

# Molecular mechanisms of viral transcription and cellular deregulation associated with the HTLV-1 Tax protein

Review Article

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**Abbreviations:** HTLV-I, human T-cell leukemia virus type-I; ATL, adult T-cell leukemia; TSP/HAM, tropical spastic paraparesis/HTLV-1 associated myopathy; LTR, long terminal repeat; CREs, cyclic AMP response elements; CREB, cAMP-response element binding protein; EMSA, electrophoretic mobility shift assays; bZIP, basic-leucine-zipper domain; bHLH, basic-helix-loop-helix domain; NF- $\kappa$ B, nuclear factor kappa B; I- $\kappa$ B, inhibitor kappa B; IKK, I- $\kappa$ B kinase; NIK, NF- $\kappa$ B-inducing kinase.

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## Summary

It is estimated that between 10 and 20 million people worldwide are infected with the human T-cell leukemia virus, type I (HTLV-I). Since HTLV-I is associated with a variety of human diseases, including an aggressive lymphoproliferative disorder named adult T-cell leukemia, infection by HTLV-I has become increasingly recognized as an important public health concern. Malignant transformation associated with HTLV-I infection is linked with the synthesis of a virally-encoded protein called Tax. In this review, we will highlight our current understanding of Tax protein function, both in its role as an activator of HTLV-I transcription and deregulator of cellular homeostasis. It is widely believed that Tax deregulation of cellular gene expression and cell cycle progression accounts for the pathogenicity associated with HTLV-I infection.

## I. HTLV-1 discovery and associated diseases

The human T-cell leukemia virus type-I (HTLV-I) was the first pathogenic human retrovirus isolated and characterized (Yoshida et al., 1982; Seiki et al., 1982; Chen et al., 1983). It was originally discovered in 1980 from two T-lymphoblast cell lines derived from a patient incorrectly diagnosed with cutaneous T-cell lymphoma (mycosis fungoides) (Poiesz et al., 1980). Both of these cell lines maintain the continuous release of mature and immature typical type C budding virus particles. Shortly after this original report, retroviral particles were also observed in a cell line obtained from a second patient who had been correctly diagnosed with adult T-cell leukemia (ATL) (Hinuma et al., 1981). Both of the original patients were eventually shown to be infected with the human T-cell leukemia/lymphoma virus, suggesting a causal

relationship between viral infection and the development of ATL (Popovic et al., 1982).

Since the publication of the first studies, HTLV-I has become widely accepted as the etiologic agent of ATL, a distinct disease entity (reviewed in Watanabe, 1997). ATL is characterized by clinical and hematological features that include unresponsiveness to radiation and chemotherapy, skin lesions due to infiltrating leukemic cells, lytic bone lesions, greater than 5% abnormal T-cells, and leukemic cells carrying the CD4<sup>+</sup> phenotype (Poiesz et al., 1981; Robert-Guroff et al., 1982; Yoshida et al., 1984). Additional evidence for the causative role of HTLV-I in ATL comes from the observation that tumor cells from patients with ATL characteristically show monoclonal or oligoclonal integration of the HTLV provirus. A second disease, referred to as tropical spastic paraparesis/HTLV-1 associated myopathy (TSP/HAM), is also tightly linked

with HTLV-1 infection (Gessain et al., 1985; Jacobson et al., 1988; Osame et al., 1986). TSP/HAM is a neurodegenerative disorder characterized by demyelination of the nerves of the spinal cord, resulting in paralysis of the lower extremities. TSP/HAM shares many similarities with multiple sclerosis, often confusing accurate diagnosis. The role HTLV-1 plays in the pathogenesis of TSP/HAM remains incompletely understood.

The T-cell transforming properties of HTLV-1 are a major focus of research, as the virus provides an excellent model system for studying oncogenesis in humans. HTLV-1 is not an acute transforming retrovirus, nor does it rely on promoter insertion mechanisms for deregulation of cellular proto-oncogenes (Seiki et al., 1984). Only a small percentage of HTLV-1 infected individuals develop ATL, and disease onset follows a latency period of several decades (Kondo et al., 1987; Murphy et al., 1989; Kawano et al., 1985). The infrequency of ATL in infected individuals indicates that HTLV-1 is necessary, but not sufficient, for leukemic transformation. The development of ATL is hypothesized to occur as a consequence of a single oncogenic transformation event resulting from long-term autocrine T-cell proliferation (reviewed in Franchini, 1995) and/or virus-dependent disruption of the normal cellular processes outlined in this review.

The regulatory protein Tax is thought to be the primary HTLV-1-encoded factor responsible for T-cell transformation (Grassmann et al., 1992; Grossman et al., 1995). Tax is required for HTLV-I replication, stimulating viral gene expression through enhancer elements in the HTLV-1 promoter (Chen et al., 1985; Cann et al., 1985; Felber et al., 1985; Sodroski et al., 1985). Tax is also very pleiotropic, as it deregulates host cell gene expression, influences apoptosis, and enhances cell-cycle progression. It is therefore not surprising that singularly, the HTLV-1 Tax protein has the ability to transform human T-lymphocytes *in vitro* and to promote tumorigenesis and leukomigenesis *in vivo* (Grassmann et al., 1992; Nerenberg et al., 1987; Grossman et al., 1995).

## **II. The HTLV-1 promoter and Tax transactivation**

The Tax protein of HTLV-I is encoded downstream of the proviral structural genes, within a region originally termed X. Mutational analysis of the various open reading frames of HTLV-1, together with transient transfection assays using a Tax expression plasmid, confirmed that Tax was the virally encoded transactivator protein (Chen et al., 1983; Cann et al., 1985; Felber et al., 1985; Sodroski et al., 1985). The 353 amino acid Tax protein is synthesized from a doubly-spliced RNA transcript, and imported back into the nucleus where it functions as a potent activator of HTLV-I transcription. This strong activation by Tax leads

to the synthesis of both the viral mRNAs that are translated into viral proteins and genomic RNA that is packaged into the virion. For these reasons, Tax transactivation plays an important role in the retroviral life cycle.

Following the discovery of Tax, and its prominent role in the viral life cycle, researchers turned to an analysis of the sequences within the HTLV-I promoter, which confer responsiveness to Tax. Early studies found that nucleotide sequences within the U3 region of the HTLV-1 long terminal repeat (LTR) carried Tax-responsive transcriptional enhancer elements (Sodroski et al., 1984; Rosen et al., 1985; Fujisawa et al., 1985; Paskalis et al., 1986). The specific Tax-responsive elements in the HTLV-1 promoter were originally identified by probing for deletions in the DNA, which resulted in abrogation of Tax transactivation. Sequence analyses quickly identified three imperfect direct repeats that were named the 21-base pair (bp) repeats (Seiki et al., 1982; Josephs et al., 1984; Rosen et al., 1985). Deletion mutagenesis of these elements identified them as the principal Tax-responsive sequences in the HTLV-1 promoter, with at least two of the three viral CREs required for efficient Tax function (Fujisawa et al., 1986; Paskalis et al., 1986; Shimotohno et al., 1986; Brady et al., 1987; Rosen et al., 1987; Jeang et al., 1988; Nakamura et al., 1989). In addition to the three 21-bp repeats, the HTLV-I promoter also carries a TATA element and several additional DNA elements which have been implicated in viral gene expression (Seiki et al., 1982; reviewed in Franklin and Nyborg, 1995).

### **A. Characterization of cellular proteins that bind the Tax-responsive promoter elements**

Although the 21-bp repeats were identified as critical for Tax transactivation, DNase I footprinting studies suggested that Tax did not directly bind to the 21 bp repeats, but rather cellular proteins provided the primary recognition of these elements (Nyborg et al., 1988; Altman et al., 1988; Jeang et al., 1988). This led to efforts aimed at identifying the cellular proteins, as it seemed likely that these proteins would serve as mediators of Tax transactivation (Jeang et al., 1988; Giam and Xu, 1989; Nakamura et al., 1989; Tan et al., 1989; Nyborg and Dynan, 1990; Beimling and Moelling, 1990; Montagne et al., 1990; Poteat et al., 1990). A clue to their identity came shortly after the discovery of the ATF/CREB family of cellular transcription factors. Members of the ATF/CREB family are characterized by a DNA binding basic region that is located immediately adjacent to a leucine zipper dimerization domain (reviewed in Montminy, 1997).

**Figure 1.** Nucleotide sequences of the three Tax-responsive viral CREs. The viral CREs are shown in **blue**, and a cellular CRE from the human chorionic gonadotropin gene promoter that is not responsive to Tax is shown in **red**. The CREB recognition element is indicated in **black**, and the conserved GC-rich viral CRE flanking sequences are underlined.

These basic-leucine zipper (bZIP) proteins bind either as homodimers or heterodimers to specific palindromic DNA sequences, called cyclic AMP response elements, or CREs (reviewed in Montminy, 1997). Since each Tax-responsive 21 bp repeat carried a CRE-like sequence centered within the repeat, it seemed plausible that members of this family of cellular proteins interacted with the Tax-responsive HTLV-I promoter elements. Specific members of the ATF/CREB family of transcription factors have been confirmed to bind the 21 bp repeat elements, interact with Tax, and possibly mediate Tax transactivation (Yoshimura et al., 1990; Zhao and Giam, 1991; Tsujimoto et al., 1991; Beimling and Moelling, 1992; Franklin et al., 1993; Suzuki et al., 1993).

Since the Tax-responsive 21 bp repeats carry CRE elements, they are now commonly referred to as viral CREs. Centered within each viral CRE is an 8-bp off-consensus CRE core sequence, which serves as the binding site for ATF/CREB proteins (viral CRE core). Immediately flanking the viral CRE core are short sequences rich in guanine and cytosine nucleotides (GC-rich flanks). Both the viral CRE core and GC-rich flanks are critical for Tax transactivation in vivo and in vitro (Jeang et al., 1988; Fujisawa et al., 1989; Montagne et al., 1990; Numata et al., 1991; Paca-Uccaralertkun et al., 1994; Brauweiler et al., 1995; Giebler et al., 1997; Lenzmeier et al., 1998). The sequences of the three HTLV-I viral CREs, as well as a cellular CRE that is not Tax-responsive, are shown in **Figure 1**.

## **B. Tax enhancement of CREB binding to the viral CREs**

Although several members of the ATF/CREB family have been demonstrated to bind the viral CREs, the transcription factor CREB (cAMP-response element

binding protein) appears to have the most prominent role in mediating Tax transactivation (Zhao and Giam, 1991; Beimling and Moelling, 1992; Franklin et al., 1993; Adya et al., 1994; Brauweiler et al., 1995; Adya and Giam, 1995; Yin et al., 1995a). The characterization of the CREB interaction with the viral CRE core led to an intense investigation on how Tax and CREB interact to enhance HTLV-1 transcription. Many researchers hypothesized that Tax transactivation resulted directly from an interaction between Tax and CREB, possibly resulting in enhancement in the transcriptional activation properties of CREB. In support of this hypothesis, several studies have shown that Tax increases the equilibrium binding affinity of CREB for the viral CRE (Zhao and Giam, 1992; Wagner and Green, 1993; Franklin et al., 1993; Anderson and Dynan, 1994; Baranger et al., 1995; Perini et al., 1995; Brauweiler et al., 1995; Yin and Gaynor, 1996a; Kwok et al., 1996). This increase in the apparent binding affinity derives, at least in part, from interactions between Tax and the bZIP segment of CREB. Consistent with this observation, Tax has also been found to enhance the DNA binding affinity of a number of related bZIP proteins (Armstrong et al., 1993; Wagner and Green, 1993; Low et al., 1994; Baranger et al., 1995; Perini et al., 1995). Although biochemical and genetic evidence suggest that Tax interacts with the leucine zipper domain, the basic DNA binding domain appears to be the primary Tax-recognition element within the CREB bZIP segment (Yin et al., 1995a, 1995b; Adya et al., 1994; Baranger et al., 1995; Perini et al., 1995). This Tax-basic region interaction may stabilize the  $\alpha$  helical structure of the parallel bZIP dimers, resulting in both enhanced DNA binding and dimerization of CREB (Baranger et al., 1995; Perini et al., 1995). These data are in agreement with other data suggesting that dimerization is tightly linked to DNA binding (Ellenberger et al., 1992; Wu et al., 1998). Furthermore, Tax increases the DNA binding affinity of a covalently cross-linked basic segment dimer, indicating that Tax interaction with just the basic amino acids is

sufficient for increased binding affinity (Baranger et al., 1995). Together, these observations are consistent with a model where Tax contributes energetically to the stability of the CREB-DNA interaction.

The Tax-induced increase in the binding affinity of CREB indicates that Tax forms a stable ternary complex with CREB and the viral CRE. The physical incorporation of Tax into the complex has been observed in electrophoretic mobility shift assays (EMSA) where the mobility of the CREB-viral CRE complex is reduced in the presence of Tax (Zhao and Giam 1991, 1992; Brauweiler et al., 1995; Goren et al., 1995; Paca-Uccaralertkun et al., 1995). Many additional approaches have been utilized to demonstrate the presence of Tax in complexes containing CREB and the viral CRE (Zhao and Giam, 1992; Wagner and Green, 1993; Kwok et al., 1996; Yin and Gaynor, 1996a, 1996b; Lenzmeier et al., 1998; Kimzey and Dynan, 1998). Together, these studies provided support for a model where Tax transactivation resulted from the increased occupancy of the transcription factor CREB on the HTLV-I promoter.

### **C. Tax interaction with the viral CREs**

The molecular interactions between Tax and CREB strongly suggest that CREB plays a pivotal role in mediating Tax transactivation. However, the functional activity of Tax also requires the short stretches of GC-rich sequences which immediately flank the viral CRE core (see **Figure 1**). These GC-rich sequences are absolutely required for Tax transactivation *in vivo* and *in vitro*, as CREs lacking the GC-rich flanking sequences, are unresponsiveness to Tax (Jeang et al., 1988; Fujisawa et al., 1989; Montagne et al., 1990; Numata et al., 1991; Seeler et al., 1993; Paca-Uccaralertkun et al., 1994; Brauweiler et al., 1995; Yin and Gaynor, 1996a; Giebler et al., 1997; Lenzmeier et al., 1998). Consistent with the Tax functional studies, the flanking sequences are also required for Tax-CREB-viral CRE ternary complex formation and Tax-dependent CREB stabilization on a viral CRE (Zhao and Giam, 1992; Paca-Uccaralertkun et al., 1994; Brauweiler et al., 1995; Kwok et al., 1996; Yin and Gaynor, 1996a, 1996b; Brauweiler et al., 1995). Until recently, the role of the GC sequences in mediating the activities of Tax remained elusive, as several studies had been unsuccessful in identifying a direct interaction between Tax and the viral CRE DNA (Nyborg et al., 1988; Altman et al., 1988; Jeang et al., 1988; Wagner and Green, 1993; Paca-Uccaralertkun et al., 1994). Although an inability to detect a Tax-DNA interaction did not preclude its existence, the absence of evidence for an interaction supported the widely held view that Tax does not bind DNA.

Recently, high-resolution DNA footprinting techniques have provided evidence that Tax directly contacts the GC-rich nucleotides within the viral CRE. Tax specifically

expanded the cleavage protection pattern of CREB from the viral CRE core into the GC-rich flanking sequences (Lenzmeier et al., 1998; Lundblad et al., 1998). This Tax-dependent expansion of the CREB footprint required the GC-rich sequences, as the expansion was not observed with a cellular CRE unresponsive to Tax (Lenzmeier et al., 1998). Protein-DNA cross-linking studies confirmed that Tax was intimately associated with the viral CRE flanking sequences, strengthening the likelihood that the changes in the cleavage protection pattern on the viral CRE were due to Tax (Lenzmeier et al., 1998; Kimzey and Dynan, 1998). Additional studies, using inosine substitution and GC-specific DNA binding drugs, provided corroborating evidence for a direct Tax interaction with the viral CRE DNA, and further showed that Tax contacted the minor groove of the DNA (Lundblad et al., 1998; Lenzmeier et al., 1998). The observation of a minor groove interaction perhaps accounts for the inability to detect the Tax-DNA interaction in previous studies. Together, these data provide a strong body of evidence supporting a direct interaction between Tax and the minor groove of the GC sequences adjacent to the CRE core, thereby defining a functional role for these viral promoter sequences.

### **D. Tax dimerization**

The apparent binding of Tax to both GC sequences flanking the viral CRE suggests that Tax exists as a dimer when incorporated into the ternary complex. In support of this idea, recent studies have provided additional evidence that Tax functions as a dimer (Tie et al., 1996; Jin et al., 1997a). Although the location of the putative Tax dimerization domain is controversial, point mutations have been identified which abrogate both Tax self-association and Tax entry into a stable Tax-CREB-viral CRE ternary complex (Tie et al., 1996; Jin et al., 1997a). Additionally, the yeast 2-hybrid assay revealed protein-protein interactions between Tax monomers *in vivo* (Tie et al., 1996; Jin et al., 1997b). Consistent with a functional role for Tax dimerization, Tax mutants deficient for self-association *in vitro* and *in vivo* were unable to activate transcription from the HTLV-1 promoter (Tie et al., 1996; Jin et al., 1997a).

While there is good evidence that Tax exists as a dimer, the role of dimerization in Tax function is still somewhat controversial. Quantitative EMSA experiments indicate that only one Tax molecule is present in the viral CRE nucleoprotein complex (Giebler et al., 1997). Additionally, it has been established that only one of the two GC-rich flanking sequences needs to be present for Tax transactivation through the viral CREs, inferring that one Tax molecule per viral CRE is sufficient for Tax transactivation (Jeang et al., 1988; Fujisawa et al., 1989; Montagne et al., 1990; Numata et al., 1991; Seeler et al., 1993; Paca-Uccaralertkun et al., 1994). Although Tax monomers may be adequate, maximal Tax transactivation

probably occurs when two Tax molecules are present on the viral CREs. Future studies which more rigorously address the stoichiometry of the viral CRE nucleoprotein complex, as well as further clarification of the nature of the Tax-Tax interaction, and its functional role in Tax transactivation, will help to solidify the role of Tax dimerization in Tax function.

### E. Coactivator recruitment by Tax

A model showing Tax incorporated into the ternary complex with CREB and the viral CRE is presented in **Figure 2**. This model illustrates the interaction between Tax and the GC-rich DNA, as well as the interaction between Tax and the bZIP segment of CREB bound to the viral CRE core. The multiple protein-protein and protein-DNA contacts confer significant stability to the nucleoprotein complex, and are believed to be critical for Tax transactivation of HTLV-I gene expression. However, this model incompletely describes the precise molecular events that lead to strong transcriptional stimulation by Tax. Recent studies suggests that Tax stabilization of CREB on the viral promoter would not result in strong transcriptional activation, as the activity of CREB is largely dependent upon phosphorylation by cAMP-dependent protein kinase A (PKA) (Gonzalez et al., 1989; reviewed in Montminy, 1997). Once CREB is phosphorylated, it directly recruits the cellular coactivator CREB binding protein (CBP) to promoters of CREB-responsive genes (Chrivia et al., 1993; Kwok et al., 1994). Since there is no evidence that Tax directly or indirectly phosphorylates CREB, a model proposing that Tax simply serves to stabilize CREB binding to the viral promoter insufficiently accounts for the strong HTLV-I transcriptional activation observed in the presence of Tax. The identification of CBP in mediating CREB-activated transcription, together with the recently discovered pleiotropic effects of CBP on many cellular transcription factor pathways, has led several groups to investigate

**Figure 2.** Model for the Tax-CREB-viral CRE ternary complex. The DNA is shown in **red** and the bZIP domain of CREB is shown in **yellow**. This model is based on the crystal structure of the yeast bZIP protein GCN4 (Ellenberger et al., 1992).

whether Tax may recruit CBP to the HTLV-I. In support of this hypothesis, published studies have now confirmed that Tax, in the context of the ternary complex, directly recruits CBP to the HTLV-I promoter to activate transcription (Kwok et al., 1996; Giebler et al., 1997).

Tax interacts with CBP with relatively high affinity, recruiting CBP to the HTLV-I promoter to form a highly stable quaternary complex (Kwok et al., 1996; Yin and Gaynor, 1996b; Laurance et al., 1997; Giebler et al., 1997; Harrod et al., 1998). CBP recruitment is dependent upon CREB in the complex, although it is independent of the phosphorylation state of CREB (Kwok et al., 1996; Laurance et al., 1997; Giebler et al., 1997). In fact, the entire amino terminus of CREB (including the PKA-phosphorylation domain), was dispensable for CBP recruitment, as the bZIP domain was sufficient for quaternary complex formation and Tax transactivation *in vivo* (Laurance et al., 1997; Giebler et al., 1997). The GC-rich sequences of the viral CREs have also been demonstrated to be critical for efficient recruitment of CBP by Tax (Kwok et al., 1996; Giebler et al., 1997; Lenzmeier et al., 1998).

**Figure 3.** Schematic illustration of the HTLV-1 promoter. For simplicity, only one of the three viral CREs is shown.

Although CBP is a very large protein (2441 amino acids), Tax has been shown to interact with a small region of CBP called the KIX domain (Kwok et al., 1996; Giebler et al., 1997). Tax specifically binds KIX amino acids 588-683, overlapping significantly with the region of KIX that is recognized by phosphorylated CREB (aa 586-665) (Yan et al., 1998; Radhakrishnan et al., 1997). These KIX amino acids fold into three  $\alpha$ -helices which come together to form a hydrophobic protein-docking pocket (Radhakrishnan et al., 1997). Although the amino acids of KIX that bind Tax and CREB are very similar, specific point mutations within KIX distinguish between the binding of Tax and phosphorylated CREB, suggesting that the precise molecular recognition of KIX by Tax and CREB are distinct (Yan et al., 1998). This observation is supported by evidence showing that the phosphorylation of CREB increases the affinity of KIX for the Tax-containing ternary complex over 14-fold. These data support the idea that KIX can recognize both Tax and phosphorylated CREB simultaneously in the context of the Tax-CREB-viral CRE complex (Giebler et al., 1997). This model is in agreement with previous studies showing that Tax transactivation was independent of, but augmented by, CREB phosphorylation (Poteat et al., 1989; Kadison et al., 1990).

Together, the above data have established a critical role for CBP in Tax transactivation. Efficient HTLV-1 transcription is likely dependent upon the stable Tax-ternary complex serving as a high affinity binding site for CBP. It is believed that the presence of CBP promotes transcriptional activation through its intrinsic and associated acetyltransferase activities (Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Yang et al., 1996; Imhof et al., 1997), and through its link to the basal transcription machinery (Abraham et al., 1993; Kwok et

al., 1994; Kee et al., 1996., Swope et al., 1996). Recent studies on Tax and CBP have begun to characterize the amino acids of Tax required for CBP recognition (Bex et al., 1998; Harrod et al., 1998), and the consequences of the Tax-CBP interaction on cellular gene expression (Colgin and Nyborg, 1998; Van Orden et al., submitted for publication). A model illustrating the interactions of CBP on the HTLV-1 promoter is shown in **Figure 3**.

### III. Consequences of Tax expression on cellular gene expression

In the HTLV-1 infected cell, Tax expression appears to dysregulate various pathways of cellular gene expression, which may explain the tight link between Tax and malignant transformation. The classes of cellular gene products whose expression may be dysregulated by Tax are presented in **Figure 4**. These include: (i) tumor suppressors (Jeang et al., 1990; Uittenbogaard et al., 1995; Feigenbaum et al., 1996); (ii) apoptosis regulators (Brauweiler et al., 1997); (iii) transcription factors (Fujii et al., 1988; Alexandre and Verrier, 1991; Alexandre et al., 1991; Duyao et al., 1992); (iv) extracellular signaling mitogens (Maruyama et al., 1987; Leung et al., 1988; Nimer et al., 1989; Kim et al., 1990; Grassman et al., 1992; reviewed in Franchini, 1995); (v) cell surface receptors (Inoue et al., 1986; Siekevitz et al., 1987; Cross et al., 1987); (vi) signal transduction kinases (Uchiumi et al., 1992; Lemasson et al., 1997); (vii) cytoskeletal components (Lilienbaum et al., 1990); (viii) DNA replication components (Ressler et al., 1997); and (ix) 5S RNAs and tRNAs (Gottesfeld et al., 1996).

**Figure 4.** Overview of the cellular processes deregulated by the HTLV-I Tax protein.

While improper expression of each of these gene products alone might contribute to a transformation event, it is likely that the global effects of Tax on cellular gene expression are responsible for Tax-mediated transformation. Some cellular genes are activated by Tax, while others are repressed by Tax. The infrequent outcome of these pleiotropic properties of Tax appears to be promotion of uncontrolled T-cell growth. This section will focus on the mechanisms by which Tax both activates and represses cellular gene expression.

### **A. Mechanisms for Tax activation of cellular gene expression**

Many cellular transcription factors have been implicated as targets of deregulation by the HTLV-1 Tax protein. Among these are the ATF/CREB family (reviewed above), nuclear factor kappa B (NF- $\kappa$ B) (reviewed below), nuclear factor Y (NF-Y) (Pise-Masison et al., 1997), Ets1 and Sp1 (Dittmer et al., 1997), nuclear factor of activated T-cells (NFAT) (Good et al., 1996;

Rivera et al., 1998), and serum response factor (SRF) (Alexandre and Verrier, 1991; Alexandre et al., 1991; Fujii et al., 1992). Interestingly, most of these transcriptional activators have also been shown to utilize the coactivators CBP and p300 (see **Figure 5**), suggesting that mechanisms of Tax deregulation of cellular gene expression may converge on alterations in the transcription properties of CBP. Of all the cellular transcription factors putatively targeted by Tax, NF- $\kappa$ B clearly plays the most significant role in Tax activation of cellular gene expression. The data associating NF-Y, Ets1, SP1, NFAT, and SRF with Tax transactivation is intriguing, but more studies aimed at defining the importance of these factors for HTLV-1 propagation and T-cell transformation are necessary before they are widely accepted as targets of Tax-deregulation. Additionally, although the ATF/CREB transcription factors are likely integral components in the mechanism of HTLV-1 transactivation by Tax, they have not been specifically implicated as targets of cellular gene deregulation by Tax.

**Figure 5.** Functional domains of the pleiotropic cellular coactivator CBP. The approximate amino acid locations of the various domains are indicated, as are the cellular proteins which interact with those respective domains.

This is probably because most cellular genes do not carry the appropriate GC-rich sequences immediately flanking the CREB binding sites. From these observations, it appears that Tax deregulation of the NF- $\kappa$ B transcription pathway may represent the most prominent pathway in Tax activation of cellular gene expression. In addition, since activate NF- $\kappa$ B proteins appear to inhibit apoptosis, Tax activation of this pathway may promote survival of the HTLV-I-infected cell, thus enhancing survival of the virus (Wang et al., 1996; reviewed in Baeuerle and Baltimore, 1996; Gilmore et al., 1996).

### **B. Tax activation of the NF- $\kappa$ B pathway**

The NF- $\kappa$ B/rel transcription factors function normally in the cell to enhance expression of genes involved in mitogen-driven proliferation (reviewed in Baeuerle and Baltimore, 1996). Each member of the NF- $\kappa$ B/rel family of transcription factors contains a rel homology domain of approximately 300 amino acids, which is critical for NF- $\kappa$ B dimerization, DNA binding, and nuclear localization (reviewed in Thanos and Maniatis, 1995). Regulation of NF- $\kappa$ B transactivation activity is by subcellular compartmentalization. The inactive form of NF- $\kappa$ B is kept in the cytoplasm by interactions with inhibitor kappa B (I- $\kappa$ B), which binds to and masks its nuclear localization signal. Activation of the NF- $\kappa$ B pathway occurs following the phosphorylation of I- $\kappa$ B by a large multiprotein kinase complex (DiDanato et al., 1997; Mercurio et al., 1997; Zandi et al., 1997). This phosphorylation event leads to the ubiquitination and proteasomal degradation of I- $\kappa$ B (Baldi et al., 1996; Chen et al., 1995), concomitant with the release of NF- $\kappa$ B for nuclear localization and activation of NF- $\kappa$ B-responsive genes (reviewed in Baeuerle and Baltimore, 1996). Although HTLV-I studies have established that Tax increases the rate of I- $\kappa$ B degradation in the cytoplasm, and enhances NF- $\kappa$ B DNA binding by increasing the fraction of NF- $\kappa$ B found in the nucleus,

little was known about how Tax activated this pathway until recently (Kanno et al., 1994; Lacoste et al., 1995; McKinsey et al., 1996; Good and Sun, 1996; Lilienbaum and Paulin, 1993; Pepin et al., 1994; Hirai et al., 1994; Suzuki et al., 1995; reviewed in Flint and Shenk, 1997).

Three hypotheses have recently been proposed for the molecular events that lead to Tax-activation of NF- $\kappa$ B-responsive genes. One study found that Tax directly interacts with two subunits of the proteasome, suggesting that proteasomal interactions are critical for maturation and nuclear localization of the active NF- $\kappa$ B dimer (Rousset et al., 1996). There is growing support for a second hypothesis which states that Tax induces I- $\kappa$ B phosphorylation, promoting release of NF- $\kappa$ B to the nucleus. Several groups have recently shown that Tax constitutively activates both of the I- $\kappa$ B kinases, IKK $\alpha$  and IKK $\beta$ , resulting in direct phosphorylation of I- $\kappa$ B (Chu et al., 1998; Uhlik et al., 1998; Geleziunas et al., 1998). This observation has been extended to show that Tax also works immediately upstream of the IKKs by activating NF- $\kappa$ B-inducing kinase (NIK), a kinase capable of IKK $\alpha/\beta$  phosphorylation and activation (Uhlik et al., 1998; Geleziunas et al., 1998). Another group has found that Tax modulates I- $\kappa$ B phosphorylation upstream of the IKKs by functionally interacting with MEKK1, which directly phosphorylates and activates IKK $\beta$ , but not IKK $\alpha$  (Yin et al., 1998). Together, these studies indicate that Tax expression increase the cytoplasmic activities of one or more of the kinases responsible for I- $\kappa$ B phosphorylation, leading to activation of the NF- $\kappa$ B pathway. Finally, a third hypothesis has been proposed where Tax also activates NF- $\kappa$ B-dependent transcription in the nucleus. CBP and the related coactivator p300 play important roles in NF- $\kappa$ B transcription (Gerritsen et al., 1997; Perkins et al., 1997; Zhong et al., 1998), and Tax has recently been shown to participate in the formation of distinct nuclear structures containing both NF- $\kappa$ B and p300 (Bex et al., 1997, 1998). These observations provide evidence that

Tax may exert an effect on NF- $\kappa$ B transcription function directly at NF- $\kappa$ B-responsive promoters. A schematic outlining the mechanisms of Tax activation of cellular gene expression through the NF- $\kappa$ B pathway is presented in **Figure 4**. It is intriguing that Tax may function in both the cytoplasm and the nucleus to activate the same cellular transcription factor pathway.

### C. Tax repression of cellular gene expression through the bHLH proteins

In 1990, it was reported for the first time that Tax represses expression of a cellular gene (Jeang et al., 1990). Transcription of the  $\beta$ -polymerase gene, which encodes a tumor suppressor important for host-cell DNA repair, was shown to be repressed in the presence of Tax. When the  $\beta$ -polymerase study was published, the details of Tax repression was not known. Several years later, members of the cellular basic-helix-loop-helix (bHLH) family of transcription factors were implicated as the targets of Tax repression (Uittenbogaard et al., 1994; Semmes et al., 1996). A bHLH binding site, called an E-box, was identified in the promoter of the  $\beta$ -polymerase gene and shown to confer Tax repression to a heterologous promoter (Uittenbogaard et al., 1994). Tax has since been shown to utilize the cellular bHLH proteins to confer repression to promoters of the p53 gene, the Bax gene, and the lck gene (Uittenbogaard et al., 1995; Brauweiler et al., 1997; Lemasson et al., 1997). Although there is strong evidence that Tax represses transcription through bHLH proteins and E-box DNA elements, the mechanism of Tax repression remains elusive. There is evidence that the protein structure of the bHLH protein c-myc is altered in Tax-expressing cell, however, Tax does not appear to physically interact with any of the bHLH proteins (Semmes et al., 1996). These data support a model where Tax indirectly influences bHLH transcription factor activity, producing repression of bHLH-regulated cellular genes. It seems plausible that Tax repression of important

genes like p53 and  $\beta$ -polymerase may be directly linked with the oncogenic properties of Tax.

### D. Tax repression of cellular gene expression through CBP

A new model for Tax repression of cellular gene expression is beginning to emerge. This model is based on the idea that Tax utilization of CBP for HTLV-I transcription reduces the concentration of available CBP for cellular transcription factor pathways. Although CBP appears to be ubiquitously expressed, the levels of intracellular CBP appear limiting (Petrij et al., 1995; Shi et al., 1998; Yao et al., 1998). Since CBP serves as a pleiotropic coactivator for a large number of structurally unrelated cellular transcription factors (see **Figure 5**), competition for limiting CBP can effectively abrogate CBP function (Arias et al., 1994; Kamei et al., 1996; Horvai et al., 1997). Although Tax interacts with the KIX domain of CBP, many cellular transcription factors also utilize this same region for recognition and recruitment of CBP. These include phosphorylated CREB, c-jun, c-myc and STAT1 (see **Figure 5**; reviewed in Janknecht and Hunter, 1996; Shikama et al., 1997; Giles et al., 1998). Because Tax shares the same CBP-docking site as several important cellular transcription factors, it has been hypothesized that Tax binding to KIX might inhibit access of other transcription factors to CBP, thus altering patterns of cellular gene expression. We have recently found that Tax effectively represses c-jun (Van Orden et al., submitted for publication) and c-myc (Colgin and Nyborg, 1998) transcription activity in vivo, and reciprocally, overexpression of these cellular transcription factors represses the transcription function of Tax. The mechanism of repression is likely through a direct competition for limiting levels of intracellular CBP, as the binding of Tax and these two cellular transcription factors to the KIX domain of CBP is mutually exclusive in vitro (Colgin and Nyborg, 1998; Van Orden et al., submitted for publication). A general model for CBP competition as a mechanism of Tax repression is presented in **Figure 6**.

**Figure 6.**  
Model for Tax repression of cellular gene expression through competition for the cellular coactivator CBP.

These observations suggest that Tax and cellular transcription factors may compete for CBP utilization in the HTLV-I infected T-cell. However, the extent and the consequence of this competition may depend upon several criteria, including the abundance of the cellular transcription factors, their relative KIX-binding affinities, and the concentration of available CBP in the cell. Tax expression in an HTLV-I-infected cell is believed to be intermittent, but that during the brief periods of Tax expression, Tax protein levels are high (0.15% of total cell protein; Slamon et al., 1985). It seems likely that during these burst periods, Tax levels would exceed those needed for optimal proviral expression, and by mass action, the high concentrations of free Tax would bind to KIX, sequestering the limiting concentrations of intracellular CBP, and altering CBP-mediated cellular gene expression.

These Tax-dependent effects on CBP function may be directly linked with cellular transformation and adult T-cell leukemia, as a prominent role for CBP in hematopoietic malignancies is emerging (reviewed in Giles et al., 1998). Chromosomal translocations involving CBP are being identified with increasing frequency in patients with treatment-related acute and chronic myeloid leukemias and myelodysplastic syndrome (Borrow et al., 1996; Giles et al., 1997; Rowley et al., 1997; Sobulo et al., 1997; Taki et al., 1997; Ida et al., 1997; Satake et al., 1997). The molecular basis of CBP translocation-associated leukemogenesis is not known; however, the available evidence strongly suggests that chromosomal translocations involving CBP result in reduced and/or defective coactivator function. Because of the pleiotropic role for CBP in cellular gene expression, it is likely that alterations in CBP function promote inappropriate regulation of cell cycle and differentiation genes. It seems plausible that Tax binding to the KIX domain may in some way mimic the deregulation that is achieved following chromosomal translocations involving CBP, with both scenarios promoting malignant transformation.

#### **IV. Tax deregulation of cell cycle checkpoints**

There is also growing evidence that Tax may play a role in leukemogenesis by circumventing cell cycle checkpoints. By physically interacting with and modulating the activity of components that regulate cell division, Tax may deregulate cell cycle progression at the G1-S phase, M-phase, and DNA damage checkpoints. A great deal of progress has recently been made on understanding the molecular basis for Tax modulation of

cell-cycle progression. These potential checkpoint interactions by Tax are summarized in **Figure 4**.

Tax appears to promote cell cycle progression at the G1-S checkpoint by directly interacting with and functionally inactivating p16<sup>INK4a</sup>, a cyclin-dependent kinase (cdk) inhibitor (Suzuki et al., 1996; Low et al., 1997). The cdks bound by p16<sup>INK4a</sup> normally promote progression from G1 phase into DNA synthesis (S-phase) by phosphorylating key regulatory proteins, some of which are transcription factors (reviewed in Sherr and Roberts, 1995). Tax precludes the binding of p16<sup>INK4a</sup> to the cdks, thereby enabling cdk kinase activity to proceed unchecked. In addition to modulating mitosis progression via inhibiting p16<sup>INK4a</sup> function, it was also recently shown that Tax directly interacts with the cyclin D-cdk4 (or cdk6) complex in vitro and in vivo (Neuveut et al., 1998). Although the molecular mechanism has not yet been defined, this interaction by Tax somehow increases the cyclin-cdk kinase activity and eventually leads to the hyperphosphorylation of the retinoblastoma (pRb) tumor suppressor protein (Neuveut et al., 1998), which has an established role in mitosis progression. Consistent with this observation, the E2F transcription factor, which is normally kept inactive by unphosphorylated pRB, is constitutively activated by Tax in a p16<sup>INK4a</sup>-independent manner (Lemasson et al., 1998). Together, the above experimental data suggest that both p16<sup>INK4a</sup> and the cyclin-cdk complexes are targets of Tax.

In addition to affecting mitotic events at the G1-S phase checkpoint, Tax was recently shown to deregulate mitosis progression by binding to and functionally inhibiting the centrosome binding protein MAD1 (Jin et al., 1998). Tax obstruction of MAD1 activity may be significant, as this causes the cell to skip the M-phase checkpoint for proper chromosomal alignment, and improper cytokinesis may proceed (Jin et al., 1998). Consistent with this hypothesis, aberrant chromosome segregation during cytokinesis, and multinucleated cells were detected in HTLV-1 transformed cells (Jin et al., 1998). These observations suggest that Tax manipulates cell cycle progression at two distinct points during mitosis.

Tax may also deregulate the cell cycle in the presence of DNA damage by functionally inactivating the tumor suppressor transcription factor p53 (Gartenhaus and Wang, 1995; Cereseto et al., 1996; Pise Masison et al., 1998a), which normally halts the cell-cycle in a DNA-damage dependent manner. There is no published evidence for a direct Tax-p53 interaction, indicating that Tax inactivation of p53 is likely indirect. Furthermore, although p53 is mutated in approximately 60% of human cancers, it is generally not mutated in HTLV-1

transformed cells. The mechanism by which Tax inhibits p53 activity is still unknown, and both transcription-dependent and transcription-independent mechanisms may be utilized. The observation that overexpression of p53 represses Tax transactivation of the HTLV-1 gene expression, indicates that Tax and p53 might compete for limiting amounts of a protein critical for both p53 and Tax transcription function (Mori et al., 1997; M. Gonzales and J.K. Nyborg unpublished data). Since p53 functionally utilizes CBP (Avantaggiati et al., 1997; Lill et al., 1997), it is plausible that Tax inhibition of p53 transcription function is simply due to competition for available CBP in the HTLV-1-infected cell. If this model is correct, Tax could promote progression through the cell cycle in the presence of DNA damage, and the resulting increase in genetic mutations could eventually lead to malignant transformation. Alternatively, the constitutive phosphorylation of p53 in HTLV-1 infected T-cells may preclude p53 interactions with the basal transcription factor TFIID (TBP), thus inhibiting p53 transcription function (Pise-Masison et al., 1998b). Future studies may help determine whether Tax modulation of p53 function is due to direct competition for CBP, or indirect manipulation of cellular signal transduction pathways.

## **V. Potential anti-viral therapeutic approaches**

The etiological link of adult T-cell leukemia (ATL) to HTLV-1 has created a field of research committed to understanding the pathogenesis associated with HTLV-1 infection. In particular, the observations that the HTLV-1-encoded Tax protein is required for viral replication, and by itself has the ability to transform cells, has led to intense studies addressing the molecular mechanisms of Tax function. A more complete understanding of how Tax activates HTLV-1 transcription, deregulates cellular gene expression through CBP, and circumvents cell cycle checkpoints will hopefully provide a foundation for the design and development of therapies aimed at the inhibition of viral replication and HTLV-1-mediated malignant transformation.

Tax transactivation of HTLV-1 is necessary for efficient viral replication. Since viral transcription also drives Tax expression, the high-level Tax expression that is probably necessary for cellular transformation is also dependent upon this process. These two observations make abrogation of HTLV-1 transcription an attractive target for therapeutic approaches. HTLV-1 transcription is very complicated because it involves intricate protein-protein and protein-DNA interactions between viral and cellular components. Studies elucidating these interactions have been challenging, but also have exposed targets for anti-Tax therapies that may inhibit Tax transactivation and Tax-mediated malignant transformation. The discovery that minor groove binding drugs like chromomycin can

preclude Tax-enhancement of CREB binding (Lenzmeier et al., 1998; Lundblad et al., 1998) and Tax recruitment of CBP (Lenzmeier et al., 1998) to the viral CREs, suggests that interruption of the Tax-DNA minor groove interaction may be a viable approach to inhibit HTLV-1 transcription. Unfortunately, the low sequence-specificity and pleiotropic effects of chromomycin (reviewed in Zimmer and Wahnert, 1996) make this molecule undesirable as an anti-Tax therapeutic. The recent development, however, of sequence-specific minor groove binding polyamides (Trauger et al., 1996; White et al., 1998; Kielhopt et al., 1998), which specifically alter gene expression in vivo (Gottesfeld et al., 1997), may provide a potentially viable therapeutic approach for inhibition of Tax transactivation through interruption of the Tax-DNA interaction.

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