV-1 U3 region (Gitlin *et al*, 1993). Transfections were performed in F9 teratocarcinoma cells, which contain CBP but require exogenous CREB and protein kinase A (PKA) for CRE-reporter activation (Montminy *et al*, 1986). The ability of Tax to augment CREB-mediated induction of the cellular CRE depended upon CREB phosphorylation, as the augmentation was prevented when PKA was omitted and was significantly decreased when the consensus PKA site in CREB was mutated (Figs. 1A,B). In contrast, Tax activated expression of the HTLV-1 reporter even in the absence of PKA (Fig. 1C). Similar results were obtained in studies utilizing the promoter proximal HTLV-1 CRE alone (data not shown.) These results suggested that Tax activates the cellular and viral CREs through different mechanisms.

We utilized a fluorescence polarization binding assay (Kwok *et al*, 1994, Lundblad *et al*, 1995, Jameson *et al* 1995) to examine the effects of Tax on DNA binding. In the absence of Tax, CREB bound poorly to the HTLV-1 CRE (K_d>100 nM; Fig. 2A). Addition of Tax lowered the K_d to 6.5 nM. Tax did not alter CREB binding to the cellular

CRE, however (K_d 4.5 nM vs. 3.2 nM; Fig. 2B). Similar data were obtained using phosphorylated CREB (data not shown). Thus, although Tax can increase the affinity of CREB for the HTLV-1 CRE, Tax activation of the somatostatin CRE must occur through another mechanism.

Because Tax activation of the cellular CRE depended upon CREB phosphorylation, we considered whether Tax could alter the affinity of the CREB co-activator CBP. In the context of the somatostatin CRE, the binding of CBP to phosphorylated CREB in the presence and absence of Tax was similar (K_d = 344 nM and 360 nM, respectively; Fig. 2C). The poor binding of CREB to the HTLV-1 CRE in the absence of Tax precluded accurate measurement of CBP affinity but, in the presence of Tax, the K_d of CBP for the phosphorylated CREB:HTLV-1 CRE complex was 150 nM (Fig. 2E). Surprisingly, Tax also allowed CBP to associate with the complex containing non-phosphorylated CREB, but only in the context of the HTLV-1 CRE (K_d=200nM; Fig. 2F). Thus, Tax increases the affinity of CREB for the HTLV-1 CRE, increases the affinity of CBP for the HTLV-1 CRE:CREB complex, and renders this latter interaction independent of CREB phosphorylation. In the context of the somatostatin CRE, non-phosphorylated CREB associated very poorly with CBP regardless of Tax (Fig. 2D).

Because Tax activation of the prototypical cellular CRE-containing gene did not appear to result from increasing CBP affinity, we asked whether Tax might bind to CBP directly. As shown in Fig. 3A, Tax binds *in vitro* to CBP residues 451-682 and the corresponding portion of the CBP homologue p300 (566-663), regions characterized previously as containing the "CREB binding domain" (Chrivia *et al*, 1993, Lundblad *et al*, 1995). Tax also bound weakly to GST-CBP fusion proteins containing residues 1099-1331 and 1680-1891 (data not shown). To test whether Tax interacts with CBP or p300 *in vivo*, we used yeast two hybrid and co-immunoprecipitation assays. In the yeast two-

hybrid assay, the combination of Lex-CBP(451-682) and VP16 Tax allowed growth of the yeast reporter strain in the absence of histidine and promoted ß-galactosidase expression (Fig.3B). We also demonstrated that p300 associates with Tax in HTLV-1 infected Hu T-102 cells (Fig.3C). To determine whether this association could augment CBP/p300-mediated transcriptional activation, we tested the ability of a Gal-CBP(451-682) fusion gene to activate a Gal-CAT reporter in F9 teratocarcinoma cells. Tax enhanced activity of the fusion gene in a dose-dependent manner, while slightly diminishing the activity of a Gal-CBP(1678-2441) fusion gene (Fig. 3D).

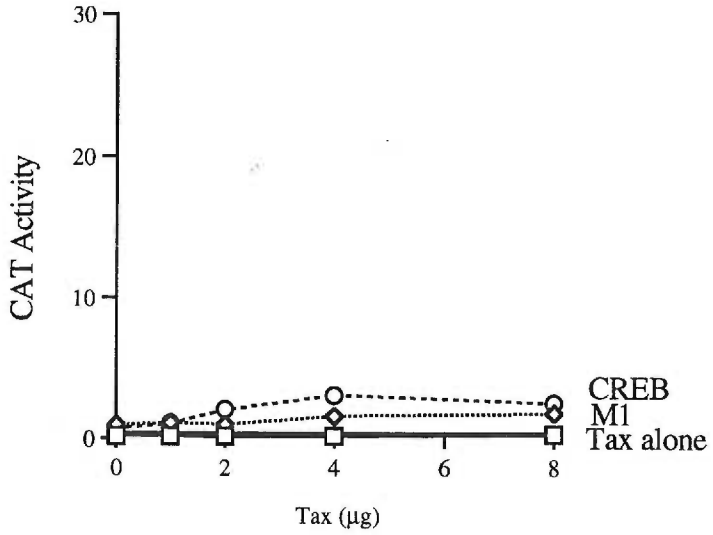
The hypothesis that CBP interacts with both Tax and phosphorylated CREB at the cellular CRE implies the formation of a quaternary complex. This possibility was tested by using an avidin-biotin complex assay. Complexes containing a biotinylated CRE, phosphorylated or non-phosphorylated CREB, CBP, and Tax were collected on avidin beads and assayed for their constituent proteins. Tax associated with the somatostatin CRE only in the presence of phosphorylated CREB and CBP (Fig. 4A). Additionally, as expected, CBP binding was detected only in the presence of phosphorylated CREB. The composition of the protein complex at the HTLV-1 CRE was notably different (Fig. 4B). In this case, Tax associated with complexes containing only DNA and CREB in a manner that was independent of CBP. Consistent with the fluorescence assays, CBP associated with the HTLV-1 CRE:non-phosphorylated CREB complex in the presence of Tax. A model describing these interactions is depicted in Fig. 4C.

Our finding that Tax increases CREB binding specifically to the HTLV-1 CRE is consistent with previous observations (Paca-Uccaralertkun *et al*, 1994, Yin *et al*, 1995, Brauweiler *et al*, 1995, Wagner *et al*, 1993). Because Tax does not alter CREB affinity for the consensus cellular CRE, it is unlikely that Tax functions primarily by increasing CREB dimerization (Perini *et al*, 1995). If this was the case, Tax should increase the affinity of

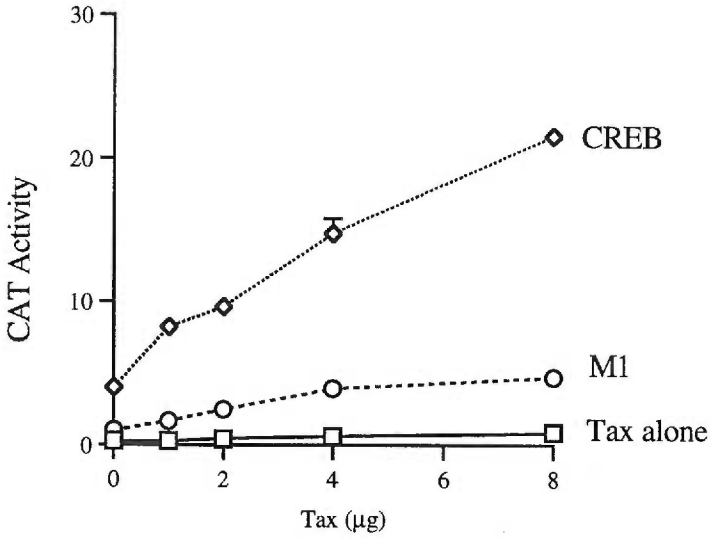
CREB for all CRE sequences. It is not known to what degree induction of the HTLV-1 CRE depends upon the individual activation properties of Tax or CBP however, and both may contribute to the ability of Tax to activate the viral LTR. The inability of Tax to activate CRE-containing cellular genes has been puzzling. Our data show that Tax can indeed potentiate expression from the prototypical cellular CRE if the PKA pathway is also activated, presumably by recruiting Tax to the CRE:phosphorylated CREB:CBP (or p300) complex. These results suggest that Tax can stimulate the viral LTR under basal conditions but activate cellular CRE-containing genes under conditions that lead to CREB phosphorylation.

Figure 1

A) Cellular CRE without PKA



B) Cellular CRE with PKA



C) pU3R without PKA

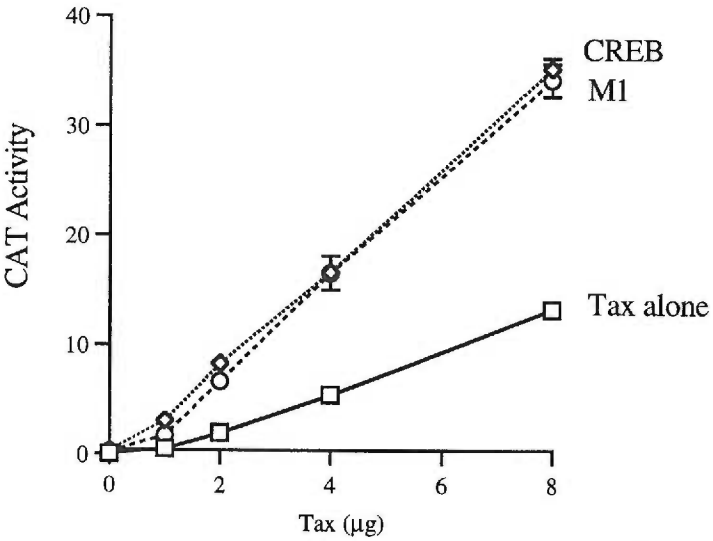
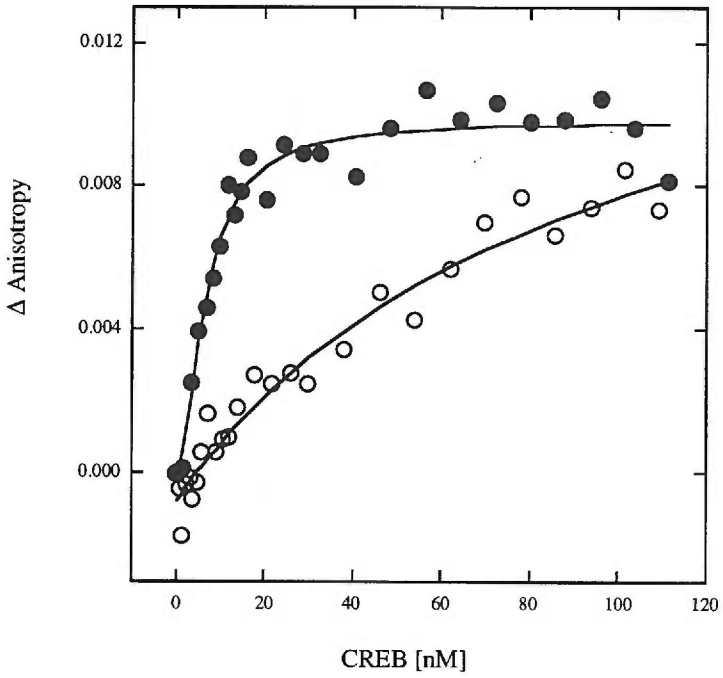


Fig. 1. Responses of the cellular CRE and HTLV-1 reporter genes. F9 teratocarcinoma cells were transfected with p(-71)SRIF-CAT (Montminy *et al*, 1986) (a,b) or pU3R-CAT (Gitlin *et al*, 1993) (c) along with RSV-CREB341 (Loriaux *et al*, 1993) or RSV-CREBM1 (in which the PKA phosphorylation site has been mutated to alanine, Loriaux *et al*, 1993), RSV-Tax (S. Marriott, unpublished), and RSV-PKA (gift from R. Maurer), as indicated. Amount of Tax vector is shown in μg. Results are expressed as CAT activity (mean±S.E., N>3), normalized for luciferase activity.

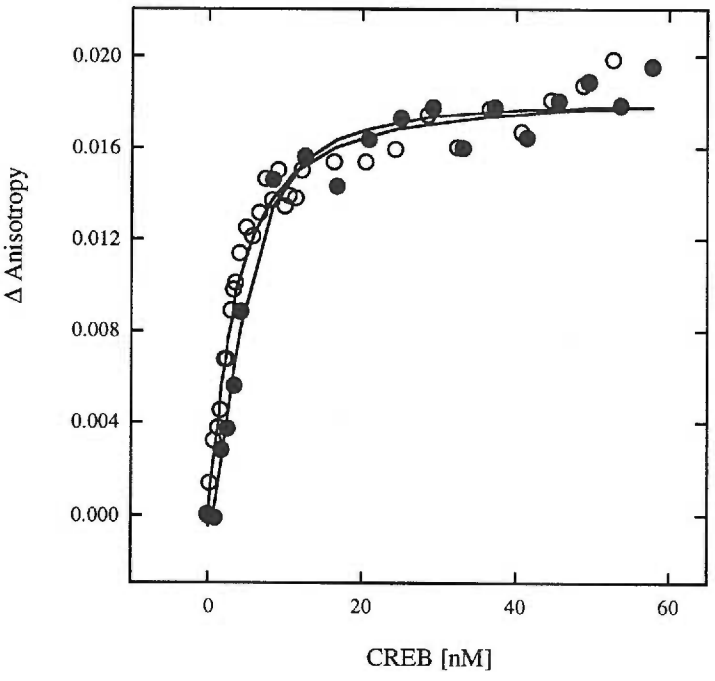
METHODS: (a,b) F9 cells were seeded with 3.5x10 (Kwok *et al*, 1994) cells per plate and were transfected by calcium phosphate precipitation (Brauweiler *et al*, 1995) with 4μg RSV-PKA as indicated, 2μg RSV-luciferase, 0-8 μg RSV-Tax, 4μg RSV-CREB or CREBM1, and 5μg p(-71)SRIF-CAT. For (c), 2μg RSV-CREB or CREBM1 and 2μg pUR3-CAT were used.

Figure 2

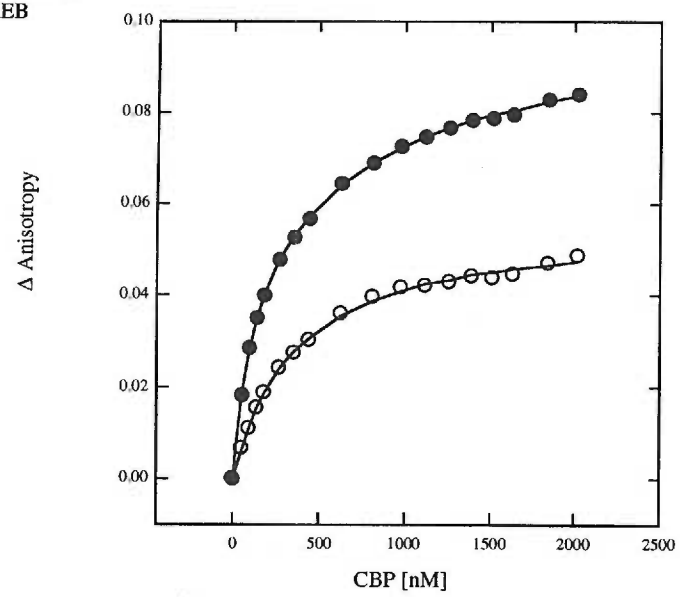
A) HTLV-1 CRE



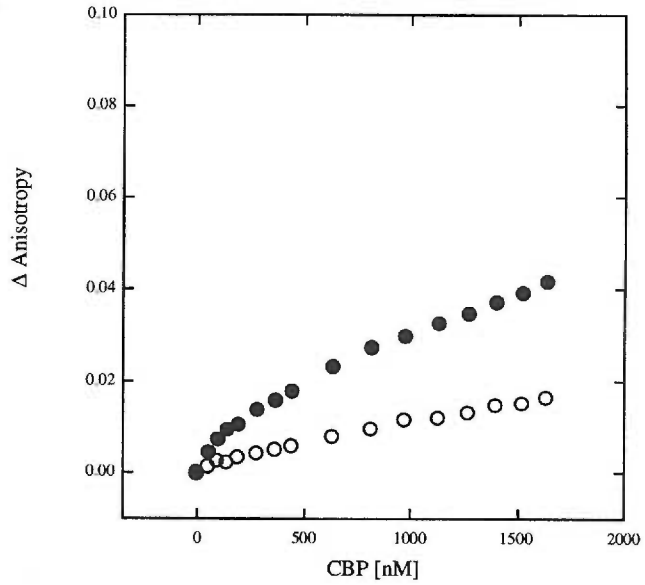
B) Cellular CRE



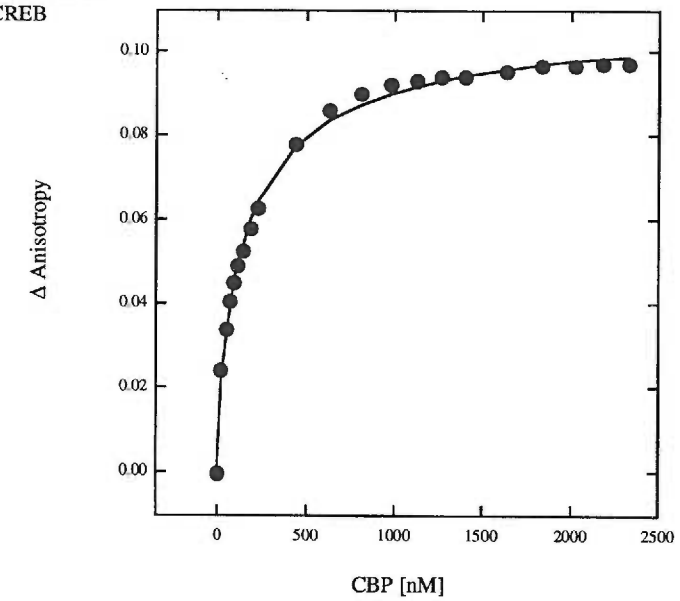
C) Cellular CRE
PCREB



D) Cellular CRE
CREB



E) HTLV-1 CRE
PCREB



F) HTLV-1 CRE
CREB

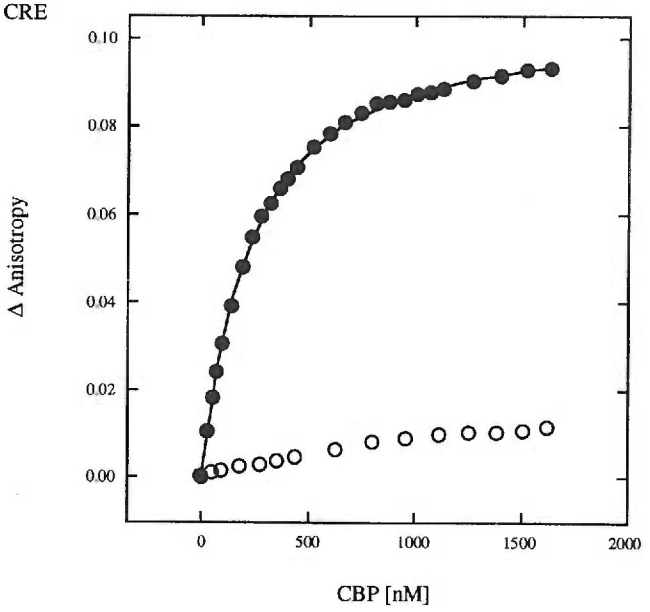
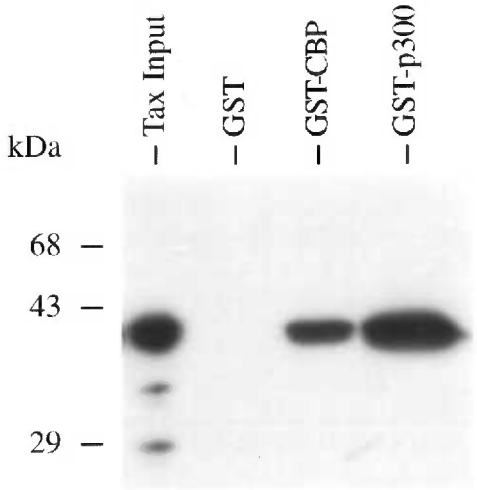


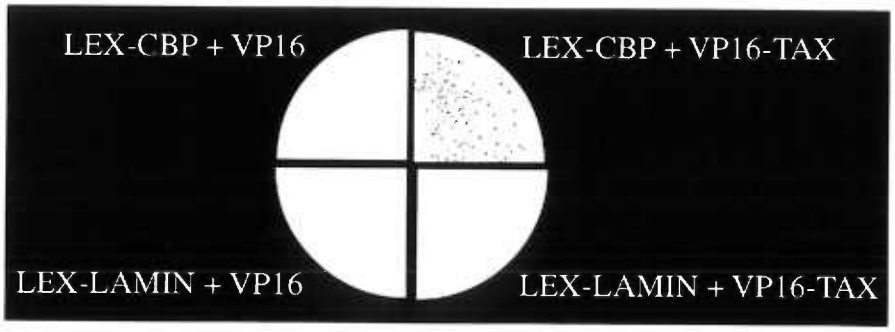
Figure 2 C-F

Fig. 2. Effects of Tax on CREB and CBP binding. (a) Fluorescence anisotropy measurements of CREB binding to the HTLV-1 CRE in the absence (open circles) and presence (closed circles) of 250nM Tax. (b) Binding of CREB to the cellular CRE in the presence and absence of Tax. (c) CBP binding to a phosphorylated CREB:cellular CRE complex in the presence or absence of Tax. (d) Binding of CPB to the non-phosphorylated CREB:cellular CRE complex. No specific binding was detected in the absence or presence of Tax. (e) CBP binding to a phosphorylated CREB:HTLV-1 CRE complex in the presence of Tax. (f) Binding of CBP to the non-phosphorylated CREB:HTLV-1 CRE complex. In the absence of Tax, there was no specific CBP binding. K_d for CBP in the presence of Tax was 200nM.

METHODS: A 28 base 5'-fluoresceinated oligonucleotide representing the sense strand of the most proximal HTLV-1 Tax response element (5'F-TCCTCAGGCGTTGACGACA ACCCCTC AC-3') and a 23 base 5'-fluoresceinated oligonucleotide representing the somatostatin CRE (5'F-CCTTGGCTGACGTCAGAGAGAGC-3') were annealed to antisense oligonucleotides. Generation of Tax, phosphorylated CREB, CBP(1-682), and fluorescence polarization measurements were as described (Kwok *et al*, 1994, Lundblad *et al*, 1995). For the CBP binding assays, fluorescein-labeled oligonucleotides (5nM) were incubated with saturating amounts (30nM) of non-phosphorylated or phosphorylated CREB and increasing amounts of CBP in the presence or absence of 250nM Tax.

Figure 3 (A and B)





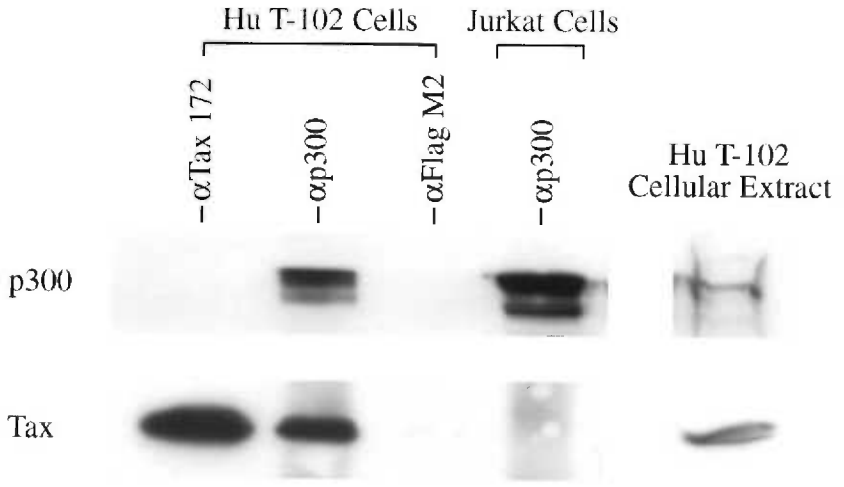

Hu T-102 Cells
Jurkat Cells
-αTax 172
-αp300
-αFlag M2
-αp300
Hu T-102
Cellular Extract
p300
Tax

Figure 3d

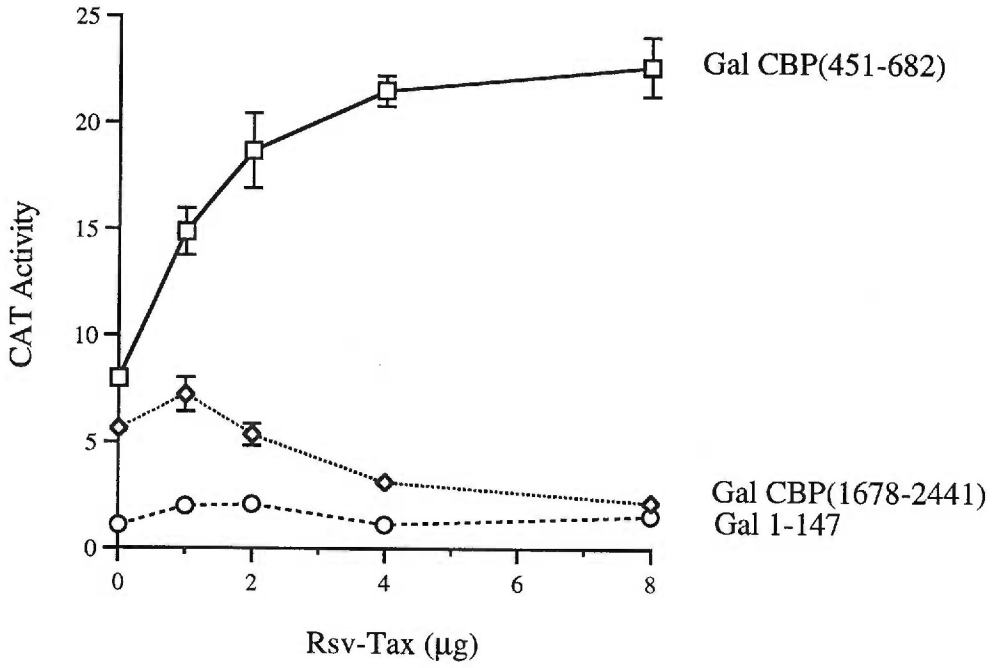

25
20
15
10
5
0
CAT Activity
0
2
4
6
8
Rsv-Tax (µg)
Gal CBP(451-682)
Gal CBP(1678-2441)
Gal 1-147

Fig. 3. Interaction between Tax and CBP. (a) *In vitro* binding. Tax input represents 10% of the protein used in the binding assay. GST represents glutathione S-transferase protein alone, GST-CBP(451-682) and GST-p300(566-663) fusion proteins are indicated. (b) Yeast two-hybrid assay. Yeast containing a Lex-CBP(451-682) fusion gene were transformed with constructs containing VP16 activation domain alone (upper left) or VP16-Tax (upper right). Interactions were identified by growth in the absence of histidine and staining in a β-galactosidase filter assay. A Lex-lamin fusion gene (lower panels) was used to test for nonspecific interactions. (c) Immunoprecipitation assay. Whole cell lysates from Hu T-102 cells were immunoprecipitated using αTax-172, αp300 (UBI, 05-256) or αFlag M2. Whole cell lysates from Jurkat cells were immunoprecipitated using αp300. The immunecomplexes were analyzed by Western blotting using either αp300 or αTax-172. The lane labeled as Hu T-102 cellular extract represents 10% of the cell extracts used in the immunoprecipitation assay. (d) *In vivo* activation. Relative CAT activity values of a Gal-CAT (Chrivia *et al*, 1993) reporter in the presence of Gal CBP(451-682), Gal CBP(1678-2441) (Kwok *et al*, 1994) Gal 1-147, and various amounts of Tax are indicated. Results are expressed as CAT activity (mean±S.E., N>3), normalized for luciferase activity. METHODS: (a) GST-pulldown assay was performed as described (Kwok *et al*, 1994, Lundblad *et al*, 1995). Tax was detected by using TAB172 monoclonal antibody (Langton *et al*, 1988) and ECL. (b) DNA encoding CBP(451-682) was cloned into BTM116 (gift from S. Hollenberg). Bait and target vectors were transformed into yeast strain L40 as described (Vojteck *et al*, 1993) and were plated onto His plates supplemented with 15mM 3-aminotriazole. A β-galactosidase filter assay (Breeden *et al*, 1985) was used to visualize interactions. (c) Cell extract preparation and conditions for immunoprecipitations and immunoblot assay have been described (Lundblad *et al*, 1995). (d) F9 cells were

transfected with Gal-CAT reporter, 4μg RSV-PKA, 2μg RSV-luciferase, and 0.5μg of either RSV-Gal CBP(451-682), RSV-GalCBP(1678-2441) or RSV-Gal 1-147, and 0-8μg of RSV-Tax, as indicated.

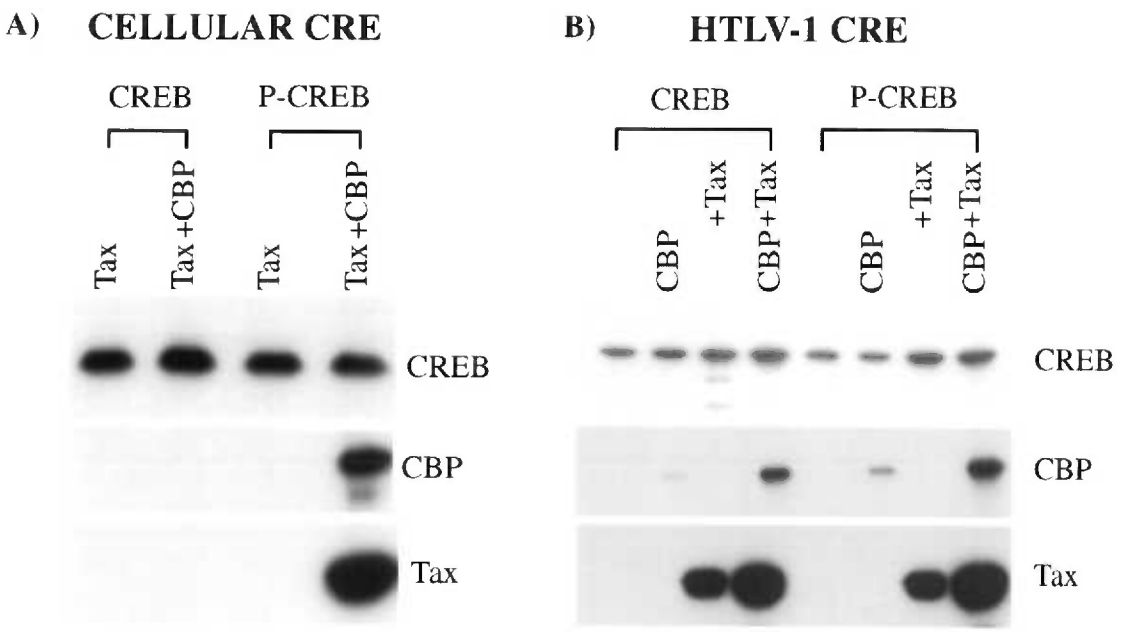

A)
CELLULAR CRE
CREB
P-CREB
Tax
Tax+CBP
Tax
Tax+CBP
CREB
CBP
Tax
B)
HTLV-1 CRE
CREB
P-CREB
CBP
+Tax
CBP+Tax
CBP
+Tax
CBP+Tax
CREB
CBP
Tax

Figure 4c

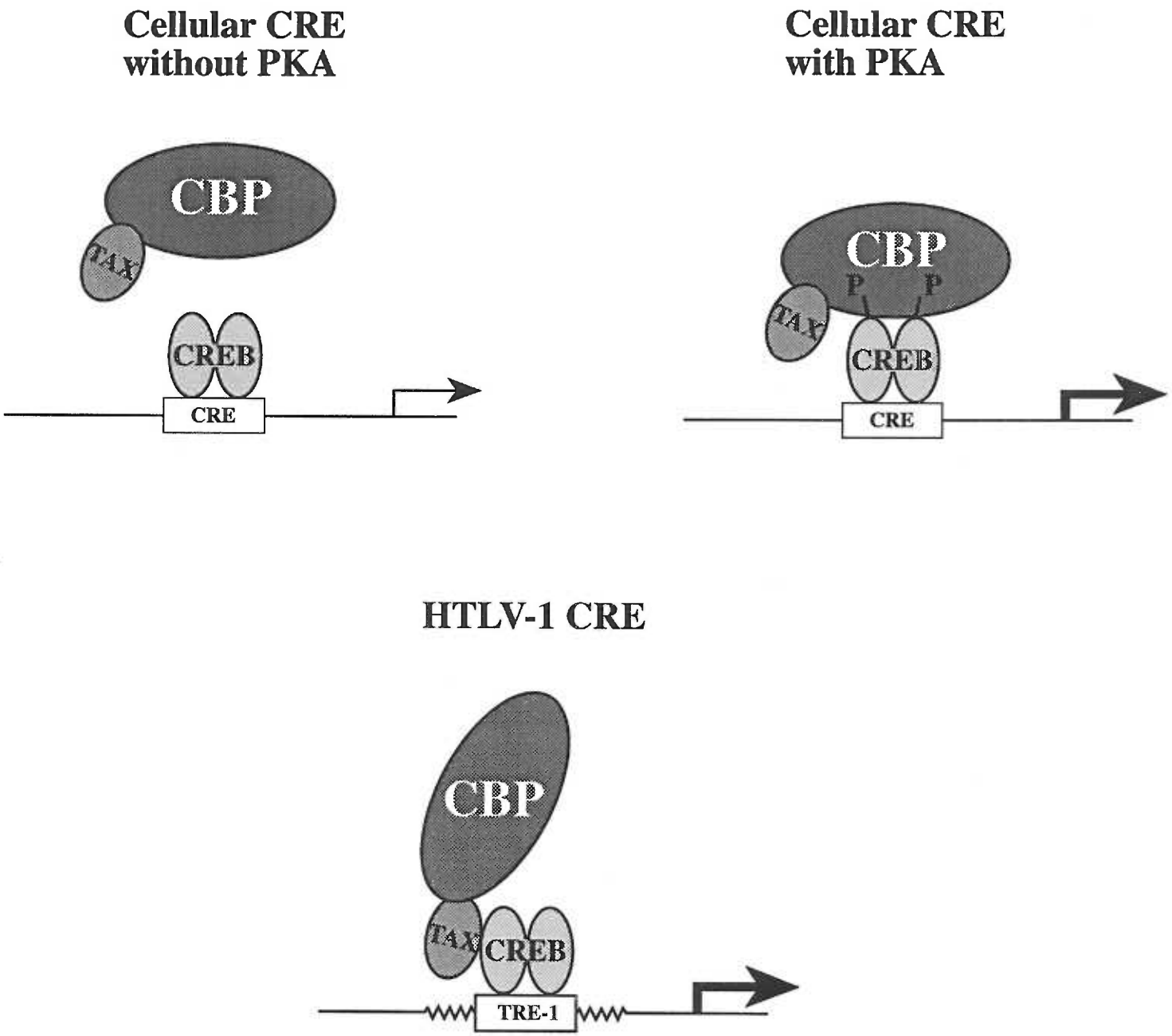

Cellular CRE
without PKA
CBP
TAX
CREB
CRE
Cellular CRE
with PKA
CBP
P
P
TAX
CREB
CRE
HTLV-1 CRE
CBP
TAX
CREB
TRE-1

Fig. 4. Avidin-biotin complex assay. (a) Mixtures of phosphorylated or non-phosphorylated CREB, CBP, and Tax were incubated in the presence of a biotinylated somatostatin CRE, collected on avidin beads, and analyzed by Western blotting. CBP binding required the inclusion of phosphorylated CREB. Tax binding required both phosphorylated CREB and CBP. (b) In the context of the HTLV-1 CRE, CBP binding was enhanced by the presence of Tax, but Tax association did not require CBP. (c) Model for Tax, CREB, and CBP binding. In the context of the cellular CRE, Tax is recruited through CBP, which requires phosphorylated CREB. In the context of the HTLV-1 CRE, CBP is recruited to the complex regardless of CREB phosphorylation, possibly through interactions with Tax rather than CREB. Tax and CREB depend upon each other for DNA binding. Jagged lines represent HTLV-1 CRE sequences proposed to contribute to CREB binding (Paca-Uccaralertkin *et al* 1994).

METHODS: A 28 base oligonucleotide (5'CGAGCCTTGGCTGACGTCAG AGAGAGCG-3') was annealed with a 36 base oligonucleotide (5'-TCGACGCT CTCTCTGACGTCAGCCAAGGCTCGAGCT-3') to form a double-stranded DNA representing the somatostatin CRE and surrounding sequences. A 31 base oligonucleotide (5'-CGAGCCTCAGGCGTTGACGACAACCCCTCAG-3') was annealed to a 39 base oligonucleotide (5'-TCGACTGAGGGGTTGTCGTCAACGCCTGAGGCTCGAGCT-3') to form a double-stranded DNA representing the promoter proximal HTLV-1 Tax response element. The 3' ends of both double-stranded DNAs were labeled with biotin-14-dATP as described (Waterman *et al*, 1988). 200ng of labeled DNA was incubated with 250nM CREB or phosphorylated CREB, 250nM Tax, and 800nM CBP(1-682) in 200ul buffer containing 50mM Tris (pH 7.6), 50mM NaCl, 0.5mM EDTA, 1mM DTT, 5mM $MgCl_2$, 0.1% Triton, 5% glycerol, 2.5mg/ml BSA, 10ug/ml Poly [d(I-C)] for 2 hours at room temperature before addition of streptavidin beads (Pierce). Mixtures were then incubated at room temperature for 1 hour and washed four times in binding buffer without BSA and

Poly [d(I-C)]. Proteins were eluted in SDS loading buffer, separated on a 10% SDS polyacrylamide gel, and detected by Western blotting and ECL (Kwok *et al*, 1994).

CHAPTER II

Differential Activation of Viral and Cellular Promoters by Human T-cell Lymphotropic Virus -1 Tax and CREM Isoforms

Megan E. Laurance, Roland P. S. Kwok, Mary S. Huang, Jane P. Richards, James R. Lundblad, and Richard H. Goodman*

Vollum Institute and Departments of Cell and Developmental Biology, Biochemistry and Molecular Biology, Pediatrics, and Internal Medicine, Oregon Health Sciences University, Portland, OR 97201

Phone: 503-494-5078 FAX: 503-494-4353

*Corresponding Author

Running Title: Activation of viral and cellular promoters by HTLV-1 Tax and CREM

SUMMARY

We have previously proposed that CREB activity is stimulated by the human T-cell lymphotropic virus (HTLV-1) protein Tax through two distinct mechanisms that are differentially dependent upon CREB phosphorylation. In this report, we test this model by examining the effects of Tax on transcriptional activation mediated by the CRE-modulator (CREM) gene products. The CREM proteins are highly homologous to CREB, particularly in their DNA-binding domains and the kinase inducible domain (KID), a region that interacts with the co-activator CBP in a phosphorylation-dependent manner. Despite the similarity to CREB, most CREM isoforms are transcriptional repressors. CREMα, in particular, lacks the glutamine-rich domains found in CREB, which are essential for transcriptional activation. In the present study, we show that the normally repressive CREMα activates the HTLV-1 and cellular CREs in the presence of Tax in a pattern identical to that of CREB -- activation of the viral element is phosphorylation-independent and activation of the cellular CRE is phosphorylation-dependent. Fluorescence polarization binding assays indicate that CREMα allows CBP to be recruited specifically to the viral CRE in a phosphorylation-independent manner. Another repressor isoform, CREM Δ(C-G), lacks both the KID and the glutamine-rich regions. This isoform activates the HTLV-1 LTR in a phosphorylation-independent manner as effectively as CREMα but does not activate the cellular CRE. These studies are consistent with the model that Tax, interacting with the basic/zipper region of the normally repressive CREM proteins, recruits CBP to the viral promoter. In contrast, Tax activation of the cellular CRE depends on the KID and its ability to interact with CBP in a phosphorylation-dependent manner.

INTRODUCTION

The human T-cell lymphotropic virus (HTLV-1) is the causative agent in adult T-cell leukemia and HTLV-1-associated myelopathy (Poiesz *et al*, 1992). The HTLV-1 genome encodes several regulatory proteins that are involved in controlling viral gene expression and pathogenesis. One such protein, the 40 kDa transactivator Tax, has been linked to cellular transformation (Felber *et al*, 1985). Precisely how activation of Tax-responsive genes leads to transformation is unknown, however. While a fusion protein containing Tax sequences linked to a heterologous DNA-binding domain is a potent transactivator of gene expression, the native Tax protein is unable to bind DNA directly (Fujii *et al*, 1994, Fujisaea *et al*, 1991). Thus, it has been proposed that the transactivating functions of Tax require its interaction with cellular DNA-binding proteins. Indeed, several basic/leucine zipper transcription factors have been shown to interact with the Tax responsive elements of the HTLV-1 promoter *in vitro*, including CREB, CREM and ATF-1 (Yoshimura *et al*, 1990, Zhao *et al*, 1992, Suzuki *et al*, 1993). Binding of these factors to the Tax responsive elements, which resemble the cAMP-regulated enhancers (CREs) of cellular genes, is markedly enhanced by the Tax protein (Kwok *et al*, 1996, Wagner *et al*, 1993, Paca-Uccaralertkin *et al*, 1994, Baranger *et al*, 1995). Furthermore, studies have shown that the HTLV-1 long terminal repeat (LTR) can be activated by protein kinase A (PKA) and the level of activation is augmented by Tax (Poteat *et al*, 1989). However, the precise molecular mechanisms through which Tax exerts its transactivation effects are not clear.

Recently, we proposed that Tax potentiates transactivation of cellular CRE-containing genes through a direct interaction with the transcriptional co-activator CREB-binding protein (CBP) (Kwok *et al*, 1996). We additionally showed that the ability of Tax to function in this manner depends upon CREB phosphorylation, as the augmentation is prevented when PKA is omitted and is significantly decreased when the consensus PKA

site in the kinase-inducible domain (KID) of CREB is mutated (Kwok *et al*, 1996). In contrast, Tax activates expression of the HTLV-1 LTR even in the absence of PKA (Kwok *et al*, 1996). These results suggest that Tax activates cellular and viral CREs through mechanisms that are differentially dependent on phosphorylation.

The structurally related transcription factors CREB, CREM, and ATF-1 are key mediators of cAMP-regulated gene transcription. While the regulatory properties of ATF-1 remain poorly defined, isoforms of CREB and CREM have well-characterized functions as activators or repressors of transcription, respectively. The CREB and CREM genes both encode an amino-terminal phosphorylation domain (kinase inducible domain; KID) flanked by glutamine-rich transcriptional activation domains (Meyer *et al*, 1993). The KID of CREB has been shown to interact with the transcriptional co-activator CBP in a phosphorylation-dependent manner (Chrivia *et al*, 1993, Kwok *et al*, 1994, Arias *et al*, 1994). Alternative splicing and selective translational initiation of CREB and CREM transcripts generate a family of proteins ranging from full-length transcriptional activators to truncated transcriptional repressors (Laoide *et al* 1993). Specifically, CREMα, which lacks the glutamine-rich domains found in activator isoforms such as CREB and CREMτ, has been shown to be a potent repressor of cAMP-activated transcription (Foulkes *et al*, 1991).

The different CREM isoforms provide valuable reagents for testing our model of Tax-activated transcription because the various functional domains in these isoforms are clearly delineated. Our model suggested that Tax activates transcription by interacting with distinct domains of the CREB transcription factor, depending on the promoter context and the presence or absence of PKA. Thus, we would predict that Tax should convert the repressor CREMα into an activator of the cellular CRE because CREMα contains a KID

and DNA binding domain that are very similar to those of CREB. Moreover, CREMα-mediated activation of the HTLV-1 LTR should occur in the absence of PKA, in a manner similar to that reported previously for CREB. The CREM Δ(C-G) isoform lacks both the glutamine-rich regions and the KID (Walker *et al*, 1994). This form is predicted to be incapable of mediating the Tax-activation of the cellular CRE but, because it contains a CREB-like basic/zipper region, it should still be able to contribute to activation of the viral LTR. By using a combination of *in vivo* transfection and *in vitro* fluorescence polarization binding assays, we show that the Tax-activation of cellular and viral CRE-containing genes is indeed mediated through different functional domains of the bZIP proteins in a manner that is differentially dependent upon phosphorylation. Our studies suggest that activation of the viral promoter occurs through the recruitment of CBP and requires, in addition to Tax, only the bZIP domain that is conserved among CREB, CREMα and CREM Δ(C-G). In contrast, activation of the cellular CRE by Tax occurs only in the presence of PKA and requires isoforms of CREB or CREM that contain the KID. Thus, recruitment of Tax to the cellular CRE depends upon a phosphorylated KID and the ability to bind to the co-activator CBP.

EXPERIMENTAL PROCEDURES

Cell culture and transfection assays: F9 teratocarcinoma cells were grown on 0.7% gelatin-coated plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin and streptomycin. Cells were seeded at 3.5 x 10^5 cells per plate 18 hours prior to transfection. DNA was prepared by alkaline lysis, purified twice in CsCl gradients, phenol extracted and ethanol precipitated prior to use. F9 cells were transfected by calcium precipitation (Brauweiler *et al*, 1995) with 4 μg RSV-PKA, 2 μg RSV-luciferase, 0-8 μg RSV-Tax, 0-8 μg RSV-CREMα, 5 μg p(-71) somatostatin-CAT (Montminy *et al*, 1986), 0-8 μg CMV-CREM Δ(C-G), and 2 μg pU3R-CAT (Gitlin *et al*, 1993), as indicated. CREMα was a gift from Dr. P. Sassone-Corsi; CREM Δ(C-G) was a gift from Dr. J. F. Habener. All other plasmids used in this study have been described previously. The total amount of DNA in each transfection was 30 μg, the balance being made up with Rc/RSV. Cells were washed, re-fed, and allowed to grow for 24 hours before harvesting. CAT activity was determined as described previously (Loriaux *et al*, 1993) and values were normalized for luciferase activity as a control for transfection efficiency. RSV-luciferase expression was not affected by introduction of CMV-CREM Δ(C-G).

Protein expression: Recombinant CREMα protein was generated through the use of the pET15b expression vector (Novagen). *E.coli* BL21(DE3) cells transformed with a plasmid containing the entire coding sequence of CREMα were grown to OD_{600} = 0.6 in Luria broth containing 2 g/L glucose and 100 μg/ml ampicillin. Cultures were induced to express the recombinant protein by the addition of isopropyl-ß-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. After 3 hours, cells were harvested and lysed by two

passages through a French press. Bacterial debris was cleared by centrifugation and the cleared supernatant was heated to 72° C for 10 minutes. Precipitated proteins were cleared by centrifugation. CREMα was purified from the supernatant over a FPLC HiTrap Q anion exchange column (Pharmacia). The minimal bZIP peptide clone was constructed from a CREB/SER vector (Richards *et al*, 1996) by engineering a transcription start site and an Nco I cleavage site at residue 282 using site-directed mutagenesis. This construct, in which the cysteine residues in the basic zipper region have been changed to serine, was subcloned into the pET15b expression vector using the Nco I/Bam HI sites. An oligonucleotide encoding a histidine-tag extension (MHHHHHHSSG) and compatible Nco I ends was ligated to the bZIP/SER vector at the Nco I site to aid in purification. *E.coli* BL21 (DE3) cells were transformed with the bZIP/SER plasmid as described above, and cells were induced with 0.8 mM IPTG. Cells were harvested by centrifugation and resuspended in 50 mM potassium phosphate buffer containing 300 mM KCl, 5% glycerol and 0.1% Triton X-100. Extracts were prepared as described above and the supernatants were heat-treated to 80°C for 10 min. The peptide was purified over a Ni-NTA resin (Qiagen, dialyzed against 25 mM Tris/HCl buffer, pH 8.0 containing 1 mM EDTA, and further purified over a FPLC Q Sepharose anion exchange column (Pharmacia). Generation of histidine-fusion Tax and CBP proteins was as previously described (Lundblad *et al*, 1995, Giam *et al*, 1986).

Phosphorylation: CREMα was phosphorylated using the purified catalytic subunit (C-subunit) of PKA (a gift from Dr. R. A. Maurer). The phosphorylation reaction was carried out in 50 mM (3-[N-Morpholino]propane-sulfonic acid) (MOPS) buffer, pH 6.8, containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, and 1 mM ATP at 30° C for 30 min. To measure the incorporation of phosphate, trace amounts of [γ-^{32}P]ATP were added to the reaction. The extent of phosphorylation was determined by trichloroacetic acid (TCA)

precipitable counts. For binding studies, excess ATP was removed by extensive dialysis against binding buffer.

Binding assays: Fluorescence polarization measurements were performed as described previously (Lundblad *et al*, 1995, Lundblad *et al*, 1996). Briefly, a 5'-fluorescein-labeled 28-base oligonucleotide representing the sense strand of the promoter proximal HTLV-1 Tax response element (5'F-TCCTCAGGCGTTGACGACAACCCCTCAC-3') and a 23-base 5'-fluoresceinated oligonucleotide representing the somatostatin CRE (5'F-CCTTGGCTGACGTCAGAGA GAGC-3) were annealed to antisense oligonucleotides. An N-terminal fragment of CBP (a.a. 1-682) was titrated in 1 ml of reaction buffer (25 mM Tris pH 7.6, 50 mM NaCl, 1 mM DTT, 0.1% Triton X-100, 5% glycerol, 6 μg BSA, 10 μg poly d(I-C), 5 mM $MgCl_2$) in borosilicate glass tubes containing 5 nM fluoresceinated HTLV-1 or somatostatin CRE saturated with either 30 nM CREMα (phosphorylated or non-phosphorylated), or 50 nM bZIP peptide, and in selected experiments, 1 μM Tax. The fluorescence anisotropy of each titration point was determined at room temperature using a PanVera Beacon Fluorescence Polarization System (PanVera Corp., Madison, WI). Four measurements of each binding condition were averaged for each determination. The apparent Kd for CBP binding was determined from a plot of the anisotropy versus CBP concentration. Binding isotherms were fitted to a simple binding model for the interaction of CBP with CREMα or bZIP peptide, including a linear non-specific binding component, by non-linear regression. Protein concentrations were determined by measurement of extinction coefficient and amino acid analysis.

RESULTS

CREMα activates the cellular CRE in the presence of Tax and PKA

CREMα, which lacks the glutamine-rich domains present in the activators CREB and CREMτ (Fig. 1), generally represses CRE-mediated transcription (Foulkes *et al*, 1991, Loriaux *et al*, 1994). Our model for Tax activation of CRE-containing genes (Kwok *et al*, 1996) suggested that CREMα should be converted into a PKA dependent activator by the addition of Tax. We tested this possibility by measuring expression of a somatostatin-CAT reporter gene in F9 teratocarcinoma cells in the presence or absence of PKA and Tax. F9 cells were chosen for these studies because they lack functional levels of CREB and PKA (Masson *et al*, 1992). As shown in Fig. 2, Tax activated expression of the reporter in a dose-dependent manner in the presence of CREMα and PKA (lanes 6-10) by over 40-fold. Importantly, this activation was eliminated in the absence of PKA (lanes 1-5) or in the absence of exogenous CREMα (data not shown). These results demonstrate that CREMα becomes an activator of the cellular CRE in the context of the viral transactivator Tax, and that this activation is PKA-dependent.

Activation of the HTLV-1 promoter through CREMα and Tax is PKA-independent.

Our model additionally predicted that CREMα should be able to mediate the Tax-activation of the HTLV-1 LTR and that this activation should not require PKA. To test this possibility, F9 cells were co-transfected with Tax and increasing amounts of CREMα in the absence of PKA. The results in Fig. 3 indicate that, in the presence of Tax, CREMα activates the HTLV-1 LTR reporter by 11-fold over basal levels (lanes 1-6). Control transfections indicate that this activation does not occur in the presence of exogenous CREMα alone (lanes 7-11). These results show that the glutamine-rich regions (lacking in CREMα) are not essential for Tax-activation of either the viral or cellular CREs. In

addition, these data confirm the differential requirement for phosphorylation in Tax-mediated activation of cellular and viral promoters.

Activation of gene expression by PKA-phosphorylated CREB has been proposed to involve the transcriptional co-activator CBP (Chrivia *et al*, 1993, Kwok *et al*, 1994, Arias *et al*, 1994). Moreover, we have proposed that CBP may participate in the PKA-independent activation of the HTLV-1 LTR mediated by Tax and CREB (Kwok *et al*, 1996). The evidence that somatostatin and HTLV-1 CRE activation by the repressor CREMα is differentially dependent on phosphorylation suggested that CBP might be involved in these processes as well.

Tax does not alter CBP binding to phosphorylated CREMα in the context of the somatostatin CRE.

The conservation of the KID regions of CREB and CREMα suggested that both factors might interact with CBP in a PKA-dependent manner. The binding of CBP to CREMα associated with a somatostatin CRE oligonucleotide was shown by fluorescence anisotropy analysis (Fig 4A). CBP binds to phosphorylated CREMα with an apparent Kd of 500 nM. In the presence of Tax, the affinity of this interaction is only minimally higher (Kd= 430 nM). As expected, the interaction of CBP with CREMα depends on phosphorylation as there is no binding of CBP to non-phosphorylated CREMα in the presence or absence of Tax (Fig. 4B). These data indicate that the phosphorylation-dependent activation of the somatostatin CRE-reporter by Tax in the presence of CREMα is probably not due to a change in the affinity of the co-activator CBP, but rather to the recruitment of Tax to the promoter via CBP.

Tax increases the affinity of CBP for CREMα and renders this interaction phosphorylation-independent in the context of the HTLV-1 CRE.

Our model suggests that CREMα and CBP allows activation of the HTLV-1 LTR by Tax in a PKA-independent manner. We characterized the assembly of this complex by fluorescence polarization binding assays. In the presence of Tax, CBP has a higher affinity for phosphorylated CREMα bound to the HTLV-1 CRE (Fig. 5A; Kd=130 nM) than the somatostatin CRE (Kd=430 nM). In addition, Tax facilitates a high affinity interaction between CBP and the non-phosphorylated form of CREMα (Fig. 5B, closed triangles, Kd=140 nM). Recruitment of CBP to the HTLV-1 CRE:non-phosphorylated CREMα complex completely depends on Tax, as there is no specific binding of CBP in the absence of Tax (Fig. 5B, open triangles). These data suggest that the PKA-independent activation seen in the context of the HTLV-1 LTR reporter may be due, in part, to recruitment of CBP. The phosphorylation-independent nature of this interaction suggests that it might involve a region of CREMα distinct from the KID.

Tax activates CREM Δ(C-G) in the context of the viral, but not the cellular CRE.

To elucidate the mechanism underlying this differential dependence on phosphorylation, we utilized a CREM isoform, CREM Δ(C-G), which contains neither the KID nor the glutamine-rich domains but has a bZIP domain that is nearly identical to that of CREB (Walker *et al*, 1994). This factor, like CREMα, is normally a transcriptional repressor. Adya *et al* have proposed that Tax interacts directly with a region of CREB shared by the CREM proteins that is amino-terminal to the basic region (Adya *et al*, 1994). Because this region is conserved in CREM Δ(C-G), we predicted that CBP should be recruited to the viral promoter through a direct interaction between Tax and the conserved bZIP domain of this isoform. Conversely, CREM Δ(C-G) would bind to the somatostatin CRE but would be unable to recruit CBP, and therefore Tax, to the cellular promoter. As a result, even though it lacks the glutamine-rich domains and KID, we predicted that CREM

Δ(C-G) would activate the viral CRE, but not the cellular CRE. We utilized an expression vector encoding CREM Δ(C-G) in functional assays to test this prediction. F9 cells were co-transfected with CREM Δ(C-G), Tax, and either the somatostatin or the HTLV-1 LTR reporter. As seen in Fig. 6A, CREM Δ(C-G) activates the HTLV-1 reporter by 25-fold in the presence of Tax (left panel, lanes 1-6). CREM Δ(C-G) is unable to activate expression of the somatostatin CRE, however, regardless of Tax (right panel, lanes 1-6). These data support a mechanism of activation in which CBP is recruited to the promoter through different domains of the CREB/CREM factors, depending on the particular promoter context.

Tax facilitates high affinity binding of CBP to the CREB bZIP peptide in an enhancer dependent manner.

Our model predicts that a minimal CREB bZIP peptide should be sufficient to allow Tax to facilitate an interaction between CBP and the HTLV-1 CRE, but should not promote CBP binding to the somatostatin CRE. Indeed, as seen in fluorescence polarization binding assays (Fig. 7, closed circles), CBP binds to the HTLV-1 CRE saturated with bZIP peptide in the presence of Tax (Kd=306 nM). There was no specific interaction between CBP and bZIP peptide in the absence of Tax (data not shown). In addition, no specific binding of CBP to the somatostatin CRE saturated with bZIP peptide was detected, regardless of the presence of Tax (Fig. 7, open circles). These biochemical data support our model for differential activation of viral and cellular CRE-containing genes. Specifically, CBP is recruited to the Tax-responsive viral CRE through direct interaction between Tax and the basic domain conserved among CREB, CREMα and CREM Δ(C-G). Recruitment of CBP, and therefore Tax, to the cellular CRE depends on the phosphorylation of the KID conserved in CREB and CREMα.

DISCUSSION

The finding that CREB participates in Tax-mediated activation of the HTLV-1 and cellular CREs, in concert with the high degree of conservation among regions of CREB and CREM, suggested that Tax might also interact with some CREM isoforms. Indeed, several lines of evidence have supported this possibility (Bantignies *et al*, 1996, Suzuki *et al*, 1993). Whether the interaction of Tax and CREM would result in activation or repression of transcription of viral and cellular CREs was unclear, however. Many of the CREM isoforms, including CREMα and CREM Δ(C-G), are known to be inhibitors of transcription (Foulkes *et al*, 1991, Walker *et al*, 1994). One model suggests that Tax functions by increasing dimerization of bZIP transcription factors, thereby enhancing binding of these factors to CREs (Wagner *et al*, 1993, Baranger *et al*, 1995). If this were the primary mode of Tax-mediated gene activation, we would expect that Tax would enhance repression by CREMα, rather than cause activation. The fact that CREMα activates both viral and cellular CRE-containing genes in the presence of Tax, and that activation of the two types of promoters is differentially dependent on phosphorylation, strongly argues for an alternative mode of action for the Tax transactivator.

The CREB transcription factor has provided a good model for understanding the role of phosphorylation in gene activation because the phosphorylation of a single residue in the activation domain markedly increases CREB-mediated gene expression. We and others have shown that phosphorylation of CREB by PKA facilitates a high affinity interaction between the KID and the CREB-binding domain of the co-activator CBP (Chrivia *et al*, 1993, Kwok *et al*, 1994, Arias *et al*, 1994). The KID is highly conserved between CREB and CREMα, suggesting that CBP could interact with the repressor upon phosphorylation by PKA. Here, we show that the binding affinity of CBP for phosphorylated CREMα is equivalent to that of CREB. Although phosphorylated CREMα

interacts with the co-activator CBP *in vitro*, the repression of cellular CRE-containing genes by CREMα *in vivo* suggests that this interaction is not sufficient for gene activation. The lack of the glutamine-rich domains in CREMα may contribute to this repression by failing to recruit other activators to the CREMα:CBP complex, or by failing to provide additional interactions with components of the basal transcriptional machinery. This report shows that phosphorylated CREMα becomes an activator of the somatostatin promoter in the presence of Tax. Presumably, recruitment of the activation domain in the Tax C-terminus (Caron *et al*, 1993, Semmes *et al*, 1995, Adya *et al*, 1995) by CBP (Kwok *et al*, 1996) allows CREMα to participate in activating the cellular promoter. The idea that CBP is an essential component of this activation process is supported by our finding that the activation domain of Tax cannot induce expression from the somatostatin CRE in the presence of CREB or CREM isoforms that are incapable of interacting with the co-activator CBP, i.e. CREB-M1 (Kwok *et al*, 1996) or CREM Δ(C-G). In contrast, CREM isoforms lacking the glutamine-rich domains such as CREMα can still participate in Tax-mediated activation of cellular CREs if they are capable of interacting with CBP.

The PKA-independent activation of the HTLV-1 CRE by Tax and CREMα may provide further insights into the mechanism of Tax action. Recent data by Adya *et al* suggests that a conserved Ala-Ala-Arg motif flanking the basic region of CREB is necessary for a direct Tax:CREB interaction (Adya *et al*, 1994). This motif is conserved in CREMα, suggesting that a direct Tax:bZIP interaction may be sufficient for activation of the HTLV-1 CRE. By utilizing another repressor isoform of CREM, CREM Δ(C-G), which contains the conserved bZIP domain but not the KID, we demonstrated that the KID was not essential for the PKA-independent viral promoter activation. Binding assays suggest that this activation is due to the ability of Tax, interacting with the minimal bZIP domain, to recruit the co-activator CBP to the viral promoter. Thus, Tax may provide a bridge between the HTLV-1 promoter and the co-activator CBP through a mechanism that does not depend on phosphorylation. The different modes of Tax action emphasize that

while Tax cannot interact directly with DNA, the sequence of viral and cellular Tax-responsive elements has a strong influence on the behavior of this transactivator.

The influence of promoter sequence on the behavior of specific transcription factors is well established (Leftsin *et al*, 1994). Indeed, while CREMα is primarily believed to be a repressor of cAMP-mediated transcription, there is some evidence that the ability of CREMα to stimulate or repress gene transcription depends upon the promoter context of the CRE sequence (Goraya *et al*, 1995). How promoter context influences CREMα activity is unclear, however. In this paper, we provide evidence that CREMα is able to activate transcription of viral and cellular promoters in the presence of Tax in a manner that is differentially dependent on phosphorylation. Specifically, this study demonstrates that while phosphorylated CREMα is able to interact with the co-activator CBP in a manner similar to CREB, this interaction does not result in activation of a cellular CRE. However, additional recruitment of the viral transactivator Tax through CBP results in a complex which promotes transcription. It remains unclear, however, whether CBP is essential for activation of the HTLV-1 CRE, or whether the activation domain of Tax functions through other co-activators or components of the basal transcription machinery (Clemens *et al*, 1996).

Figure 1.

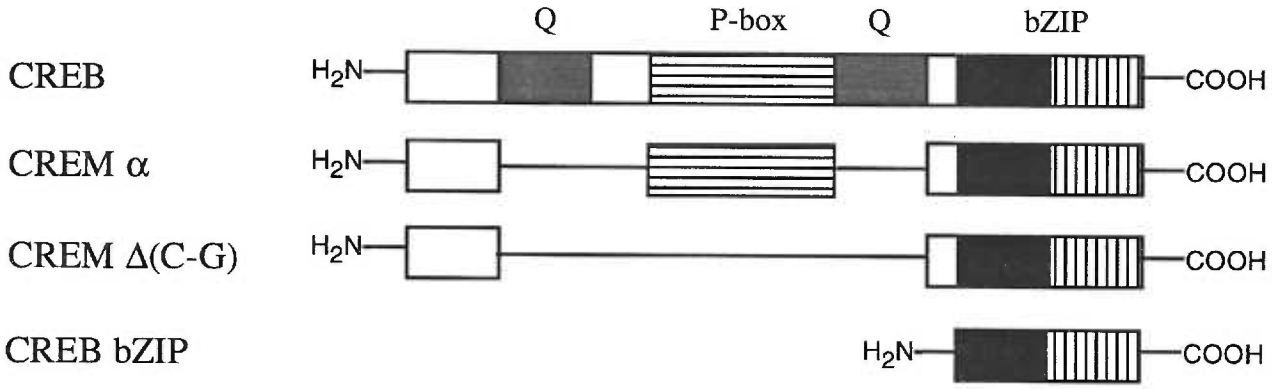


Fig. 1. Schematic representation of modular domains of CREB and CREM proteins. A diagrammatic depiction of regions of homology between CREB and CREM isoforms used in this study. The glutamine-rich domains are shaded gray; kinase inducible domain marked by horizontal stripes; basic region by solid black; and leucine zipper by vertical stripes.

Figure 2.

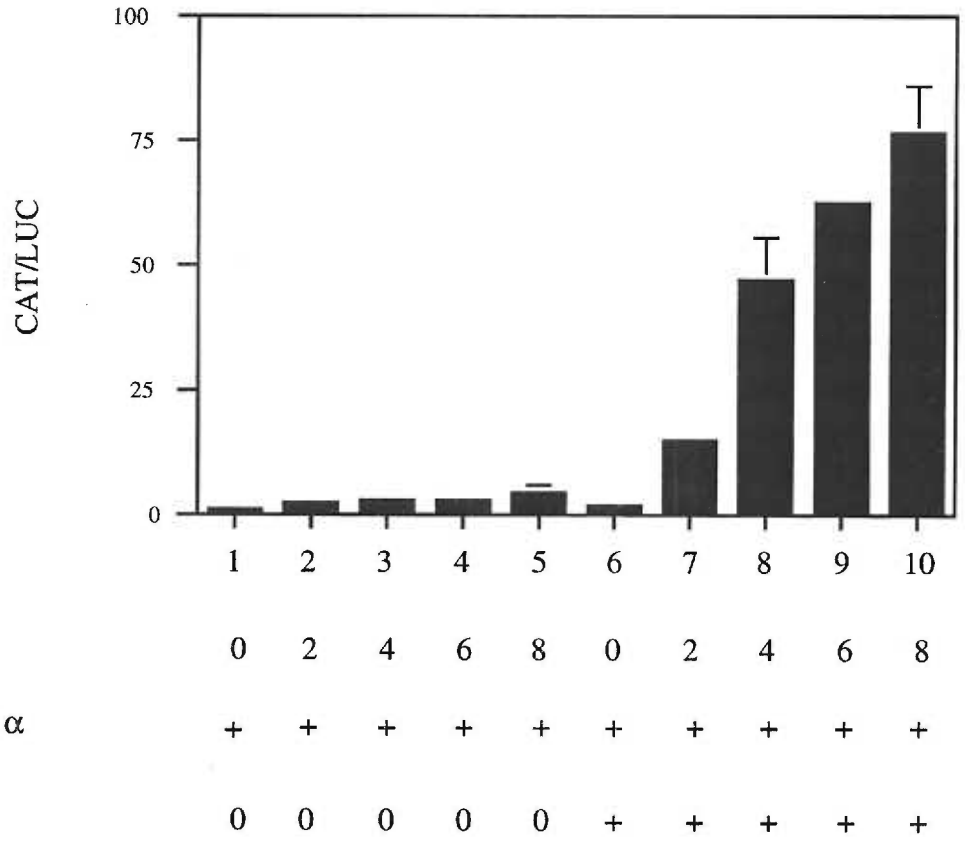
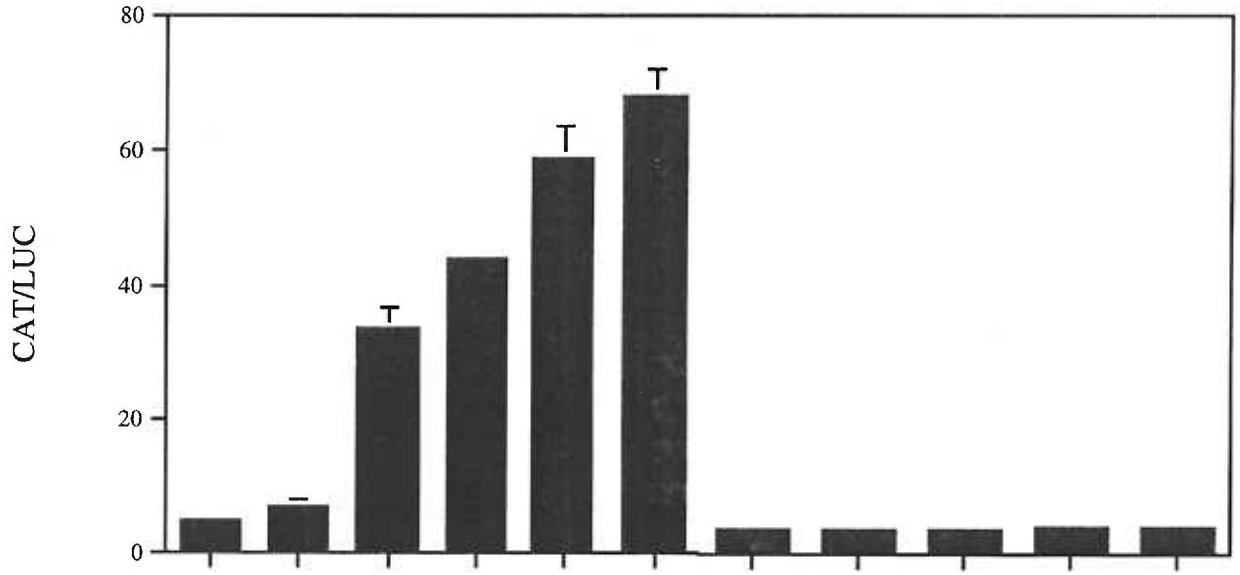

100
75
50
25
0
CAT/LUC
lane
1 2 3 4 5 6 7 8 9 10
Tax
0 2 4 6 8 0 2 4 6 8
CREM α
+ + + + + + + + + +
PKA
0 0 0 0 0 + + + + +

Fig. 2. Activation of the somatostatin promoter by CREMα. F9 teratocarcinoma cells were transfected with 5 μg of p(-71) SRIF-CAT, 2 μg RSV-luciferase, 4 μg RSV-CREMα, 0-8 μg RSV-Tax, and 4 μg RSV-PKA, as indicated. Results are expressed as CAT activity normalized for luciferase activity. Values represent the mean +/- standard deviation, n=3.

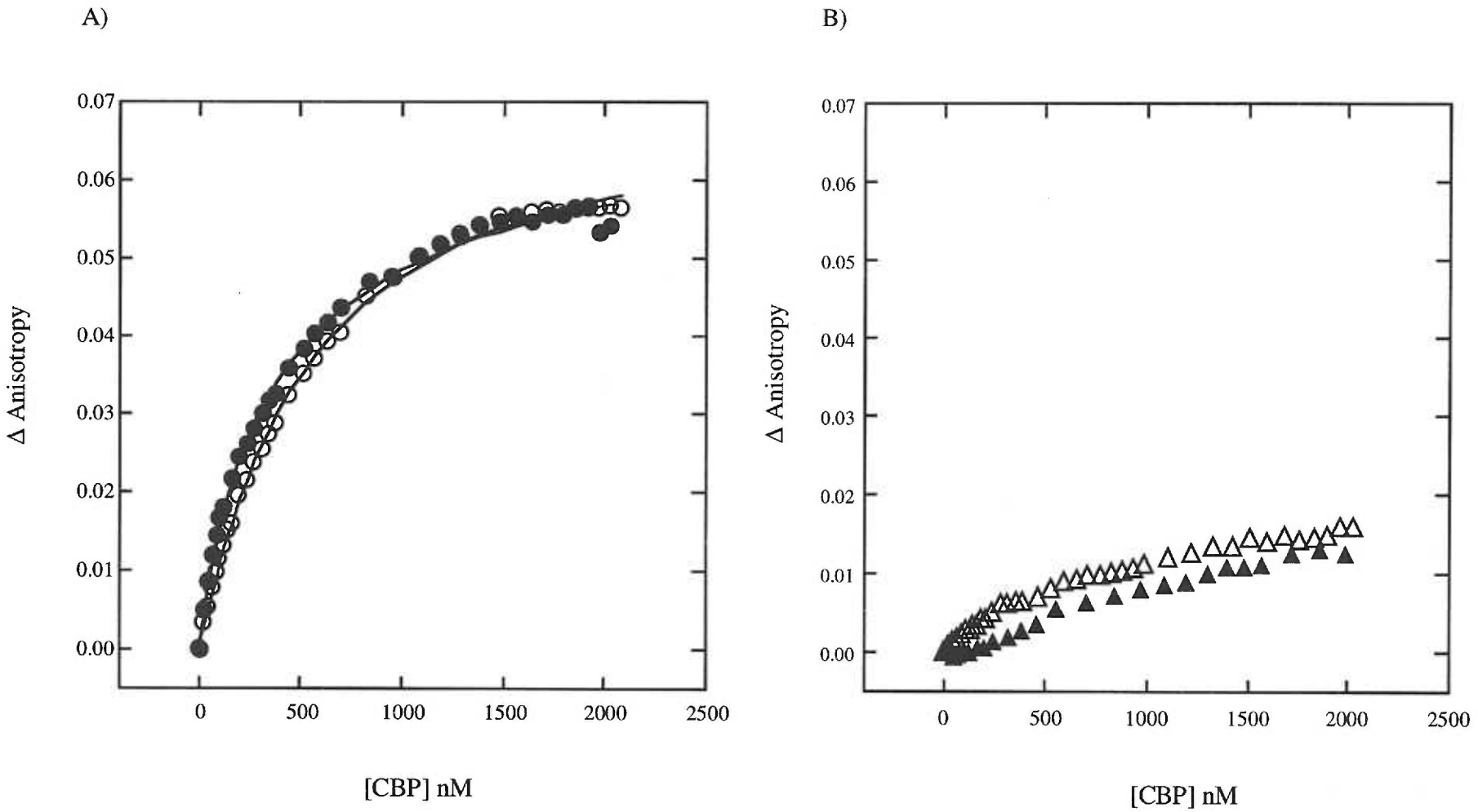
Figure 3.



lane	1	2	3	4	5	6	7	8	9	10	11
Tax	0	+	+	+	+	+	0	0	0	0	0
CREM α	0	0	1	2	4	8	0	1	2	4	8

Fig. 3. Activation of the HTLV-1 promoter by CREMα. F9 teratocarcinoma cells were transfected with 2 μg pU3R-CAT, 2 μg RSV-luciferase, 0-8 μg RSV-CREMα, and 1 μg RSV-Tax, as indicated. Results are expressed as CAT activity normalized for luciferase activity. Values represent the mean +/- standard deviation, n=3.

Figure 4.


A)
B)
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0.04
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Δ Anisotropy
0
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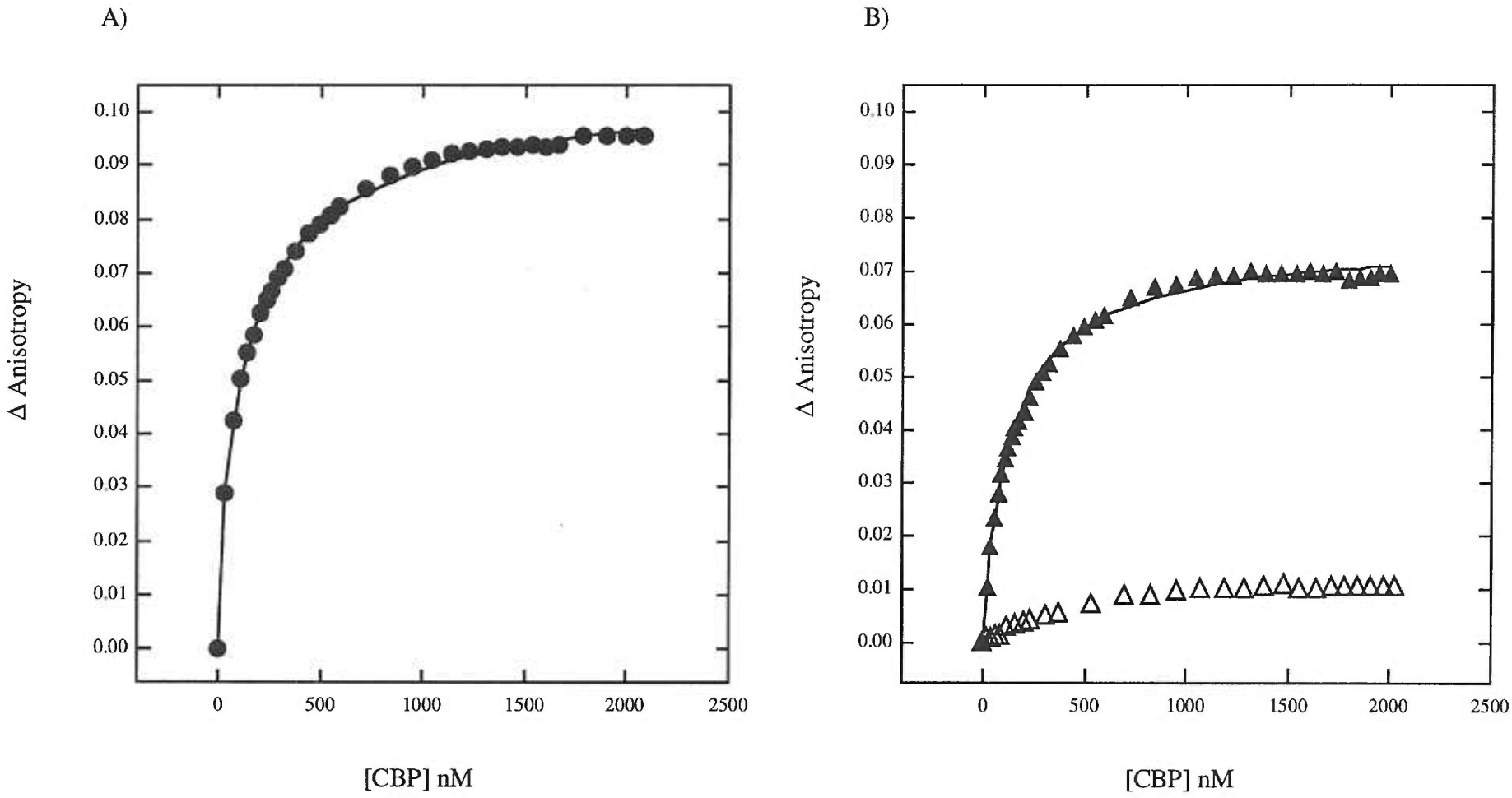
1500
2000
2500
[CBP] nM

Fig. 4. Binding of CBP to CREMα on the somatostatin CRE. Binding was monitored by fluorescence anisotropy assays. A fluorescein-labeled oligonucleotide containing the somatostatin CRE was saturated with 30 nM phosphorylated (left panel) or non-phosphorylated CREMα (right panel). CBP (a.a. 1-682) was titrated in the presence (filled symbols) or absence (open symbols) of 1 μM Tax.

Figure 5.


A)
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Δ Anisotropy
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500
1000
1500
2000
2500
[CBP] nM

Fig. 5. Binding of CBP to CREMα on the HTLV-1 CRE. Binding was monitored by fluorescence anisotropy assays. A fluorescein-labeled oligonucleotide containing the promoter proximal 21-base-pair Tax-responsive-element (TRE) from the HTLV-1 LTR was saturated with 30 nM phosphorylated (left panel) or non-phosphorylated (right panel) CREMα. CBP was titrated in the presence (filled symbols) or absence (open symbols) of 1 μM Tax.

Figure 6.

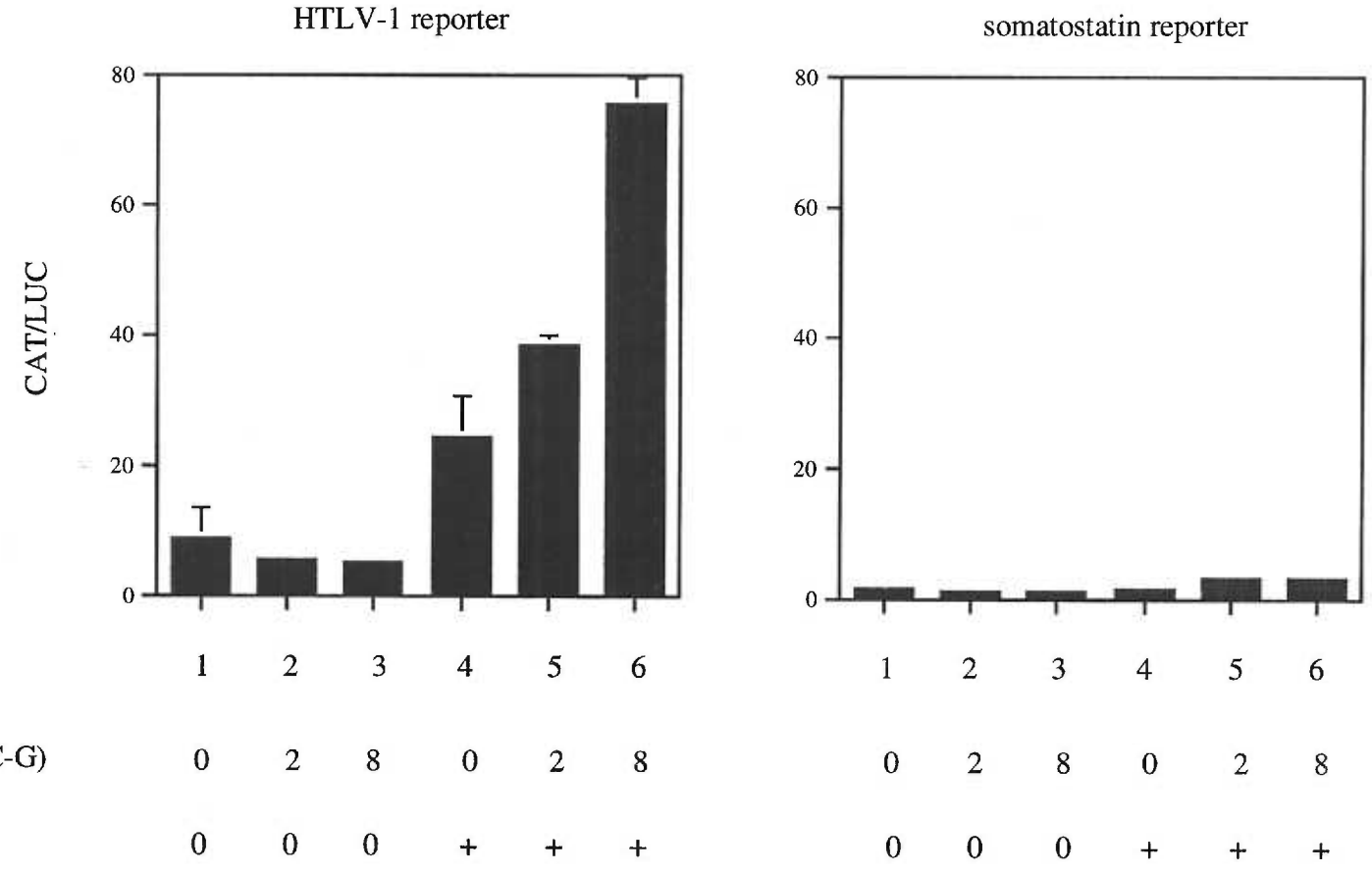
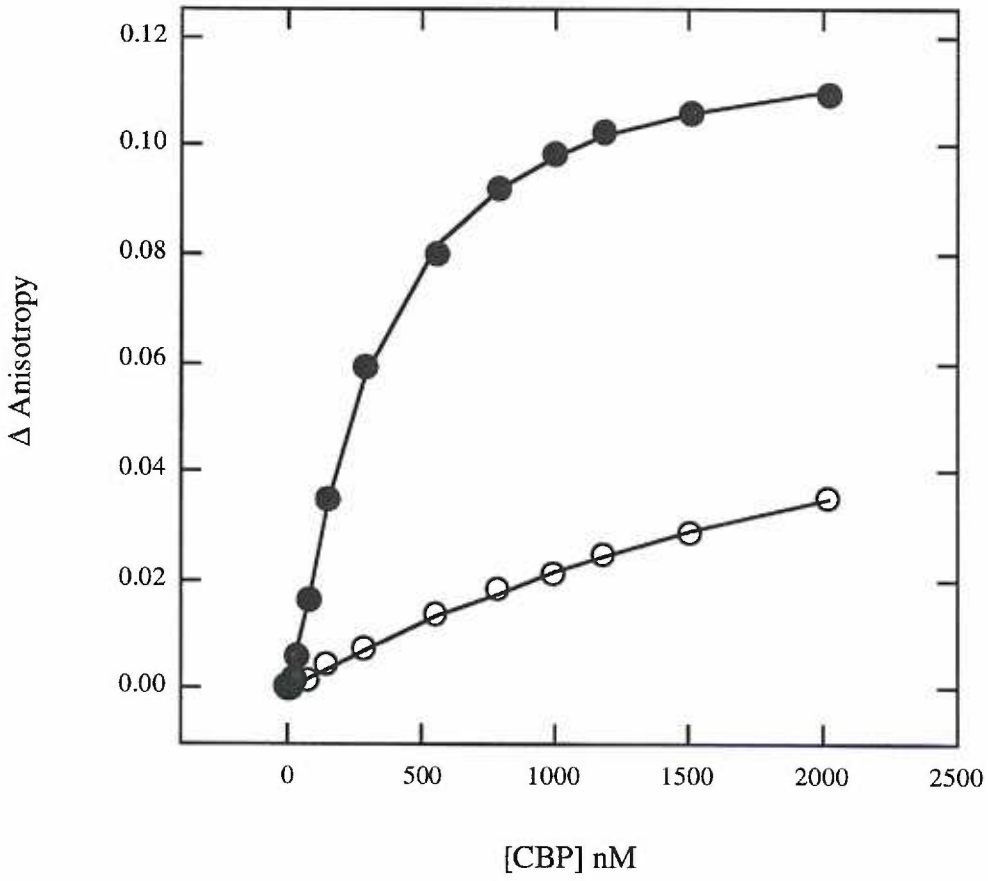

HTLV-1 reporter
somatostatin reporter
CAT/LUC
80
60
40
20
0
lane
1 2 3 4 5 6
CREM Δ(C-G)
0 2 8 0 2 8
Tax
0 0 0 + + +

Fig. 6. Activation of the HTLV-1 and somatostatin promoters by CREM Δ(C-G). F9 teratocarcinoma cells were transfected with 2 μg pU3R-CAT (left panel) or 5 μg p(-71) SRIF-CAT (right panel), 2 μg RSV-luciferase, 0-8 μg CMV-CREM Δ(C-G), and 1 μg RSV-Tax, as indicated. Results are expressed as CAT activity normalized for luciferase activity. Values represent the mean +/- standard deviation, n=3.

Figure 7.


0.12
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Δ Anisotropy
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[CBP] nM

Fig. 7. Binding of CBP to CREB bZIP peptide on the somatostatin and HTLV-1 CREs. Binding was monitored by fluorescence anisotropy assays. A fluorescein labeled HTLV-1 TRE (filled symbols) or somatostatin CRE (open symbols) was saturated with 50 nM CREB bZIP peptide. CBP was titrated in the presence of 1 μM Tax.

CHAPTER III

Discussion, Conclusions, and Future Directions

DISCUSSION

The characterization of Tax-mediated activation of viral and cellular CREs began with functional analysis using transient transfection assays. These studies were done to determine whether a) Tax activates a cellular CRE, b) and if so, is this activation is PKA dependent. In this thesis, the somatostatin CRE was used as the primary model for cellular CREs. This is a reasonable approach in light of oligonucleotide selection experiments demonstrating that CREB selects the consensus somatostatin CRE (Paca-Uccaralertkun *et al*, 1994). These studies were done in the context of undifferentiated F9 cells, a cell line well characterized with respect to cAMP-responsive genes (Kwok *et al*, 1994). These studies demonstrated that Tax-mediated activation of the cellular CRE through CREB was indeed PKA dependent. Tax transactivation of a cellular CRE was not entirely unexpected given that CREs mediate Tax dependent transactivation of cellular genes such as c-fos (Armstrong *et al*, 1993) and Krox-20 and Krox-24 genes (Alexandre *et al*, 1991).

The high degree of conservation among regions of CREB and CREM, prompted a similar characterization of this repressor in the context of Tax, and both the viral and cellular promoters. While Tax had been shown to recruit CREM to the HTLV-I TREs (Bantignies *et al*, 1996, and Suzuki *et al*, 1993), whether the interaction of Tax and CREM would result in activation or repression of transcription of viral and cellular CREs remained unclear. Many of the CREM isoforms, including CREMα and CREM Δ(C-G), are known to be inhibitors of transcription (Foulkes *et al*, 1991, and Walker *et al*, 1994). The model put forth by Green *et al* (Wagner *et al*, 1993, Baranger *et al*, 1995) suggests that Tax functions by increasing dimerization of bZIP transcription factors, thereby enhancing binding of these factors to CREs. If this were the primary mode of Tax-mediated gene activation, Tax would have been expected to enhance repression by CREMα, rather than cause activation. The fact that CREMα activates both viral and cellular CRE-containing

genes in the presence of Tax, and that activation of the two types of promoters is differentially dependent on phosphorylation, argued for an alternative mode of action for the Tax transactivator.

CREB and CREM are highly homologous, particularly in their DNA-binding domains and the kinase inducible domain (KID), a region that interacts with the co-activator CBP in a phosphorylation-dependent manner. CREMα lacks the glutamine-rich domains found in CREB that are essential for transcriptional activation. The kinase-inducible domain (KID) of CREMα can be activated in *trans* by the glutamine-rich portion of CREB (Brindle *et al*, 1994), suggesting that CREMα at least has the potential to participate in transcriptional activation. Additionally, previous studies have demonstrated that CREB:CREMα heterodimers are transcriptionally active in a phosphorylation-dependent manner (Loriaux *et al*, 1994).

The data in this thesis shows that the conserved KID interacts with CBP *in vitro* upon phosphorylation by PKA. While it has been demonstrated that binding to CBP is insufficient for transactivation of cellular CREs (Sun *et al*, 1995), recruitment of the additional activation domain in Tax via the CBP:Tax complex appears to be sufficient to activate the cellular CRE. Indeed, although phosphorylated CREMα interacts with the co-activator CBP *in vitro*, the repression of cellular CRE-containing genes by CREMα *in vivo* suggests that this interaction is not sufficient for gene activation. The lack of the glutamine-rich domains in CREMα may contribute to this repression by failing to recruit other activators to the CREMα:CBP complex, or by failing to provide additional interactions with components of the basal transcriptional machinery. Indeed, one report has indicated that the glutamine-rich domain of CREB interacts directly with Drosophila TAF110, a component of the TFIID basal transcriptional complex (Ferrari *et al*, 1994).

CREM Δ(C-G) lacks both the KID and the glutamine-rich regions. This isoform activates the HTLV-1 LTR in a phosphorylation-independent manner but does not activate the cellular CRE. These studies suggest that Tax, interacting with the basic/zipper region

of CREM, recruits CBP to the viral promoter. Tax activation of the cellular CRE depends on the KID and its ability to interact with CBP in a phosphorylation-dependent manner. Presumably, recruitment of the activation domain in the Tax C-terminus (Caron *et al*, 1993, Semmes *et al*, 1995, and Adya *et al*, 1995) by CBP (Kwok *et al*, 1996) allows CREMα to participate in activating the cellular promoter. The idea that CBP is an essential component of this activation process is supported by data in this thesis demonstrating that the activation domain of Tax cannot induce expression from the somatostatin CRE in the presence of CREB or CREM isoforms that are incapable of interacting with the co-activator CBP, i.e. CREB-M1 (Kwok *et al*, 1996) or CREM Δ(C-G). In contrast, CREM isoforms lacking the glutamine-rich domains can still participate in Tax-mediated activation of cellular CREs if they are capable of interacting with CBP.

The finding that HTLV-1:CREB or CREMα complexes bind to CBP in the absence of phosphorylation was unexpected, but consistent with the observation that activation of the HTLV-1 LTR does not depend upon stimulation of the PKA pathway. The differential dependence of the viral and cellular CREs for PKA activation may function to limit the aberrant activation of cellular CRE-containing genes by Tax. Thus, Tax would be expected to stimulate the viral LTR under basal conditions and only activate cellular genes under conditions that lead to CREB phosphorylation. In this sense, attenuation of the activation of the HTLV-I CRE would appear to be under the control of Tax rather than dephosphorylation, as is the case with cAMP mediated activation.

One mechanism for selective model of Tax activation (Adya *et al*, 1994) suggests that Tax may modulate the binding of CREB to the HTLV-1 CRE by interacting with the G-C sequences and an Ala-Ala-Arg sequence adjacent to the CREB DNA binding domain. While the role of the G-C rich regions flanking the TRE is not completely understood, it is likely that Tax facilitates efficient binding of CREB to a CRE structurally altered by these sequences. In support of this model, phasing analysis (Darnell *et al*, 1990) indicate that CREB induces modest but reproducible directed DNA bending of the 21-base pair repeat

TRE and a cellular CRE flanked by the G-C rich regions, but not in the somatostatin CRE. Thus, a conformational change in the 21-bp repeat and the hybrid CRE, which is dependent on the flanking G-C rich regions, could result in a low affinity CREB binding site. While Tax does not appear to alter the CREB-induced DNA bending angle, it may alter the CREB conformation to permit high affinity binding of CREB to the 21-bp repeat.

The conserved Ala-Ala-Arg amino acid sequence in CREB is largely conserved in CREMα and CREMΔ(C-G), which might explain why Tax also increases CREMα binding, and is able to activate the HTLV-I LTR through these proteins. This thesis does not address whether Tax increases the affinity of related CRE-binding proteins, such as ATF-1 and ATF-2, as well. These proteins lack the Ala-Ala-Arg sequence so their binding might be expected not to be influenced by Tax. Indeed, the existing data on the specificity of Tax-enhanced binding to HTLV-I TREs remains unresolved. For example, while Giam *et al* demonstrated that the Ala-Ala-Arg sequence, absent in ATF-1 and ATF-2, was necessary for this mode of Tax action, others have used similar methods to show enhanced binding of these factors to HTLV-I TREs (Nyborg *et al*, Greene *et al*)
Additional recruitment of the viral transactivator Tax to a transcriptionally inactive CREM: CBP interaction results in a transcriptionally competent complex. It remains unclear, however, whether activation domains in CBP are essential for activation of the HTLV-1 CRE, or whether the activation domain of Tax functions through other co-activators or components of the basal transcription machinery (Clemens *et al*, (1996).

While the Tax:CBP interaction occurs independent of DNA, the resulting effect on transcriptional activity is highly dependent on the sequence of DNA to which that complex is recruited. Indeed, the influence of promoter sequence on transcriptional outcome is well established (Leftsin *et al*, 1994). In this sense, Tax and CBP are mutually dependent transcriptional activators: in the context of the HTLV-I promoter, CBP is dependent on Tax interacting with the bZIP factor bound to the enhancer, while Tax is dependent on a CBP:KID interaction in the context of a cellular CRE. It is interesting to note that Tax has

consistently targeted members of the bZIP family of transcription factors that have been shown to be directly involved in cAMP signaling, namely CREB, CREM and ATF-1. In addition, each of these proteins has been shown to bind to CBP upon phosphorylation, suggesting that Tax has specifically targeted bZIP proteins which interact with the co-activator CBP.

While the data from this thesis suggests that the outcome of Tax-mediated activation is highly dependent on the enhancer sequence, it remains possible that the binding of CREB to other cellular CREs might be influenced by Tax. I have tested CREB binding to the low affinity tyrosine aminotransferase CRE, however, and have found that it is also insensitive to the addition of Tax. Thus, it is more likely that Tax increases the binding of CREB specifically to the low affinity HTLV-1 element flanked by the G-C rich sequences. Cellular CREs typically lack these sequences and thus are not affected directly by Tax.

This model conflicts with the results of Green *et al* (1994, 1996) who suggest a direct effect of the dimerization of many bZIP factors, and consequently on binding to viral and cellular elements. Recent data from Lundblad *et al* (manuscript in preparation) suggests, however, that the equilibrium constant of bZIP dimers is much lower (nM) than the uM range originally suggested (Richards *et al*, 1996). These studies additionally showed no effect of Tax and the dimerization of bZIP factors.

The search for cellular homologues of Tax based on sequence comparison alone has been unsuccessful. However, the identification of functional homologues of Tax which would influence binding of bZIP proteins to cAMP responsive elements is a worthwhile pursuit. Indeed, a modification of the yeast two-hybrid assay has allowed isolation of co-factors in drosophila whose interactions with the Ftz gene product require their binding to DNA. This strategy proved effective in an elegant study by Yu *et al* (1997), and Gulchet *et al*, (1997) demonstrating the necessity of the co-factor Ftz-F1 in recruiting the homeodomain factor Ftz to Ftz enhancer elements. The influence of Tax on binding site selectivity of basic/leucine zipper factors, and the co-dependence of Tax and CBP is

reminiscent of the function of these homeodomain proteins which interact with HOX proteins to increase DNA-binding affinity.

While genetic analysis of Tax mutants has suggested that cellular-CRE containing genes account in part for Tax-mediated transformation (Smith and Greene, 1991), it is unlikely that transformation is mediated solely through the CREB/CRE pathway. It is more probable that the HTLV-I virus, through expression of the viral regulatory protein Tax, provides some initial alteration in cell metabolism which pre-disposes the development of ATL. Subsequently, the rearrangement or altered expression of cellular oncogenes may provide the "second-hit" leading to development of ATL. Indeed, chromosomal abnormalities such as trisomy 3 and trisomy 7 have been observed in patients with acute ATL. In addition, there appears to be a frequent abnormal rearrangement in the long arm of chromosome 6 in ATL patients from northern Japan. Critical regulatory and cellular oncogenes which are located on these three chromosomes include RAF, ROS, MAS, SRC/YES-related oncogene, TCR-β, TCR-γ, PDGF, ERB, EGF receptor.

The demonstration that Tax modulates the transcriptional activity of bHLH proteins suggests another mode of deregulation of cellular gene expression by HTLV-I. It is interesting to speculate that Tax may repress a bHLH protein analogous to MyoD involved in determining T-cell phenotype, leading to cellular proliferation associated with HTLV-1 infection. Data demonstrating repression of β-polymerase through bHLH protein suggests that Tax may inhibit transcriptional activation by directly sequestering bHLH proteins. Thus, Tax repression of β-polymerase gene expression may result in a deficiency of DNA repair, followed by progression of malignant transformation.

It is interesting to consider that CBP may participate in the cell transformation pathways utilized by other oncogenic viruses. Lundblad *et al* (1995) have reported previously that the 12S E1A protein of adenovirus binds to CBP and blocks its function as a transcriptional co-activator (see also Arany *et al*, 1995). This interaction occurs through the third zinc finger motif of CBP, a region that is clearly distinct from the Tax-interacting

domain. The human papillomavirus E6 and E7 proteins also appear to be capable of stimulating cellular CRE sequences (Morosov *et al*, 1994), so it is possible that CBP and/or p300 may contribute to their actions as well. CBP has also been shown to interact with an immediate early gene product of cytomegalovirus, IE86 (Spector *et al*, 1996). The functional consequences of this interaction have yet to be elucidated. Tax and E1A are unable to bind DNA directly, and must target transcription factors which provide and anchor to the promoter. It is interesting to note that CBP has been targeted by both of these transforming viruses, suggesting a role for CBP in oncogenesis.

The recent finding that CBP mutations are associated with the Rubinstein-Taybi syndrome (Petrij *et al*, 1995), a syndrome featuring mental retardation, developmental abnormalities, and a propensity for malignancy (Miller and Rubinstein, 1995), further implicates CBP in cell transformation events.

CONCLUSIONS

The results presented in Chapters I and II have led to the following conclusions:

1. Tax-mediated activation of the HTLV-I LTR by CRE-binding proteins occurs independent of PKA.
2. Tax-mediated activation of the somatostatin promoter by CRE-binding proteins is dependent on PKA.
3. Tax enhances the binding of basic/leucine zipper transcription factors to the HTLV-I Tax-responsive element, but not to the cellular CRE.
4. Tax enhances binding of the transcriptional co-activator CBP to the bZIP:HTLV-I TRE complex in the absence of phosphorylation by PKA.
5. Tax binds to CBP *in vivo*.
6. The minimal bZIP domain conserved among CREB and CREM isoforms is sufficient to recruit the Tax:CBP complex to the HTLV-I promoter.
7. PKA phosphorylation of the kinase-inducible-domain of CREB and CREM isoforms is necessary recruitment of the Tax:CBP complex to the somatostatin promoter.

FUTURE DIRECTIONS

While the results presented in this thesis have elucidated several mechanisms by which the viral transactivating protein Tax is able to activate both viral and cellular CRE-containing genes, several questions remain. For example, it is be important to address the individual contributions provided by the distinct transactivation domains of Tax and CBP in the context of the viral and the cellular promoter. In order to address this question, it would be appropriate to specifically disrupt the Tax:CBP interaction and test the consequences of this disruption both functionally and biochemically. In order to create Tax mutants which specifically disrupt the Tax:CBP interaction, but allow Tax to retain other transactivation properties, the yeast "split-hybrid" assay will be utilized. This variation of the yeast two-hybrid assay allows for positive selection of mutations which disrupt a specific protein:protein interaction. Selection for a Tax mutant which is able to recruit CREB to viral DNA, but unable to bind to CBP, will further elucidate the relative importance of CBP in activation of viral and cellular CREs through Tax. These studies are being pursued by members of the Goodman lab.

In order to further address the mechanism by which Tax recruits CRE-binding proteins specifically to the viral G-C rich-flanked CREs, and not to cellular CREs, structural analysis by means of x-ray crystallography will be performed. These studies will clearly lend understanding as to the conformational differences between HTLV-I CRE:bZIP:Tax , and HTLV-I CRE:bZIP complexes. It is these differences which likely allow the stable interaction of Tax with the bZIP:DNA complex, and the further recruitment of the co-activator CBP. In addition, structural studies of Tax alone will lend some understanding as to the functional domains of this protein. These studies are being pursued by members of the Goodman, and Brennan labs.

REFERENCES

Adam, E., Kerkhofs, P., Mammerickx, M., Burny, A., Kettmann, R., and Willems, L. (1995) The CREB, ATF-1, and ATF-2 transcription factors from bovine leukemia virus-infected B lymphocytes activate viral expression. J. of Virol. (70) 1990-1999.

Adya, N., and Giam, C.-Z. (1995) Distinct regions in human T-cell lymphotropic virus type I Tax mediate interactions with activator protein CREB and basal transcription factors. J. of Virol. (69) 1834-1841.

Adya, N., Zhao, L.-J., Huang, W., Boros, I., and Giam, C.-Z. (1994) Expansion of CREB's DNA recognition specificity by Tax results from interaction with Ala-Ala-Arg at positions 282-284 near the conserved DNA-binding domain. Proc. Natl. Acad. Sci. USA (91) 5642-5646.

Alexandre, C., Chranay, P., and Verrier, B. (1991) Transactivation of KROX-20 and KROX-24 promoters by the HLTV-I Tax protein through common regulatory elements. Oncogene (6) 1851-1857.

Alexandre, C., and Verrier, B. (1991) Four regulatory elements in the human c-fos promoter mediate transactivation by HTLV-I Tax protein. Oncogene (6) 543-551.

Altman, R., Harrich, D., Garcia, J.A., and Gaynor, R.B. (1988) Human T-cell leukemia virus types I and II exhibit different Dnase I protection patterns. J. of Virol. (62) 1339-1346.

Arany, Z., Sellcro, W.R., Livingston, D.M., and Eckner, R. (1995) E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. Nature (374) 81-84.

Arany, Z., Newsome, D., Oldread, E., Livingston, D.M., and Eckner, R. (1995) A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. Nature (374) 81-84.

Arias, J., Alberts, A.S., Brindle, P., Claret, F.X., Smeal, T., Karin, M., Feramisco, J., Montminy, M (1994) Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. Nature (370) 226-229.

Arima, N., Molitor, J.A., Smith, M.R., Kim, J.H., Daitoku, Y., and Greene, W.C. (1991) Human T-cell leukemia virus type I Tax induces expression of the Rel-related family of kB enhancer-binding proteins: evidence for a pretranslational component of regulation. J. of Virol. (65) 6892.

Armstrong, A.P., Franklin, A.A., Uittenbogaard, M.N., Giebler, H.A., and Nyborg, J.K. (1993) Pleiotropic effect of the human T-cell leukemia virus Tax protein on the DNA binding activity of eukaryotic transcription factors. Proc. Natl. Acad. Sci. USA (90) 7303-7307.

Avantaggiati, M.L., Carbone, M., Graessmann, A., Nakatani, Y., Howard, B., and Levine, A.S. (1996) The SV40 large T antigen and adenovirus E1a oncoproteins interact with distinct isoforms of the transcriptional co-activator, p300. EMBO Journal (15) 2236-2248.

Ballard, D.W., Bohnlein, E., Lowenthal, J.W., Wano, Y., Franza, B.R., and Greene, W.C. (1988) HTLV-I Tax induces cellular proteins that activate the κB element in the IL-2 receptor a gene. Science (241) 1652-1655.

Bannister, A.J., Oehler, T., Wilhelm, D., Angel, P., and Kouzarides, T. (1995) Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. Oncogene (11) 2509-2514.

Bannister, A.J., and Kouzarides, T. (1996) The CBP co-activator is a histone acetyltransferase. Nature (384) 641-643.

Bannister, A.J., and Kouzarides, T. (1995) CBP-induced stimulation of c-Fos activity is abrogated by E1A. EMBO Journal (14) 4758-4762.

Bantignies, F., Rousset, R., Desbois, S., and Jalinot, P. (1996) Genetic characterization of transactivation of the human T-cell leukemia virus type 1 promoter: binding of Tax to Tax-responsive element 1 is mediated by the cyclic AMP-responsive members of the CREB/ATF family of transcription factors. Mol. and Cell. Biol. (16) 2174-2182.

Baranger, A.M., Palmer, C.R., Hamm, M.K., Giebler, H.A., Brauweiler, A., Nyborg, J.K., and Schepartz, A. (1995) Mechanism of DNA-binding enhancement by the human T-cell leukaemia virus transactivator Tax. Nature (376) 606-608.

Bhattacharya, S., Eckner, R., Grossman, S., Oldread, E., Arany, Z., D'Andrea, A., and Livingston, D.M. (1996) Cooperation of Stat2 and p300/CBP in signaling induced by interferon-α. Nature (383) 344-347.

Bisotto, S., Minorgan, S., and Rehfuss, R.P. (1996) Identification and characterization of a novel transcriptional activation domain in the CREB-binding protein. J. Biol. Chem. (271) 17746-17750.

Bodor, J., Walker, W., Flemington, E., Spetz, A.-L., and Habener, J.F. (1995) Modulation of Tax and PKA-mediated expression of HTLV-1 promoter via cAMP response element binding and modulator proteins CREB and CREM. FEBS Letters (377) 413-418.

Bodor, J., Spetz, A.-L., Strominger, J.L., and Habener, J.F. (1996) cAMP inducibility of transcriptional repressor ICER in developing and mature human T lymphocytes. Proc. Natl. Acad. Sci. USA (93) 3536-3541.

Borrow, J., Stanton, V.P. Jr., Andresen, J.M., Becher, R., Behm, F.G., Chaganti, R.S.K., Civin, C.I., Disteche, C., Dube, I., Frischauf, A.M., Horsman, D., Mitelman, F., Volinia, S., Watmore, A.E., and Housman, D.E. (1996) The translocation t(8;16) (p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nature Genetics (14) 33-41.

Bosselut, R., Duvall, J.F., Gegonne, A., Bailly, M., Hemar, A., Brady, J., and Ghysdael, J. (1990) The product of the c-ets-1 proto-oncogene and the related Ets2 protein act as transcriptional activators of the long terminal repeat of human T cell leukemia virus HTLV-I. EMBO Journal (9) 3137-3144.

Bosselut, R., Lim, F., Romond, P.-C., Frampton, J., Brady, J., and Ghysdael, J. (1992) Myb proteins bind to multiple sites in the human T cell lymphotropic virus type I long terminal repeat and transactivates LTR-mediated expression. Virology (186) 764-769.

Brady, J., Jeang, K.-T., Duvall, J., and Khoury, G. (1987) Identification of p40x-responsive regulatory sequences within the human T-cell leukemia virus type I long terminal repeat. J. of Virol. (61) 2175-2181.

Brauweiler, A., Garl, P., Franklin, A.A., Geibler, H.A., and Nyborg, J.K. (1995) A molecular mechanism for human T-cell leukemia virus latency and Tax transactivation. J. Biol. Chem. (270) 12814-12822.

Brindle, P., Nakajima, T., and Montminy, M. (1995) Multiple protein kinase A-regulated events are required for transcriptional induction by cAMP. Proc. Natl. Acad. Sci. USA (92) 10521-10525.

Brown, D.A., Xu, X., and Nerenberg, M. (1996) Genomic footprinting of HTLV type 1 and HIV type 1 in human T cell lines. AIDS Research and Human Retroviruses (12) 829-832.

Cann, A.J., Rosenblatt, J.D., Wachsman, W., Shaw, N.P., and Chen, I.S.Y. (1985) Identification of the gene responsible for human T-cell leukemia virus transcriptional regulation. Nature (318) 571-574.

Caron, C., Rousset, R., Beraud, C., Moncollin, V., Egly, J.-M., and Jalinot, P. (1993) Functional and biochemical interaction of the HTLV-1 Tax1 transactivator with TBP. EMBO Journal (12) 4269-4278.

Chakravarti D., LaMorte V.J., Nelson M.C., Nakajima T., Schulman I.G., Juguilon H., Montminy, M., and Evans R.M. (1996) Role of CBP/P300 in nuclear receptor signalling Nature 383, 99-103.

Chen, I.S.Y., Slamon, D.J., Rosenblatt, J.D., Shah, N.P., Quan, S.G., and Wachsman, W. (1985) The x gene is essential for HTLV replication. Science (229) 54058.

Chrivia, J.C., Kwok, R.P.S., Lamb, N., Hagiwara, M., Montminy, M.R., and Goodman, R.H. (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature (365) 855-859.

Clapham, R., Nagy, K., Cheingsong-Popov, R., Exley, M., and Weiss, R.A. (1983) productive infection and cell-free transmission of human T-cell leukemia virus in a nonlymphoid cell line. Science (222) 1125-1127.

Clemens, K.E., Piras, G., Radonovich, M.F., Choi, K.S., Duvall, J.F., DeJong, J., Roeder, R., and Brady, J.N. (1996) Interaction of the human T-cell lymphotropic virus type 1 Tax transactivator with transcription factor IIA. Mol and Cell. Biol. (16) 4656-4664.

Comb, M., Birnberg, N.C., Seasholtz, A., Herbert, E., and Goodman, H.M. (1986) A cyclic-AMP-and phorbol ester-inducible DNA element. Nature (323) 353.

Cross, S.L., Feinberg, M.B., Wolf, J.B., Holbrook, N.J., Wong-Staal, F., and Leonard, W.J. (1987) Regulation of the human interleukin-2 receptor a chain promoter:

activation of a nonfunctional promoter by the transactivator gene of HTLV-I. Cell (49) 47-56.

Cvekl, A., Kashanchi, F., Sax, C.M., Brady, J.N., and Piatigorski, J. (1995) Transcriptional regulation of the mouse αA-crystallin gene: activation dependent on a cyclic AMP-responsive element (DE1/CRE) and a Pax-6-binding site. Mol and Cell. Biol (15) 653-660.

Dai, P., Akimura, H., Tanaka, Y., Hou, D.X., Yasukawa, T., Kanei-Ishii, C., Takahashi, T., Ishii, S. (1995) CBP as a transcriptional coactivator of c-Myb. Genes and Development (10) 528-540.

D'Arcangelo, G., Curran, T. (1995) Smart transcription factors. Nature (376) 292-293.

Duvall, J.F., Kashanchi, F., Cvekl, A., Radonovich, M.F., Piras, G., and Brady, J.N. (1995) Transactivation of the human T-cell lymphotropic virus type 1 Tax_1-responsive 21-base-pair repeats requires holo-TFIID and TFIIA. J, of Virol. (69) 5077-5086.

Duyao, M.P., Kessler, D.J., Spicer, D.B., Bartholomew, C., Cleveland, J.L., Siekevits, M., and Sonenshein, G.E. (1992) Transactivation of the c-myc promoter by human T cell leukemia virus type 1 tax is mediated by NFκB. J. Biol. Chem. (267) 16288-16291.

Eckner, R., Yao, T.P., Oldread, E., and Livingston D.M. (1996) Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. Genes and Development (10) 2478-2490.

Fann, N., Gavalchin, J., Paul, B., Wells, K.H., Lane, M.J., and Poiesz, B.J. (1992) Infection of peripheral blood mononuclear cells and cell lines by cell-free human T-cell lymphoma/leukemia virus type I. J. Clin. Microbiol. (30) 905-910.

Felber, B.K., Paskalis, H., Kleinman-Ewing, C., Wong-Staal, F., and Pavlakis, G.N. (1985) The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. Science (229) 675-679.

Foulkes, N.S., Borrelli, E., and Sassone-Corsi, P. (1991) CREM gene: use of alternative DNA-binding domains generates multiple agonists of cAMP-induced transcription. Cell (64) 739-749.

Franklin, A.A., Kubik, M.F., Uittenbogaard, M.N., Brauweiler, A., Utaisincharoen, P., Matthews, M.-A. H., Dynan, W.S., Hoeffler, J.P., and Nyborg, J.K. (1993) Transactivation by the human T-cell leukemia virus Tax protein is mediated through enhanced binding of activating transcription factor-2 (ATF-2) ATF-2 response and cAMP element-binding protein (CREB). J. Biol. Chem. (268) 21225-21231.

Fujii, M., Sassone-Corsi, P., and Verma, I.M. (1988) c-fos promoter trans-activation by the tax_1 protein of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA (85) 8526-8530.

Fujii, M., Tsuchiya, H., and Seiki, M. (1991) HTLV-I tax has distinct but overlapping domains for transcriptional activation and for enhancer specificity. Oncogene (6) 2349-2352.

Fujii, M., Miki, T., Mori, T., Matsuda, T., Matsui, M., Nomura, N., and Seiki, M. (1991) HTLV-I Tax induces expression of various immediate early serum responsive genes. Oncogene (6) 1023-1029.

Fujii, M., Tsuchiya, H., Chuhjo, T., Akizawa, T., and Seiki, M. (1992) Interaction of HTLV-I Tax1 with $p67^{srf}$ causes the aberrant induction of cellular immediate early genes through CArG boxes. Genes and Development (6) 2066-2076.

Fujii, M., Tsuchiya, H., Chuhjo, T., Minamino, T., Miyamoto, K.-I., and Seiki, M. (1994) Serum response factor has functional roles both indirect binding to the CArG box and in the transcriptional activation function of human T-cell leukemia virus type I Tax. J of Virol. (68) 7275-7283.

Fujisawa, J., Seiki, M., Kiyokawa, T., and Yoshida, M. (1985) Functional activation of the human T-cell leukemia virus type I by transacting factor. Proc. Natl. Acad. Sci. USA (82) 2277-2281.

Fujisawa, J.-I., Seiki, M., Sato, M., and Yoshida, M. (1986) A transcriptional enhancer sequence of HTLV-I is responsible for *trans*-activation mediated by $p40^{x}$ of HTLV-I. EMBO Journal (5) 713-718.

Fujisawa, J.-I., Toita, M., Yoshimura, T., and Yoshida, M. (1991) The indirect association of human T-cell leukemia virus *tax* protein with DNA results in transcriptional activation. J. of Virol. (65) 4525-4528.

Gazzolo, L., and Duc Dodon, M. (1987) Direct activation of resting T lymphocytes by human T-lymphotropic virus type I. Nature (326) 714-717.

Gegonne, A., Bosselut, R., Bailly, R.A., and Ghysdael, J. (1993) Synergistic activation of the HTLV-I LTR ets-reponsive region by transcription factors ets1 and Sp1. EMBO Journal (12) 1169-1178.

Georgakopolous, T., and Thireos, G. (1992) Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. EMBO Journal (11) 4145-4152.

Gessain, A., Barin, F., Vernant, J.C., Gout, O., Maurs, L., and Calendar A. (1985) Antibodies to human T-lymphotropic virus type I in patients with tropical spastic paraparesis. Lancet (ii) 407-410.

Giam, C.-Z., Nerenberg, M., Khoury, G. and Jay, G. (1986) Expression of the complete human T-cell leukemia virus type I pX coding sequence as a functional protein in *Escherichia coli*. Proc. Natl. Acad. Sci. USA (83) 7192-7196.

Giam, C.-Z., and Xu, Y.-L. (1989) HTLV-I tax gene product activates transcription via pre-existing cellular factors and cAMP responsive element. J. Bio. Chem. (264) 15236-15241.

Gitlin, S.D., Dittmer, J., Reid, R.L., and Brady, J.N. (1993) The molecular biology of human T-cell leukemia viruses. In Human Retroviruses, B.R. Cullen editor, Oxford University Press, pp159-192.

Gonzalez, G.A., and Montminy, M.R. (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell (59) 675-680.

Goren, I., Semmes, O.J., Jeang, K.-T., and Moelling, K. (1995) The amino terminus of Tax is required for interaction with the cyclic AMP response element binding protein. J. of Virol. (69) 5806-5811.

Gottesfield, J.M., Johnson, D.L., and Nyborg, J.K. (1996) Transcriptional activation of RNA polymerase III-dependent genes by the human T-cell leukemia virus type 1 Tax protein. Mol. Cell. Biol. (16) 1777-1785.

Grassmann, R., Dengler, C., Muller-Fleckenstein, I., Fleckenstein, B., McGuire, K., Dokhelar, M.-C., Sodroski, J.G. and Haseltine, W.A. (1989) Transformation to continuous growth of primary human T lymphocytes by human T-cell leukemia virus type I X-region genes transduced by a *Herpesvirus saimiri* vector. Proc. Natl. Acad. Sci. USA (86) 3351-3355.

Greene, W.C., Bohnlein, E., and Ballard, D.W. (1989) HIV-1, HTLV-1 and normal T-cell growth: transcriptional strategies and surprises. Immunology Today (10) 272-278.

Greene, W.C., Leonard, W.J., Wano, Y., Svetlik, P.B., Peffer, N.J., Sodroski, J.G., Rosen, C.A., Goh, W.C., and Haseltine, W.A. (1986) Trans-activator gene of HTLV-II induces IL-2 receptor and IL-2 cellular gene expression. Science (232) 877-880.

Gulchet, A., Copeland, J.W.R., Erdelyi, M., Hlousek, D., Zavorsky, P., Ho, J., Brown, S., Percival-Smith, A., Krause, H.M., and Ephrussi, A. (1997) The nuclear receptor homologue Ftz-F1 and the homeodomain protein Ftz are mutually dependent cofactors. Nature (385) 548.

Hennekam, R.C.M., Tilanus, M., Hamel, B.C.J., Voshart-van Heeren, H., Mariman, E.C.M., van Beersum, S.E.C., van den Boogaard, M.-J.H., and Breuning, M.H. (1993) Deletion at Chromosome 16p13.3. as a cause of Rubinstein-Taybi syndrome: clinical aspects. American Journal of Human Genetics (52) 255-262.

Hinrichs, S.H., Nerenberg, M., Reynolds, R.K., Khoury, G., and Jay, G. (1987) A transgenic mouse model for human neurofibromatosis. Science (237) 1340-1343.

Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M., and Roeder, R. (1993) The p250 subunit of native TATA box-binding factor TFIID is the cell-cycle regulatory protein CCG1. Nature (362) 179-181.

Ho, D.D., Rota, T.R., and Hirsch, M.S. (1984) Infection of human endothelial cells by human T-lymphotropic virus type I. Proc. Natl. Acad. Sci. USA (81) 7588-7590.

Hoffmann, P.M., Dhibjalbut, S., Mikovits, J.A., Robbins, D.S., Wolf, A.L., Bergey, G.K., Lohrey, NC, Weislow, OS, and Ruscetti, FW. (1992) Human T-cell leukemia virus type I infection of monocytes and microglial cells in primary human cultures. Proc. Natl. Acad. Sci. USA (89 11784-11788.

Inoue, J., Seiki, M., Tanaguchi, T., Tsuru, S., and Yoshida, M. (1986) Induction of interleukin 2 receptor gene expression by p40x encoded by human T-cell leukemia virus type I. EMBO Journal (5) 2883-2888.

Inoue, J., Yoshida, M., and Seiki, M. (1987) Transcriptional (p40x) and post-transcriptional (p27x-III) regulators are required for the expression and replication of human T-cell leukemia virus type I genes. Proc. Natl. Acad. Sci. USA (84) 3652-3657.

Jacobson, S., Shida, H., McFarlin, D.E., Fauci, A.S., and Koenig, S. (1990) Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. Nature (348) 245.

Janknecht, R., Nordheim, A. (1996) MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. Biochemical and Biophysical Research Communications (228) 831-837.

Janknecht, T., and Nordheim, A. (1996) Regulation of the c-fos promoter by the ternary complex factor Sap-1a and its coactivator CBP. Oncogene (12) 1961-1969.

Jeang, K.-T., Widen, S.G., Semmes, O.J., and Wilson, S.H. (1990) HTLV-I transactivator protein, Tax, is a transrepressor of the human b-polymerase gene. Science (247), 1082.

Jeang, K.-T., Chiu, R., Santos, E., and Kim, S.-J. (1991) Induction of the HTLV-1 LTR by Jun occurs through the Tax-responsive 21-bp elements. Virology (181) 218-227.

Josephs, S.F., Wong-Staal, F., Manzari, V., Gallo, R.C., Sodroski, J.G., Trus, M.D., Perkins, D., Patarca, R., and Haseltine, W.A. (1984) Long terminal repeat structure of an American isolate of type I human T-cell leukemia virus. Virology, (139) 340.

Kalyanaraman, V.S., Sarngadharan, M.G., Robert-Guroff, M., Miyoshi, I., Blayney, D., Golde, D., and Gallo, R.C. (1982) a new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science (218) 571.

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.-C., Heyman, R.A., Rose, D.W., Glass, C.K., Rosenfeld, M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell (85) 403-414.

Kee, B.L., Arias, J., and Montminy, M.R. (1996) Adaptor-mediated recruitment of RNA polymerase II to a signal-dependent activator. J. of Biol. Chem. (271) 2373-2375.

Kimata, J.T., and Ratner, L. (1991) Temporal regulation of viral and cellular gene expression during human T-lymphotropic virus type I-mediated lymphocyte immortalization. J. of Virol. (65) 4398-4407.

Kirchbaum-Stenger, K., Poiesz, B., Keller, P., Ehrlich, G., Gavalchin, J., Davis, B.H., and Moore, J.L. (1987) Specific adsorption of HTLV-I to various target human and animal cells. Blood (70) 1301-1311.

Kirshenbaum, L.A., Schneider, M.D. (1995) The cardiac cell cycle, pocket proteins, and p300. TCM (5) 230-235.

Kronke, M., Leonard, W.J., Depper, J.M., and Greene, W.C. (1985) Deregulation of interleukin-2 receptor gene expression in HTLV-I induced adult T-cell leukemia. Science (228) 1215-1217.

Kukolj, G., and Skalka, A.M. (1995) Enhanced and coordinated processing of synapsed viral DNA ends by retroviral integrases in vitro. Genes and Development (9) 2556-2567.

Kwok, R.P.S., Lundblad, J.R., Chrivia, J.C., Richards, J.P., Bachinger, H.P., Brennan, R.G., Roberts, S.G.E., Green, M.R., and Goodman, R.H. (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature (370) 223-226.

Kwok, R.P.S., Laurance, M.E., Lundblad, J.R., Goldman, P.S., Shih, H-M, Connor, L.M., Marriott, S.J., and Goodman, R.H. (1996) Control of cAMP-regulataed enhancers by the viral transactivator Tax through CREB and the co-activator CBP. Nature (380) 642-646.

Lacoste, J., Cohen, L., and Hiscott, J. (1991) NF-κB activity in T cells stably expressing the Tax protein of human T cell lymphotropic virus type I. Virology (184) 553.

Laoide, B.M., Foulkes, N.S., Schlotter, F., and Sassone-Corsi, P. (1993) The functional versatility of CREM is determined by its modular structure. EMBO Journal (12) 1179-1191.

Larkin, J., Sinnott, J.T., Weiss, J., and Holt, D.A. (1990) Human T-cell lymphotropic virus -type I. Infect. Control, Hosp. Epoidemiol. (11) 314-318.

Laurance, M.E., Kwok, R.P.S., Huang, M.S., Richards, J.P., Lundblad, J.R., and Goodman, R.H. (1997) Differential activation of viral and cellular promoters by human T-cell lymphotropic virus-1 Tax and cAMP-responsive element modulator isoforms. J of Biol. Chem. (272) 2646-2651.

Lee, J.-S., Galvin, K.M., See, R.H., Eckner, R., Livingston, D., Moran, E., and Shi, Y. (1995) Relief of YY1 transcriptional repression by adenovirus E1A is mediated by E1A-associated protein p300. Genes and Development (9) 1188-1198.

Lee, J.-S., Zhang, X., and Shi, Y. (1996) Differential interactions of the CREB/ATF family of transcription factors with p300 and adenovirus E1A. J. of Biol. Chem. (271) 17666-17674.

Leung, K., and Nabel, G.J. (1988) HTLV-I transactivator induces interleukin-2 receptor expression through an NF-κB like factor. Nature (333) 776-778.

Lilienbaum, A., Dodon, M.D., Alexandre, C., Gazzolo, L., and Paulin, D. (1990) Effect of human T-cell leukemia virus type I Tax protein on activation of the human vimentin gene. J. of Virol. (64) 256-263.

Lilienbaum, A., and Paulin, D. (1993) Activation of the human vimentin gene by the Tax human T-cell leukemia virus I. J. Biol. Chem. (268) 2180-2188.

Lindholm, P.F., Marriott, S.J., Gitlin, S.D., Bohan, C.A., and Brady, J.N. (1990) Induction of nuclear NF-kB DNA binding activity after exposure of lymphoid cells to soluble Tax1 protein. New. Biol. (2) 1034.

Lindholm, P.F., Reid, R.L., and Brady, J.N. (1992) Extracellular Tax1 protein stimulates tumor necrosis factor-β and immunoglobulin kappa light chain expression in lymphoid cells. J. of Virol. (66) 1294.

Low K.G., Chu, H.-M., Tan, Y., Schwartz, P.M., Daniels, G.M., Melner, M.H., and Comb, M.J. (1994) Novel interactions between human T-cell leukemia virus type I Tax and activating transcription factor 3 at a cyclic AMP-responsive element. Mol. and Cell. Biol. (14) 4958-4974.

Lundblad, J.R., Kwok, R.P.S., Laurance, M.E., Harter, M.L., and Goodman, R.H. (1995) Adenoviral E1A-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. Nature (374) 85-88.

Lundblad, J.R., Laurance, M.E., and Goodman, R.H. (1996) Fluorescence polarization analysis of protein-DNA and protein-protein interactions. Mol. Endocrinology (10) 607-612.

Manns, A., and Blattner, W.A. (1991) The epidemiology of the human T-cell lymphotropic virus type I and type II: etiologic role in human disease. Transfusion (31) 67-75.

Marriott, S.J., Lindholm, P.F., Reid, R.L., and Brady, J.N. (1991) Soluble HTLV-I Tax1 protein stimulates proliferation of human peripheral blood lymphocytes. New Biol. (3) 678.

Marriott, S.J., Boros, I., Duvall, J.F., and Brady, J.N. (1989) Indirect binding of human T-cell leukemia virus type I tax1 to a responsive element in the viral long terminal repeat. Mol. and Cell. Biol. (9) 4152-4160.

Marriot, S.J., Lindholm, P.F., Gitlin, S.D., Brown, K.M., Rodonovich, M.F., Duvall, J.F., and Brady, J.N. (1990) Molecular analysis of trans-activation in the HTLV-I LTR. In *Human retroviruses* (ed. J.E. Groopman, I.S.Y Chen, M. Essex, and R.A. Weiss), pp 13-22. Wiley/Liss, New York.

Maruyama, M., Shibuya, H., Haraa, H., Hatakeyama, M., Seiki, M., Fujita, T., Inoue, J., Yoshida, M., and Tanaguchi, T. (1987) Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-I encoded p40x and T3/Ti complex triggering. Cell (48) 343-350.

Masson, N., Ellis, M., Goodbourn, S., and Lee, KA. (1992) Cyclic AMP response element-binding protein and the catalytic subunit of protein kinase A are present in F9 embryonal carcinoma cells but are unable to activate the somatostatin promoter. Mol. and Cell. Biol. (12) 1096-1106.

Meyer, T.E., and Habener, J.F. (1993) Cyclic adenosine 3', 5'-monophosphate reponse element binding protein CREB and related transcription-activating deoxyribonucleic acid binding proteins. Endocrine Reviews (14) 269.

Migone, T.-S., Lin, J.-X., Cereseto, A., Mulloy, J.C., O'Shea, J.J., Franchini, G., and Leonard, W.J. (1995) Constitutively activated Jak-STAT pathway in T-cells transformed with HTLV-1. Science (269) 79-81.

Miller, R.W., and Rubenstein, J.H. (1995) Tumors in Rubenstein-Taybi syndrome. American Journal of Medical Genetics (56) 112-115.

Missero, C., Calutti, E., Eckner, R., Chin, J., Tsai, L.H., Livingston, D.M., and Dotto, G.P. (1995) Involvement of the cell-cycle inhibitor Cip1/WAF1 and the E1A associated p300 protein in terminal differentiation. Proc. Natl. Acad. Sci. USA (92) 5451-5455.

Mitchell, P.J, and Tjian, R. (1989) Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science (245) 371-378.

Miyatake, S., Deiki, M., Malefijt, R.D., Keike, T., Fujisawa, J., Takebe, Y., Nishida, J., Shlomai, J., Yokota, T., Yoshida, M., Arai, K., and Arai, N. (1988) Activation of T cell-derived lymphokine genes in T cells and fibroblasts: effects of human T cell leukemia virus type 1 p40x protein and bovine papilloma virus encoded E2 protein. Nucleic Acids Research (16) 6547-6566.

Miyazaki, T., Liu, Z.-J., and Taniguchi, T. (1996) Selective cooperation of HTLV-1-encoded $p40^{tax-1}$ with cellular oncoproteins in the induction of hematopoietic cell proliferation. Oncogene (12) 2403-2408.

Molgaard, C.A., Eisenman, P.A., Ryden, L.A., and Golbeck, A.L. (1989) Neuro-epidemiology of human T-lymphotropic virus type-I associated tropical spastic paraparesis. Neuroepidemiology (8) 109.

Montminy, M.R., Sevarino, K.A., Wagner, J.A., Mandel, G., and Goodman, R.H. (1986) Identification of a cyclic-AMP responsive element within the rat somatostatin gene. PNAS USA (83) 6682-6686.

Muchardt, C., Seeler, J.S., Nirula, A., Gong, S., and Gaynor, R. (1992) Transcription factor AP-2 activates gene expression of HTLV-I. EMBO Journal (11) 2573-2581.

Murphy, E.L., and Blattner, W.A. (1988) HTLV-I associated leukemia: a model for chronic retroviral diseases. Ann. Neurol., (23 suppl.), S174.

Nakajima, T., Fukamizu, A., Takahashi, J., Gage, F.H., Fisher, T., Blenis, J., and Montminy, M.R. (1996) The signal-dependent coactivator CBP is a nuclear target for pp90RSK. Cell (86) 465-474.

Nerenberg, M., Hinrichs, S.H., Reynolds, R.K., Khoury G., and Jay, G. (1987) The *tat* gene of human T-lymphotropic virus type I induces mesenchymal tumors in transgenic mice. Science (237) 1324-1329.

Newbound, G.C., Andrews, J.M., O'Rourke, J.P., Brady, J.N., and Lairmore, M.D. (1996) Human T-cell lymphotropic virus type 1 Tax mediates enhanced transcription in CD4+ lymphocytes. J. of Virol. (70) 2101-2106.

Nicholas, J., and Nevins, J.R. (1991) Distinct DNA targets for *trans*-activation by HTLV1 *tax* and Adenovirus E1A. Virology (182) 156-167.

Niewiewsk, S., Daenke, S., Parker, C.E., Taylor, G., Weber, J., Nightingale, S., and Bangham, C.R.M. (1995) Naturally occurring variants of human T-cell leukemia virus type I Tax protein impair its recognition by cytotoxic T lymphocytes and the transactivation function of Tax. J. of Virol. (69) 2649-2653.

Nowak, M.A., Bangham, C.R.M. (1996) Population dynamics of immune responses to persistent viruses. Science (272) 74-79.

Nyborg, J.K., and Dynan, W.S. (1990) Interaction of cellular proteins with the human T-cell leukemia virus type 1 transcriptional control region: purification of cellular proteins that bind the 21-base pair repeat elements. J. of Biol. Chem. (265) 8230-8236.

Nyborg, J.K., Dynan, W.S., Chen, I.S.Y., and Wachsman, W. (1988) Binding of host-cell factors to DNA sequences in the long terminal repeat of human T-cell leukemia virus type I: implications for viral gene expression. Proc. Natl. Acad. Sci. USA (85) 1457-1461.

Nyborg, J.K., Matthews, M.-A. H., Yucel, J., Walls, L., Golde, W.T., Dynan, W.S., and Wachsman, W. (1990) Interaction of host cell proteins with the human T-cell leukemia virus type I transcriptional control region: II. a comprehensive map of protein-binding sites facilitates construction of a simple chimeric promoter responsive to the viral tax_2 gene product. J. Biol. Chem. (265) 8237-8242.

Oelgeschlager, M., Janknecht, R., Krieg, J., Schreek, S., and Luscher, B. (1996) Interaction of the co-activator CBP with Myb proteins: effects on Myb-specific transactivation and on the cooperativity with NF-M. EMBO Journal (15) 101-110.

Paca-Uccaralertkun, A., Zhoa, L.-J., Adya, N., Cross, J.V., Cullen, B.R., Boros, I.M., and Giam, C.-Z. (1994) In vitro selection of DNA elements highly responsive to the human T-cell lymphotropic virus type I transcriptional activator, Tax. Mol. and Cell. Biol. (14) 456-462.

Parker, D., Ferreri, K., Takajima, T., LaMorte, V.J., Evans, R., Koerber, S.C., Hoeger, C., and Montminy, M.R. (1996) Phosphorylation of CREB at Ser-133 induces complex formation with CREB-binding protein via a direct mechanism. Mol. and Cell. Biol. (16) 694-703.

Paskalis, F., Felber, B.K., and Pavlakis, G.N. (1986) Cis-acting sequences responsible for the transcriptional activation of human T-cell leukemia virus Type I constitute a conditional enhancer. Proc. Natl. Acad. Sci. USA (83) 6558-6562.

Paul, N.L., Lenardo, M.J., Novak, K.D., Sarr, T., Tang, W.-L., and Ruddle, N.H. (1990) Lymphotoxin activation by human T-cell leukemia virus infected cell lines: role for NF-κB. J. of Virol. (64) 5412.

Perini, G., Wagner, S., and Green, M.R. (1995) Recognition of bZIP proteins by the human T-cell leukaemia virus transactivator Tax. Nature (376) 602-605.

Perkins, N.D., Felzien, L.K., Betts, J.C., Leung, K., Beach, D.H., and Nabel, G.J. (1997) Regulation of NF-κB by the cyclin-dependent kinases associated with the p300 coactivator. Science (275) 523-527.

Peterson, C.L., and Herskowitz, I. (1992) Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. Cell (68) 573-583.

Petrij, F., Giles, R.H., Dauwerse, H.G., Saris, J.J., Hennekam, R.C.M., Masuno, M., Tommerup, N., van Ommen, G.-J.B., Goodman, R.H., Peters, D.J.M., and Breuning, M.H. (1995) Rubenstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature (376) 348-351.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D., and Gallo, R.C. (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA (77) 7415-7419.

Ponting, C.P., Blake, D.J., Davies, K.E., Kendrick-Jones, J., and Winder, S.J. (1996) ZZ and TAZ: new putative zinc fingers in dystrophin and other proteins. Trends. Biochem. Sci. (21) 11-13.

Poteat, H.T., Kadison, P., McGuire, K., Park, L., Park, R.E., Sodroski, J.G., and Haseltine, W.A. (1989) Response of the human T-cell leukemia virus type I long terminal repeat to cyclic AMP. J. of Virol (63) 1604-1611.

Pozzatti, R., Vogel, J., and Jay, G . (1990) The human T-lymphotropic virus I tax gene can cooperate with the ras oncogene to induce neoplastic transformation in cells. Mol. and Cell. Biol. (10) 413-417.

Pras, E., Aksentijevich, I.A., Gruberg, L., Balow, J.E., Prosen, L., Dean, M., Steinberg, A.D., Pras, M., and Kastner, D.L. (1992) Mapping of a gene causing familial Mediterranean fever to the short arm of chromosome 16. New England Journal of Medicine (326) 1509-1513.

Richards, J.P., Bachinger, H.P., Goodman, R.H., and Brennan, R.G. (1996) Analysis of the structural properties of cAMP-responsive element-binding protein (CREB) and phosphorylated CREB. J. of Biol. Chem. (271) 13716-13723.

Rosen, C.A., Park, R., Sodroski, J.G., and Haseltine, W.A. (1987) Multiple sequence elements are required for regulation of human T-cell leukemia virus gene expression. Proc. Natl. Acad. Sci. USA (84) 4919-4923.

Roth, S.Y. (1996) Something about silencing. Nature Genetics (14) 3-4.

Rousset, R., Desbois, C., Bantignies, F., and Jalinot, P. (1996) Effects on NF-κB/p105 processing of the interaction between the HTLV-1 transactivator Tax and the proteosome. Nature (381) 328-331.

Ruben, A., Poteat, H., Tan, T., Kawakami, K., Roeder, R., Haseltine, W., and Rosen, C.A. (1988) Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-I tax gene product. Science (241) 89-92.

Ruben, S., Perkins, A., and Rosen, C.A. (1989) Activation of NF-kB by the HTLV-I trans-activator protein tax requires an additional factor present in lymphoid cells. New Biol. (1) 275.

Ruddell, A. (1995) Transcription regulatory elements of the avian retroviral long terminal repeat. Virology (206) 1-7.

Saggioro, D., Forino, M., Penzo, A., Pesce, M., Oliviero, S., and Chieco-Bianchi, L. (1994) Tax-induced HTLV-1 LTR transcriptional activation is modulated by phosphorylation. Biochemical and Biophysical Research Communications (205) 666-673.

Schreck, R., and Baeuerle, P.A. (1990) NF-κB as inducible transcriptional activator of the granulocyte-macrophage colony-stimulating factor gene. Mol. and Cell. Biol. (10) 1281.

Seiki, M., Eddy, R., Shows, T.B., and Yoshida, M. (1984) Nonspecific integration of the HTLV-I provirus genome into adult T-cell leukaemia cells. Nature (309) 640-642.

Seiki, M., Hattori, S., Hirayama, Y., and Yoshida, M. (1983) Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. Proc. Natl. Acad. Sci. USA (80) 3618-3622.

Seiki, M., Inoue, J., Takada, T., and Yoshida, M . (1986) Direct evidence that p40x of human T-cell leukemia virus type I is a transacting transcriptional activator. EMBO Journal (5) 561-565.

Semmes, O.J., and Jeang, K.-T. (1992) Mutational analysis of human T-cell leukemia virus type I tax: regions necessary for function determined with 47 mutant proteins. J. of Virol. (66) 7183-7192.

Semmes, O.J., and Jeang, K.-T. (1995) Definition of a minimal activation domain in the human T-cell leukemia virus type I Tax. J. of Virol. (69) 1827-1833.

Semmes, O.J., Barrett, J.F., Dang, C.V., and Jeang, K.-T. (1996) Human T-cell leukemia virus type I Tax masks c-myc function through a cAMP-dependent pathway. J. Biol. Chem. (271) 9730-9738.

Shih, H.-M., Goldman, P.S., DeMaggio, A.J., Hollenberg, S.M., Goodman, R.H., and Hoekstra, M.F. (1996) A positive genetic selection for disrupting protein-protein interactions: Identification of CREB mutations that prevent association with the co-activator CBP. Proc. Natl. Acad. Sci. USA (93) 13896-13901.

Shimotohno, K., Takahashi, Y., Shimizu, N., Gojobori, T., Golde, D.W., Chen I.S.Y., Miwa, M., and Sugimura, T. (1985) Complete nucleotide sequence of an infectious clone of human T-cell leukemia virus type II: an open reading frame for the protease gene. Proc. Natl. Acad. Sci. USA (82) 3101.

Shimotohno, K., Takano, M., Teruuchi, T., and Miwa, M. (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.

Shnyreva, M., and Munder, T. (1996) The oncoprotein Tax of the human T-cell leukemia virus type 1 activates transcription via interaction with cellular ATF/CREB factors in *Saccharomyces cerevisiae*. J. of Virol. (70) 7478-7484.

Short, J.M., Wynshaw-Boris, A., Short, H.P., and Hanson, R.W. (1986) Characterization of the phosphoenolpyruvate carboxykinase (GTP) promoter regulatory region. J. Biol. Chem. (261) 9721.

Smith M.R., and Greene, W.C. (1990) Identification of HTLV-1 *tax trans*-activator mutants exhibiting novel transcriptional phenotypes. Genes and Development (4) 1875-1885.

Smith, M.R., and Greene, W.C. (1991) Type I human T cell leukemia virus tax protein transforms rat fibroblasts through the cyclic adenosine monophosphate response element binding protein/activating transcription factor pathway. J. Clin. Invest. (88) 1038-1042.

Smith, C.L., Onate, S.A., Tsai , M.J., and O'Malley, B.W. (1996) CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. Proc. Natl. Acad. Sci. USA (93) 8884-8.

Sodroski, J.G., Rose, C.A., and Haseltine, W.A. (1984) *Trans*-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science (225) 381-385.

Sodroski, J. (1992) The human T-cell leukemia virus (HTLV) transactivator (Tax) protein. Biochemica et Biophysica Acta (1114) 19-29.

Sun, P., and Maurer, R.A. (1995) An inactivating point mutation demonstrates that interaction of cAMP response element binding protein (CREB) with the CREB binding protein is not sufficient for transcriptional activation. J. of Biol. Chem. (270) 7041-7044.

Suzuki, T., Kitao, S., Matsushime, H., and Yoshida, M. (1996) HTLV-1 Tax protein interacts with cyclin-dependent kinase inhibitor $p16^{INK4A}$ and counteracts its inhibitory activity towards CDK4. EMBO Journal (15) 1607-1614.

Suzuki, T., Fujisawa, J.-I., Toita, M., and Yoshida, M. (1993) The trans-activator Tax of human T-cell leukemia virus type 1 (HTLV-1) interacts with cAMP-responsive element (CRE) binding and CRE modulator proteins that bind to the 21-base-pair enhancer of HTLV-1. Proc. Natl. Acad. Sci. USA (90) 610-614.

Suzuki, T., Hirai, H., Fujisawa, J.-I., Fujita, T., and Yoshida, M. (1993) A trans-activator Tax of human T-cell leukemia virus type 1 binds to NF-κB p50 and serum response factor (SRF) and associates with enhancer DNAs of the NF-κB site and CArG box. Oncogene (8) 2391-2397.

Swope, D.L., Mueller, C.L., and Chrivia, J.C. (1996) CREB-binding protein activates transcription through multiple domains. J. of Biol. Chem. (271) 28138-28145.

Tabor, E., Gerety, R.J., Cairns, J., and Bayley, A.C. (1990) Did HIV and HTLV originate in Africa? JAMA (264) 691-692.

Tamkun, J.W., Deuring, R., Scott, M.P., Kissinger, M., Pattatucci, A.M., Kaufmann, T.C., and Kennison, J.A. (1992) brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell (68) 561-572.

Tan, T.-H., Horikoshi, M., and Roeder, R. (1989) Purification and characterization of multiple nuclear factors that bind to the TAX-inducible enhancer within the human T-cell leukemia virus type 1 long terminal repeat. Mol. and Cell. Biol. (9) 1733-1745.

Tanaka, A., Takahashi, C., Yamaoka, A., Nosaka, T., Maki, M., and Hatanaka, M. (1990) Oncogenic transformation by the Tax gene of human T-cell leukemia virus type I in vitro. Proc. Natl. Acad. Sci. USA (87) 1071-1075.

Tanimura, A., Teshima, H., Fujisawa, J.-I., and Yoshida, M. (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. of Virol. (67) 5375-5382.

Tendler, C.L., Greenberg, S.J., Blattner, W.A., Manns, A., Murphy, E., Fleisher, T., Hanchard, B., Morgan, O., Burton, J.D., Nelson, D.L., and Waldmann, T.A. (1990) Transactivation of interleukin 2 and its receptor induces immune activation in human T-cell lymphotropic virus type-I associated myelopathy: pathogenic implications and a rationale for immunotherapy. Proc. Natl. Acad. Sci. USA (87) 5218.

Tie, F., Adya, N., Greene, W.C., and Giam, C.-Z. (1996) Interaction of the human T-lymphotropic virus type 1 Tax dimer with CREB and the viral 21-base-pair repeat. J. of Virol. (70) 8368-8374.

Trono, D. (1995) HIV accessory proteins: leading roles for the supporting cast. Cell (82) 189-192.

Tsuchiya, H., Fuji, M., Tanada, Y., Tozawa, H., and Seiki, M. (1994) Two distinct regions form a functional activation domain of the HTLV-I trans-activator Tax1. Oncogene (9) 337-340.

Uittenbogaard, M.N., Armstrong, A.P., Chiaramello, A., and Nyborg, J.K. (1994) Human T-cell leukemia virus type 1 Tax protein represses gene expression through the basic helix-loop-helix family of transcription factors. J. of Biol. Chem. (269) 22466-22469.

Uittenbogaard, M.N., Giebler, H.A., Reisman, D., and Nyborg, J.K. (1995) Transcriptional repression of *p53* by human T-cell leukemia virus type I Tax protein. J. of Biol. Chem. (270) 28503-28506.

Varmus, H. (1988) Retroviruses. Science (240) 1427-1435.

Walker, W.H., Sanborn, B.M., and Habener, J.F. (1994) An isoform of transcription factor CREM expressed during spermatogenesis lacks the phosphorylation domain and repressed cAMP-induced transcription. Proc. Natl. Acad. Sci. USA (91) 12423-12427.

Wang, H.-G. H., Moran, E., and Yaciuk, P. (1995) E1A promotes association between p300 and pRB in multimeric complexes required for normal biological activity. J. of Virol. (69) 7917-7924.

Wagner, S. and Green M.R. (1994) DNA-binding domains: targets for viral and cellular regulators. Current Opinion in Cell Biology (6) 410-414.

Wagner, S., and Green, M.R. (1993) HTLV-1 Tax protein stimulation of DNA binding of bZIP proteins by enhancing dimerization. Science (262) 395-399.

Wong-Staal, F., and Gallo, R.C. (1985) Human T-lymphotropic retroviruses. Nature (317) 395-403.

Xu, X., Brown, D.A., Kitajima, I., Bilakovics, J., Fey, L.W., and Nerenberg, M.I. (1994) Transcriptional suppression of the human T-cell leukemia virus type I long terminal repeat occurs by an unconventional interaction of a CREB factor with the R region. Mol. and Cell. Biol. (14) 5371-5383.

Xu, X., Kang, S.-H., Heidenreich, O., Brown, D.A., and Nerenberg, M.I. (1996) Sequence requirements of ATF2 and CREB binding to the human T-cell leukemia virus type 1 LTR R region. Virology (218) 362-371.

Yamaoka, S., Inoue, H., Sakurai, M., Sugiyama, T., Hazama, M., Yamada, T. and Hatanaka, M. (1995) Constitutive activation of NF-κB is essential for transformation of rat fibroblasts by the human T-cell leukemia virus type I Tax protein. EMBO Journal (15) 873-887.

Yang, X.-J., Ogryzko, V.V., Nishikawa, J.-I., Howard, B.H., and Nakatani, Y. (1996) A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature (382) 319-324.

Yin, M.-J., Paulssen, E., and Gaynor, R.B. (1996) cAMP-response element-binding protein induces directed DNA bending of the HTLV-I 21-base pair repeat. J. Biol. Chem. (271) 4781-4790.

Yin, M.-J., and Gaynor. R.B. (1996) HTLV-1 21 bp repeat sequences facilitate stable association between Tax and CREB to increase CREB binding affinity. J. Mol. Biol. (264) 20-31.

Yin, M.-J., Paulssen, E.J., Seeler, J.S., and Gaynor, R.B. (1995) Protein domains involved in both in vivo and in vitro interactions between human T-cell leukemia virus type I Tax and CREB. J. of Virol. (69) 3420-3432.

Yin, M.-J., Paulssen, E., Seeler, J., and Gaynor, R.B. (1995) Chimeric proteins composed of Jun and CREB define domains required for interaction with the human T-cell leukemia virus type 1 Tax protein. J. of Virol. (69) 6209-6218.

Yin, M.-J., and Gaynor, R.B. (1996) Complex formation between CREB and Tax enhances the binding affinity of CREB for the human T-cell leukemia virus type 1 21-base-pair repeats. Mol. and Cell. Biol. (16) 3156-3168.

Yip, M.T. and Chen, I.S.Y. (1990) Modes of transformation by the human T-cell leukemia viruses. Mol. Bio. Med. (7) 33-44.

Yoshida, M., Miyoshi, I., and Hinuma, Y. (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.

Yoshida, M. (1995) HTLV-1 oncoprotein Tax deregulates transcription of cellular genes through multiple mechanisms. J. Cancer Res. Clin. Oncol. (121) 521-528.

Yoshimura, T., Fujisawa, J.-I., and Yoshida, M. (1990) Multiple cDNA clones encoding nuclear proteins that bind to the *tax*-dependent enhancer of HTLV-1: all contain a leucine zipper structure and basic amino acid domain. EMBO Journal (9) 2537-2542.

Yu, Y., Li, W., Yussa, M., Han, W., Perrimon, N., and Pick, L. (1997) The nuclear hormone receptor HtZ-F1 is a cofactor for the Drosophila homeodomain protein Ftz. Nature (385) 552.

Yuan, W., Condorelli, G., Caruso, M., Felsani, A., and Giordano, A. (1996) Human p300 protein is a coactivator for the transcription factor MyoD. J. Biol. Chem. (271) 9009-9013.

Zhao, L.-J., and Giam, C.-Z. (1991) Interaction of the human T-cell lymphotropic 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.

Zhao, L.-J., and Giam, C.-Z. (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.

Zhang, J.J., Vinkemeier, U., Gu, W., and Darnell, J.E. Jr. (1996) Two contact regions between Stat1 and CBP/p300 in interferon gamma signaling. Proc. Natl. Acad. Sci. USA (93) 15092-15096.