DISSERTATION

CHROMATIN-DEPENDENT MECHANISMS OF CBP/p300 COACTIVATOR FUNCTION IN HTLV-I TAX TRANSACTIVATION

Submitted by
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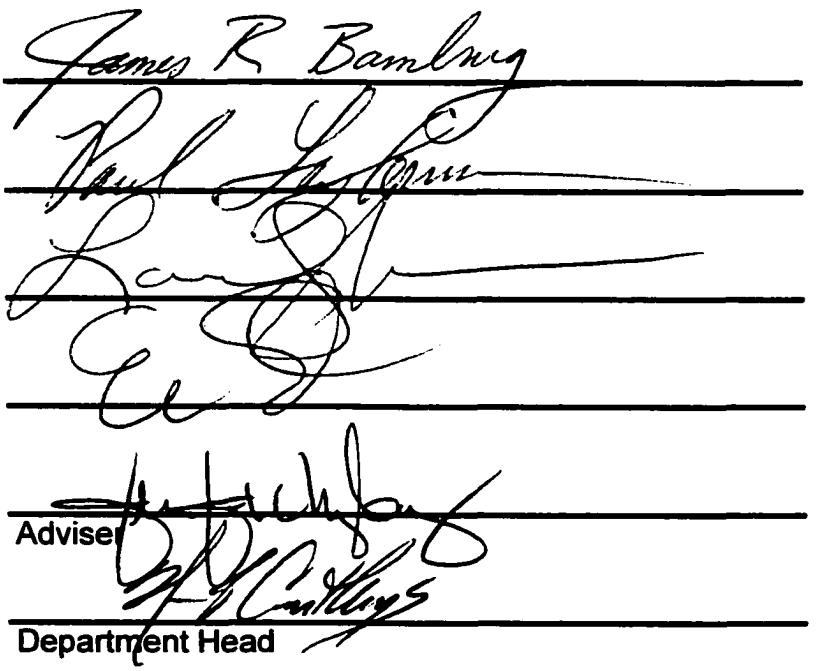

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ABSTRACT OF DISSERTATION

CHROMATIN-DEPENDENT MECHANISMS OF CBP/p300 COACTIVATOR FUNCTION IN HTLV-I TAX TRANSACTIVATION

Efficient transcription of the human T-cell leukemia virus (HTLV-I) genome requires Tax, a virally encoded oncogenic transcription factor, complexed with the cellular transcription factor CREB and the coactivators p300/CBP. To examine the role of Tax transactivation in a chromatin context, we used chromatin assembled with recombinant core histones. Using this chromatin-based transcription system, we typically saw greater than 100-fold activation of transcription in the presence of Tax, CREB and p300. We found that transcription from chromatin templates was dependent on acetyl CoA. To test the hypothesis that this effect was due to the role of acetyl CoA in histone tail acetylation, we generated chromatin templates selectively lacking amino terminal histone tails. Compared with wild type chromatin, these tailless chromatin templates demonstrated enhanced transcriptional activation by Tax and CREB, with significantly reduced dependence on acetyl CoA. However, Tax/CREB activation from tailless chromatin templates retained a substantial requirement for acetyl CoA. Additionally, Tax transactivation from tailless templates was refractory to stimulation by endogenous p300, suggesting that tail deletion and acetylation are functionally equivalent. Importantly, p300 did not stimulate

transcription from unassembled DNA templates, indicating that p300 functions by a chromatin-dependent mechanism in Tax-transactivation.

We also investigated the mechanism of CBP/p300 recruitment to the HTLV-I promoter. Tax has been characterized previously to interact with the KIX domain of CBP/p300, but this interaction has not been dissected in a functional context. Using polypeptides to inhibit CBP/p300 recruitment to the DNA-bound Tax/CREB complex, we found that the KIX domain is a primary mediator of CBP/p300 coactivator function on the HTLV-I promoter. Additionally, we found that Tax/CREB transcription strongly requires CBP/p300, since the polypeptides potently inhibited the function of CBP/p300 endogenous to the nuclear extract used in these studies.

We used Lys-CoA, a selective inhibitor of CBP/p300 acetyltransferase function, to further investigate the contribution of endogenous CBP/p300 in Tax-transactivation. Our data suggest that CBP/p300 may act in concert with other acetyltransferases to acetylate histone tails during Tax- transactivation. Furthermore, we discovered that CBP/p300 acetyltransferase activity is required on tailless chromatin templates, and is targeted at an unknown substrate whose acetylation is critical to Tax-transactivation.

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CHAPTER 1
INTRODUCTION TO HTLV-I TRANSCRIPTIONAL REGULATION

1.1 HUMAN T-CELL LEUKEMIA VIRUS TYPE I

Human T-cell leukemia virus, type I (HTLV-I) was discovered in 1980, and is the first human retrovirus to be identified (173). It is estimated that 10-20 million people worldwide are infected with the virus, with heavy distribution in endemic areas including Japan and the Caribbean basin. Although the majority of persons infected with HTLV-I will be lifelong, asymptomatic carriers of the virus, viral infection is strongly associated with the pathogenesis of two distinct diseases: adult T-cell leukemia (ATL) and tropical spastic paraperesis/HTLV-I-associated myelopathy (TSP/HAM). HTLV-I is considered to be an exogenous virus, as it is not transmitted to the children of infected people through the germline. The transmission of HTLV-I requires direct cell-cell contact; HTLV-I can be transmitted through sexual contact and contact with contaminated blood, but the major route of transmission appears to be mother-to-child either in utero or through breast feeding.

1.1a VIRAL GENOME AND LIFE CYCLE

HTLV-I is a member of the retrovirus family, which includes lentiviruses such as HIV-1, and betaretroviruses such as mouse mammary tumor virus.

HTLV-I has recently been classified as a member of the deltaretrovirus genus within the retrovirus family, and is closely related to HTLV-II (which is not associated with disease), as well as Simian T-cell lymphotropic virus and Bovine leukemia virus (reviewed in 26). HTLV-I infects mature $CD4^+$ and $CD8^+$ T-cells, via interaction with an unknown cell surface receptor. The HTLV-I virion contains two copies of the single stranded viral RNA genome, which direct the production of proteins necessary to the life cycle of the virus (34, 188, 190). As with other prototypical retroviruses, the HTLV-I genome encodes for structural proteins including the nucleocapsid (NC), capsid (CA), and matrix (MA) proteins in the *gag* region, and the transmembrane (TM/gp 21) and surface (SU/gp 46) glycoproteins within the *env* region (Fig. 1.1). The *pol* region of the genome encodes for reverse transcriptase (RT), integrase (IN) required for integration of the viral DNA into the host genome, and protease (which cleaves polycistronic viral proteins). The viral genome also encodes for a unique transcriptional activator Tax (123, 198), and Rex, a protein involved in mRNA trafficking (191).

The viral genome features identical long terminal repeat (LTR) regions at each end of the genome that contain promoter sequences important for the regulation of viral transcription (34, 95, 169, 188, 190). The U3 region of the LTR contains three 21 bp repeat sequences that are highly conserved relative to each other and among various isolates of the virus (95, 169). These sequences are called viral CREs, because they resemble cellular CRE (cyclic AMP response element) enhancers within their core sequences. The viral CREs are responsive to the viral transcription factor Tax, and are critical for the expression of viral

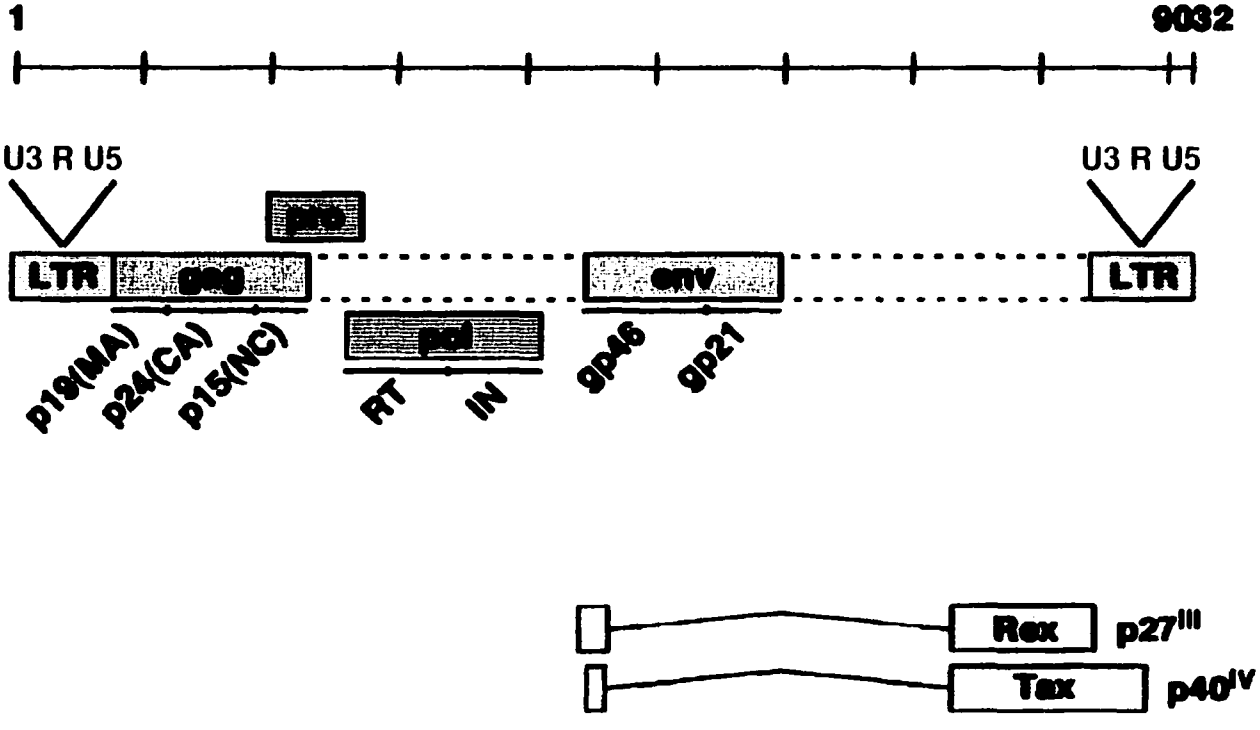


Figure 1.1. **The HTLV-I genome.** Proteins encoded by the viral genome, arranged relative to the coordinates of their respective coding sequences, are illustrated. This figure has been modified from Grant et al. 2002 (66).

genes. Mutagenesis studies have revealed that at least two of the three viral CREs must be intact in order to mediate activated viral transcription (169).

Once inside the cell, the viral RNA genome is reverse transcribed, and the resulting double stranded DNA inserts randomly into the chromosomal DNA of the host (189, 234). Following integration, the virus is thought to undergo an extended period of latent infection, with viral propagation achieved mostly via a passive mechanism through mitotic division of the infected cell. The second phase of the HTLV-I life cycle, involving the activation of viral gene expression leading to production of infectious virions, is triggered by an ill-defined cellular stimulus, and may be associated with the development of ATL or TSP/HAM.

1.1b HTLV-I AND ADULT T-CELL LEUKEMIA

HTLV-I was originally isolated from cell lines derived from a person incorrectly diagnosed with cutaneous T-cell lymphoma/mycosis fungiodes (173). Since that discovery, evidence of viral infection has been found repeatedly in people diagnosed with adult T-cell leukemia (ATL) and in people living in areas endemic for HTLV-I infection that are asymptomatic carriers of the virus (81, 98, 174, 178, 181, 234). Evidence of monoclonal integration of the viral genome in ATL transformed cells demonstrates unequivocally that HTLV-I is the causative agent of the leukemia (235). ATL develops in only a very small percentage (1-3%) of people infected with HTLV-I. This observation, and the finding that development of the disease typically follows decades of latent viral infection, suggests that HTLV-I infection per se is insufficient for initiating malignant

transformation. The mechanism by which HTLV-I causes ATL is unclear, but is believed to involve widespread Tax-mediated disruption of cellular regulatory processes, including signal transduction, DNA repair, and apoptosis, leading to cellular transformation (reviewed in 233). ATL is characterized by uncontrolled proliferation of transformed T-lymphocytes having lobulated or flower shaped nuclei, and the infiltration of leukemic cells to produce skin and bone lesions. ATL is a highly aggressive and fatal leukemia subtype that is unresponsive to most chemotherapy regimens, although there has been some progress in chemotherapy development in recent years (19).

1.1c HTLV-I AND TROPICAL SPASTIC PARAPERESIS

A second disease, tropical spastic paraperesis/HTLV-I associated myelopathy (TSP/HAM) has been strongly correlated with HTLV-I infection (reviewed in 66). Unlike ATL, this disease involves progressive degeneration of motor function as a result of demyelination of the central nervous system. The precise mechanism by which HTLV-I causes TSP/HAM is unclear. Clinical observations suggest that TSP/HAM is not caused directly by the overproliferation of infected T-cells, but rather by a strong immune response to infected cells that causes chronic inflammation in the central nervous system. For example, TSP/HAM patients develop autoantibodies directed against neurons, but asymptomatic people infected with HTLV-I and uninfected people do not (130). A recent study demonstrated that the HTLV-I-related autoimmune response observed in TSP/HAM patients is directed against hnRNP-A1, a

cellular protein involved in mRNA transport (131). Interestingly, anti-Tax antibodies recognize hnRNP-A1, despite the two proteins having no obvious amino acid sequence similarity. Autoantibodies against hnRNP-A1 were shown to inhibit neuron firing in vitro, suggesting a correlation between HTLV-I infection, autoimmunity and neurological damage. It is likely that additional autoantibodies, with cross-reactivity to HTLV-I proteins, will be discovered which play a role in TSP/HAM pathology. However, at the present time it is not known what factors determine whether a person infected with HTLV-I will develop TSP/HAM, ATL, or be a lifelong, asymptomatic carrier of the virus.

1.2 TAX-MEDIATED ACTIVATION OF HTLV-I TRANSCRIPTION

The mechanism by which HTLV-I mediates leukemogenesis differs from those of other oncogenic viruses. HTLV-I integrates randomly into the host cell's genome; as a result the virus does not consistently disrupt any particular gene that would directly cause ATL development. Unlike other transforming viruses, HTLV-I does not encode any proteins that resemble cellular oncogenes. Instead, the HTLV-I Tax protein is responsible for the development of ATL, as several in vitro and in vivo studies demonstrate that the Tax gene alone is sufficient to immortalize cells, induce the development of a variety of tumors, and cause leukemia (39, 67, 68, 158).

The 353 amino acid Tax protein is essential for transactivation of the HTLV-I LTR, leading to the expression of viral genes (27, 33, 46, 49, 200, 201). However, Tax alone cannot interact with the LTR to stimulate viral transcription

(105, 239). Instead, Tax participates in specific protein-protein and protein-DNA interactions to build a complex of cellular and viral proteins that will direct the high level expression of the viral genome.

1.2a TAX-CREB INTERACTIONS

Early studies of the HTLV-I LTR showed that it contains multiple viral CRE enhancer elements that bind several cellular proteins. These proteins were later characterized to be members of the ATF/CREB family of transcription factors (5, 48, 89, 148, 160). Of the ATF/CREB family members, the role of CREB in HTLV-I transcriptional regulation has been studied in the greatest detail. CREB is a 43 kDa protein that recognizes and binds CRE enhancer sequences of genes involved in the cAMP signaling pathway. The transcriptional activity of CREB is regulated by protein kinase A through phosphorylation at serine 133 (149). In addition to the kinase-inducible domain, CREB has several other functional domains that are important for DNA binding, dimerization, and interaction with general transcription factors (reviewed in 195).

In HTLV-I gene activation, CREB dimers recognize and bind an 8 bp sequence located within the core of each viral CRE, which represents an off-consensus CRE site (7, 239). The binding affinity of CREB for the viral CRE is much weaker than for the consensus CRE, due to single base pair changes within one of the half sites of the viral CRE core (25). However, Tax strongly increases the binding affinity of CREB for the viral CRE, through direct protein-protein interactions with CREB (7, 25, 48, 230, 231, 239, 240). Quantitative

electrophoretic mobility shift assays have demonstrated that Tax enhances CREB binding to the viral CRE by stimulating CREB dimerization, presumably through interactions with the basic leucine zipper domain of CREB (2, 7, 18, 48, 58, 207, 220, 231, 232, 239). Tax does not appear to interact with the DNA binding domain of CREB (207). The amino terminus of Tax, as well as amino acids within the carboxy terminus, appears to be critical for mediating Tax-CREB interactions (1, 65, 231). Interestingly, phosphorylation of amino acids 300 and 301 within the carboxy-terminal CREB-interacting domain of Tax has been shown to be critical for HTLV-I activation in vivo, but the kinase responsible for Tax phosphorylation has not been identified (21). A schematic illustrating the functional domains of Tax and CREB is presented in figure 1.2.

Although Tax stimulates CREB binding to the viral CRE, it has no effect on the affinity of CREB for cellular CRE sequences, and does not stably interact with the CREB-cellular CRE complex (25, 117, 230, 232). This indicates that the protein-protein interactions between Tax and CREB are necessary, but not sufficient, for the formation of the high affinity ternary complex on the HTLV-I promoter. Instead, direct interactions between Tax and GC-rich DNA sequences flanking the viral CRE core appear to be important for stabilizing the ternary complex, and are critical for Tax function in vitro and in vivo (7, 25, 50, 127, 148, 166, 232).

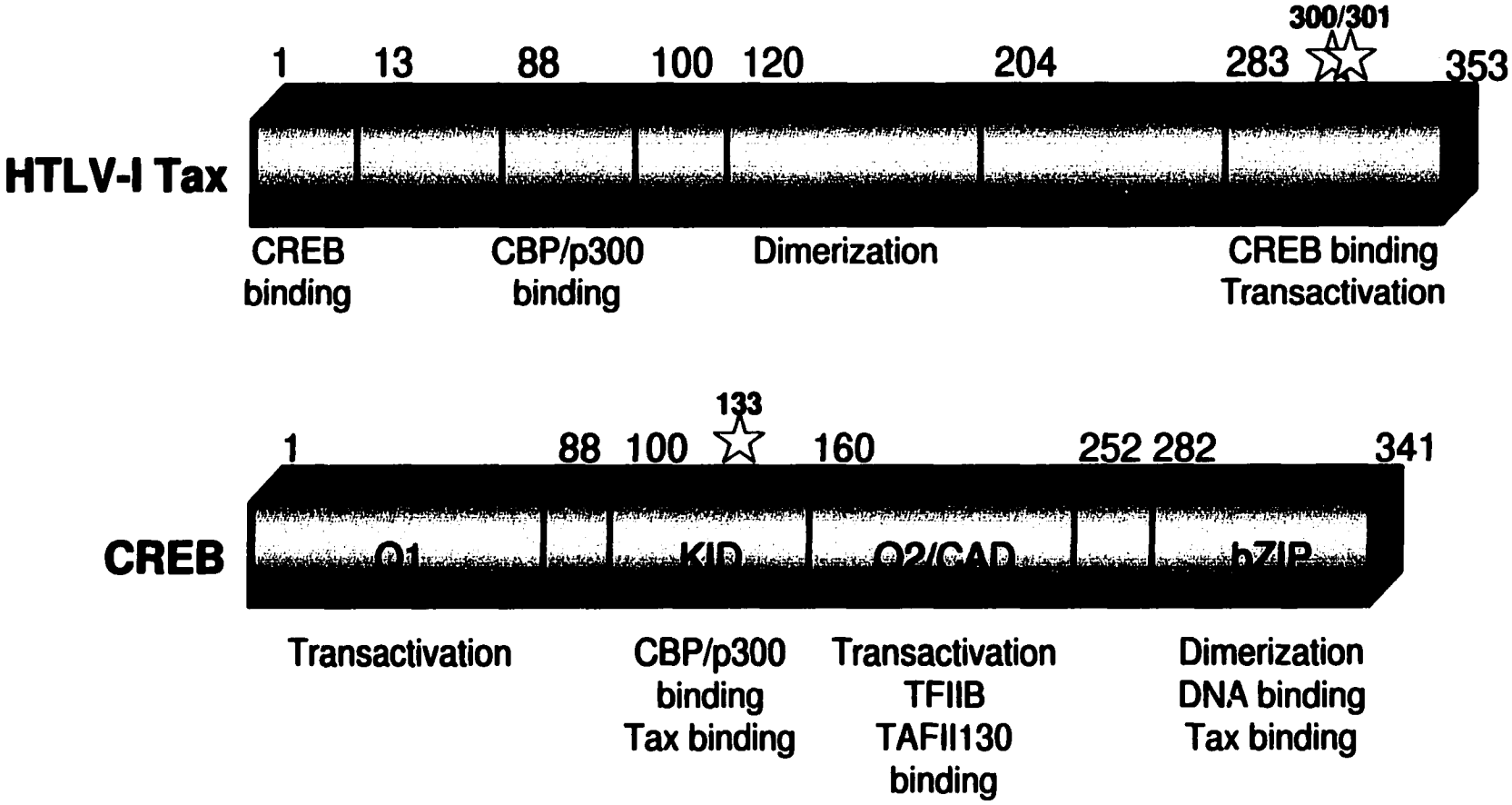


Figure 1.2. **Functional domains of Tax and CREB.** The approximate amino acid coordinates of the functional domains of Tax and CREB are illustrated, together with sites of phosphorylation*.

1.2b TAX-DNA INTERACTIONS

Within the viral CRE, the GC residues flanking the 8 bp viral CRE core sequence are necessary for Tax-mediated formation of the ternary complex and Tax-activated transcription. The Tax protein directly binds the viral CREs at these GC residues, but does not bind within the CRE core (104, 105, 126, 127, 142). Tax binding is inhibited using sequence-specific minor groove binding drugs, indicating that unlike CREB, which binds the CRE sequence in the major groove, Tax contacts the viral CRE flanking sequences within the minor groove of the DNA (126, 127, 142). Tax contacts flanking sequences both upstream and downstream of the CRE core, indicating that Tax may bind the viral CRE as a dimer (105, 126, 127). This hypothesis is strengthened by observations that Tax can dimerize in solution, and that dimerization is important for Tax-dependent complex formation and transcriptional activation (93, 94, 208). However, Tax activation apparently requires only one of the two flanking sequences (50, 126, 127, 148). Lacking structural data, the question of whether a single molecule of Tax recognizes both flanks of the viral CRE, or whether Tax binds the DNA as a dimer, is unresolved.

Tax participates in protein-protein interactions with CREB, and protein-DNA interactions with the viral CRE to form a ternary complex that binds the HTLV-I promoter with high affinity. However, formation of this complex alone is insufficient for mediating high levels of viral transcription. Instead, the cellular coactivators CBP/p300 are implicated as required factors for the activation of Tax/CREB transcription.

1.3 THE CBP/p300 COACTIVATORS

The 2,414 amino acid protein p300 was originally identified by co-immunoprecipitation to be a binding partner for the viral oncoprotein E1A (45, 203). The 2,441 amino acid protein CBP was later independently identified through interaction with CREB (37). Soon after these initial characterizations, it became apparent that CBP and p300 have highly homologous amino acid sequences, exhibit a significant degree of evolutionary conservation, and share a number of functional motifs (11). These observations have resulted in CBP and p300 being considered functionally interchangeable in the literature. However, despite their high degree of sequence homology, CBP and p300 are not absolutely functionally identical. This is particularly apparent in studies of CBP and p300 knockout mice, which exhibit distinct phenotypes according to the relative dose of each protein (115).

CBP/p300 participate in the regulation of a number of genes through direct binding of a multitude of transcriptional activators (Fig. 1.3). The promoters that utilize CBP/p300 are involved in diverse cellular processes; therefore, it is not surprising that CBP/p300 have important roles in regulating embryonic development, cellular proliferation and differentiation, DNA damage repair and apoptosis (78, 109, 115, 135, 163, 177, 180, 209, 228, 236). Additionally, mutations of CBP/p300 have been strongly linked to certain leukemia subtypes (59, 82, 107, 108, 120, 167, 184, 199), and to Rubinstein-Taybi syndrome, a CBP/p300 haploinsufficiency disorder in which affected persons are not only

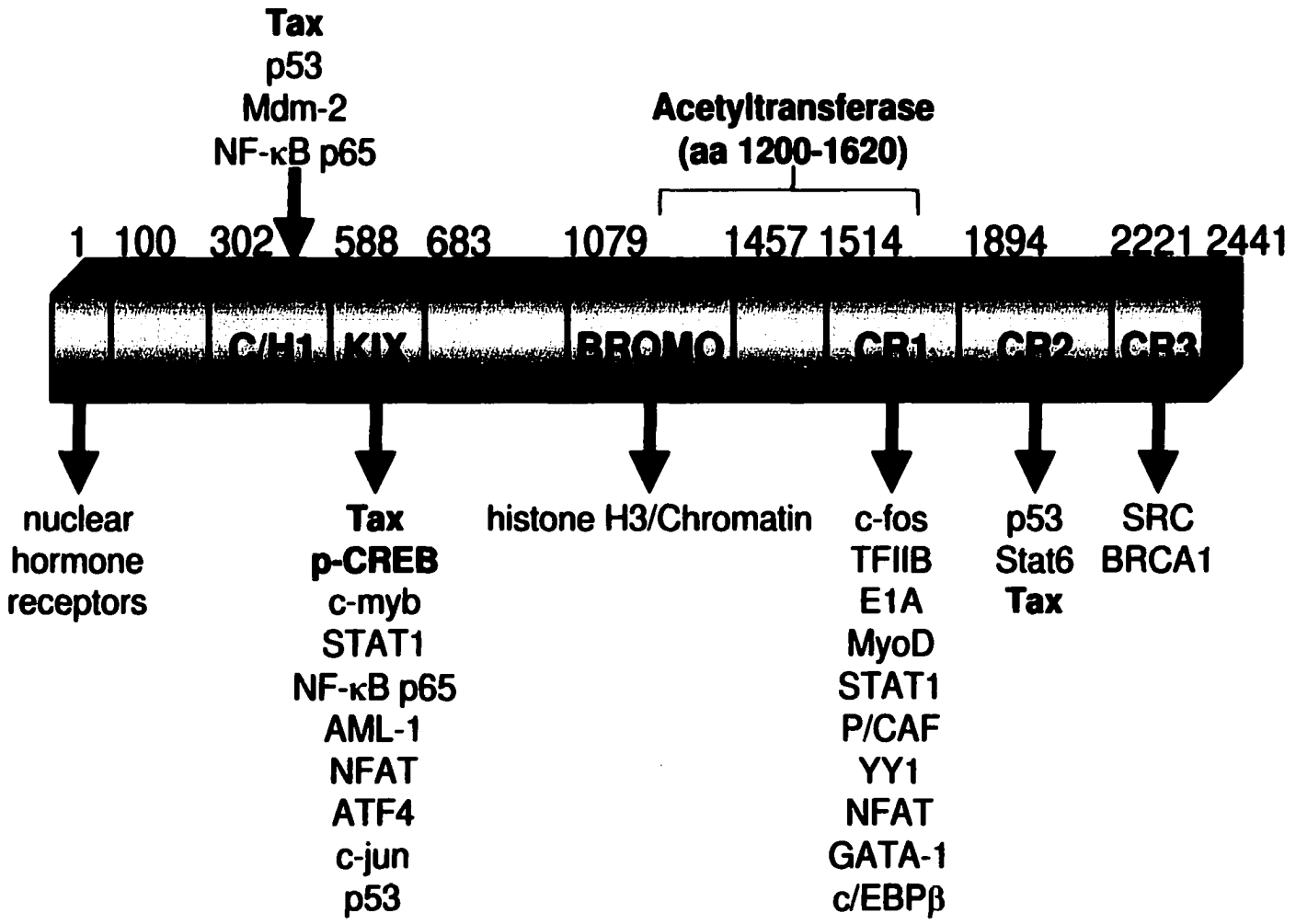


Figure 1.3. **Functional interactions of CBP/p300.** Due to the high degree of homology between CBP and p300, only the amino acid coordinates of the functional domains of CBP are represented.

developmentally abnormal, but also highly susceptible to tumor development (60, 153, 162, 172).

CBP/p300 are coactivators, as they activate transcription at a diverse number of promoters through interactions with DNA-bound transcription factors, rather than directly binding enhancer elements at target promoters (10, 45, 118, 141). The following sections summarize the details of CBP/p300 interactions with Tax and CREB, and the known functions of CBP/p300 that may contribute to robust Tax transactivation of the HTLV-I genome.

1.3a CBP/p300 INTERACTIONS WITH TAX AND CREB

Tax directly interacts with several domains of CBP/p300. The Tax-KIX interaction, involving amino acids 588-683 of CBP (aa566-663 of p300), was the first to be identified and remains the best characterized of all the Tax-CBP/p300 interactions (22, 58, 77, 117). The isolated KIX domain of CBP/p300 binds to Tax/CREB complexes on the viral CRE and acts to stabilize the ternary complex (58, 77, 117, 207, 225). Several lines of evidence suggest that direct Tax-KIX interactions mediate the binding of CBP/p300 to the Tax/CREB/viral CRE DNA complex. Purified Tax specifically binds KIX in solution (22, 117, 225). Additionally, amino acid substitutions in Tax (K88A and V89A), which do not affect Tax-CREB interactions, abolish the ability of Tax to bind KIX and to activate Tax-dependent transcription in vitro and vivo (77, 116, 126). CBP/p300-CREB interactions require phosphorylation of CREB at ser-133 by protein kinase A, while KIX binding to the ternary complex is CREB phosphorylation-

independent (58, 117, 225). Similarly, while activation of CREB-responsive genes by CBP/p300 is dependent on CREB phosphorylation (12), activation of the HTLV-I genome by Tax does not require CREB phosphorylation (117, 118).

Direct CREB-KIX interactions may also be involved in Tax/CREB transactivation, however. For example, transcription from the HTLV-I LTR is activated upon stimulation of the cAMP-response pathway, which results in CREB phosphorylation (50, 89, 212). In addition, KIX binds to the ser-133 phosphorylated CREB/Tax complex with >10-fold-higher affinity than to the CREB/Tax complex (58). These observations suggest that CREB and Tax may interact with KIX simultaneously, or that the phosphorylation of CREB somehow alters the conformation of the complex to enhance the interaction between Tax and KIX. The question of whether Tax and ser-133 phosphorylated CREB co-occupy the KIX binding site is unresolved. Interestingly, Tax also strongly interacts with the C/H1 domain of CBP/p300, which is located immediately adjacent to KIX at amino acids 302-451 of CBP (124). Perhaps an extended region consisting of the contiguous C/H1 and KIX domains allows for simultaneous, direct interactions of both ser-133 phosphorylated CREB and Tax with CBP/p300 at the HTLV-I promoter.

In addition to the interactions with the C/H1 and KIX domains, Tax also directly binds a carboxy-terminal region of CBP/p300 called CR2 (aa1892-2212). This interaction appears to be distinct from the interaction between Tax and KIX, since a point mutant of Tax (K88A) that does not bind KIX binds CR2 with an affinity comparable to wild type Tax. Conversely, a Tax point mutant (Y312E)

that does not bind CR2 binds the contiguous C/H1-KIX domains of CBP with wild-type affinity (187). It is unclear whether a single molecule of Tax can form multiple simultaneous contacts with full-length CBP/p300. However, it is intriguing to consider that multiple interaction domains within CBP/p300 may allow the coactivators to act as bridging factors between the repeated viral CREs on the HTLV-I promoter by contacting Tax/CREB in more than one viral CRE-bound complex (Fig. 1.4).

1.3b INTERACTIONS BETWEEN CBP/p300 AND THE GENERAL TRANSCRIPTION MACHINERY

Recruitment of CBP/p300 by transcription factors to target promoters results in high levels of transcriptional activation. CBP/p300 may stimulate transcription via several mechanisms, including recruitment of components of the basal transcription apparatus. Interactions between CBP/p300 and RNA polymerase II have been demonstrated by a number of methods, including chromatographic co-fractionation, co-immunoprecipitation, and in vivo co-localization (36, 99, 155, 157, 219, 229). A region of the carboxy terminus of CBP, composed of amino acids 1805-1890, is responsible for polymerase binding (36, 155, 157). Interestingly, CBP/p300 interact specifically with the hypophosphorylated, or nonelongating, form of the polymerase (36, 219). The interactions between CBP/p300 and RNA polymerase II appear to occur in the context of the large RNA polymerase II holoenzyme complex; CBP/p300 make additional contacts with other components of this complex, including TFIIE and

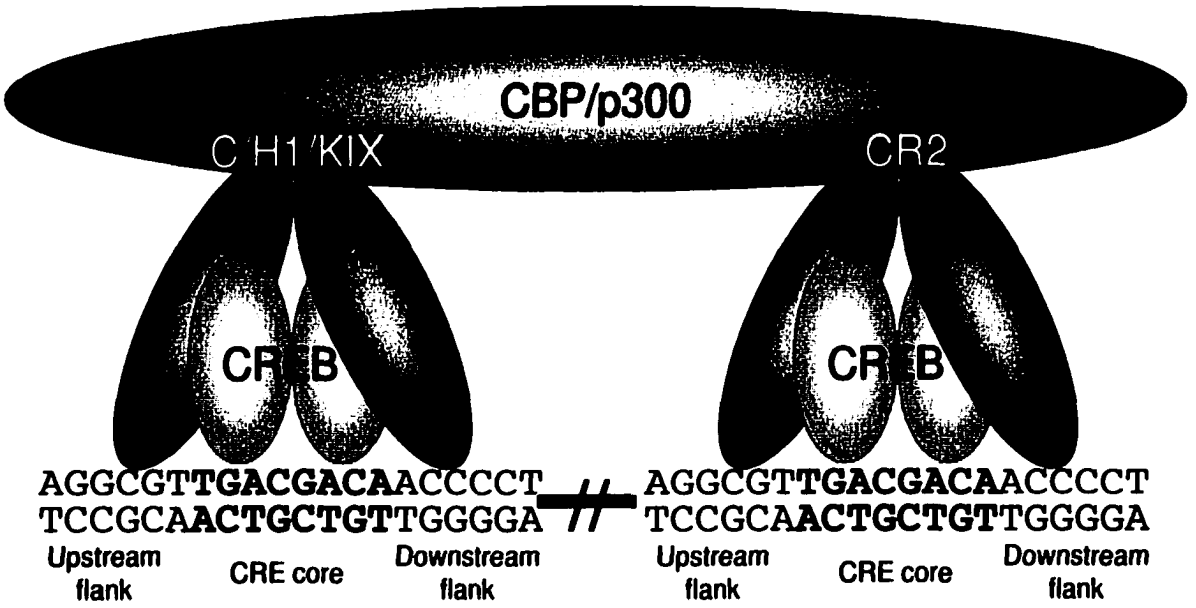


Figure 1.4. **Model for bipartite binding of CBP/p300 to the HTLV-I LTR.** Potential interactions between Tax/CREB complexes, bound to their respective sequences on the viral CREs, with distinct domains of CBP/p300.

SRB7 (103, 155, 229). While one group has reported a direct interaction between CBP and TFIIB by GST-pulldown assay (118), other groups have not observed an interaction between the two proteins (99, 103). Several studies have suggested that the interactions between CBP/p300 and the holoenzyme result in enhanced recruitment of RNA polymerase II, and efficient assembly of preinitiation complexes, at target promoters (112, 155, 229). In this way, CBP/p300 may act as "bridging factors" to allow communication between transcriptional activators bound to upstream enhancer elements and the basal apparatus assembled at the core promoter.

1.3c CBP/p300 ACETYLTRANSFERASE ACTIVITY

The intrinsic acetyltransferase activity of CBP/p300 is extremely well characterized, and is thought to contribute significantly to CBP/p300 coactivator function on promoters. CBP/p300 were initially discovered to be histone-specific acetyltransferases (or HATs) that target multiple residues on the amino terminal regions of the core histone proteins H2A, H2B, H3 and H4. CBP/p300 acetylate histones both free in solution, and when complexed with DNA in the form of nucleosomes (16, 161, 185). In addition, CBP/p300 directly bind other acetyltransferase proteins including P/CAF (p300/CBP-associated factor) (226) and SRC-1 (steroid receptor coactivator-1) (202) and thereby possess associated as well as intrinsic enzymatic activity.

The acetyltransferase activity of CBP/p300 is integral to its coactivator function in many transcription systems, including ser-133 phosphorylated CREB

(13), thyroid hormone receptor (132, 133), the pituitary-specific factor Pit-1 (224), and estrogen receptor (113), among others. However, not all promoters that utilize CBP/p300 require acetyltransferase function, perhaps suggesting that CBP/p300 recruitment of RNA polymerase II holoenzyme is the primary role of CBP/p300 in those contexts (see for example 113, 132, 144).

Several studies have shown that CBP/p300 acetyltransferase activity is directly targeted to promoters by DNA-bound transcription factors, and that this recruitment is correlated with increased levels of histone acetylation (31, 102, 114, 137). In addition, Martinez-Balbas et al. (144) demonstrated that tethering the isolated acetyltransferase domain of CBP to a promoter (via a Gal4-CBP HAT fusion) was sufficient to mediate transcriptional activation. Interestingly, a recent study reported a direct interaction between the bromodomain of p300 and promoter templates, suggesting that binding of transcriptional activators may not be the only mechanism by which CBP/p300 acetyltransferase activity is targeted to genes (143).

In addition to the well-characterized HAT activity of CBP/p300, there is a growing list of CBP/p300 "FAT" (factor acetyltransferase) substrates, including, EKLF (237), HMG(I)Y (152), HIV-1 Tat (101),the p50 subunit of NF-kB (51), p53 (69), and the general transcription factors TFIIEβ and TFIIF (84), among others. Acetylation can alter the DNA-binding and protein-protein interaction properties of targeted factors. However, in many cases, the functional consequences of factor acetylation are unknown. Because CBP/p300 can modify both histones and transcription factors, one open question is whether the coactivators are

primarily HATs or FATs. The answer will likely vary according to whether the acetylation of transcription factors is required for activation at a given promoter, which might give primacy to the FAT function of CBP/p300 in that context.

Interestingly, several studies have demonstrated that CBP/p300 have no coactivator function on naked DNA (13, 112, 114, 119, 137, 154). This suggests that unique components of the physiological chromatin DNA template, such as histones, are required for CBP/p300 activity.

1.4 ROLE OF CHROMATIN IN GENE EXPRESSION

The eukaryotic genome is hierarchically packaged into highly compact DNA-protein complexes that are ultimately organized to form chromosomes (Fig. 1.5A). The characteristics of higher order chromatin structures, and their roles in the regulation of gene expression, are poorly understood. However, the fundamental unit of DNA packaging, the nucleosome, has been studied in great detail. The nucleosome is composed of two dimers of the core histones H2A and H2B, complexed with a tetramer of the core histones H3 and H4; the resulting octamer assembles with approximately 146 bp of DNA to form a nucleosome core particle (Fig. 1.5B). When nucleosomes are arrayed on a DNA template, linker DNA exists between tandem nucleosomes that generates a repeat length of approximately 200 bp of DNA per nucleosome (although this exact repeat length varies among species). This conformation is often referred to as an "11 nm fiber". In addition to the four core histones, linker histones (such as histone

H1) associate with the linker DNA, and are thought to be critical for mediating the packaging transition from an extended 11 nm fiber to a more compact 30 nm chromatin fiber. The following sections will focus on the impact of nucleosomes on transcriptional activation in the context of regulatory processes involving the amino termini of the core histone proteins.

1.4a ROLE OF HISTONE TAILS IN CHROMATIN STRUCTURE AND TRANSCRIPTIONAL REGULATION

Each of the four core histone proteins is composed of two domains: a highly-structured histone fold domain, or core globular domain, and the amino terminal histone tail, an unstructured region that extends beyond the nucleosome core (138) and is defined by susceptibility to trypsin digestion (23) (Fig. 1.5B). It is hypothesized that the major contribution of the core globular domain is to maintain the structural integrity of the nucleosome core particle. The amino terminal tails appear to play important roles in regulating transitions between the 11 nm nucleosomal array and higher order chromatin structures that may be important for the regulation of gene expression.

Chromatin is generally repressive to transcription. Several in vitro studies have shown that as nucleosomes are deposited onto a DNA template, the transcriptional activity of the template decreases dramatically relative to naked DNA (74, 75, 121, 165, 213). This decrease in transcription is not due to the inability of an elongating RNA polymerase to negotiate the chromatin template, but rather appears to be the result of decreased initiation and decreased rate of

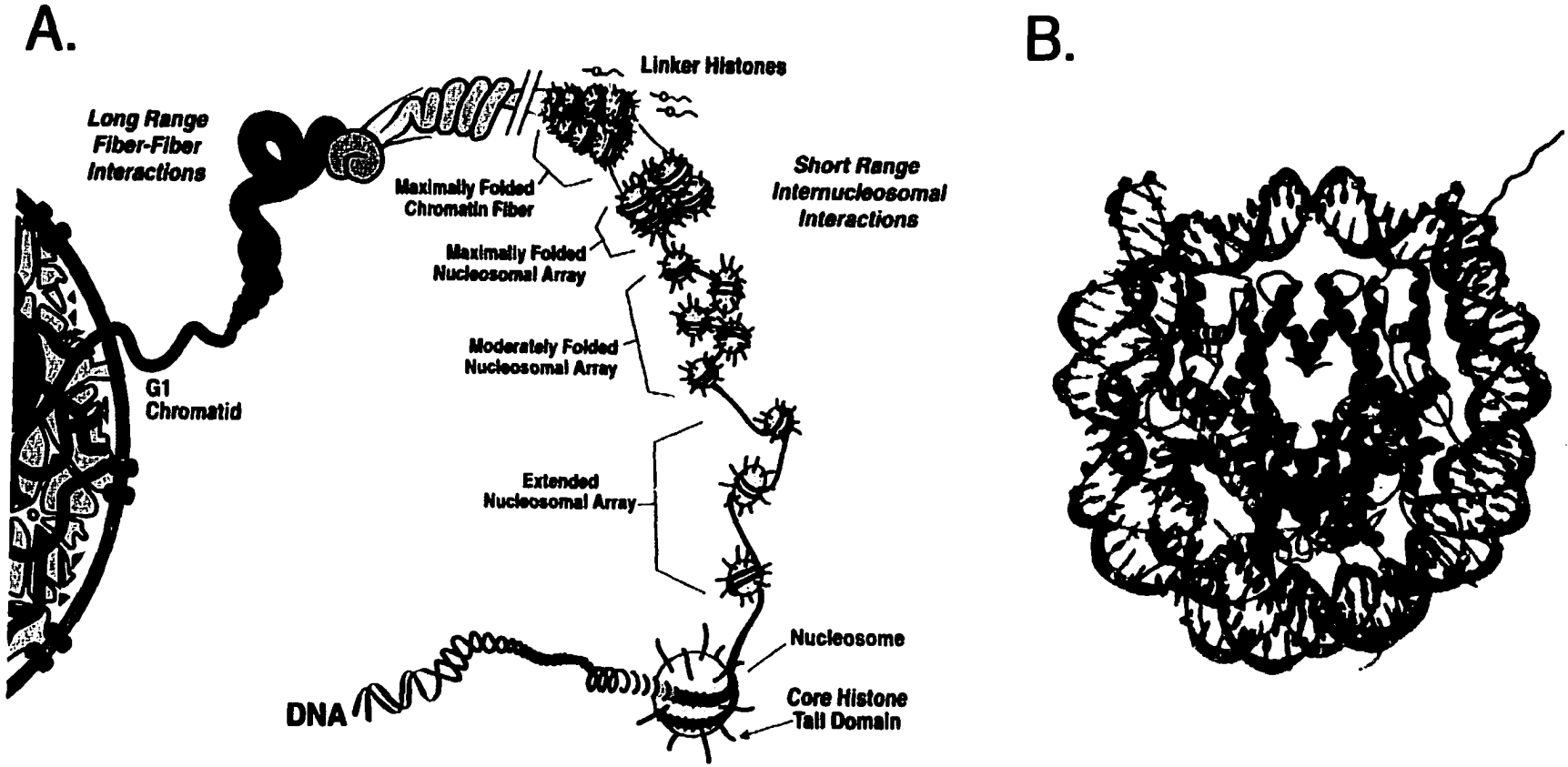


Figure 1.5. **Chromatin organization and structure.** A. Schematic representation of the hierarchical packaging of cellular DNA into chromatin. This illustration was taken from Hansen, 2002 (72). B. Crystal structure of the nucleosome core particle, as published in Luger et al., 1997 (138).

elongation, leading to lower overall processivity of the enzyme (74, 165, 179). A possible basis for transcriptional inhibition by chromatin is that histones limit the accessibility of the promoter DNA for binding by transcriptional activators. Nucleosomes have repeatedly been shown to inhibit the binding of transcription factors to target genes, including TBP (62, 83, 193), TFIIA (62), and TFIIIA (122). For example, nucleosomes inhibit the binding of TBP and TFIIA to the TATA element even when the sequence is located in the linker region of the DNA (62). Specifically, the histone tails appear to play a key regulatory role in transcriptional activation. For example, removal of the amino terminal histone tails (by trypsinization) increases the processivity and elongation rate of RNA polymerase (179). Additionally, trypsinization of the histone tails leads to greater accessibility of the DNA flanking the core particle, and facilitates transcription factor binding (62, 122, 175, 179, 217). Importantly, these effects are not due to dissociation of nucleosomes from the DNA template, since tail removal does not affect nucleosome structure, nor does it affect the stability of protein-DNA interactions or protein-protein interactions within the nucleosome core (14, 96, 151, 222).

Transcriptional activation from chromatin templates is facilitated by a process called chromatin remodeling. Chromatin remodeling is thought to play a role in transcriptional initiation by allowing for the transient exposure of nucleosome-free DNA sequences that can then bind transcriptional activators (156). Chromatin remodeling enzymes use ATP to catalyze the movement of nucleosomes on DNA, and are usually components of large, multisubunit

complexes. Multiple chromatin remodeling complexes have been identified in various organisms, including humans (hSWI/SNF, NURD and RSF), yeast (ySWI/SNF and RSC), and *Drosophila* (ACF, CHRAC, NURF), among others (reviewed in 106). Interestingly, the activities of some, but not all, chromatin remodeling complexes appear to require direct interactions with histone amino terminal tails. For example, the ACF, CHRAC and NURF complexes, which all share the ISWI ATPase as a catalytic subunit, have reduced remodeling activity on trypsinized chromatin templates and appear to require specific interaction with the histone H4 tail in order to catalyze nucleosome sliding (24, 38, 44, 56). In contrast, the RSC (yeast) and SWI/SNF (yeast and human) complexes, which do not contain ISWI, demonstrate equivalent remodeling activity on trypsinized and intact chromatin (24, 70). Together these studies suggest that histone tails may play important roles in chromatin remodeling processes. Interestingly, the proteolytic removal of the histone H2B allows uncatalyzed nucleosome sliding in vitro, suggesting potentially differential roles for particular histone tails on chromatin templates (71).

Histone tails also play important structural roles in DNA packaging (for review, see 72). The movement from an 11 nm nucleosomal array to a 30 nm fiber species is thought to reflect the transition from an open chromatin conformation in which linker DNA is accessible to a highly compacted chromatin template that is not permissive to access by the general transcription machinery. In fact, when the transition from the 11 nm array to the 30 nm fiber is recapitulated in vitro using high concentrations of magnesium chloride, high

levels of transcriptional repression are observed (75). The amino terminal histone tails appear to mediate internucleosomal contacts that are thought to be essential for the formation of the 30 nm fiber, since trypsinized nucleosomal arrays are unable to form highly compacted structures as measured by analytical ultracentrifugation (28, 47, 54, 150, 186, 210).

Overall, these studies demonstrate that histone tails play significant roles in mediating the structural transitions of the chromatin fiber and facilitating access of the transcriptional machinery to the DNA template. In the physiological environment, however, there is no mechanism for selectively and reversibly removing the histone tails in order to regulate these processes. Instead, the current evidence supports a model for the regulation of histone function through specific post-translational modification of the core histone tails.

1.4b REGULATION OF GENE EXPRESSION THROUGH HISTONE TAIL ACETYLATION

The histone tails in vivo are subject to a complex array of post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (reviewed in 63) (Fig. 1.6). These modifications have direct consequences on histone function, and may work in a combinatorial fashion to alter the transcriptional or structural properties of a chromatin template (90, 205). Among these modifications, histone acetylation has been studied in the greatest detail, and is the most closely associated with transcriptional activation.

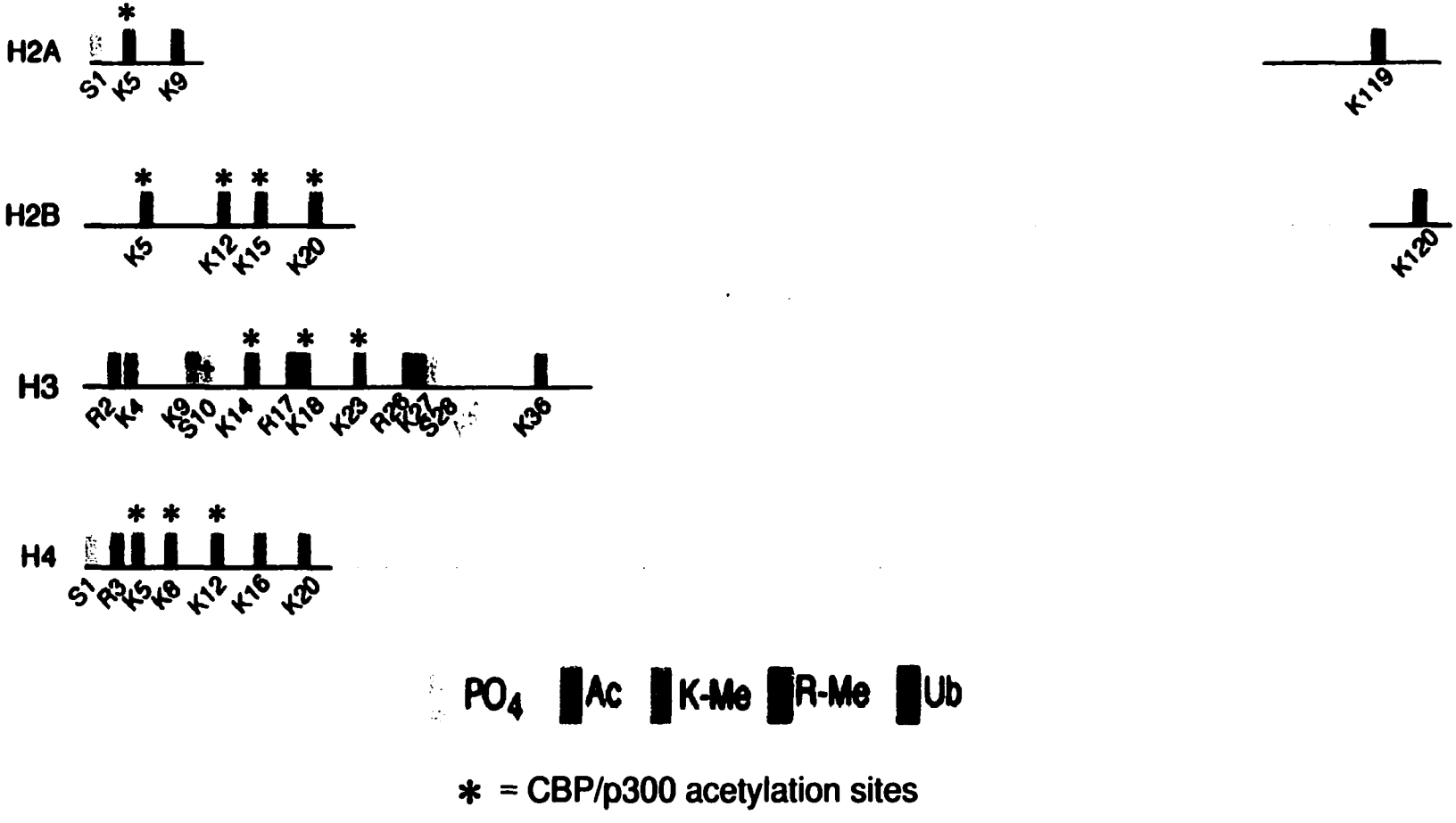


Figure 1.6. **Post-translational modifications of core histones.** The various amino acids that are post-translationally modified on the four core histones are illustrated. PO_4 = phosphorylation, Ac = acetylation, K-Me = lysine methylation, R-Me = arginine methylation, Ub = ubiquitination. In some cases, the effect of the modifications on transcription (+ activating, - repressive) is noted. Residues acetylated by CBP/p300 are indicated. This schematic was adapted from Goll and Bestor, 2002 (63).

Since 1964, histone acetylation has been strongly correlated with activated transcription (4). Since this original observation, there has been a significant amount of evidence demonstrating that reversible histone acetylation is a fundamental mechanism for transcriptional regulation. In addition to the large number of acetyltransferase enzymes (such as CBP/p300) that have been characterized as transcriptional coactivators (reviewed in 183), several histone deacetylases have been identified that act as transcriptional repressors by removing acetyl groups from histone amino terminal tails (reviewed in 159).

Interestingly, recent evidence suggests that highly acetylated chromatin templates may have properties similar to templates assembled with tailless histones. For example, templates containing hyperacetylated nucleosomes are more transcriptionally active than hypoacetylated nucleosomal templates in vitro (136, 147, 179, 211, 213). Like trypsinization of histones, hyperacetylation specifically enhances the elongation rate of RNA polymerase, and increases polymerase processivity (179). Additionally, hyperacetylated chromatin is more transcriptionally active than hypoacetylated chromatin in vivo (4).

Several factors may contribute to the increased transcriptional activity observed on hyperacetylated chromatin. Biophysical studies have revealed that hyperacetylated nucleosomal arrays are incapable of achieving the states of higher order folding and compaction that are correlated with transcriptional repression (55, 211, 221). Additionally, histone acetylation increases the ability of transcriptional activators to bind DNA (122, 193, 216). Like histone tail removal, acetylation does not appear to affect nucleosome stability or structure

(15, 134, 151, 164), and does not alter the positioning of nucleosomes on DNA (71, 122, 134, 147, 213). Furthermore, histone acetylation is not required for chromatin assembly (129). This suggests that histone acetylation may affect protein-protein interactions that are critical for transcriptional activation. This may specifically affect the transcriptional apparatus, since histone acetylation neither increases nor decreases the activity of a number of chromatin remodeling enzymes tested in vitro (24, 129). A possible exception is the human chromatin remodeling factor RSF, since histone acetylation has been shown to improve the ability of RSF to catalyze the formation of nucleosomal arrays (136).

Together, these studies demonstrate that histone tail acetylation may act in several ways to alter the transcriptionally repressive properties of chromatin and facilitate gene activation, and that these effects may be mechanistically similar to the consequences of removing histone amino terminal tails. For my thesis research, I set out to compare the effects of histone tail acetylation and histone tail removal on transcription in order to gain an understanding of the mechanisms of CBP/p300 coactivator function in Tax-transactivation on chromatin templates. To achieve this objective, I have utilized a fully recombinant chromatin assembly system that includes recombinant core histones expressed both as wild-type and amino terminal tail deletion mutants. The data presented herein demonstrate that transcriptional activation by Tax and p300 on wild type chromatin templates correlates with histone tail acetylation; however, tailless templates are also responsive to Tax. This supports the findings of previous studies, summarized above, which suggest that histone tail

deletion and acetylation may be functionally equivalent. Tax-mediated recruitment of the CBP/p300 via the KIX domain is required for high levels of Tax-transactivation in vitro. Furthermore, CBP/p300 acetylation of an unknown substrate, in addition to histone tail acetylation, is required for Tax-activated transcription. Further characterization of this CBP/p300 acetylation substrate, and investigation into the role of histone tails in the formation of transcriptionally active complexes at the HTLV-I promoter, will extend our fundamental knowledge of mechanisms of regulated gene expression on chromatin templates.

CHAPTER 2
p300-MEDIATED TAX TRANSACTIVATION FROM RECOMBINANT CHROMATIN: HISTONE TAIL DELETION MIMICS COACTIVATOR FUNCTION

Chapter two describes a study of the coactivator function of p300 in Tax/CREB transcriptional activation, examined on recombinant chromatin templates assembled with wild type core histones and histone tail deletion mutants. This work reflects a collaboration between the Luger, Laybourn and Nyborg laboratories. I performed all of the experiments shown in this chapter. W.L. Kraus provided the p300 and p300MutAT2 baculoviruses, and Karolin Luger provided all purified recombinant histone octamers. This work has been published in the journal *Molecular and Cellular Biology*, and is presented here exactly as it appeared in the journal. However, data cited as "data not shown" in the publication are provided here as supplemental figures. The citation for the publication is:

Georges, S.A., W.L. Kraus, K. Luger, J.K. Nyborg and P.J. Laybourn. 2002. p300-mediated Tax transactivation from recombinant chromatin: Histone tail deletion mimics coactivator function. Mol. Cell. Biol. **22:**127-137.

2.1 ABSTRACT

Efficient transcription of the human T-cell leukemia virus (HTLV-I) genome requires Tax, a virally encoded oncogenic transcription factor, in complex with the cellular transcription factor CREB and the coactivators p300/CBP. To examine Tax transactivation in vitro, we used a chromatin assembly system that included recombinant core histones. The addition of Tax, CREB, and p300 to the HTLV-I promoter assembled into chromatin activated transcription several hundred-fold. Chromatin templates selectively lacking amino terminal histone tails demonstrated enhanced transcriptional activation by Tax and CREB, with significantly reduced dependence on p300 and acetyl CoA. Interestingly, Tax/CREB activation from the tailless chromatin templates retained a substantial requirement for acetyl CoA, indicating a role for acetyl CoA beyond histone acetylation. These data indicate that during Tax transcriptional activation, the amino terminal histone tails are the major targets of p300 and that tail deletion and acetylation are functionally equivalent.

2.2 INTRODUCTION

Tax is a regulatory oncoprotein produced by the human T-cell leukemia virus type-I (HTLV-I) that is required for high level expression of the viral genome. The mechanism of Tax activation of HTLV-I transcription has been intensely studied over the past several years, and many of the molecular events that lead to Tax transactivation have recently been characterized (128). The HTLV-I promoter carries three highly conserved 21 bp enhancer elements, called

viral CREs, which are critical to Tax-activated transcription. The cellular transcription factor CREB binds to the core viral CRE sequence, and Tax associates with CREB through protein-protein interactions (1, 48, 65, 239). Tax also forms protein-DNA interactions with GC-rich minor groove DNA sequences that immediately flank the CREB binding site, further stabilizing the nucleoprotein complex (105, 127, 142). The Tax-containing ternary complex serves as a high affinity binding site for the recruitment of the multifunctional cellular coactivator p300/CBP (58, 117). Tax recruits the coactivator through multiple interactions with both the amino and carboxy terminal regions of p300/CBP (58, 77, 97, 117, 124, 187). The interaction between Tax and p300/CBP likely tethers the coactivator to the HTLV-I promoter, leading to high level transcription of the viral genome.

CBP and its paralog p300 are very large, highly conserved coactivator proteins that serve as central mediators in the regulation of gene expression in metazoans (64, 215). p300/CBP are conserved from *C. elegans* to humans, and appear to be involved in essentially all known pathways regulating gene expression. These include signal dependent and independent activation, cellular differentiation programs, and the execution of programmed cell death (61, 64). p300/CBP are utilized by many structurally distinct transcription factors, such as c-fos, MyoD and YY1, that represent nearly all known classes of DNA binding proteins (17, 53, 180).

The p300/CBP coactivators may function in a variety of ways to activate transcription. There is evidence that both p300 and CBP are intrinsic

components of the RNA polymerase II holoenzyme complex and are therefore brought to target promoters simultaneous with polymerase recruitment (103, 157, 229). Perhaps consistent with these observations, several studies have demonstrated that p300 acts to enhance the formation of productive transcriptional initiation complexes (41, 112, 133). Recent studies have also suggested that p300/CBP may stabilize components of the general transcription machinery, including TFIIB and TBP (40, 118). Perhaps the best characterized coactivation property of p300 and CBP is their intrinsic histone acetyltransferase (HAT) activity (16, 161). Both p300 and CBP have been shown to acetylate lysine residues in the amino terminal tails of all four core histones (H2A, H2B, H3 and H4), both free in solution, and assembled into chromatin (113, 161). Following recruitment to a promoter, these coactivators have been shown to locally hyperacetylate the amino terminal tails of nucleosomal histones assembled on the target promoter (114, 168). Although all four histones are acetylated by p300/CBP, studies have suggested that the H3 and H4 amino-terminal tails are the preferred substrates (16, 161).

Each of the four core histones consists of two structural and functional domains. The first is an α-helical "histone fold" domain that participates in formation of the histone octamer, the protein core of nucleosome. The second domain is the apparently unstructured, lysine rich amino-terminal tail. In native chromatin, these tails interact with DNA and α-helical regions of histones located outside of their nucleosome of origin. Histone tails also participate in determining nucleosome structure and DNA sequence accessibility, however removal or

acetylation of the N-terminal tails does not significantly affect histone octamer stability or nucleosome formation (14, 15, 134, 164, 197, 222). It appears that the primary structural role of the histone tails is the formation of higher order folding of the chromatin fiber, which compacts and organizes the DNA in chromosomes (28, 55, 73, 146, 197, 210). It is through their role in chromatin compaction that the histone tails exert their greatest repressive effect on transcription factor access and transcriptional activation (73-75, 211). This repression is believed to be reversed through coactivator acetylation of histone tails. This nucleosomal modification is believed to increase the accessibility of the promoter DNA to binding by regulatory proteins and/or components of the basal transcription machinery; a critical and perhaps rate-limiting step in gene activation (62, 122, 216).

The HTLV-I genome is naturally integrated into chromatin following retroviral infection, and thus transcriptional activation by Tax requires interaction with the viral CRE enhancer elements as they exist in a chromatin environment. We designed this study to analyze the mechanistic role of p300 in Tax/CREB-mediated transcriptional activation, in a chromatin context. We wished to distinguish between the functions of p300 as an acetyltransferase and as a mediator of basal factor recruitment. We also investigated the acetyl CoA requirement in Tax transcriptional activation. Furthermore, we wanted to define the point in the HTLV-I transcription process at which histone tails exert their greatest regulatory effect. To address these questions, we utilized chromatin templates assembled with recombinant core histones. This system provides a

powerful means to examine activator-coactivator interactions in a biochemically defined chromatin context. The recombinant histones in our system lack post-translational modifications, and can be manipulated to form chromatin templates lacking any or all of the histone amino terminal tails.

We found that nucleosome assembly strongly repressed transcription from the HTLV-I promoter, however, the addition of Tax/CREB countered the repression in an acetyl CoA dependent fashion, leading to a ~100-fold increase in RNA synthesis. The addition of exogenous p300 further increased transcriptional activation to a level greater than 300-fold over basal. Removal of the amino terminal histone tails enhanced Tax/CREB transcriptional activation, however, the mutant chromatin templates exhibited a reduced dependence on p300 HAT activity. This suggests that histone tail deletion functionally mimics acetylation in the transactivation process. We also examined the role of acetyl CoA in transcriptional activation from the wild type and tail mutant chromatin templates. Although tailless templates displayed decreased sensitivity to acetyl CoA when compared to wild type chromatin, transcriptional activation remained significantly dependent on the cofactor. Together, these results indicate that robust Tax transactivation is dependent upon the presence of chromatin and acetyl CoA, and is potentiated by the addition of the coactivator p300. These studies reveal the critical role of the histone tails in coactivator-mediated transcriptional activation, and suggest that the primary role for p300 in Tax/CREB transcriptional activation is the acetylation of the core histone tails.

2.3 RESULTS

2.3a TRANSCRIPTIONAL ACTIVATION BY TAX/CREB AND p300 ON UNASSEMBLED TEMPLATES IN VITRO

Several previous studies have suggested that the HTLV-I Tax protein utilizes the coactivators p300/CBP to mediate transcriptional activation from the viral promoter (58, 77, 97, 117, 124, 187). We were interested determining the mechanistic role of p300 in Tax/CREB-mediated transcriptional activation. Figure 2.1 shows the purified recombinant Tax, CREB, and p300 proteins used in this study (panels A-C). We tested the transcriptional activity of these proteins on unassembled DNA templates. We used the p4TxRE/G-less circular plasmid DNA template (Fig. 2.1D, schematic), which carries four copies of the Tax-responsive viral CRE driving synthesis of a 380 nt guanine-less transcript (8). Transcription was examined in the presence of nuclear extracts prepared from the CEM cell line (HTLV-I negative human T-lymphocyte). Figure 2.1D shows that the addition of Tax and CREB stimulated transcription from the p4TxRE/G-less template 4.5-fold. We analyzed p300 activity on a wide range of Tax/CREB levels, and found that the coactivator produced only modest effects on activated transcription (an average of 1.6-fold above that of Tax/CREB alone) (Fig. 2.1D, lane 4; Fig. 2.6). The addition of exogenous p300 had no effect on basal transcription (Fig. 2.7). Consistent with other studies, these results suggest that p300 may not function in the absence of chromatin (112, 114).

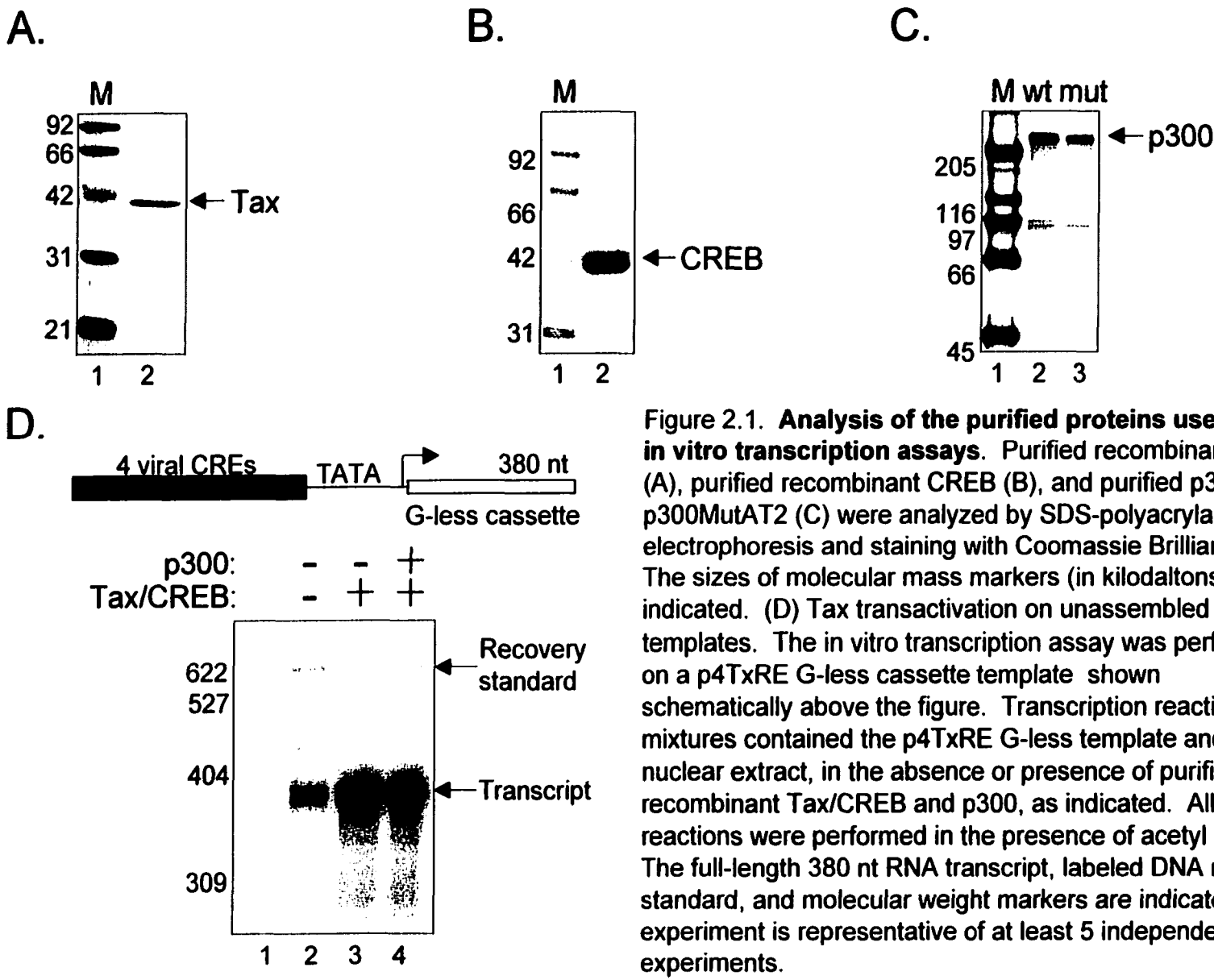


Figure 2.1. **Analysis of the purified proteins used in the in vitro transcription assays**. Purified recombinant Tax (A), purified recombinant CREB (B), and purified p300 and p300MutAT2 (C) were analyzed by SDS-polyacrylamide gel electrophoresis and staining with Coomassie Brilliant Blue. The sizes of molecular mass markers (in kilodaltons) are indicated. (D) Tax transactivation on unassembled DNA templates. The in vitro transcription assay was performed on a p4TxRE G-less cassette template shown schematically above the figure. Transcription reaction mixtures contained the p4TxRE G-less template and CEM nuclear extract, in the absence or presence of purified, recombinant Tax/CREB and p300, as indicated. All reactions were performed in the presence of acetyl CoA. The full-length 380 nt RNA transcript, labeled DNA recovery standard, and molecular weight markers are indicated. This experiment is representative of at least 5 independent experiments.

2.3b RECOMBINANT CHROMATIN ASSEMBLY SYSTEM

To test whether p300/CBP utilization by Tax might require a chromatin context, we developed a biochemically defined system for assembling chromatin templates from purified, recombinant proteins. The recombinant *Drosophila* chromatin assembly proteins NAP-1 and ACF (88), together with recombinant *Xenopus* (r*Xenopus*) core histones (140), provide the foundation for this system. NAP-1 is an H2A-H2B histone chaperone (85), and ACF is a two-subunit complex containing Acf1 and ISWI. The ACF complex acts catalytically in chromatin assembly and in the nucleosome remodeling that accompanies transcriptional activation (86). These assembly proteins are sufficient for the ATP-dependent formation of evenly spaced nucleosomal arrays (86, 88). Although these histone assembly/remodeling proteins are of *Drosophila* origin, they can be used with histones from several sources due to the evolutionary conservation of the histone proteins (P.J. Laybourn, unpublished observations). In addition, the *Drosophila* assembly system is compatible with transcriptional activators and nuclear extracts derived from human cells (112, 113, 145, 154, 170, 196). As a source of nucleosomes in the assembly reaction, we have chosen to use recombinant *Xenopus* core histones. These histones are expressed in *E. coli* and are therefore unmodified and better suited than native histones for studies examining the HAT activity of p300 in Tax-activated transcription. Furthermore, a recombinant chromatin assembly system enables characterization of the role of the core histone tails in transcriptional repression, activation and coactivator function, through selective histone tail deletions.

Finally, the templates are free of histone variants that are known to be present in native core histone preparations (223). The purified chromatin assembly factors Acf1, ISWI and NAP-1, and the r*Xenopus* core histones, are shown in figure 2.2 (panels A-C).

We used DNA topological analysis to determine whether our recombinant assembly factors efficiently deposit r*Xenopus* histone octamers onto the HTLV-I transcription template. Figure 2.2D compares the ability of native *Drosophila* core histones versus r*Xenopus* core histones to assemble chromatin onto the p4TxRE/G-less template (lanes 3-10). In the presence of the assembly factors, increasing ratios (w/w) of the core histones to the DNA produced a concomitant increase in DNA supercoiling, indicating that nucleosomes were deposited onto the template. Figure 2.2D shows that at a r*Xenopus* histone/DNA ratio of 1.2:1 (w/w), the DNA template was fully assembled into chromatin (lane 9). The highest r*Xenopus* histone/DNA ratio, 1.6:1, produces an inhibition of the apparent supercoiling by interfering with DNA topoisomerase I activity (Fig. 2.2D, lane 10).

We were next interested in determining whether increasing nucleosome density produced an associated increase in repression of basal transcription. An in vitro transcription assay was performed following chromatin assembly with the r*Xenopus* core histones (Fig. 2.2E). The core histone/DNA ratio that gave complete nucleosome assembly in the supercoiling assay (1.2:1; Fig. 2.2D, lane 9) produced strong repression of basal HTLV-I transcription (Fig. 2.2E, lane 5). These results indicate that our recombinant system efficiently forms chromatin and is functional for repression of basal transcription in vitro.

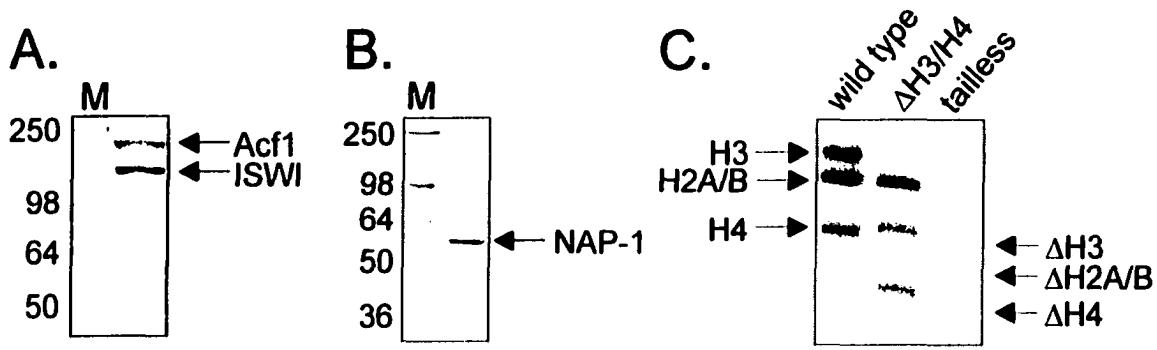


Figure 2.2A-C. **Analysis of the purified proteins used in the chromatin assembly assay**. Purified recombinant ACF (consisting of Acf1 and ISWI) (A), purified recombinant dNAP-1 (B), and purified recombinant r*Xenopus* core histones (C) were analyzed by SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue. The sizes of molecular mass markers (in kilodaltons) are indicated.

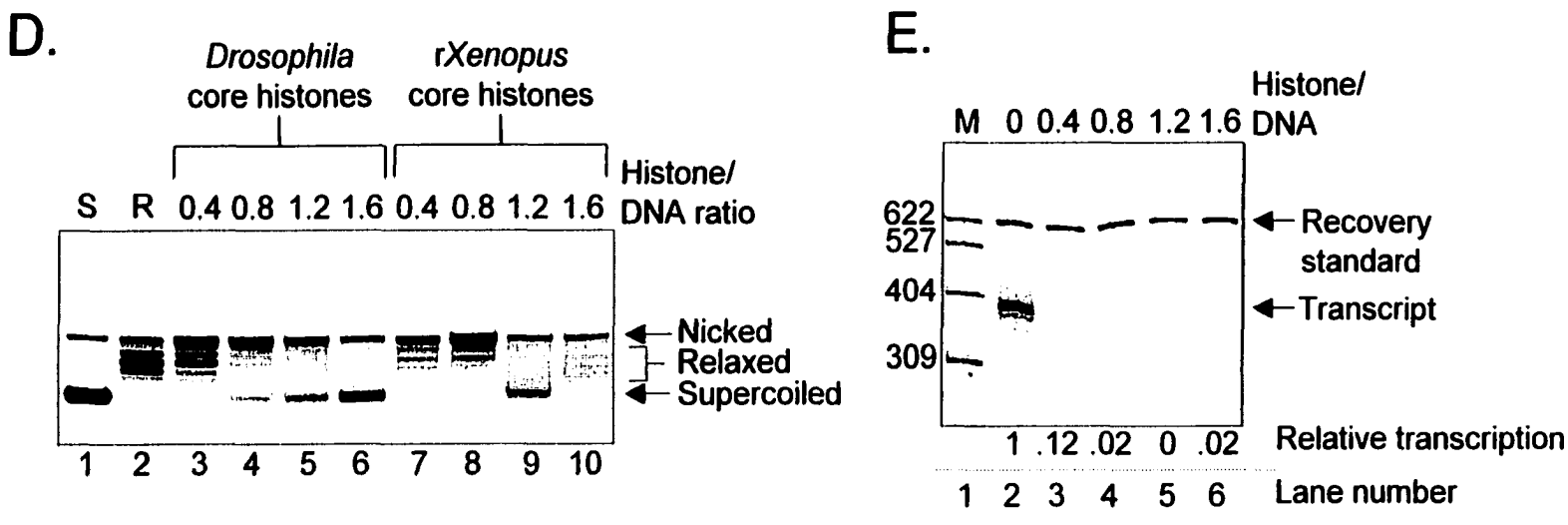


Figure 2.2 D-E. **Analysis of chromatin assembly and transcription**. (D) One-dimensional DNA topological assays comparing chromatin templates assembled with native *Drosophila* and recombinant *Xenopus* core histones, in the presence of ACF and NAP-1. The DNA topoisomers were resolved on an agarose gel, and the DNA stained with ethidium bromide. The supercoiled (S), relaxed (R), and nicked DNA populations, and the histone/DNA ratio, are indicated. Relaxed (R) DNA samples normally consist of multiple topoisomers (+2 to –2 supercoils) that resolve as multiple bands. A negative image of the ethidium-stained gel is shown to enhance visibility of the bands. Topoisomerase I-treated p4TxRE/G-less plasmid DNA was assembled with increasing amounts of purified, native *Drosophila* core histones (lanes 3-6), or recombinant *Xenopus* core histones (lanes 7-10). (E) Basal transcription from the p4TxRE/G-less template was assayed in the presence of increasing amounts of r*Xenopus* core histones. Naked (unassembled) DNA was assayed as a positive control for basal transcription (lane 2). Molecular weight size markers, recovery standard, and full length G-less transcripts are indicated.

2.3c TRANSCRIPTIONAL ACTIVATION BY TAX AND p300 IN A CHROMATIN CONTEXT

We next tested whether the p4TxRE/G-less template, assembled into chromatin with the unmodified r*Xenopus* histones, could support Tax/CREB and p300-mediated transcriptional activation. Figure 2.3 shows that the addition of Tax and ser133-phosphorylated CREB (pCREB) together strongly activated transcription ~100-fold (lane 7). Transcription was also stimulated by Tax and acetyl CoA alone, presumably through interactions with endogenous CREB present in the nuclear extract (Fig. 2.3, lane 5). Exogenous p300 addition enhanced activated transcription by Tax/pCREB an additional 3-fold, leading to an overall activation level of ~300-fold over basal transcription (Fig. 2.3, lane 9). This fold activation by p300 is consistent with other studies (41, 112-114). We did not detect p300 stimulation of transcription in the absence of added Tax/pCREB (Fig 2.7). Comparable Tax/pCREB and p300 stimulatory effects were observed on transcription templates assembled with native *Drosophila* core histones (Fig. 2.8). Transcriptional stimulation in the presence of the activators and coactivator was absolutely dependent upon the presence of acetyl CoA in the reactions (Fig. 2.3, lanes 4-9); however, acetyl CoA had no detectable effect on basal transcription (Fig. 2.3, lanes 2-3). These results are in agreement with several other studies showing that acetyl CoA is required for transcriptional activation within a chromatin context (41, 92, 114). The overall degree of Tax/CREB/p300 activation is far greater than that observed on unassembled templates. This result, in a recombinant chromatin environment, is similar to the

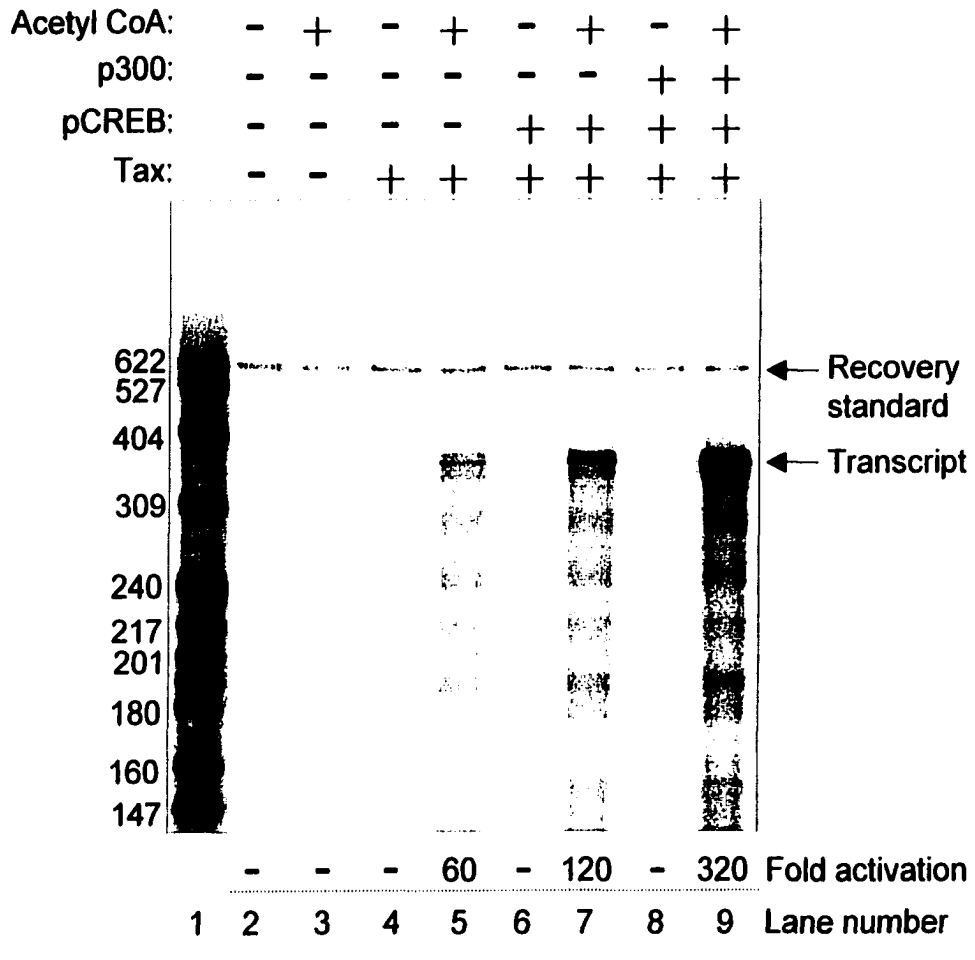


Figure 2.3. **Transcriptional activation from recombinant chromatin templates is dependent on acetyl CoA.** Transcriptional activation on p4TxRE/G-less chromatin templates was analyzed in the presence of ser133-phosphorylated CREB (pCREB), Tax, and p300, in the presence and absence of acetyl CoA. Molecular weight size markers, recovery standard, and full length G-less transcripts are indicated. Basal transcription levels, although not visible in this exposure, were measurable and upon subtraction of the background signal generated a nonzero number that was set equal to 1 in terms of relative transcription. *In this experiment, protein kinase A-phosphorylated CREB was used. However, we have found essentially no difference in the transcriptional activity of Tax in the presence of CREB versus pCREB. We have therefore used only unmodified CREB in subsequent experiments.

strong Tax/CREB-dependent activation of HTLV-I transcription and p300-mediated coactivation that is seen in vivo (27, 46, 110, 192, 200).

2.3d DELETION OF HISTONE TAILS ABOLISHES p300 FUNCTION

The amino terminal histone tails are thought to play a prominent role in the formation of higher order chromatin structure. Post-translational modification of the histone tails has been hypothesized to participate in the conversion of transcriptionally silent, highly compacted chromatin to a more relaxed chromatin that is accessible to the transcriptional machinery. We investigated whether the histone tails would be required for transcriptional repression as well as for the high levels of transcriptional activation mediated by Tax/CREB and p300. We expressed and purified each of the four r*Xenopus* histones carrying amino terminal deletions. The sites of proteolytic cleavage on nucleosomal histones have been previously characterized (23); we used these sites to define our deletion mutants. We then combined our histone tail mutants to generate the two types of histone octamers used in this study. The first, which we call "ΔH3/H4", contains wild-type H2A and H2B assembled together with the H3 and H4 tail deletion mutants (H3Δ1-27 and H4Δ1-19) (see Fig. 2.2C, lane 2). The second, which we call "tailless", contains deletion mutations in all four core histones (H2AΔ1-12,119-128, H2BΔ1-23, H3Δ1-27, H4Δ1-19) (see Fig. 2.2C, lane 3).

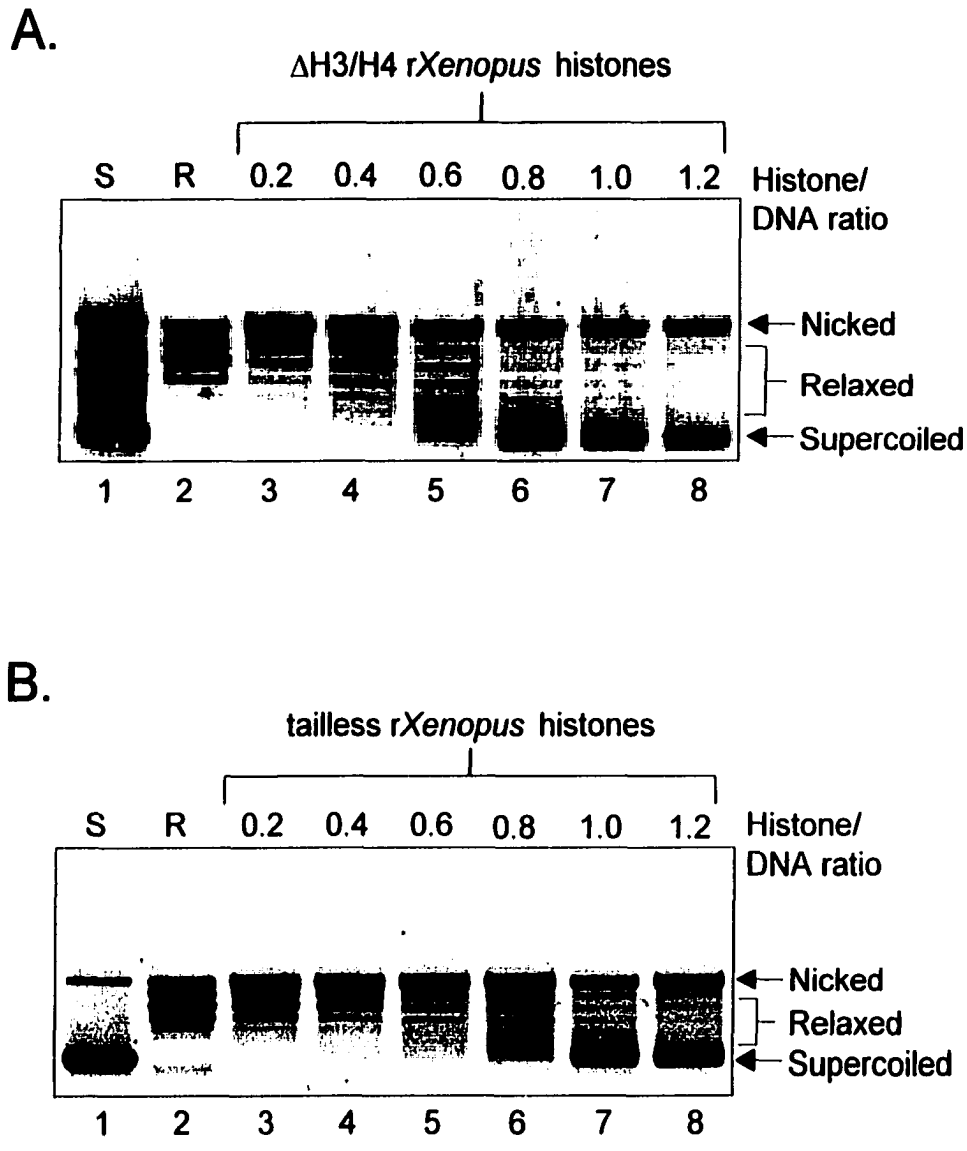


Figure 2.4. **One-dimensional DNA topological analysis of histone mutants.** Topoisomerase I-treated p4TxRE G-less cassette templates were assembled with ΔH3/H4 (A) and fully tailless (B) recombinant *Xenopus* core histones, in the presence of ACF and NAP-1. The DNA topoisomers were resolved on an agarose gel, and the DNA was stained with ethidium bromide. The supercoiled (S), relaxed (R), and nicked DNA populations, and the histone/DNA ratio, are indicated. A negative image of the ethidium-stained gel is shown to enhance visibility of the bands.

DNA topological analysis demonstrated that both mutant octamers assembled onto the p4TxRE/G-less plasmid template, with a degree of assembly comparable to that observed with wild-type octamer (Fig. 2.4). This is consistent with previous studies using both native, proteolyzed and recombinant histone tail deletion mutants that have demonstrated that removing the histone tails does not adversely affect nucleosome assembly, structure or stability (14, 15, 38, 79, 134, 151).

We tested whether the mutant histones affected Tax/CREB and p300-mediated transcriptional activation. Nucleosomal templates formed with wild-type, ΔH3/H4 and tailless histone octamers each strongly repress basal transcription relative to transcription from naked DNA templates (Fig. 2.5A, lanes 2, 3, 8, 13). Thus, the levels of transcriptional repression were not significantly affected by the deletion of the H3/H4 tails or of all four histone tails (Fig. 2.5A, compare lanes 3, 8, 13). The addition of Tax/CREB produced robust transcriptional activation from both chromatin templates carrying the histone tail deletions, with a 1.7-fold increase in activation relative to wild-type chromatin (Fig. 2.5A, lanes 4, 9, 14). The level of Tax/CREB activation from fully tailless templates was still 30-fold greater than that on free DNA templates (see Fig. 2.1). We then compared the role of p300 in Tax/CREB activated transcription using these mutant chromatin templates. Exogenous p300 activated Tax/CREB transcription approximately 4-fold from the wild type templates (Fig. 2.5A, lanes 4-5). In contrast, chromatin formed with histones carrying the H3/H4 tail deletions supported less than a 2-fold enhancement by p300, and the fully

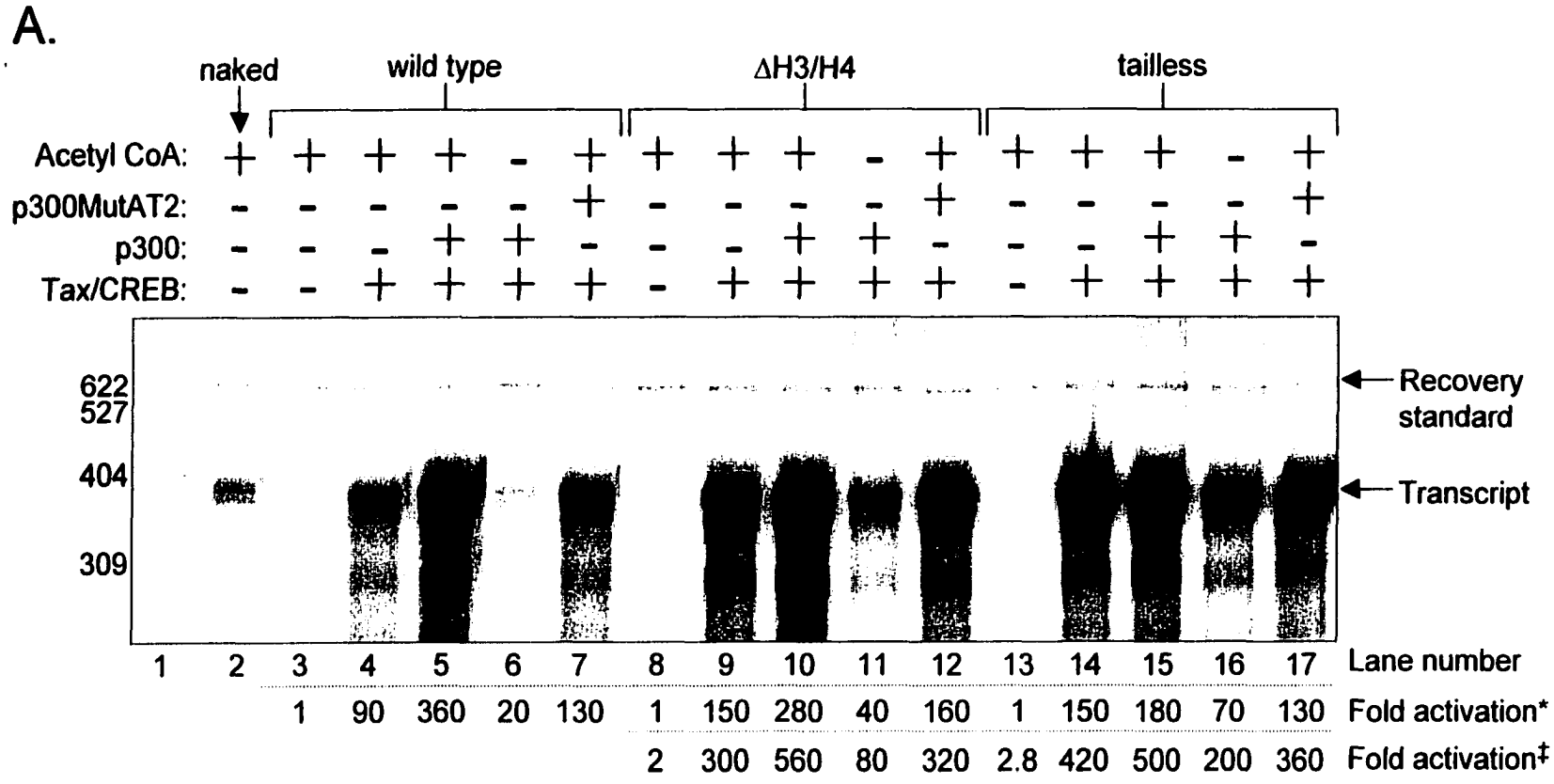


Figure 2.5A. **Analysis of transcriptional activity of chromatin templates assembled with *rXenopus* histone tail deletion mutants.** In vitro transcription assay of tail mutant chromatin templates. The p4TxRE/G-less template was assembled into chromatin using wild-type, ΔH3/H4, or tailless r*Xenopus* histone octamers in the presence of NAP-1 and ACF. Transcriptional repression and activation on the assembled templates was analyzed in the presence of CREB, Tax, wild-type p300 and the HAT-defective p300 mutant p300MutAT2, as indicated. Transcription was assayed in the presence and absence of acetyl CoA. Molecular weight size markers, recovery standard, and full length G-less transcripts are indicated. *Fold activation calculated within each chromatin type. ‡Fold activation calculated relative to basal transcription from wild-type chromatin.

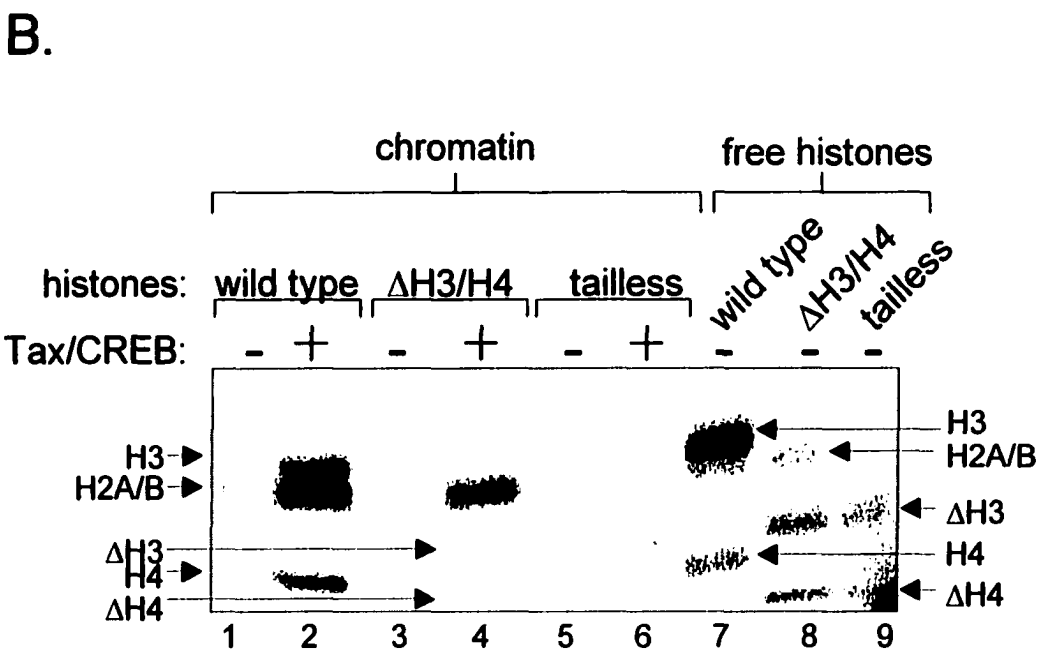


Figure 2.5B. **Analysis of acetylation states of chromatin templates assembled with r*Xenopus* histone tail deletion mutants.** In vitro acetylation assay of recombinant histones. Wild-type (lanes 1, 2), ΔH3/H4 (lanes 3, 4), or tailless histones (lanes 5, 6) were assembled into chromatin on the p4TxRE/G-less template in the presence of NAP-1 and ACF. Both assembled chromatin templates and free core histones (lanes 7-9) were acetylated in vitro in the presence of p300 and ^{14}C-acetyl CoA. Tax and CREB were added to the acetylation reactions, as indicated.

tailless histones were essentially unaffected by p300 addition (Fig. 2.5A, lanes 9-10 and lanes 14-15). The ~2-fold p300 stimulation from the ΔH3/H4 chromatin may be mediated through p300 acetylation of the intact H2A and H2B tails on these templates (see Fig. 2.5B, lane 4).

To distinguish the role of acetylation from the role of basal factor recruitment by p300 in transcriptional activation, we tested a p300 mutant that is compromised for acetyltransferase function. This protein, called p300MutAT2, carries six point mutations located within the acetyltransferase domain and has been found to have <1% of the acetyltransferase activity of the wild-type p300 (113). An SDS-PAGE of the purified p300MutAT2 is shown in figure 2.1C (lane 3). We tested the transcriptional activation by p300MutAT2 in the presence of Tax and CREB on wild-type, ΔH3/H4 and tailless chromatin templates. Relative to wild-type p300, transcriptional coactivation by p300MutAT2 is reduced on all templates (Fig. 2.5A, compare lanes 5, 10, and 15 with lanes 7, 12, and 17). RNA synthesis levels produced by Tax/CREB/p300MutAT2 are approximately equal to that observed with Tax/CREB in the absence of added coactivator (Fig. 2.5A, compare lanes 4, 9 and 14 with lanes 7, 12 and 17). This data further supports the idea that the transcriptional stimulation by p300 is largely due to the intrinsic histone acetyltransferase activity of the coactivator.

2.3e AN ACETYL COA REQUIREMENT BEYOND HISTONE TAIL ACETYLATION

We have already established a strong dependence on acetyl CoA for Tax/CREB and p300-mediated transcriptional activation from wild-type chromatin templates (see Fig. 2.3). Since we have observed that p300 functions primarily through its intrinsic HAT activity in Tax transactivation, we were interested in assessing the requirement for acetyl CoA in transcription from the mutant chromatin templates. As shown in figure 2.5A, we find a strong, but not complete, dependence on the addition of acetyl CoA for Tax/CREB and p300 transcriptional activation on the wild-type, ΔH3/H4 and tailless templates. However, when the histone tails are progressively deleted, there is a concomitant decrease in the dependence on acetyl CoA for transcriptional activation on these templates (Fig. 2.5A, compare lanes 6, 11, 16). For example, on wild type templates, transcription appears to be 95% dependent on acetyl CoA (Fig. 2.5A, compare lanes 5 and 6), whereas on tailless templates transcription is 60% dependent on acetyl CoA (Fig. 2.5A, compare lanes 15 and 16). This observation demonstrates that histone tail acetylation promotes transcriptional activation and that acetylation becomes less important as the histone tails are removed. It is noteworthy that there is a significant acetyl CoA requirement for transcriptional activation even in the absence of tails.

2.3f TAX/CREB STIMULATION OF p300 HAT ACTIVITY

Recent studies have demonstrated that histone acetylation by p300 requires targeted recruitment by activators at promoter elements (114, 168). To directly measure the level and specificity of p300-dependent acetylation of the histones assembled into the chromatin templates and to determine the dependence upon Tax and CREB, we performed p300 acetylation assays on chromatin templates using ^{14}C-labeled acetyl CoA. Figure 2.5B shows that the addition of Tax/CREB strongly enhanced acetylation of all four core histones by p300, as assembled into chromatin (lanes 1, 2). However Tax and CREB do not stimulate p300-mediated acetylation of free histones (Fig. 2.9).

As expected, acetylation of H3 and H4 was significantly reduced on chromatin formed with the ΔH3/H4 histones, without affecting H2A and H2B acetylation (Fig. 2.5B, lanes 3, 4). We did not detect significant acetylation of chromatin formed with the fully tailless histones (Fig. 2.5B, lanes 5, 6). As a positive control for p300 activity and histone acetylation, we also assayed free wild type, ΔH3/H4 and tailless histones in the absence of Tax and CREB (Fig. 2.5B, lanes 7-9). Our results confirm that p300 acetylates the amino terminal tails of the r*Xenopus* core histones assembled into chromatin. Additionally, p300 acetylation of histone tails on chromatin is strongly stimulated by Tax and CREB. This result, together with Figure 2.5A, directly correlates core histone acetylation with p300 function in Tax/CREB-mediated activation of the HTLV-I promoter.

2.4 DISCUSSION

The HTLV-I viral genome is naturally integrated into chromatin following retroviral infection, and thus transcriptional activation by Tax requires interaction with the viral CREs and stimulation of preinitiation complex formation in a chromatin environment. In this study, we have developed a biochemically-defined chromatin template to examine the functional role of p300 in Tax transcriptional activation. To generate this template, we assembled recombinant core histones into nucleosomes on a Tax-responsive HTLV-I template DNA. We selected recombinant *Xenopus* core histones as our source of nucleosomes, as these histones are expressed in *E. coli*, and therefore do not carry post-translational modifications that might obscure or alter coactivator acetyltransferase function in the transcription assay. In addition, we can readily examine the role of histone tails in transcriptional regulation by forming octamers that selectively lack any or all of the tails. Finally, since the template is assembled in a defined system, it is free of histone variants that are known to be present in native core histone preparations (223). Our results indicate that the primary function of p300 in Tax/CREB activated transcription from the HTLV-I promoter is to counteract repression by the core histone tails through acetylation. By examining the activity of chromatin templates selectively lacking core histone tails, we observe that tail deletion functionally mimics acetylation, and in fact abrogates the requirement for p300 for maximal transcriptional activation.

We found that the recombinant histones efficiently assemble into nucleosomes on the template DNA, resulting in strong repression of basal

transcription. This is consistent with numerous studies demonstrating that nucleosomes are repressive to basal transcription in vitro (223). The addition of Tax and CREB reversed nucleosomal repression in an acetyl CoA-dependent fashion, and strongly stimulated RNA synthesis. This derepression and activation combined to increase RNA synthesis nearly 100-fold, which was further augmented 3 to 4-fold by the addition of the coactivator p300. Together, the activator/coactivator complex gave a greater than 300-fold activation, relative to transcription observed in the absence of activators from the chromatin assembled HTLV-I promoter. This degree of activation is far greater than that observed on unassembled templates, and is comparable to the level of HTLV-I Tax transactivation observed in vivo (27, 46, 110, 192, 200), suggesting that our chromatin-based transcription system accurately recapitulates critical aspects of viral gene regulation within the environment of an infected T-cell. Using a p300 mutant, we demonstrate that p300 primarily functions as a histone acetyltransferase in HTLV-I transcription, as mutations in the HAT domain abrogate the ability of p300 to augment Tax transactivation. This result is in agreement with previous work showing that p300 HAT mutants fail to activate Tax-dependent transcription from the HTLV-I promoter in vivo (91). Consistent with this observation, wild-type p300-mediated activation is strongly dependent on the presence of acetyl CoA in the transcription reactions. Finally, our in vitro HAT assay on the assembled templates reveals a strong dependence on Tax and CREB for efficient p300 acetylation of the four core histone tails. This effect is likely due to the targeted recruitment of p300 to the chromatin template by the

Tax/CREB complex. Overall, we observe that maximal transcriptional activity (360-fold activation) from the wild type chromatin template is p300 and acetyl CoA dependent. Furthermore, Tax and CREB strongly stimulate the HAT activity of p300. Together, these data provide direct functional evidence supporting a role for p300 HAT activity in mediating Tax transactivation.

We also examined the role of the core histone tails in Tax/CREB and p300-mediated activation of the HTLV-I promoter. We generated mutant chromatin templates lacking either the H3/H4, or all four, core histone tails. We found that progressive removal of the amino terminal histone tails resulted in modest derepression of basal transcription and enhanced activation by the Tax/CREB complex. The level of Tax/CREB activation from fully tailless templates was 30-fold greater than that on free DNA templates. This effect is primarily due to the tailless nucleosomes still retaining significant ability to repress basal transcription while lacking any ability to inhibit Tax/CREB transcriptional activation. We found that Tax/CREB activation from tail deletion templates was refractory to stimulation by p300. Specifically, p300 addition modestly enhanced transcription from the ΔH3/H4 chromatin templates, and had essentially no effect on the fully tailless chromatin templates. The ~2-fold p300 stimulation on the ΔH3/H4 chromatin was likely mediated through acetylation of the H2A and H2B tails, which were present (and acetylated) on the ΔH3/H4 chromatin templates. It is interesting to note that the absolute levels of Tax/CREB activation from the ΔH3/H4 and tailless chromatin templates were comparable to that observed from the wild type templates in the presence of

Tax/CREB *and* p300. This suggests that tail removal obviates the requirement for the coactivator. Together these observations suggest that all four histone tails serve as p300 acetylation targets in transcriptional activation.

Acetyl CoA has previously been shown to be required for transcriptional activation from chromatin, however, its exact function in this process remains to be fully characterized (41, 92, 114). It has been widely speculated that acetyl CoA is primarily utilized for histone tail acetylation during transcriptional activation. Yet, here we show that transcription in the absence of histone tails still exhibits a substantial requirement for acetyl CoA. This result suggests the involvement of other acetyltransferases in HTLV-I transcription, since addition of p300 had no effect on transcriptional activation from tailless templates. The basis for the acetyl CoA requirement remains to be characterized, but could reflect a number of recent observations concerning the role of acetyl CoA during the transactivation process. Acetyl CoA has been shown to enhance basal transcription on non-chromatin templates through a mechanism not involving acetyltransferase activity (52). Components of the RNA polymerase I and II general transcription machinery, such as UBF, TFIIE and TFIIF, can also be acetylated, but the functional consequences of acetylation are unknown (32, 84, 171). Acetylation can increase the protein-DNA and protein-protein binding affinities of a variety of transcription factors, including EKLF, MyoD and c-Myb (32, 176, 238). Unfortunately, we have found no evidence that Tax and CREB are acetylated. In summary, this is the first study to separate the requirement for acetyl CoA in histone tail acetylation from other acetylation targets in Tax

transcriptional activation. Our observations have significant implications for the role of acetyl CoA as a critical cofactor in transcription, beyond the process of histone tail acetylation.

The primary consequence of core histone tail deletion, which functionally mimics tail acetylation, during Tax/CREB transcriptional activation may be to reduce the chromatin barrier to activator function. It is possible that deletion of the histone tails increases the accessibility of the core promoter to preinitiation complex formation. It is also possible that deletion of histone tails serves to facilitate elongation by RNA polymerase II. For example, histone tail removal has been shown to enhance the elongation rate of T7 RNA polymerase through nucleosomes (179). However, the primary effect we observed is on activator function. The degree to which histone tail deletion contributes to enhanced regulatory protein binding, enhanced RNA polymerase recruitment, or enhanced rates of elongation, remains to be elucidated.

Although p300 functions primarily to acetylate core histone tails in Tax activated transcription, this does not appear to be generally true for all activators that utilize p300/CBP. For example, investigation of a panel of transcription factors demonstrated a differential requirement for p300 HAT activity during transcriptional activation (113). Because of the multifunctional nature of the p300/CBP coactivators, we have considered the possibility that p300 may have additional, HAT-independent functions in mediating Tax transcriptional activation that cannot be detected in our system. A recent study indicates that p300 forms a stable, template committed complex with chromatin, perhaps through

interaction with histone H3 (143). This association of p300 with chromatin may serve to bring the coactivator into the vicinity of target genes, with promoter localization achieved following stable association with promoter-bound activators. Our ΔH3/H4 and tailless chromatin may be unable to carry out this first step in p300 recruitment, and therefore the additional activation functions of p300, such as recruitment and/or stabilization of the general transcription machinery, would not be detected using the mutant chromatin templates. A second possibility is that p300 is present in the RNA polymerase II holoenzyme complex, and that specific p300 activation functions, distinct from histone acetylation, accompany the RNA polymerase II holoenzyme that is supplied by the nuclear extract (103, 157, 229). The contribution of endogenous p300 in the nuclear extract used in these studies remains to be fully characterized.

In summary, these studies represent a major advance in our understanding of Tax and p300-mediated transcriptional activation function, and have the clear potential to extend our fundamental knowledge of coactivator-mediated transcription in a chromatin environment. The observation that progressive deletion of the histone tails is inversely proportional to coactivator utilization and acetyl CoA dependence is a direct demonstration of the connection between p300 acetyltransferase activity, histone tail acetylation and transcriptional activation. This supports the idea that histone tail deletion and acetylation are functionally equivalent during the transcriptional activation process. Finally, we uncover a prominent role for acetyl CoA beyond histone acetylation during the transactivation process.

2.5 MATERIALS AND METHODS

2.5a PURIFICATION OF RECOMBINANT PROTEINS.

The four core histones, both as wild-type and deletion mutant proteins, were individually expressed in *E. coli* and purified to homogeneity as previously described (139). Histone octamers were prepared by denaturation, then renaturation in high salt, followed by purification of the octamers by gel filtration and ion exchange chromatography. *Drosophila* NAP-1 (dNAP-1) (His_6-tagged) was expressed from recombinant baculovirus (85) in Sf9 cells cultured in spinner flasks. The protein was purified by Ni^{++}-agarose batch binding and elution followed by Source 15Q column chromatography (87). We co-expressed FLAG-tagged ISWI and Acf1 from baculovirus in Sf9 cells, and purified the complex by anti-FLAG affinity batch binding and elution as previously described (88). Full length, recombinant CREB (48) was expressed and purified as previously described (58). Tax was expressed from the $pTaxH_6$ expression plasmid (240), and purified as previously described (58). His_6-tagged p300 wild-type and p300MutAT2 proteins were expressed from recombinant baculoviruses in Sf9 cells and purified as previously described (113).

2.5b CHROMATIN ASSEMBLY AND TOPOLOGICAL ASSAYS.

Chromatin templates were assembled as previously described (87). Briefly, histone octamers were incubated with an 8-fold (w/w) excess of dNAP-1 on ice for 30 min. The core histone/dNAP-1 mixture was then added together

with ACF (Acf1/ISWI; 0.87 fmol per ng of core histone octamer) and DNA to provide the empirically determined optimal core histone to DNA ratio. The p4TxRE-G-less plasmid DNA used in the assembly reactions carried 4 tandem copies of the HTLV-I promoter proximal, viral CRE, cloned upstream of the HTLV-I core promoter (8). Following the addition of the DNA, ATP (3 mM), creatine phosphokinase (1 µg/ml), and phosphocreatine (30 mM) were added in a 70 µl reaction containing 10 mM HEPES (K^+), pH 7.6, 50 mM KCl, 5 mM $MgCl_2$, 5% (v/v) glycerol, and 1% (w/v) polyethylene glycol. Assembly reactions were incubated for 4-18 hours at 27°C. For topological assays, the p4TxRE-G-less plasmid DNA template was relaxed prior to assembly with 0.05 units of topoisomerase I (purified recombinant *Drosophila* topoisomerase I) per µg of DNA for 60 min at 37°C in the presence of 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 0.5 mM EDTA, and 30 µg/ml bovine serum albumin. Immediately prior to assembly into chromatin an additional 0.29 units of topoisomerase I per µg of DNA was added to the DNA template. Assembly reactions were stopped by the addition of 2 mM Na-EDTA, pH 8, 100 mM Tris-HCl pH 7.5, 1% SDS, 2 mM $CaCl_2$ and 16 µg proteinase K, and the samples were incubated at 45°C for 30 min. The samples were extracted, precipitated and analyzed on a 1% agarose gel, and the degree of supercoiling was visualized by ethidium bromide staining.

2.5c *IN VITRO* TRANSCRIPTION.

The supercoiled p4TxRE/Gless plasmid template was assembled into chromatin using NAP-1, ACF and *rXenopus* histones, at a histone:DNA ratio that had been empirically determined to give full chromatin assembly. Following assembly, preinitiation complexes were formed on 75 fmol of the plasmid DNA in the absence or presence of Tax (8 nM), CREB (8 nM), p300 (3 nM), and p300 MutAT2 (3 nM). All reactions contained 100 μM acetyl CoA unless otherwise indicated. CEM (HTLV-I negative human T-lymphocyte) nuclear extract (70 μg) (43) was added immediately following the addition of the transcriptional activators. Following a 60 minute preincubation reaction, RNA synthesis was initiated by the addition of 250 μM ATP and CTP, and 12 μM UTP plus 0.8 μM ^{32}P-α-UTP (3000 Ci/mmol). Transcription from unassembled templates was performed as described above, but without the chromatin assembly step. Transcription reactions were processed and analyzed as previously described (126). Molecular weight markers (radiolabeled *Hpa* II digested pBR322) were used to estimate the size of the RNA products.

2.5d *IN VITRO* ACETYLATION ASSAY.

The p4TxRE/G-less plasmid template was assembled into chromatin using r*Xenopus* histones (2 μg), NAP-1, and ACF as described above. Chromatin assembly was performed at empirically determined histone:DNA mass ratios. p300 (12 nM) and ^{14}C-acetyl CoA (0.9 mM; 57mCi/mmol) were added following chromatin assembly, along with 400 nM Tax and CREB where

applicable, in a solution containing 50 mM Tris, pH 8, 10% glycerol, 10 mM sodium butyrate, 1 mM DTT, 1 mM PMSF. Samples were incubated at 30°C for 60 minutes and protein was precipitated by methanol/chloroform extraction and analyzed by 18% SDS-PAGE.

2.6 ACKNOWLEDGMENTS

We thank James Kadonaga for the Acf1, ISWI, and dNAP-1 baculoviruses, Stephanie Price, Jes Kuruvilla, Sriwan (Apple) Wongwisansri, Holli A. Giebler, Raji Edayathumangalam, Stephanie Ebbesen, Robert Suto, and Jeanne Mick for their significant contributions to this research. This study was supported by grants from the National Institutes of Health/NCI (CA-87540) to J.K.N. and P.J.L., (CA-55035) to J.K.N, the Colorado Cancer League to P.J.L and J.K.N., a Career Award in the Biomedical Sciences from the Burroughs Wellcome Fund and a grant from the National Institutes of Health/NIDDK (DK58110) to W.L.K.

SUPPLEMENTAL FIGURES

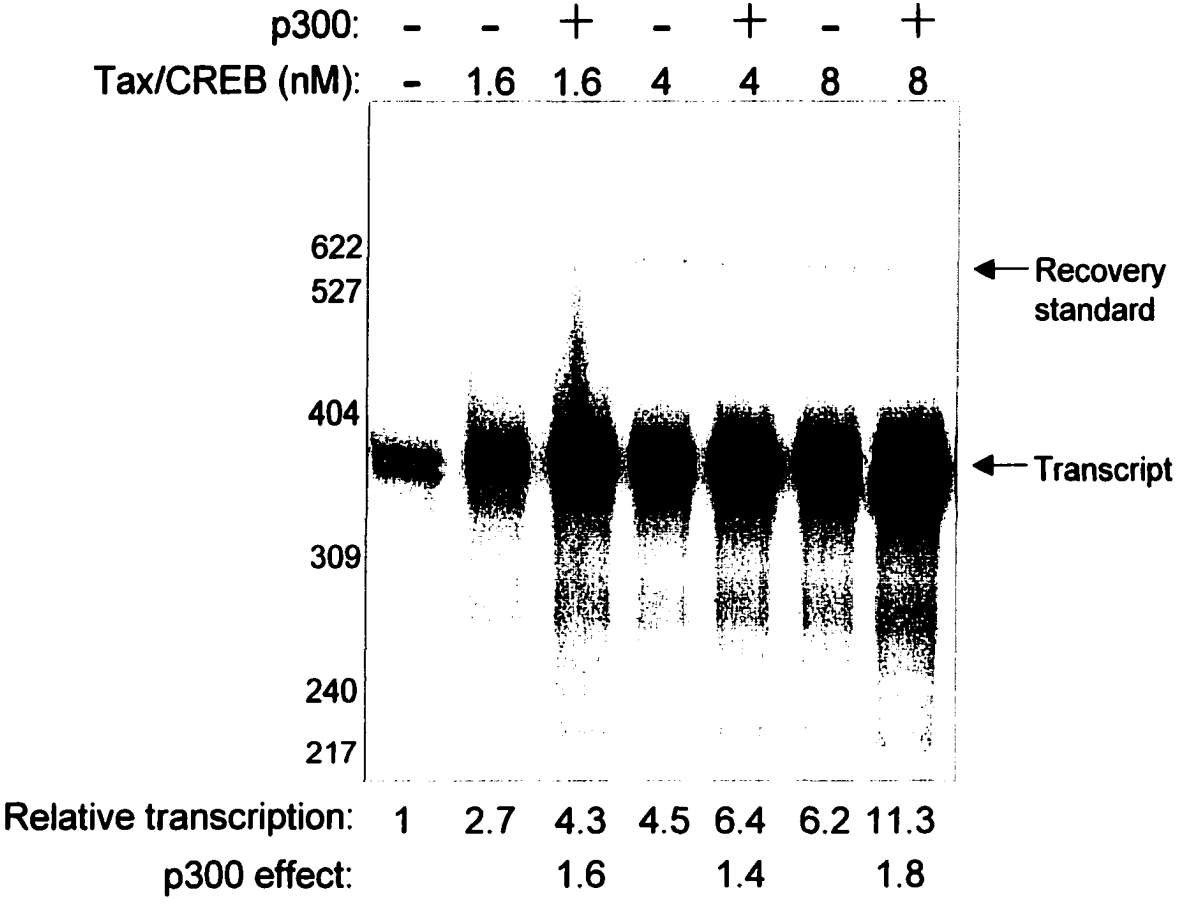


Figure 2.6. **Analysis of p300 function on unassembled DNA templates.** *Transcriptional activation on* unassembled p4TxRE/G-less templates was analyzed in the presence of CREB, Tax, and p300 as indicated, in the presence of acetyl CoA. Molecular weight size markers, recovery standard, and full length G-less transcripts are indicated.

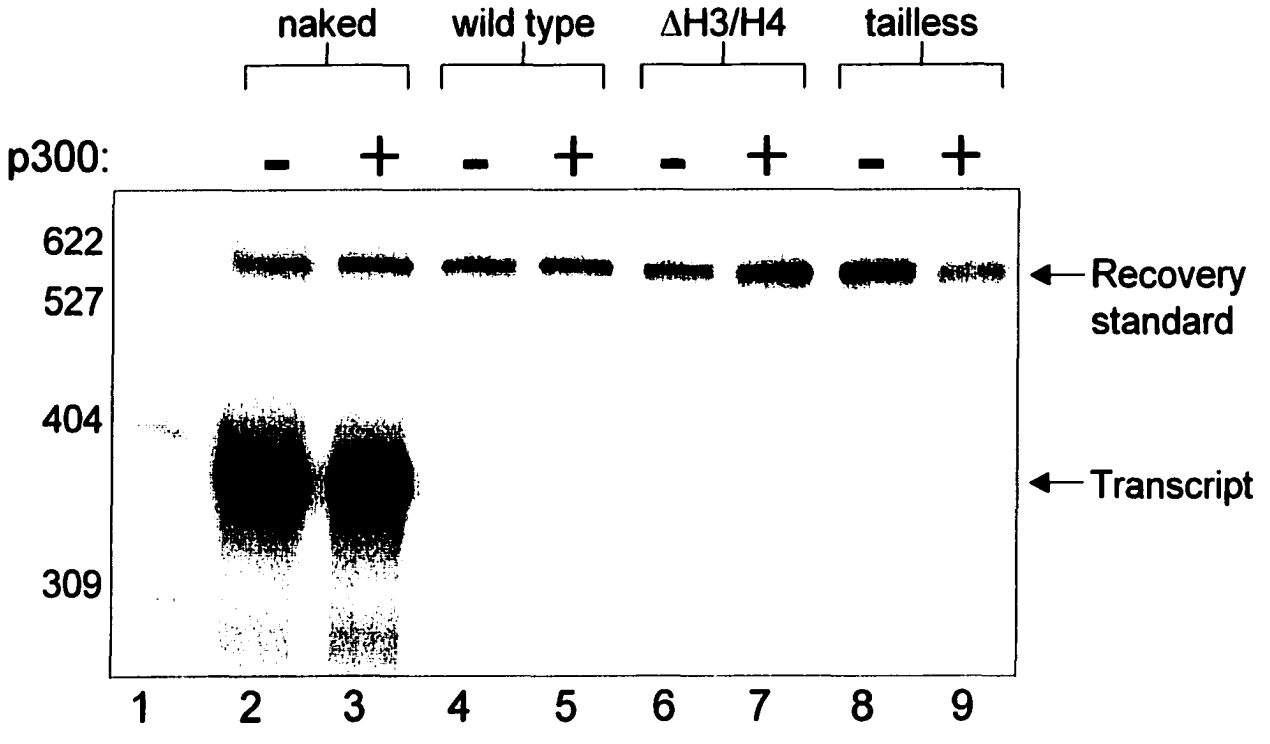


Figure 2.7. **p300 does not activate transcription in the absence of Tax.** The p4TxRE/G-less template was assembled into chromatin using wild-type, ΔH3/H4, or tailless r*Xenopus* histone octamers in the presence of NAP-1 and ACF. Unassembled templates were also assayed, as indicated. Repression of basal transcription on the assembled templates, and transcriptional activation, was analyzed in the presence of wild-type p300, as indicated. Transcription was assayed in the presence of acetyl CoA. Molecular weight size markers, recovery standard, and full length G-less transcripts are indicated.

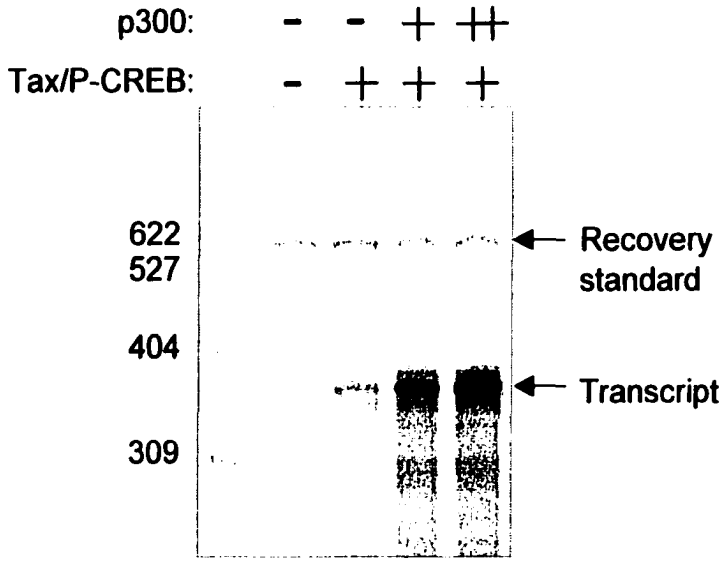


Figure 2.8. **p300 enhances Tax-activated transcription on native *Drosophila* chromatin templates.** In vitro transcription assay of chromatin templates. The p4TxRE/G-less template was assembled into chromatin using native *Drosophila* histone octamers (source: *Drosophila* embryos), in the presence of NAP-1 and ACF. Transcriptional repression and activation on the assembled templates was analyzed in the presence of CREB, Tax and wild-type p300, as indicated. Transcription was assayed in the presence of acetyl CoA. Molecular weight size markers, recovery standard, and full length G-less transcripts are indicated.

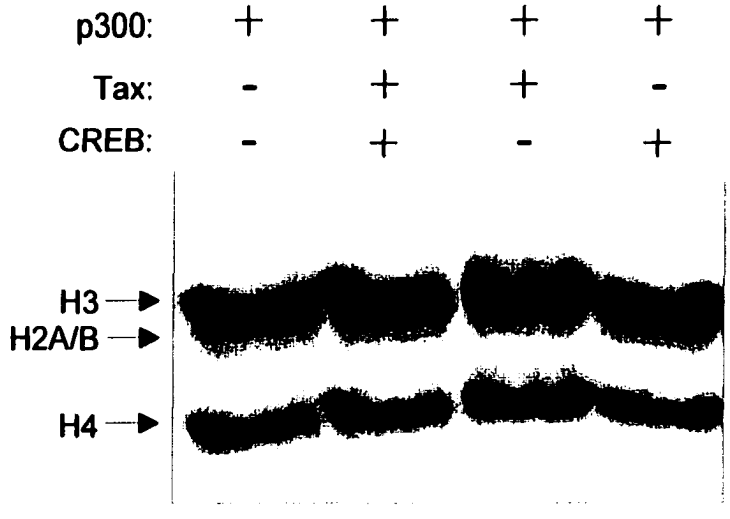


Figure 2.9. **Tax and CREB do not stimulate p300-mediated acetylation of free histones.** In vitro acetylation assay of wild-type, recombinant *Xenopus* histones. Free histones were acetylated in vitro in the presence of p300 and ^{14}C-acetyl CoA. Tax and CREB were added to the acetylation reactions, as indicated.

CHAPTER 3
TAX RECRUITMENT OF CBP/p300, VIA THE KIX DOMAIN, REVEALS A POTENT REQUIREMENT FOR ACETYLTRANSFERASE ACTIVITY THAT IS CHROMATIN-DEPENDENT AND HISTONE TAIL-INDEPENDENT

Chapter three describes a study of the contribution of the KIX domain to the recruitment of CBP/p300 in Tax-activated transcription. This study utilizes various CBP/p300-selective inhibitors to address the important question of exogenous versus endogenous CBP/p300 activities in our nuclear extract-based in vitro transcription system. Additionally, this study further investigates the role of CBP/p300 acetyltransferase activity in Tax-transactivation. This work reflects a collaboration between the Luger, Laybourn, and Nyborg laboratories. Phil Cole provided the Lys-CoA reagent, and Karolin Luger provided all purified recombinant histone octamers. Holli A. Giebler contributed the experiments shown in figure 3.3D and figure 3.4C. I conducted the remainder of the experiments presented in this chapter. Data cited as "data not shown" in the manuscript are provided here as supplemental figures. This work has been submitted to the journal *Molecular and Cellular Biology* for publication. The citation for the manuscript is:

Georges, S.A., H.A. Giebler, P.A. Cole, K. Luger, P.J. Laybourn and J.K. Nyborg. 2002. Tax recruitment of CBP/p300, via the KIX domain, reveals a potent requirement for acetyltransferase activity that is chromatin-dependent and histone tail-independent. Submitted.

3.1 ABSTRACT

Robust transcription of the human T-cell leukemia virus, type I (HTLV-I) genome requires the viral transactivator Tax. Although Tax has been shown previously to interact with the KIX domain of CBP/p300 in vitro, the precise functional relevance of this interaction remains unclear. Using two distinct approaches to interrupt the physical interaction between Tax and KIX, we find that Tax-transactivation from chromatin templates is strongly dependent on CBP/p300 recruitment via the KIX domain. Additionally, we find that the functional contribution of CBP/p300 to Tax-transactivation resides, perhaps exclusively, in the intrinsic acetyltransferase activity of the coactivators. These studies unexpectedly uncover a specific requirement for CBP/p300 acetyltransferase activity on chromatin templates assembled with nucleosomes *lacking* their amino terminal tails. Together, these data indicate that the KIX domain of CBP/p300 is essential for targeting the acetyltransferase activity of the coactivator to the Tax/CREB complex. Significantly, these observations reveal the presence of a CBP/p300 acetyltransferase target that functions specifically on chromatin templates, is independent of the histone tails, and is critical to Tax-transactivation.

3.2 INTRODUCTION

The human T-cell leukemia virus type-1 (HTLV-I)-encoded Tax protein is required for high level transcription of the viral genome (for review, see 233).

Tax stimulates HTLV-I transcription through interaction with three conserved 21 bp repeat DNA elements, called viral CREs, located in the transcriptional control region of the virus. These sequences serve as binding sites for Tax in complex with the cellular transcription factor CREB (or other members of the ATF/CREB family of transcription factors) (1, 48, 65, 239). Tax binds to the viral CRE sequences through protein-DNA interactions with GC-rich minor groove sequences (105, 126, 127, 142), and protein-protein interactions with CRE-bound CREB (1, 65). The formation of this Tax/CREB promoter-bound complex is critical for the recruitment of the multifunctional cellular coactivators CBP/p300 (58, 77, 97, 117, 225).

CBP and p300 are very large paralogous proteins that coordinate highly regulated gene expression in metazoans. Several conserved domains in CBP and p300 serve as binding sites for a wide variety of structurally unrelated cellular and viral transcription factors. These domains in CBP/p300 include two cysteine-histidine rich domains (CH1 and CH3), the SRC-interacting domain, and the KIX domain (for recent reviews, see 30, 64). The transcription factor-coactivator interactions mediated by these domains facilitate recruitment of the coactivators to target promoters. Subsequent to recruitment by the transcription factors, CBP/p300 appear to stimulate transcription through multiple, distinct mechanisms. For example, there is evidence that CBP/p300 may directly stabilize components of the general transcription machinery at target promoters (40, 118). Additionally, CBP/p300 have intrinsic acetyltransferase activity that targets multiple lysine residues present on the four core histone tails (16, 161). A significant body of evidence suggests that histone tail acetylation weakens

internucleosomal interactions, which may lead to a more relaxed chromatin structure, providing greater transcription factor/RNA polymerase access at target promoters (9, 55, 73). Additionally, combinations of differentially acetylated histone tails may serve as recognition sites for the assembly of factors that facilitate transitions from transcriptionally silent to transcriptionally active chromatin (90, 205). Although the evidence for histone acetylation by CBP/p300 is strong, the precise mechanistic events that couple transcriptional activation with acetylation of promoter proximal nucleosomes are unknown.

The HTLV-I Tax protein has previously been shown to bind at three of the four major transcription factor interaction domains in CBP/p300: CH1, KIX and the carboxy-terminal SRC-interacting domain (58, 77, 117, 124, 187, 225). The interaction between Tax and KIX has been studied in the greatest detail, and is the only Tax-CBP/p300 interaction that has been reported to occur with the Tax-containing, promoter-bound ternary complex (Tax/CREB/viral CRE DNA) (58, 77, 117, 225). This observation suggests that the ternary complex interaction with KIX may play a major role in CBP/p300 recruitment to the HTLV-I promoter. However, little is known about the functional significance of this protein-protein interaction.

In this study, we explore the transcriptional relevance of the Tax-KIX interaction in Tax-transactivation from chromatin-assembled templates in vitro. Using polypeptides specifically designed to inhibit CBP/p300 association with the Tax/CREB/viral CRE complex, we demonstrate that the KIX domain of CBP/p300 is critical for mediating coactivator function at the HTLV-I promoter. The

inhibition is specific to Tax-transactivation on chromatin templates, and, unexpectedly, is independent of the four core histone amino terminal tails. We extend these studies to examine the contribution of the acetyltransferase function of the coactivators on Tax-transactivation from the HTLV-I promoter. We find that the CBP/p300-selective acetyltransferase inhibitor Lys-CoA inhibits Tax-transactivation from chromatin templates to the same levels observed with the polypeptide inhibitors. These observations indicate that CBP/p300 play a prominent role in Tax-transactivation from chromatin templates, and that the acetyltransferase activity of the coactivators provides the major functional contribution to transcriptional activation in vitro. Interestingly, a strong CBP/p300-specific acetyltransferase requirement was also observed on chromatin templates prepared from tailless histones. These data indicate that CBP/p300 participate in additional chromatin-specific, tail-independent acetylation events during transcriptional activation by Tax.

3.3 RESULTS

3.3a THE KIX DOMAIN OF CBP/p300 MEDIATES COACTIVATOR FUNCTION IN TAX-TRANSACTIVATION FROM CHROMATIN TEMPLATES

High level transcription of the HTLV-I genome requires the binding of the virally-encoded Tax protein, in complex with the cellular transcription factor CREB, to the viral CRE promoter elements. This promoter-bound complex is

believed to recruit the cellular coactivators CBP/p300 via interactions with the KIX domain. The physical interaction between the KIX domain, of both CBP and p300, and the Tax/CREB complex has been well characterized (22, 58, 77, 117, 225). However, the precise functional relevance of this interaction in Tax-transactivation remains unclear.

In this study, we utilized a chromatin-based transcription system that includes recombinant core histones (6, 57) to characterize the mechanisms of Tax-activated transcription. Transcription reactions were performed in the presence of acetyl CoA and nuclear extract prepared from CEM cells (HTLV-I negative human T-lymphocyte). We used the p4TxRE/G-less template, which carries four copies of the Tax-responsive viral CRE driving synthesis of a 380 nt guanine-less transcript (8). This template was chosen because the four viral CREs are the only known activator binding sites in the promoter region of the plasmid. Therefore, the transcriptional read-out should be fully dependent upon the addition of Tax/CREB. This template was responsive to Tax/CREB, both as free DNA and assembled into recombinant chromatin (Fig. 3.1A, lanes 9, 10, and 12, 13). Exogenous p300 further activated Tax/CREB transcription only from the p4TxRE/G-less template, in a chromatin-specific manner (Fig. 3.1A, compare lanes 10, 11 with lanes 13, 14). In contrast, a template containing only the HTLV-I core promoter sequence (-52 to +1, relative to the transcription start site; designated p-52/G-less) and lacking the viral CREs, was minimally responsive to Tax/CREB and Tax/CREB/p300 (Fig. 3.1A, lanes 1-5). Additionally, this core promoter template was unresponsive to Tax/CREB and

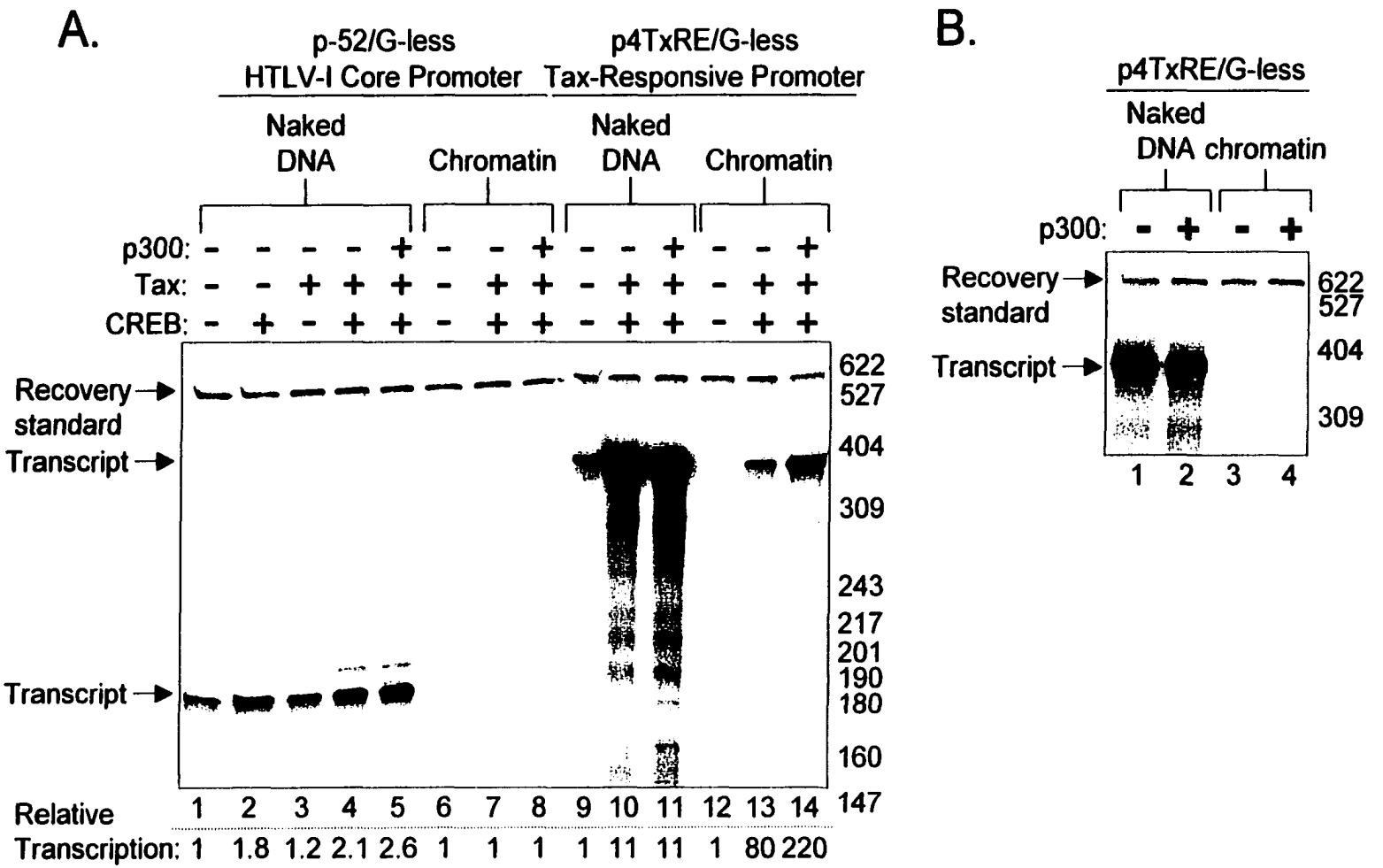


Figure 3.1. **p300 coactivator function requires the viral CREs and Tax/CREB for HTLV-I transcription.** The p-52/G-less and p4TxRE/G-less cassette templates were assayed both as free DNA, and as DNA assembled into chromatin using recombinant *Xenopus* histones. Transcription reactions contained CEM nuclear extract, and purified recombinant p300, Tax, CREB, Tax/CREB, or Tax/CREB/p300 as indicated. Recovery standard, size markers, and the position of the full-length transcript are indicated. (A) The Tax-responsive viral CRE enhancer elements are required for transcriptional activation. Relative transcription was calculated separately for each set of templates, with basal transcription set equal to 1. (B) p300 coactivator function requires Tax/CREB for HTLV-I transcriptional activation.

Tax/CREB/p300 when assembled into chromatin (Fig. 3.1A, lanes 6-8). Importantly, p300 coactivator function required Tax/CREB, suggesting that p300 cannot function independently of promoter-bound activators in this system (Fig. 3.1B).

In this study, we were interested in evaluating the role of CBP/p300, and specifically the contribution of the KIX domain, in Tax-mediated transcriptional activation from chromatin templates in vitro. To biochemically characterize the role of KIX in CBP/p300 recruitment by the Tax/CREB complex, we expressed and purified two polypeptides designed to specifically block the physical interaction between promoter-bound Tax and CBP/p300. A schematic showing the relevant polypeptide interactions predicted to block coactivator recruitment is shown in figure 3.2. The first polypeptide, KIX, carries CBP amino acids 588-683 (CBP and p300 share 93% homology within the KIX domain). This represents the minimal region of KIX required for high affinity binding to Tax (225). We hypothesized that the binding of KIX to the Tax/CREB/viral CRE complex would block CBP/p300 recruitment to the HTLV-I promoter (Fig. 3.2A). The second polypeptide, SREBP-1a, carries the first 50 amino acids of SREBP-1 (sterol regulatory element binding protein-1), and has previously been shown to bind to the KIX domain of CBP (154). We hypothesized that SREBP-1a would bind to the KIX domain of CBP/p300, thus preventing association of CBP/p300 with the Tax/CREB/viral CRE complex (Fig. 3.2B).

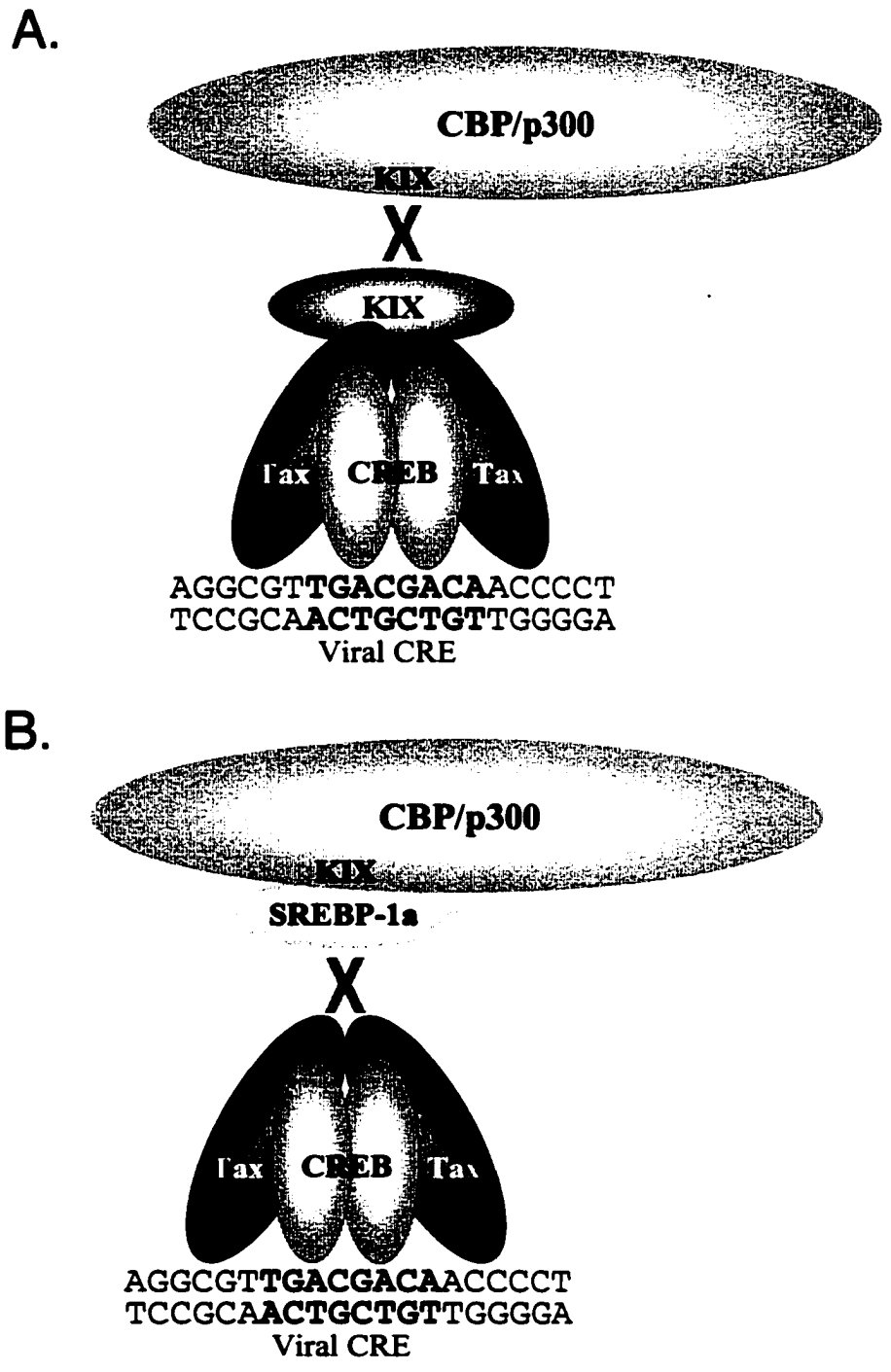


Figure 3.2. **Strategies for the disruption of CBP/p300 recruitment to the Tax-responsive HTLV-I promoter.** (A) KIX binding to the promoter-bound Tax/CREB complex blocks the recruitment of full-length CBP/p300. (B) SREBP-1a binding to the KIX domain of full-length CBP/p300 prevents binding of the coactivators to the Tax/CREB/viral CRE DNA complex.

We first asked whether addition of the KIX domain to in vitro chromatin transcription reactions would inhibit Tax/CREB/p300-mediated transcriptional activation. Figure 3.3A shows that the addition of recombinant, purified Tax/CREB and Tax/CREB/p300 strongly activated HTLV-I transcription (lanes 2-4). As hypothesized, the addition of purified GST-KIX to the transcription reactions significantly diminished Tax-transactivation in a dose-dependent manner (Fig. 3.3A, lanes 4-6). As a negative control, we tested a KIX mutant (GST-KIXmut), carrying CBP amino acids 597-719. We have previously shown that deletion of amino acids 588-597 abolishes KIX interaction with Tax (225). The addition of GST-KIXmut to the transcription reactions had only a modest effect on Tax/CREB/p300 transcriptional activation (Fig. 3.3A, lanes 7, 8).

Because the addition of KIX reduced activated transcription to levels below that observed with Tax/CREB alone, we were interested in testing the effect of KIX on Tax/CREB-activated transcription in the absence of exogenous p300. Figure 3.3B shows that GST-KIX strongly inhibited transcription in the absence of the recombinant coactivator, suggesting that endogenous CBP/p300 present in the CEM nuclear extract participates in Tax-activated transcription from the chromatin template. Interestingly, the addition of GST-KIX or GST-KIXmut to non-chromatin, or naked, p4TxRE/G-less templates had minimal effect on Tax/CREB-activated transcription (Fig. 3.3C). These data are consistent with previous studies showing that p300 does not activate transcription on naked DNA (13, 57, 112, 114, 137) (Fig 3.3C, lane 3).

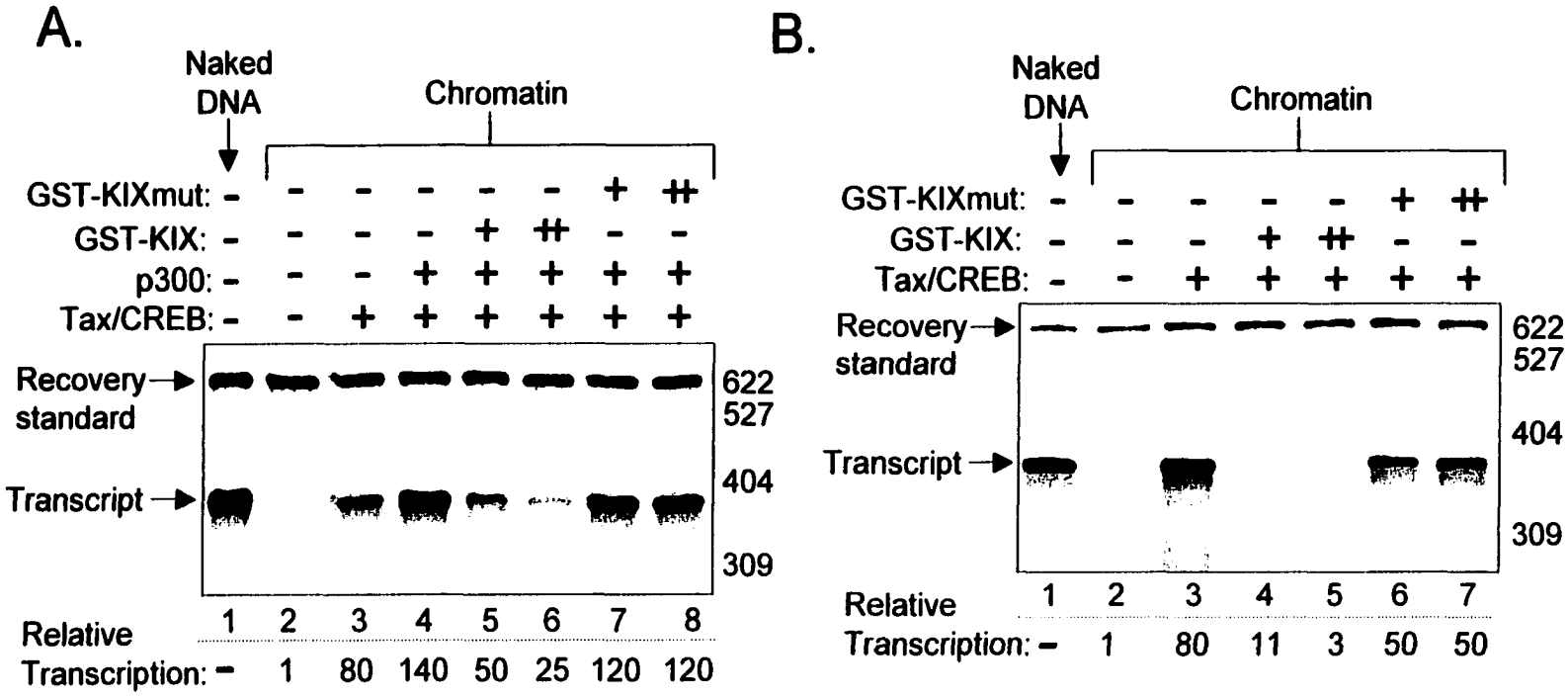
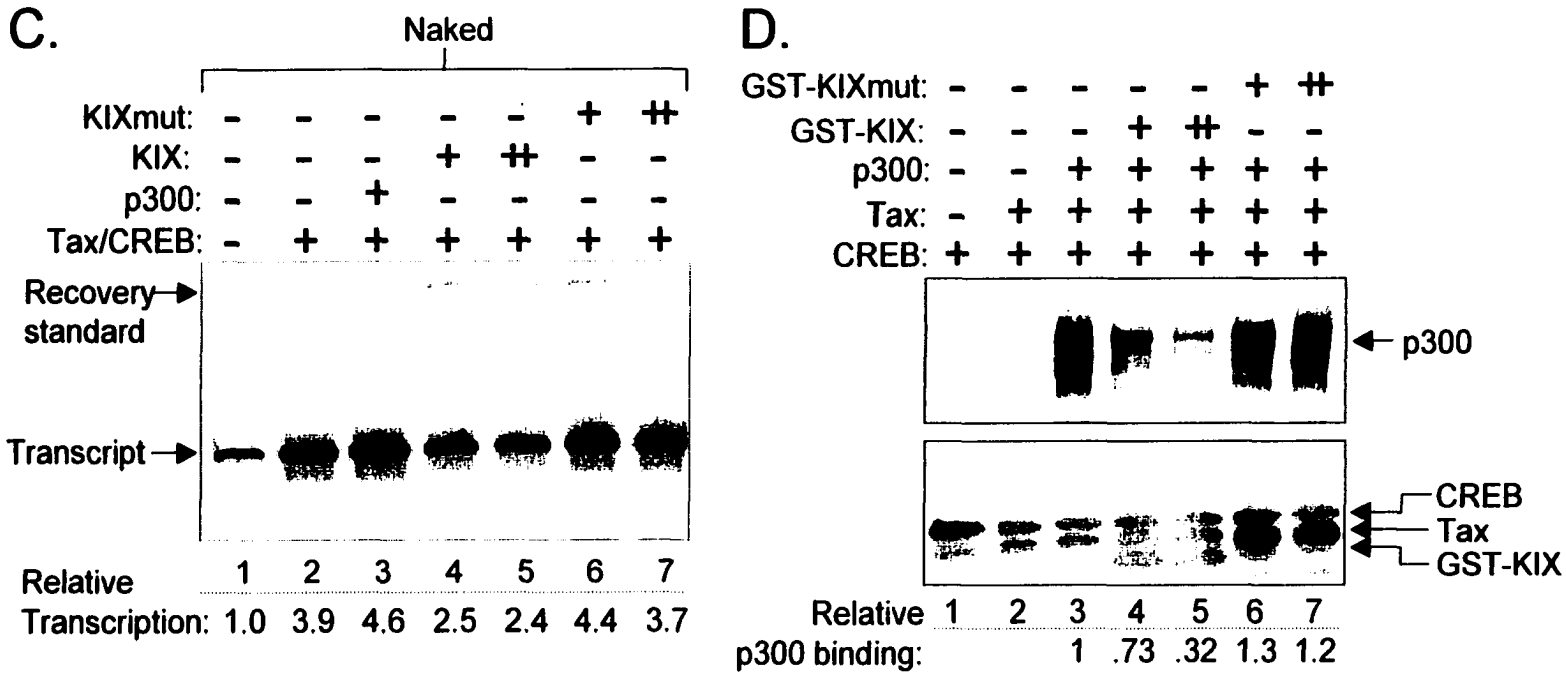


Figure 3.3A-B. **The KIX peptide inhibits Tax-activated transcription on chromatin templates.** (A) KIX inhibits Tax/CREB/p300-mediated transcription. The p4TxRE-G-less cassette template was assembled into chromatin using recombinant *Xenopus* histones. Transcription reaction mixtures contained CEM nuclear extract, and purified recombinant Tax/CREB, or Tax/CREB/p300, as indicated. GST-KIX (aa 588-683) and GST-KIXmut (aa597-719) were added to the transcription reactions at equimolar (+), or five-fold molar excess (++), relative to the Tax/CREB complex (see Materials and Methods). Recovery standard, size markers, and the position of the full-length transcript are indicated. (B) KIX inhibits Tax/CREB transcriptional activation in the absence of exogenous p300. Transcription reactions were performed as described in panel A, except in the absence of exogenous p300.



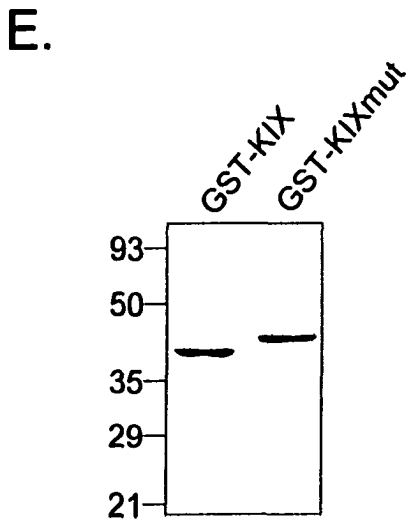


Figure 3.3C-E. **Analysis of KIX function.** (C) KIX does not affect Tax/CREB transcriptional activation on naked DNA templates. In vitro transcription reactions were performed on the unassembled p4TxRE-G-less template. Transcription reaction mixtures contained CEM nuclear extract, and purified Tax/CREB, p300, GST-KIX or GST-KIXmut, as indicated. (D) KIX disrupts p300 binding to the Tax/CREB/viral CRE DNA complex. Binding reactions contained a DNA fragment carrying a single viral CRE linked to a streptavidin-agarose bead. CREB, Tax, and p300 were added to the immobilized viral CRE DNA, as indicated. GST-KIX or GST-KIXmut were added at 0.5X molar (+), or equimolar (++) amounts, relative to the Tax/CREB complex. Reactions were analyzed by Western blot. For this experiment, ser133-phosphorylated CREB was used. This experiment was contributed by Holli A. Giebler. (E) Analysis of purified proteins. Purified recombinant GST-KIX and GST-KIXmut were analyzed by SDS-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue. The sizes of molecular mass markers (in kilodaltons) are indicated.

To test directly whether KIX blocks p300 recruitment to Tax/CREB/viral CRE complexes, we performed a biotin-streptavidin agarose DNA pull-down assay using an immobilized DNA fragment carrying a single viral CRE binding site for the Tax/CREB complex. We used a naked DNA fragment in this assay, since p300 has previously been shown to interact directly with chromatin (143). As shown in figure 3.3D, full-length p300 forms a quaternary complex with Tax and CREB on the viral CRE DNA (lane 3). Addition of GST-KIX to the binding reaction displaced p300 from the complex in a dose-dependent fashion (Fig. 3.3D, lanes 3-5). Displacement of p300 from the Tax/CREB/DNA complex likely occurred as a consequence of GST-KIX binding, as GST-KIX association with the DNA-bound fraction correlated directly with p300 dissociation (Fig. 3.3D, lanes 3-5). As expected, GST-KIXmut was unable to compete with p300 for binding to the Tax/CREB/DNA complex (Fig. 3.3D, lanes 6-7). An SDS-PAGE showing purified GST-KIX and GST-KIXmut used in these assays is presented in figure 3.3E. Together, these data support a model where KIX inhibits Tax/CREB/p300-mediated transcription via disruption of CBP/p300 binding to the Tax/CREB/viral CRE DNA complex (Fig. 3.2A).

Although the KIX peptide repression data shown above are consistent with inhibition of CBP/p300 recruitment, it is also possible that the tight binding of KIX to the Tax/CREB complex simply blocks access of another coactivator or ancillary factor to the HTLV-I promoter. To more specifically define a role for CBP/p300 in Tax-transactivation, we next tested whether the addition of purified GST-SREBP-1a could similarly repress activated transcription from the

chromatin-assembled HTLV-I promoter. Since SREBP-1a binds directly to the CBP/p300 KIX domain, it is a highly specific inhibitor of KIX function (154). Figure 3.4A shows that addition of GST-SREBP-1a to the chromatin-based transcription reactions inhibited both Tax/CREB-activated transcription (lanes 2-4) and Tax/CREB/p300-activated transcription (lanes 6-8). GST-SREBP-1a-mediated transcriptional inhibition was weaker in the presence of exogenously added p300 (Fig. 3.4A, lanes 6-8), consistent with the idea that saturation of CBP/p300 KIX domain is required. Since SREBP-1a was expressed as a GST fusion, we used GST alone as a negative control. GST had no effect on Tax/CREB, or Tax/CREB/p300-activated transcription (Fig. 3.4A, lanes 5 and 9). Similar to our observation with the GST-KIX polypeptide, addition of GST-SREBP-1a, or GST alone, had minimal effect on Tax/CREB-activated transcription on naked templates (Fig. 3.4B). Finally, the addition of GST-SREBP-1a to immobilized biotin-streptavidin DNA pull-down reactions containing Tax, CREB, p300, and the viral CRE DNA, inhibited p300 association with the Tax/CREB/DNA complex in a dose-dependent fashion (Fig. 3.4C). Unlike KIX, SREBP-1a did not replace p300 in the Tax/CREB/viral CRE DNA complex (Fig. 3.9), consistent with our model for SREBP-1a inhibition (Fig. 3.2B). The purified GST-SREBP-1a and GST used in these experiments are shown in figure 3.4D. The SREBP-1a used in these experiments was functional for binding to full-length p300, as shown by a GST pull-down assay (Fig. 3.4E). Together, these data suggest that KIX and SREBP-1a similarly inhibit Tax-transactivation by physically blocking coactivator access to the Tax/CREB complex bound to the

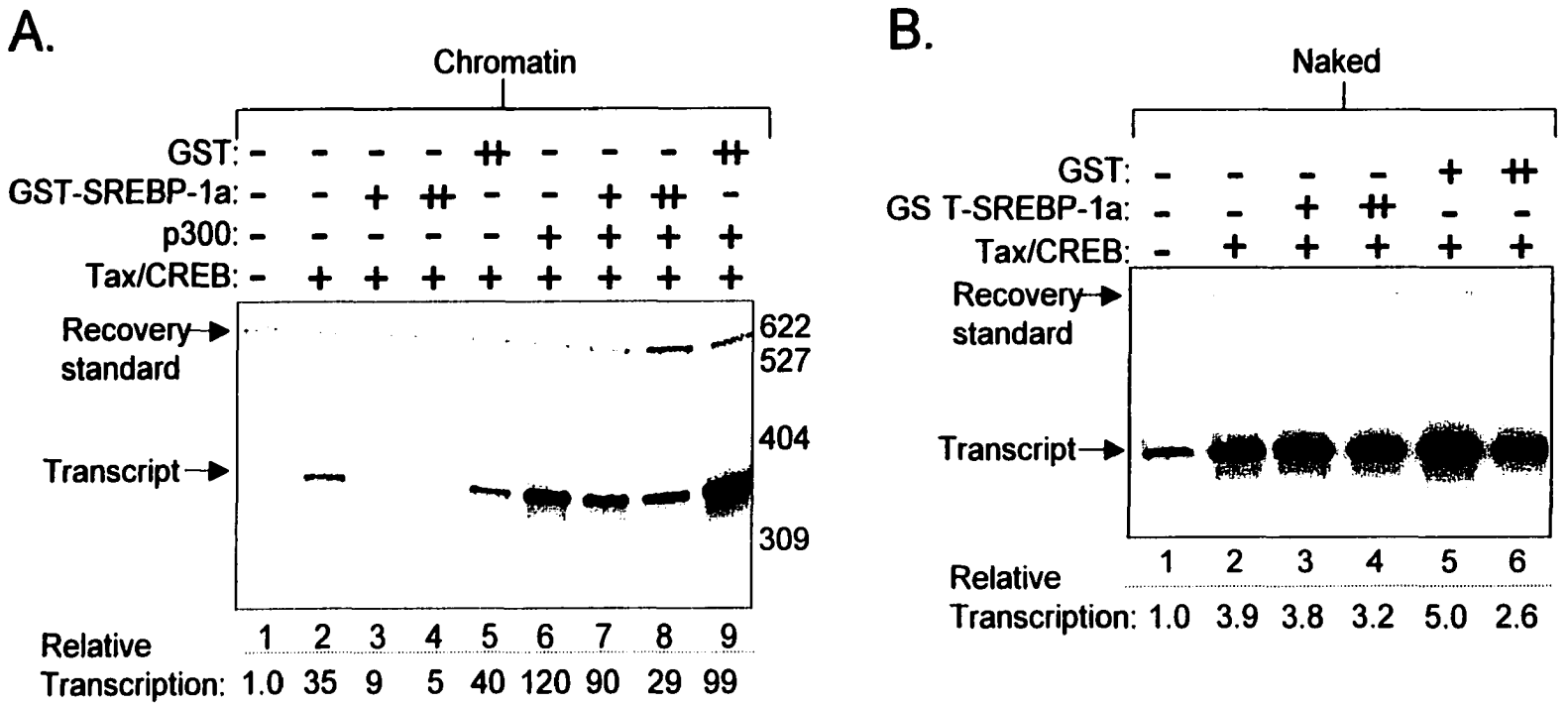


Figure 3.4A-B. **The SREBP-1a peptide inhibits Tax-activated transcription on chromatin templates.** (A) SREBP-1a inhibits Tax/CREB/p300-mediated transcription. Transcription reactions contained the chromatin-assembled p4TxRE-G-less template, CEM nuclear extract, and Tax/CREB, or Tax/CREB/p300, as indicated. GST-SREBP-1a and GST alone were added to the transcription reactions at equimolar (+), or five-fold molar excess (++), relative to the Tax/CREB complex, as indicated. Recovery standard, size markers, and the position of the full-length transcript are indicated. (B) SREBP-1a does not affect Tax/CREB transcriptional activation on naked DNA templates. Transcription reactions contained the unassembled p4TxRE-G-less template, CEM nuclear extract, Tax/CREB, SREBP-1a or GST, as indicated.

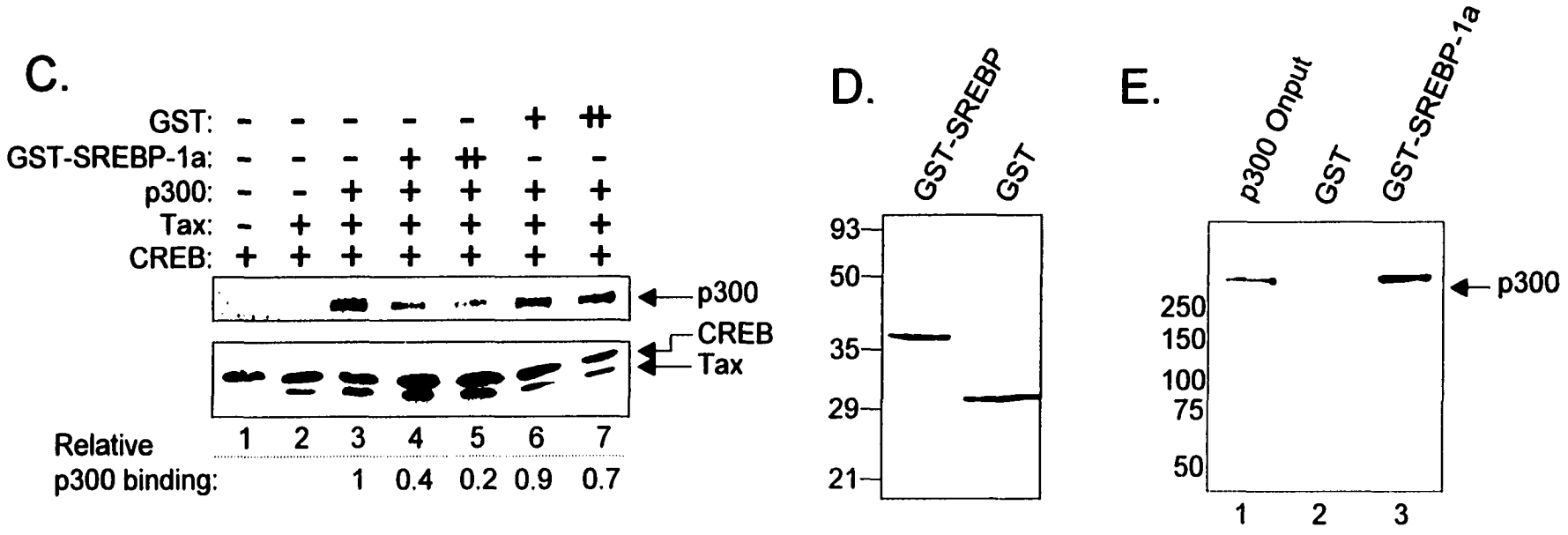


Figure 3.4C-E. **The SREBP-1a peptide inhibits p300 binding to the Tax/CREB/viral CRE DNA complex.** (C) SREBP-1a inhibits p300 binding to the Tax/CREB/viral CRE DNA complex. Streptavidin-agarose DNA pull-down reactions were performed as described in figure 3.2D. GST-SREBP-1a or GST were added at equimolar molar (+) or 5X molar excess (++), relative to the Tax/CREB complex, as indicated. Reactions were analyzed by Western blot. This experiment was contributed by Holli A. Giebler. (D) Analysis of purified proteins used in the transcription and DNA binding reactions. Purified recombinant GST-SREBP-1a and GST were analyzed by SDS-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue. The sizes of molecular mass markers (in kilodaltons) are indicated. (E) GST-SREBP-1a interacts with p300. The GST pull-down assay contained purified recombinant p300, and GST or GST-SREBP-1-a bound to glutathione agarose, as indicated. p300 onput (10%) is shown in lane 1. Reactions were analyzed by Western Blot.

HTLV-I promoter. Furthermore, the strong transcriptional inhibition produced by KIX and SREBP-1a in the absence of exogenous p300 strongly suggests that Tax-activated transcription in a chromatin context in vitro is highly dependent upon CBP/p300 provided by the nuclear extract. Western blot analysis of the CEM nuclear extracts reveals the presence of approximately 0.7 pmol of CBP/p300 in each transcription reaction (Fig. 3.10). This level of CBP/p300 in the nuclear extract is likely sufficient for the strong Tax-transactivation observed in these assays, and may account for the relatively modest stimulation observed in the presence of exogenous p300.

Next, we examined the specificity of KIX and SREBP-1a function by performing a p300 "add-back" experiment. We reasoned that if KIX and SREBP-1a inhibited transcription by blocking Tax/CREB access to the coactivator, then addition of exogenous p300 would overcome the observed effect. Figure 3.5 shows that the GST-KIX inhibition was modestly relieved by the addition of recombinant p300 (lanes 5-6). However, GST-SREBP-1a inhibition was fully reversed in the presence of the highest amount of added coactivator (Fig. 3.5, lane 9). This difference in the degree of transcriptional response to exogenous p300 in the presence of KIX and SREBP-1a is not unexpected, as the two polypeptides mediate transcriptional inhibition via distinct mechanisms (see Fig. 3.2). It is possible that a slow off-rate of GST-KIX from the Tax/CREB complex inhibits access to the exogenous p300. Alternatively, SREBP-1a transcriptional inhibition likely occurs via direct stoichiometric binding to endogenous CBP/p300,

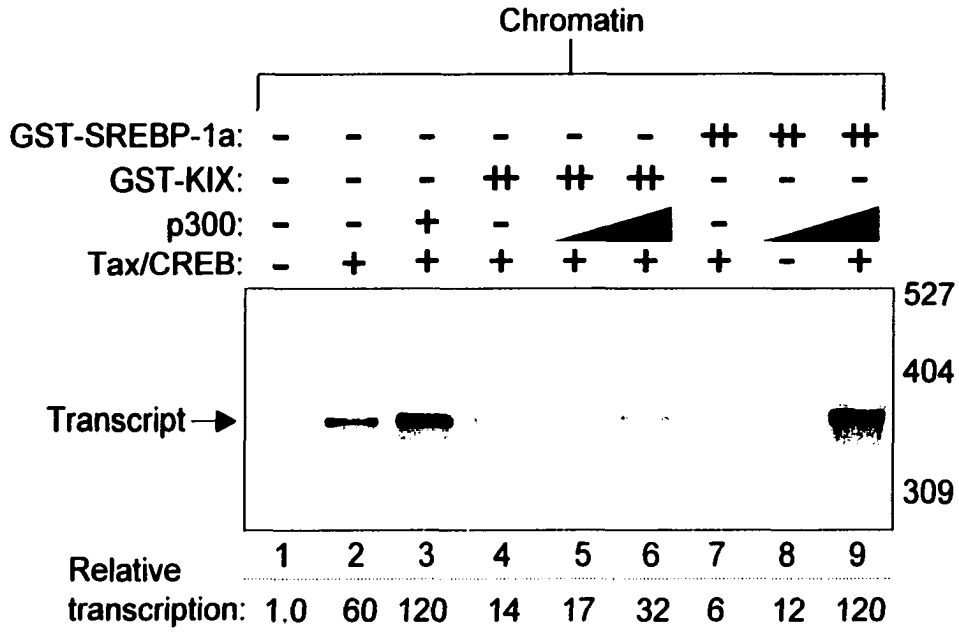


Figure 3.5. **Exogenous p300 relieves KIX and SREBP-1a-mediated inhibition of Tax-activated transcription.** Transcription reactions contained the chromatin-assembled p4TxRE-G-less template, CEM nuclear extract, and Tax/CREB, as indicated. GST-KIX and GST-SREBP-1a were added to the indicated transcription reactions at five-fold molar excess (++), relative to the Tax/CREB complex. Recombinant p300 was added at 0.05 pmol and 0.1 pmol. Size markers and the position of the full-length transcript are indicated. Full-length G-less transcripts are indicated.

therefore, the excess p300 should be available for recruitment to the Tax/CREB/viral CRE complex.

3.3b THE CBP/p300-SELECTIVE ACETYLTRANSFERASE INHIBITOR LYS-COA INHIBITS TAX-TRANSACTIVATION ON CHROMATIN TEMPLATES

The experiments presented above support a prominent role for CBP/p300 in Tax/CREB-activated transcription from Tax-responsive chromatin templates. Since these experiments reveal a significant contribution of endogenous CBP/p300 present in our transcription system, we were interested in selectively testing whether the acetyltransferase function of endogenous CBP/p300 was responsible for Tax/CREB transcriptional activation. We have previously observed that activation of Tax transcription by exogenous p300 required intact acetyltransferase activity, as a p300 acetyltransferase mutant was inactive for transcriptional stimulation relative to wild type p300 (57). However, these experiments did not address the contribution of endogenous CBP/p300 acetyltransferases in mediating Tax function.

To specifically evaluate the contribution of endogenous acetyltransferase activity in our transcription system, we tested the effect of the selective CBP/p300 inhibitor Lys-CoA on Tax/CREB-activated transcription. Lys-CoA has previously been shown to inhibit p300 (and by extension CBP) both in vitro and in vivo, with a greater than 100-fold selectivity for p300 relative to P/CAF (13, 114, 119, 137, 177). Figure 3.6A shows that the addition of 50 μM Lys-CoA to Tax-activated chromatin transcription reactions containing 10 μM acetyl CoA

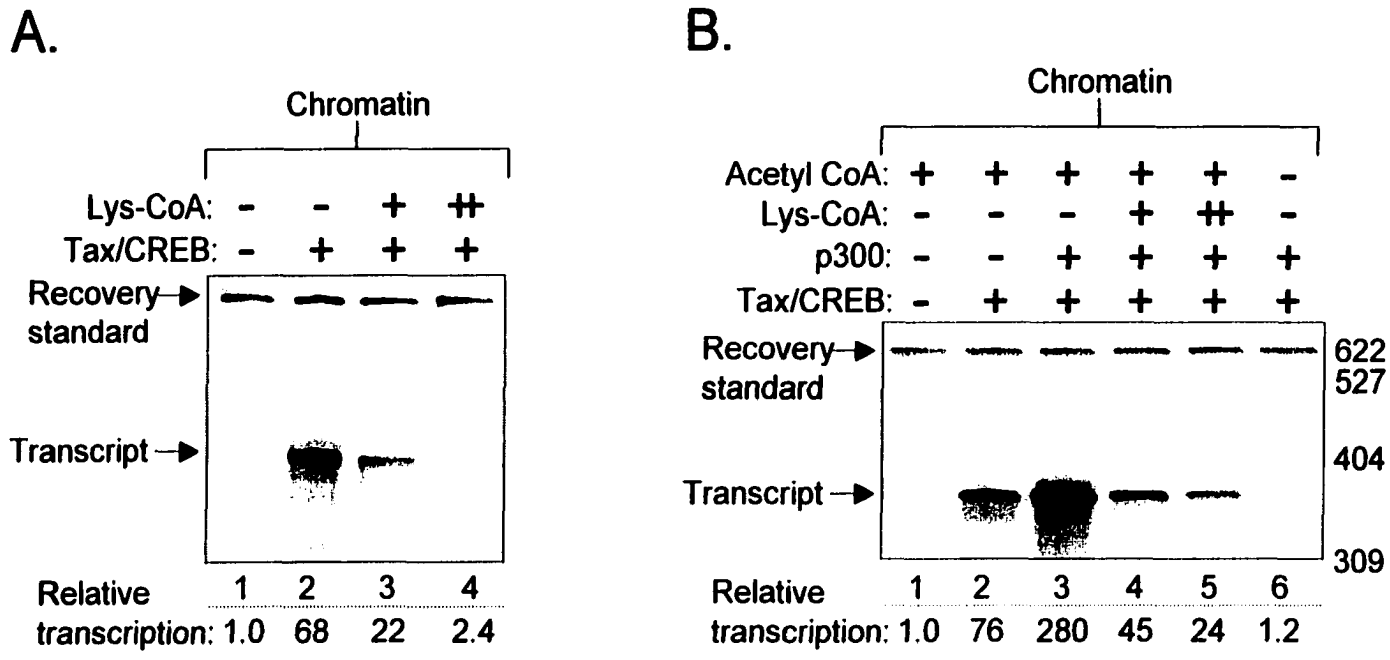


Figure 3.6A-B. **The CBP/p300-selective HAT inhibitor Lys-CoA inhibits Tax-activated transcription on chromatin templates.** (A) Transcription reactions contained the chromatin-assembled p4TxRE-G-less template, CEM nuclear extract, and Tax/CREB, as indicated. Lys-CoA was added to the transcription reactions at final concentrations of 10 μM (+) and 50 μM (++), as indicated. Acetyl CoA was added to a final concentration of 10 μM in all samples. Recovery standard and the position of the full-length transcript are indicated. (B) Lys-CoA inhibits Tax-activated transcription in the presence of exogenous p300. Transcription reactions were performed as described in panel A, except in the presence of exogenous p300, as indicated. Lane 6 shows Tax/CREB–activated transcription in the absence of acetyl CoA.

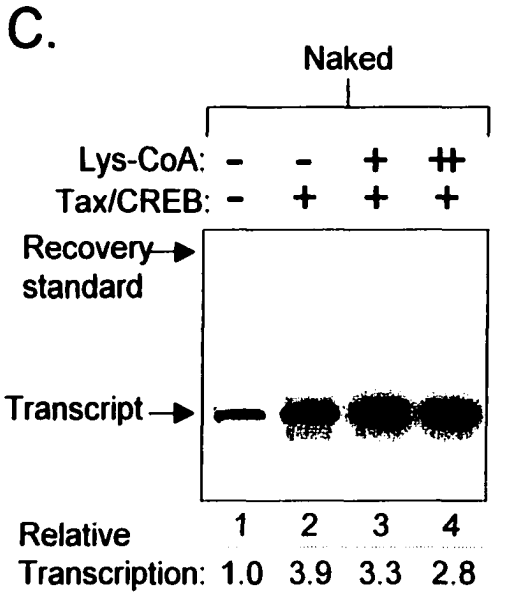


Figure 3.6C. **The CBP/p300-selective HAT inhibitor Lys-CoA does not inhibit Tax-activated transcription on naked DNA templates.** Transcription reactions were performed on the unassembled p4TxRE-G-less template. Transcriptional activation was analyzed in the presence of Tax/CREB, 10 μM acetyl CoA, and 10 μM (+) or 50 μM (++) Lys-CoA, as indicated.

reduced RNA synthesis by greater than 90% (lane 4), consistent with a previous observation (46). The concentrations of Lys-CoA used in these experiments have previously been shown to selectively inhibit p300 acetyltransferase activity by >90% (119). We next tested Lys-CoA inhibition in the presence of Tax/CREB and exogenous p300 and found that Lys-CoA inhibited transcription to a degree similar to that observed with Tax/CREB alone (Fig. 3.6B, lanes 4-5). As expected, Tax/CREB/p300-activated transcription was essentially abolished in the absence of acetyl CoA (Fig. 6B, lane 6). Lys-CoA had minimal effect on Tax/CREB-activated transcription from naked DNA templates (Fig. 3.6C).

The Lys-CoA inhibition data strongly suggest that Tax/CREB transcriptional activation associated with CBP/p300 recruitment results primarily from the intrinsic acetyltransferase activity associated with the coactivator(s). If this is correct, then it would follow that the transcriptional inhibition observed in the presence of the KIX and SREBP-1a polypeptides results from an inhibition of targeted p300 histone acetyltransferase (HAT) activity. To test this idea directly, we performed an in vitro HAT assay in the presence of Tax/CREB and KIX. Figure 3.7 shows that, as expected, Tax/CREB addition increased the p300-dependent acetylation of histones assembled on the Tax-responsive p4TxRE/G-less template (lanes 1, 2). However, Tax/CREB did not increase p300-dependent acetylation of histones assembled on the p-52/G-less plasmid template lacking the Tax-responsive viral CRE enhancer elements (Fig. 3.11). The addition of GST-KIX reduced histone acetylation to levels observed in the absence of added activators, presumably through inhibition of p300 recruitment

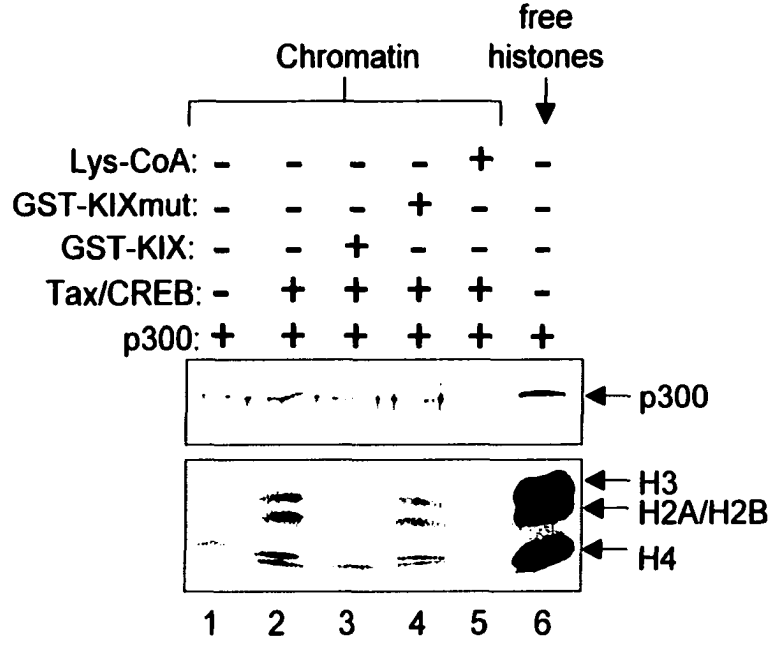


Figure 3.7. **KIX disrupts the recruitment of p300 HAT activity to chromatin templates in a Tax-dependent manner.** In vitro acetylation assay. Both chromatin- assembled templates and free histones were acetylated in vitro in the presence of p300 and ^{14}C-acetyl CoA. Tax/CREB, GST-KIX and GST-KIXmut were added to the indicated acetylation reactions. The CBP/p300 specific HAT inhibitor lysyl CoA was added to a final concentration of 50 μM, as indicated. GST-KIX and GST-KIXmut were each added at five-fold molar excess relative to the Tax/CREB complex.

(Fig. 3.7, lane 3). This inhibition was specific to chromatin, as free histone acetylation and p300 autoacetylation were unaffected by GST-KIX (Fig. 3.7, lane 3, and Fig. 3.12). GST-KIXmut, which is defective for Tax/CREB binding, had minimal effect on p300 HAT activity (Fig. 3.7, lane 4). As expected, 50 μM Lys-CoA completely inhibited p300 acetyltransferase activity (Fig. 3.7, lane 5). Together, these data indicate that the primary mechanism of CBP/p300 transcriptional co-activation on the HTLV-I promoter is through the acetyltransferase activity of the coactivators.

3.3c HISTONE TAIL DELETION REVEALS AN UNKNOWN TARGET FOR CBP/p300 ACETYLTRANSFERASE FUNCTION

The data presented thus far support a role for the KIX domain in Tax/CREB recruitment of CBP/p300 to the HTLV-I promoter. We also show data indicating that CBP/p300 cooperate with Tax to activate transcription of the p4TxRE/G-less chromatin template via their intrinsic acetyltransferase activity. Since the histone tails are established targets of coactivator acetylation during transcriptional activation, we reasoned that deletion of the histone tails would render the transcription system insensitive to CBP/p300. To test this hypothesis, we used "tailless" histones carrying amino terminal deletions (H2A Δ1-12, and 119-128; H2B Δ1-23; H3 Δ1-26; H4 Δ1-19) to assemble chromatin on the p4TxRE/G-less template as previously described (57). All of the previously identified targets of CBP/p300 acetylation are deleted in our tailless histones (185). We tested whether chromatin composed of tailless histones was sensitive

to KIX/SREBP-1a inhibition of Tax/CREB-mediated transcriptional activation. As shown before, nucleosomal templates formed with tailless histones were strongly activated by Tax/CREB, and insensitive to exogenous p300 (Fig. 3.8A, lanes 1-3) (see 57). Unexpectedly, the addition of GST-KIX, but not GST-KIXmut, inhibited Tax/CREB transcriptional activation over 80% on the tailless templates (Fig. 3.8A, lanes 4-7). Similar transcriptional inhibition was observed with GST-SREBP-1a, both in the presence and absence of exogenous p300 (Fig. 3.8B). These results were surprising, as the tailless histones carry deletions of the regions encompassing the previously characterized sites of acetylation. We confirmed the specificity of the effects of KIX and SREBP-1a on tailless chromatin by performing a p300 "add-back" experiment (Fig. 3.8C). Consistent with the results on wild type chromatin templates (Fig. 3.5), we found that inhibition by GST-KIX is highly stable and not responsive to exogenous p300, while inhibition by GST-SREBP-1a was reversed by exogenous p300. To determine if KIX and SREBP-1a-mediated transcriptional inhibition was associated with disrupted recruitment of the acetyltransferase activity of CBP/p300, we tested the effect of Lys-CoA on Tax-activated transcription from the tailless chromatin templates. Figure 3.8D shows that 50 μM Lys-CoA inhibited transcription by up to 80%. Interestingly, this effect was indistinguishable from the level of inhibition observed in the absence of acetyl CoA, suggesting that acetyl CoA is utilized exclusively by CBP/p300 on these templates. Together, these data strongly support a role for CBP/p300 acetyltransferase activity in Tax/CREB-dependent activation of transcription

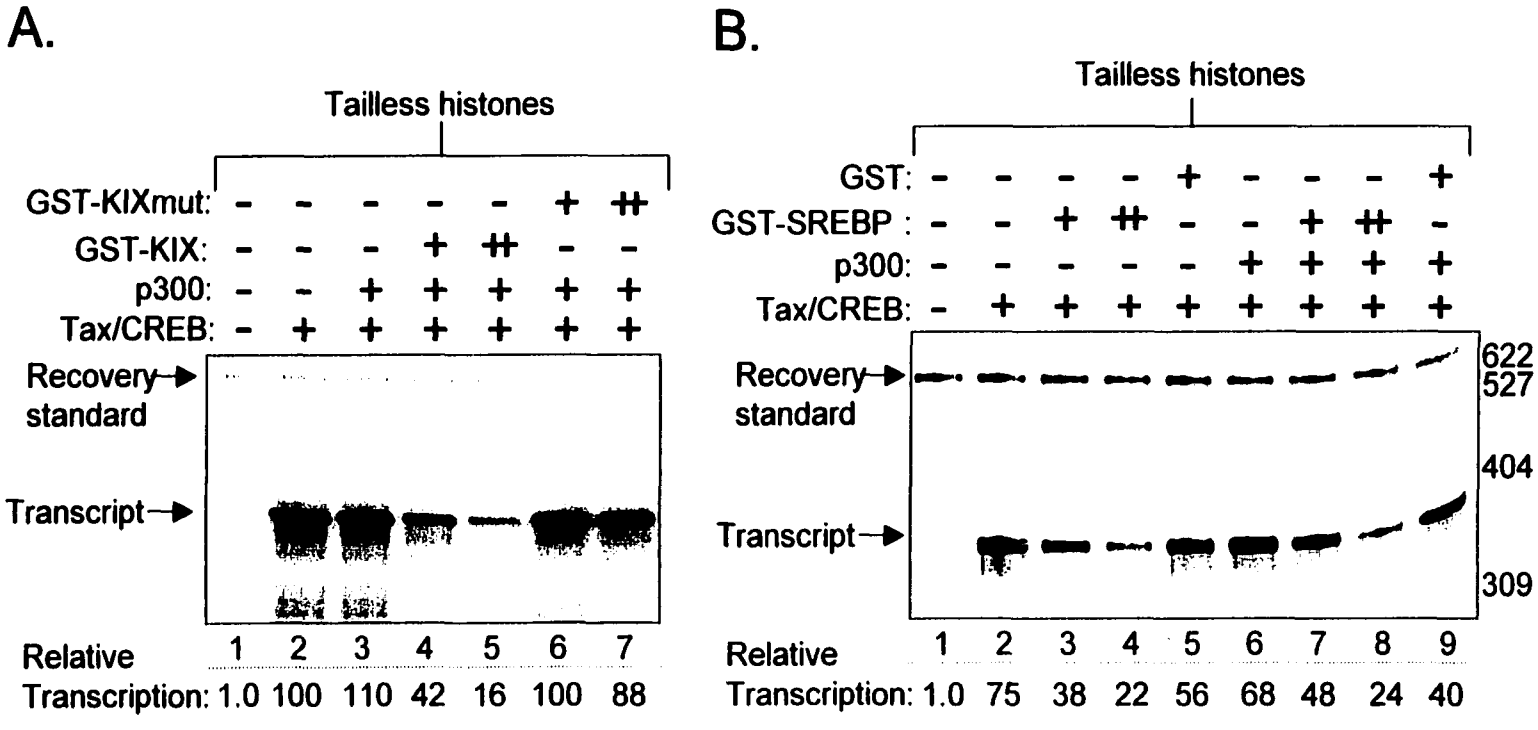


Figure 3.8A-B. **Inhibition of p300 function on chromatin templates lacking histone amino-terminal tails.** Transcription reactions contained the p4TxRE-G-less template assembled into chromatin using tailless histones, CEM nuclear extract, and Tax/CREB, as indicated. Recovery standard and full-length G-less transcripts are indicated. (A) The KIX polypeptide specifically inhibits Tax/CREB transcription on tailless chromatin. GST-KIX or GST-KIXmut were added at equimolar (+), or five-fold molar excess (++), relative to the Tax/CREB complex, as indicated. (B) SREBP-1a specifically inhibits Tax/CREB transcription on tailless chromatin. GST-SREBP-1a and GST were added at equimolar (+), or five fold molar excess (++), relative to the Tax/CREB complex, as indicated.

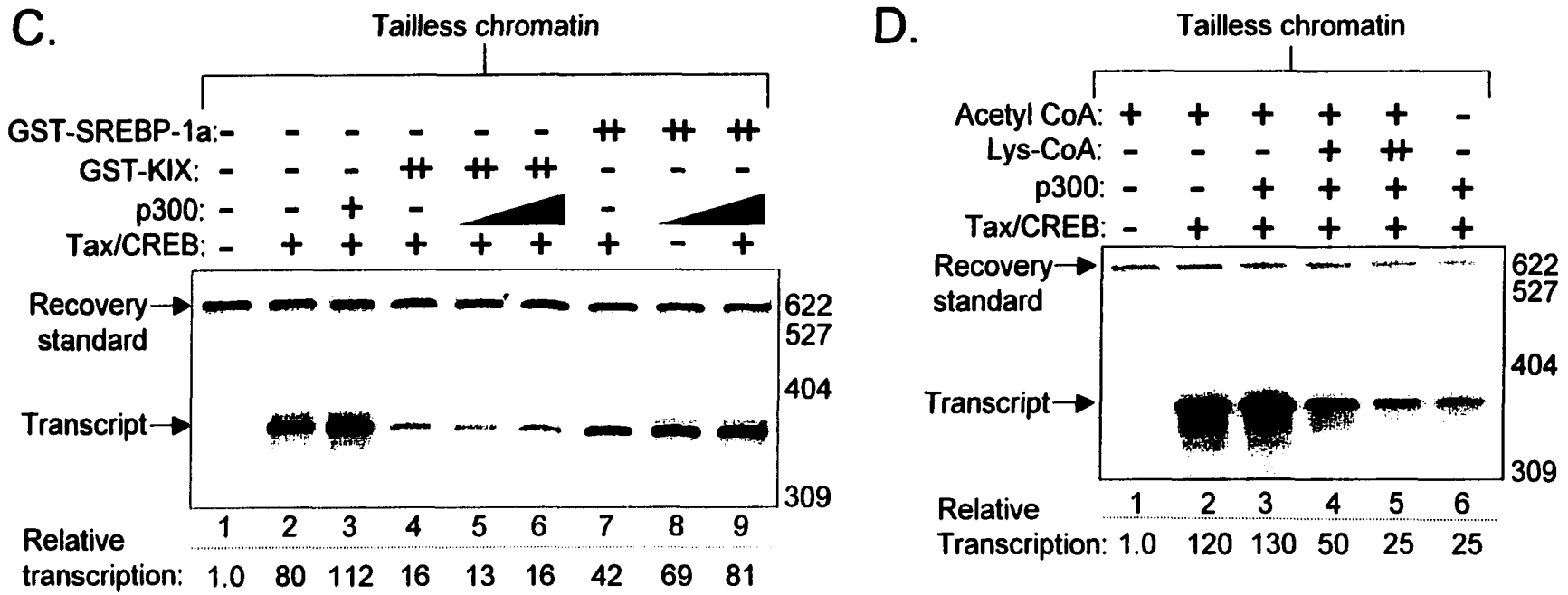


Figure 3.8C-D. **Inhibition of p300 function on chromatin templates lacking histone amino-terminal tails.** In vitro transcription assay. Transcription reactions contained the p4TxRE-G-less template assembled into chromatin using tailless histones, CEM nuclear extract, and Tax/CREB, as indicated. (C) Exogenous p300 relieves SREBP-1a-mediated inhibition of Tax/CREB transcription on tailless chromatin. GST-KIX and GST-SREBP-1a were added to the indicated transcription reactions at five-fold molar excess (++), relative to the Tax/CREB complex. Recombinant p300 was added at 0.05 pmol and 0.1 pmol. (D) Lys-CoA inhibits Tax-activated transcription on chromatin templates lacking histone amino-terminal tails. For this experiment, acetyl CoA was added to a final concentration of 10 μM in samples 1-5; acetyl CoA was not added to sample 6. Lys-CoA was added to samples at final concentrations of 10 μM (+) and 50 μM (++). Recovery standard and full-length G-less transcripts are indicated.

from tailless nucleosomal templates. Since the CBP/p300 inhibitors had a minimal effect on transcription from naked DNA templates, these data suggest that chromatin-related acetylation substrates, distinct from the histone tails, may be targeted by CBP/p300 to promote transcriptional activation from the HTLV-I promoter in vitro.

3.4 DISCUSSION

Several previous studies have characterized a physical interaction between the KIX domain of CBP/p300 and the viral transcriptional activator protein Tax (58, 77, 117, 225). Here, we provide the first direct evidence that KIX-mediated Tax recruitment of CBP/p300 promotes strong Tax-transactivation from chromatin templates. Using specific polypeptide inhibitors of the Tax-KIX interaction (GST-KIX and GST-SREBP-1a), we find that Tax recruitment of CBP/p300 present in the nuclear extract is a critical step in mediating Tax-transactivation in a chromatin context, accounting for greater than 80% of the observed Tax stimulation in this system. The SREBP-1a inhibition is counteracted by the addition of recombinant p300 to the transcription reactions, providing strong evidence that CBP/p300 are the primary endogenous coactivators that mediate Tax-transactivation. The effect of the coactivators is absolutely dependent on chromatin, as Tax-transactivation from unassembled (naked) templates is refractory to the stimulatory effects of acetyl CoA and p300, and is not inhibited by the polypeptides.

We also investigated the specific role of the intrinsic acetyltransferase activity of CBP/p300 in Tax-transactivation. As previously shown, we find that Tax enhances nucleosomal histone acetylation by recruiting p300 to chromatin templates (57, 137). We find that the addition of KIX peptide dramatically inhibits this effect, directly coupling Tax recruitment of CBP/p300, via the KIX domain, with nucleosome acetylation and transcriptional activation. We find that the p300-selective inhibitor Lys-CoA significantly inhibits Tax-activated transcription, reducing transcription to the same levels observed with the KIX-specific polypeptide inhibitors. This observation is consistent with a prominent role for a CBP/p300 acetyltransferase activity in transcriptional initiation, as previously suggested (112, 114). These data provide further evidence showing that CBP/p300 are the prominent coactivators mediating Tax transcription function, and importantly, that the acetyltransferase activity intrinsic to CBP/p300 provides the dominant transcriptional stimulatory activity associated with coactivator recruitment to the Tax-responsive promoter.

Although we observe residual Tax-transactivation in the presence of Lys-CoA, transcription is abolished in the absence of acetyl CoA. While this may represent incomplete Lys-CoA inhibition of endogenous CBP/p300, it is also possible that acetyltransferases unrelated to CBP/p300 participate in Tax-transactivation from chromatin templates. This observation is not surprising, as a broad range of coactivators and ancillary factors utilize acetyl CoA to activate transcription (204). The most likely candidates for these additional coactivators are proteins that acetylate lysine residues on the amino terminal histone tails that

are distinct from those lysines targeted by CBP/p300. For example, SRC-1 acetylates lysine 9 of histone H3, while Tip60 and MORF acetylate lysines 12 and 16 of histone H4 (29, 204). Acetylation of these lysine residues, in addition to CBP/p300-specific acetylation, may be required for optimal transcriptional activation by Tax. Although SRC-1 forms a complex with CBP/p300 at certain promoters (102, 227), a role for SRC-1 in HTLV-I transcription has not been described. It is also possible that additional acetyltransferases having overlapping substrate specificity with CBP/p300 cooperate to acetylate key lysine residues on the amino terminal histone tails. For example, lysine 14 of histone H3 is acetylated not only by CBP/p300, but also by GCN5, P/CAF, SRC-1, and $TAF_{II}250$ (204). Of these acetyltransferases, P/CAF has been shown to interact with Tax in both vitro and in vivo, and to activate HTLV-I transcription in transient transfection assays (76, 91). Based on these data, it is conceivable that P/CAF cooperates with CBP/p300 in histone tail acetylation, and thus participates in Tax-transactivation of HTLV-I transcription.

We previously reported that the amino terminal histone tails are required for Tax-transactivation in response to exogenously added p300, and that tail deletion rendered the chromatin templates insensitive to recombinant p300 addition, while remaining moderately sensitive to acetyl CoA (57). In the previous study, however, the role of endogenous CBP/p300 in Tax-transactivation was not addressed. In the present study, we make the unexpected observation that the acetyltransferase activity required for Tax-transactivation on the tailless chromatin templates is contributed by CBP/p300 that is present in the nuclear

extract. As with wild type chromatin templates, we observe potent inhibition of Tax-activated transcription from the tailless chromatin templates with both CBP/p300-selective Lys-CoA and the polypeptide inhibitors. Interestingly, Lys-CoA inhibits transcription to the same level as that observed in the absence of acetyl CoA. These data indicate that CBP/p300 play a prominent and perhaps exclusive role in Tax-transactivation from tailless chromatin templates, and that the acetyltransferase activity provides the coactivator function in this context.

It seems paradoxical that tailless chromatin templates require CBP/p300 acetyltransferase activity and yet are unresponsive to exogenous p300. However, responsiveness to exogenous p300 may be a function of the number of potential acetylation sites present in the transcription system. For example, previous characterization of CBP/p300 suggests that wild-type chromatin templates contain up to 22 CBP/p300 acetylation targets per nucleosome, in addition to other potential CBP/p300 acetyltransferase targets present in the nuclear extract (185). Although we have found a substantial concentration of endogenous CBP/p300 in our CEM nuclear extract, it appears that the endogenous CBP/p300 may be limiting. Therefore, Tax-transactivation from wild-type chromatin templates is enhanced by exogenous p300. However, in the absence of histone tails, the number of potential CBP/p300 acetylation substrates present in the transcription system is dramatically reduced. Therefore, endogenous CBP/p300 acetyltransferase activity may be saturating for strong Tax-transactivation from tailless chromatin templates; consequently, exogenous p300 has no effect. Consistent with the idea that a reduced number

of acetylation substrates leads to a reduced requirement for acetyltransferase enzyme, we have previously observed that tailless chromatin templates have a reduced requirement for acetyl CoA in Tax-transactivation compared with wild type chromatin templates (see 57, and data herein).

In contrast to our findings on both wild type and tailless chromatin templates, the inhibitors of CBP/p300 (GST-KIX, GST-SREBP-1a, and Lys-CoA) did not significantly affect transcription from the naked DNA templates. These observations reveal the presence of a CBP/p300 acetyltransferase target that functions specifically on chromatin templates, is independent of the histone tails, and is critical to Tax-transactivation. This unknown acetylation target of CBP/p300 may be a component of the general transcription machinery and/or chromatin remodeling factor that is acetylated to facilitate negotiation of chromatin templates during transcriptional activation. Although it is possible that Tax, and/or CREB, may be the acetylation target in this context, we have found no evidence for p300 acetylation of the free proteins (Fig. 3.12). Since the p4TxRE/G-less transcription template used in these assays carries, in addition to the viral CREs, only the minimal HTLV-I promoter (-52 to +1), it is also unlikely that the acetylation target is an uncharacterized transcription factor that binds to this simplified promoter. Based on these observations, the evidence indicates that the acetylation target of CBP/p300 is a chromatin-affiliated protein, or chromatin itself. An intriguing possibility is that the nucleosomal core is itself acetylated by CBP/p300. In the experiments presented herein, we used a histone H3 deletion mutant that carried a lysine residue at the amino terminal end

may in turn promote stable association of the general transcription machinery with the promoter DNA, and the initiation of HTLV-I transcription.

The role of the histone amino terminal tails in p300-mediated transcriptional activation has recently been investigated using Gal4-VP16 and recombinant core histones (6). Similar to the HTLV-I Tax chromatin transcription system, GAL4-VP16 displayed acetyl CoA-dependent and p300-dependent activation. However, deletion of the histone tails potently attenuated GAL4-VP16 transcriptional activation (6), in sharp contrast to our observations with Tax (57), and data herein). This apparent dichotomy in the behavior of tailless nucleosomes may be attributable to differences in the activators used in the assay (Gal4-VP16 vs. Tax), or differences in DNA template design. The GAL4-responsive promoter is flanked by multiple 5S repeats, whereas the construct used in our assays is not. The disparity between our data and that of An et. al. (6) is likely due to uncharacterized chromatin-based processes by which different transcriptional activators require histone tails, or histone tail modifications, to promote activated transcription.

The discrepancy in transcriptional activities on the tailless chromatin templates discussed above raises fundamental questions as to the precise function of the histone tails during transcriptional activation. Several previous studies have shown that templates containing tailless histones are more accessible to binding by transcriptional activators (62, 175, 218), and are generally more transcriptionally active than intact chromatin templates (35, 80, 179, 218). This may reflect the ability of tailless chromatin to adopt a less

condensed structure than intact chromatin (28, 47, 111, 210; reviewed in 72). However, in vivo, histone tails are subject to a complex array of highly conserved post-translational modifications, in addition to acetylation, that appear to be intimately involved in regulated gene expression (20). These distinct histone tail modifications apparently serve as an epigenetic code to signal the binding of transcriptional regulatory proteins that are involved in transitions between active and silent chromatin states (90, 205). Based on the complexity of the histone code, it appears paradoxical that our tailless chromatin templates are functionally indistinguishable from wild type chromatin templates with respect to transcriptional activation by Tax. One explanation for this paradox is that, in our in vitro chromatin system, wild type and tailless templates exist in a fully relaxed chromatin state and therefore are permissive to activators, especially strong activators such as Tax. If histone tail modifications function primarily to regulate transitions between higher-ordered structures in vivo, it is not unexpected that the chromatin templates used in these assays are exempt from regulation according to the "histone code". Therefore, histone tail deletion may represent the "ultimate" activating modification of chromatin.

In summary, the data presented in this study demonstrate a prominent and perhaps exclusive role for the KIX domain in CBP/p300 recruitment by the HTLV-I Tax protein. We find that Tax-transactivation from chromatin templates is largely dependent upon CBP/p300 present in the nuclear extract, and that the acetyltransferase activity is the prominent functional property of the coactivators responsible for mediating transcriptional activation. By specifically inhibiting

CBP/p300 enzymatic activity, we have discovered a mode of CBP/p300 transcriptional regulatory function that is acetyl CoA-dependent and histone tail-independent. We find that CBP/p300 acetyltransferase activity is directed not only at the histone tails, but also at an undefined target whose acetylation is critical to the activation of viral gene expression in a chromatin context. Our data provides insight into novel mechanisms of HTLV-I transcriptional regulation through CBP/p300, and may also have significant implications for CBP/p300 function in a broad range of promoter contexts.

3.5 MATERIALS AND METHODS

3.5a PURIFICATION OF RECOMBINANT PROTEINS.

The four *Xenopus laevis* core histones, both as wild-type and amino terminal deletion mutant proteins, were individually expressed in *E. coli* and purified to homogeneity as previously described (139, 140). The amino acid co-ordinates of the histone deletion mutants are: H2A (Δ1-12 and 119-128), H2B (Δ1-23), H3 (Δ1-26), and H4 (Δ1-19). *Drosophila* NAP-1 (85), ISWI and Acf1 (88) were expressed and purified as previously described (57). Full-length, recombinant CREB (48) and Tax (240) were expressed and purified as previously described (58). His_6-tagged p300 was expressed and purified as previously described (113). Recombinant glutathione-S-transferase (GST), and the GST fusion proteins GST-KIX (CBP aa588-683) (58), GST-KIXmut (CBP

aa597-719) (225), and GST-SREBP-1a (SREBP-1 aa1-50) (154), were expressed and purified as previously described.

3.5b CHROMATIN ASSEMBLY AND *IN VITRO* TRANSCRIPTION.

Chromatin templates were assembled as previously described, at core histone to DNA ratios that were empirically determined to give complete chromatin assembly (57). The 3.2 kbp p4TxRE/G-less plasmid DNA used in the transcription assays carried 4 tandem copies of the HTLV-I promoter proximal viral CRE cloned upstream of the HTLV-I core promoter, and drove the synthesis of a 380 nt transcript (8). The p-52/G-less plasmid DNA carried –52 to +1 (relative to the transcription start site) of the natural HTLV-I core promoter, and drove the synthesis of a 190 nt transcript. This plasmid contained a TATA box, but no Tax-responsive viral CRE sequences. Following chromatin assembly, 75 fmol of the chromatin-assembled template were incubated in the absence or presence of 8 nM purified recombinant Tax, 8 nM CREB, and 3 nM p300, as previously described (57, 126). For experiments using unassembled, or naked DNA templates, samples were prepared as above, but without the chromatin assembly step. All reactions contained 100 μM acetyl CoA unless otherwise indicated. Molecular weight markers (radiolabeled *Hpa* II digested pBR322) were used to estimate the size of the RNA products.

3.5c BIOTIN-STREPTAVIDIN DNA PULL-DOWN ASSAY.

Double stranded DNA fragments containing a single viral CRE (and biotinylated at the 5'-end of one strand, [Integrated DNA Technologies]) were annealed and bound to streptavidin agarose (Novagen) in TM buffer (50 mM Tris, 100 mM KCl, 12.5 mM $MgCl_2$, 1 mM EDTA, 20% glycerol, 0.1% Tween-20, 2 mM DTT, and 1 mM PMSF). The HTLV-I promoter-proximal viral CRE sequence used in this assay was modified to contain a consensus CRE site to enhance CREB binding. The sequence of the DNA fragment is:

5'-GAAGATCTCTCAGGCGT**TGACGTCA**ACCCCTCACCAGATCTT-3'

The core CRE region is indicated in bold, and the conserved GC-rich flanks are underlined. The immobilized viral CRE DNA (2 pmol) was incubated with Tax (10 pmol), protein kinase A-phosphorylated CREB (10 pmol), p300 (10 pmol), GST-KIX or GST-KIXmut (5 or 10 pmol), GST-SREBP-1a or GST (10 or 50 pmol) as indicated in the relevant figures. The reactions were incubated in binding buffer (25 mM Tris, 50 mM KCl, 6.25 mM $MgCl_2$, 0.5 mM EDTA, 10% glycerol, 0.05% Tween-20, 50 mM NaCl, 2 mM DTT, and 1 mM PMSF) for 1 hour at 4°C, washed three times in binding buffer, resuspended in SDS sample dyes, boiled, and analyzed by 5-20% SDS-PAGE. Bound proteins were detected by Western blot analysis, using an antibody mixture of anti-GST (to detect GST-KIX, GST-KIXmut, GST and GST-SREBP-1a; Sigma), anti-His_6 (to detect Tax and p300; Santa Cruz), and anti-CREB (Santa Cruz).

3.5d GST PULL-DOWN ASSAY.

The GST pull-down assay was performed by incubating 10 pmol of GST or GST-SREBP-1a with 15 µl of swollen glutathione agarose (Sigma) in 400 µl of 0.5X Superdex buffer (12.5 mM HEPES pH 7.9, 6.25 mM $MgCl_2$, 75 mM KCl, 5 µM $ZnSo_4$, 0.5 mM EDTA, 10% glycerol, 0.05% NP-40) at 4°C for 2 h. The beads were then washed twice with 0.5X Superdex buffer and incubated with 2 pmol p300 (in a total volume of 400 µl of 0.5X Superdex buffer) at 4°C for 18 h. After washing twice with 0.5X Superdex, the beads were resuspended in SDS sample dyes, boiled, and analyzed by 5-20% SDS-PAGE. Bound protein was detected by Western Blot analysis, using an antibody against p300 (N-15, Santa Cruz).

3.5e *IN VITRO* ACETYLATION ASSAY.

The p4TxRE/G-less plasmid template was assembled into chromatin using *Xenopus* histones (2 µg), NAP-1, and ACF as previously described (57). Chromatin assembly was performed at empirically determined histone to DNA mass ratios. p300 (12 nM) and ^{14}C-acetyl CoA (0.9 mM; 57 mCi/mmol) were added following chromatin assembly, together with 400 nM Tax and CREB where applicable, in a solution containing 50 mM Tris, pH 8, 10% glycerol, 10 mM sodium butyrate, 1 mM DTT, 1 mM PMSF. Samples were incubated at 30°C for 60 minutes and protein was precipitated by methanol/chloroform extraction and analyzed by 18% SDS-PAGE.

3.6 ACKNOWLEDGEMENTS.

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SUPPLEMENTAL FIGURES

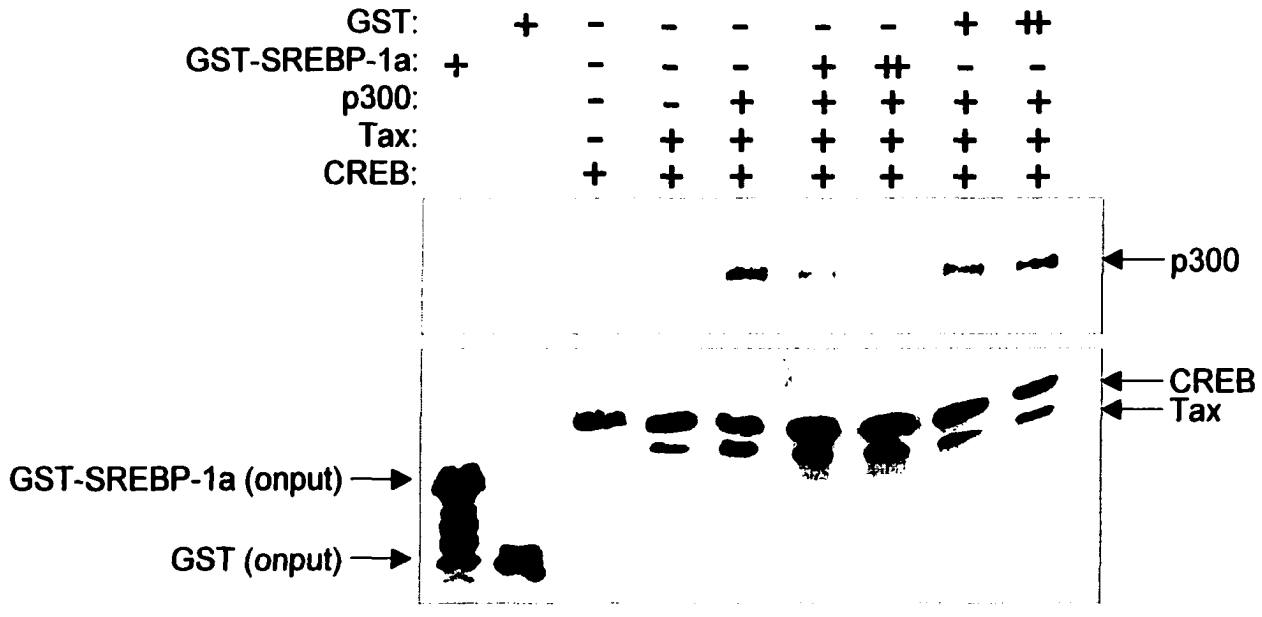


Figure 3.9. **The SREBP-1a peptide does not bind the Tax/CREB/viral CRE DNA complex.** SREBP-1a inhibits p300 binding to the Tax/CREB/viral CRE DNA complex, but does not itself bind to the complex. Streptavidin-agarose DNA pull-down reactions were performed as described in figure 3.3D. GST-SREBP-1a or GST were added at equimolar molar (+) or 5X molar excess (++), relative to the Tax/CREB complex, as indicated. Reactions were analyzed by Western blot. Shown is an expanded version of the same experiment that is presented in figure 3.4C. This experiment was contributed by Holli A. Giebler.

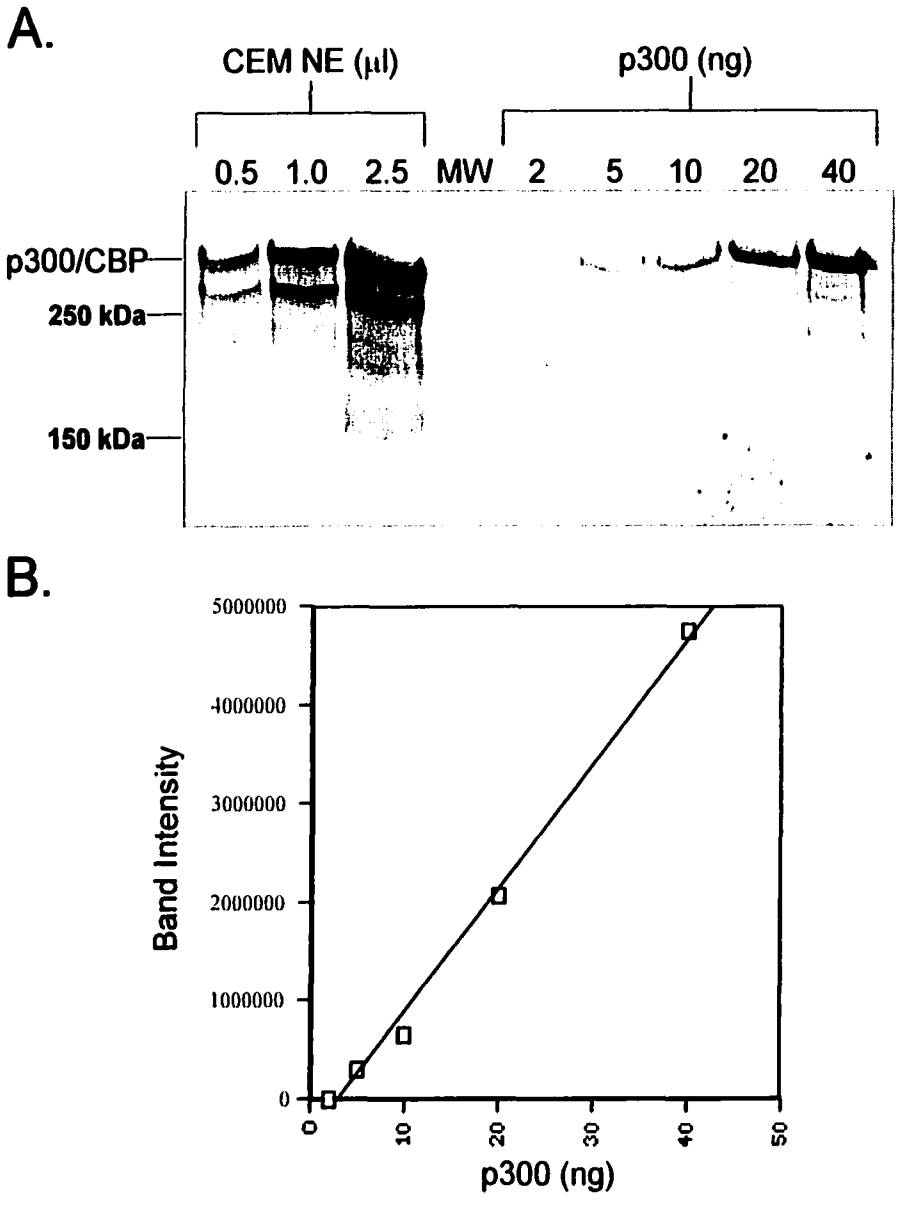


Figure 3.10. **Quantitative estimation of endogenous p300/CBP in CEM nuclear extract.** (A) Western blot of CEM nuclear extract and purified, recombinant p300. Proteins were resolved by 5% SDS-PAGE, and detected using an antibody that recognizes both CBP and p300 (α-CBP-451, Santa Cruz). (B) p300 standard curve. Western blot band intensities were measured using Image Quant software, and the values for the recombinant p300 bands were used to generate a standard curve. The best fit equation for the curve was:

band intensity = [126805(ng p300)] -390839

The Image Quant values for CBP/p300 bands in the CEM nuclear extract (A) were used to in the above equation to solve for the quantity of CBP/p300 present in those samples. Although CBP and p300 do not have the same molecular weight (265 kDa vs. 300 kDa, they did not resolve on this gel. Only the upper band in the CEM NE samples was quantitated, as this band reflects the specific CBP/p300 proteins. These values were averaged to give ng p300/µl extract. This value in turn was used to estimate that each transcription reaction (see Results) contains approximately 0.7 pmol of endogenous CBP/p300.

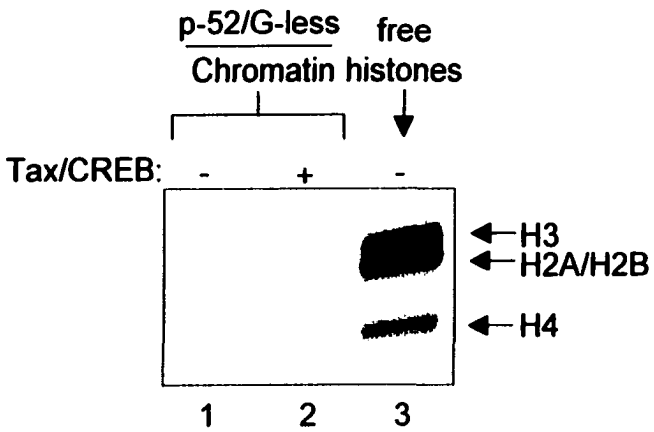
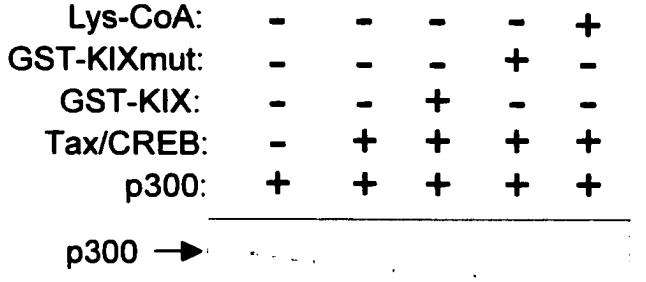


Figure 3.11. **Tax and CREB do not enhance p300 acetylation of chromatin on templates lacking the viral CREs.** In vitro acetylation assay. Both chromatin- assembled templates and free histones were acetylated in vitro in the presence of p300 and ^{14}C-acetyl CoA. Tax and CREB were added to the indicated acetylation reactions.



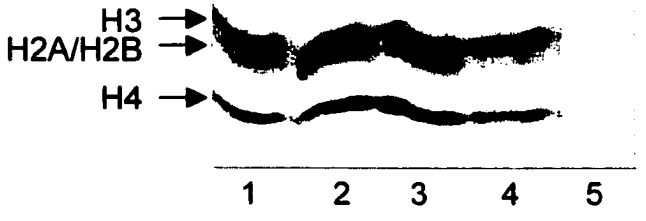


Figure 3.12. **Free histone acetylation is unaffected by GST-KIX or Tax/CREB.** In vitro acetylation assay. Free histones were acetylated in vitro in the presence of p300 and ^{14}C-acetyl CoA. Tax/CREB, GST-KIX, and GST-KIXmut were added to the indicated acetylation reactions. The CBP/p300 specific HAT inhibitor lysyl CoA was added to a final concentration of 50 μM, as indicated. GST-KIX and GST-KIXmut were each added at five-fold molar excess relative to the Tax/CREB complex.

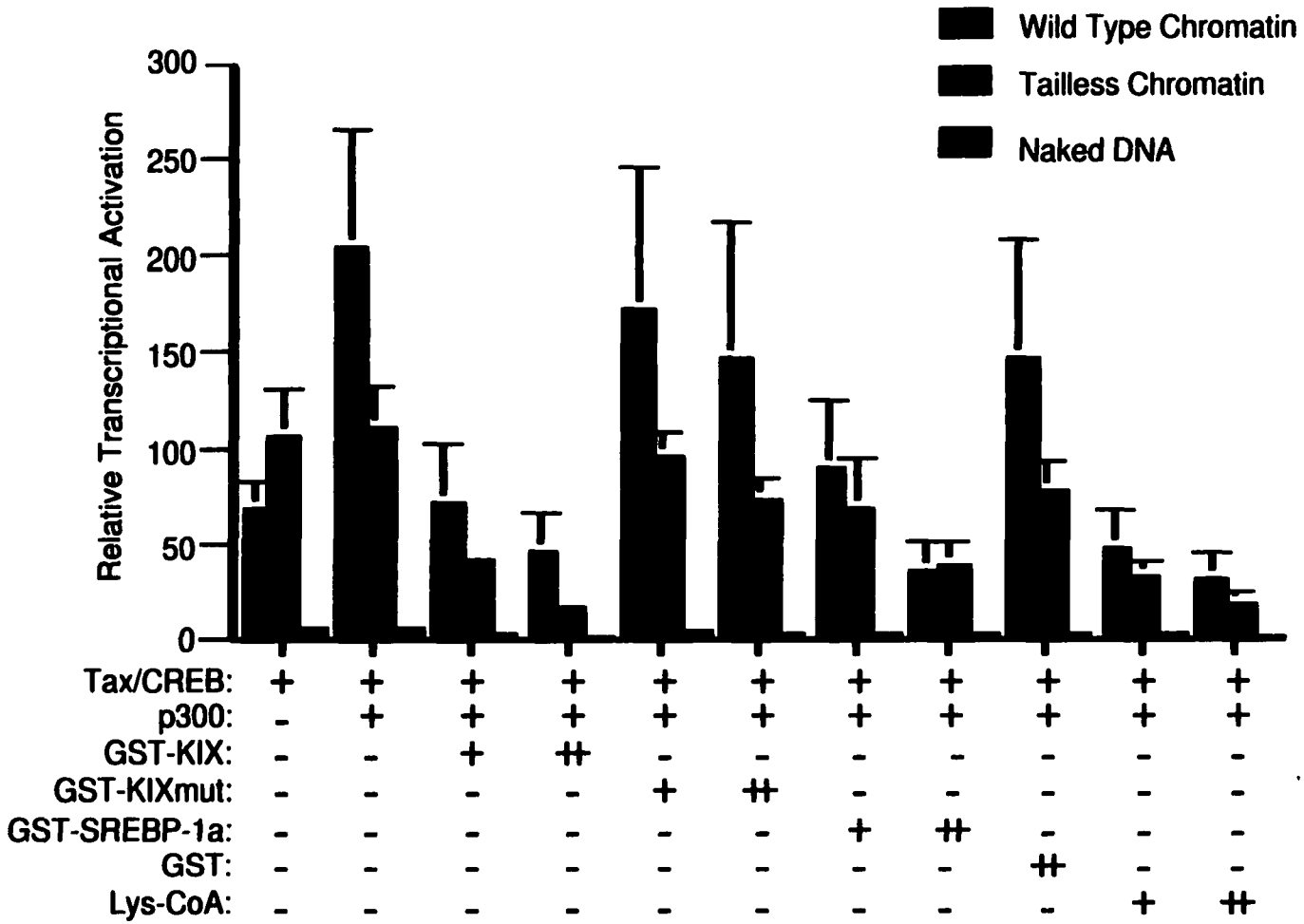


Figure 3.13. **Analysis of inhibitor function on Tax-transactivation from different transcription templates.** Relative levels of transcriptional activation from wild type chromatin, tailless chromatin, and naked DNA templates, under various conditions, are shown as mean values above. For Tax/CREB and p300 values, N>3; for all other conditions, N=3, except for data on naked DNA, for which only two experimental data sets were available. Error bars, indicating standard deviation, are shown.

CHAPTER 4
FUTURE DIRECTIONS

The data presented here reveal that Tax-transactivation from chromatin templates requires the acetyltransferase activities of the CBP/p300 coactivators. Unlike previous studies of Tax function, we have used recombinant chromatin templates to investigate the role of histone amino terminal tails in the regulation of Tax-transactivation. We show that transcriptional activation by Tax and p300 correlates with histone amino-terminal tail acetylation. However, we find that the acetyltransferase activity of CBP/p300 is also required for Tax-transactivation on tailless chromatin templates. This observation suggests that one or more additional CBP/p300 acetylation targets may be relevant to HTLV-I transcription.

Histone tails have been well characterized to regulate the binding of transcriptional activators to DNA sequences. For example, several studies have shown that deletion of histone tails facilitates the association of transcription factors with DNA (62, 122, 175, 179, 217). In agreement with these observations, we have found that tailless chromatin templates are highly responsive to Tax and promote high levels of transcription. However, it is possible that histone tails are required for the binding of certain transcription factors to their cognate sequences. For example, a group using the GAL4-VP16

activator has found that tailless recombinant chromatin, as well as recombinant chromatin in which lysine residues of the histone tails have been mutated, are refractory to transcriptional activation (6). In addition, another study found that proteolytic removal of histone tails from chromatin templates inhibited histone tail-acetylation dependent TBP recruitment to the IFNβ enhancer (3). Together, these studies suggest that acetylated histone tails may function as "docking sites" for the binding of transcription factors in certain promoter systems. Although our transcriptional data on tailless chromatin suggests that Tax does not require histone amino terminal tails in order to bind the HTLV-I promoter, this has not yet been tested directly.

Here, we have used polypeptide inhibitors to disrupt the recruitment of CBP/p300 by Tax. We find that these polypeptides inhibit endogenous CBP/p300 as well as exogenous p300, suggesting that nearly all Tax-transactivation is CBP/p300 dependent. However, these inhibitors do not distinguish between CBP and p300. CBP and p300 share a high degree of amino acid sequence similarity, and also exhibit a substantial redundancy in function. Both CBP and p300 have been shown to interact with Tax and function as coactivators in HTLV-I transcription. For example, both CBP and p300 have been localized to HTLV-I promoter-bound complexes in infected cells in vivo (125). However, CBP and p300 may also have distinct roles in Tax-transactivation. For example, a Tax mutant that interacts with p300, but not CBP, was found to be defective for Tax-transactivation in vivo (22). In contrast, we find that p300 strongly stimulates Tax-transactivation in vitro. Since both

CBP and p300 are endogenous to the nuclear extract used in these transcription studies, it is possible that both coactivators are available for Tax-transactivation. It is therefore unclear whether both CBP and p300 are involved in HTLV-I transcription, or whether one of the coactivators plays a more prominent, or perhaps exclusive, role in this process.

In future work, we would like to address the issues presented above, as they raise important questions regarding the regulation of HTLV-I transcription. Here, we submit initial strategies for future studies of the mechanisms of Tax-transactivation from chromatin templates.

4.1 WHAT ARE THE CHROMATIN-DEPENDENT, HISTONE TAIL-INDEPENDENT CBP/p300 ACETYLATION TARGETS IN TAX-TRANSACTIVATION?

We have found that Tax-transactivation from tailless chromatin templates requires CBP/p300 acetyltransferase activity and acetyl CoA, albeit to a lesser degree compared to transcription from wild type chromatin templates. Since these templates are lacking the histone amino terminal tails that have previously been characterized to be major substrates for CBP/p300 acetyltransferase activity, our observations raise the possibility that other CBP/p300-specific acetylation targets are relevant to HTLV-I transcription. We have not found any evidence that Tax or CREB are acetylated by p300. However, the globular domains of the core histones are intriguing candidates for acetylation by CBP/p300 acetylation. We have performed in vitro acetylation experiments on

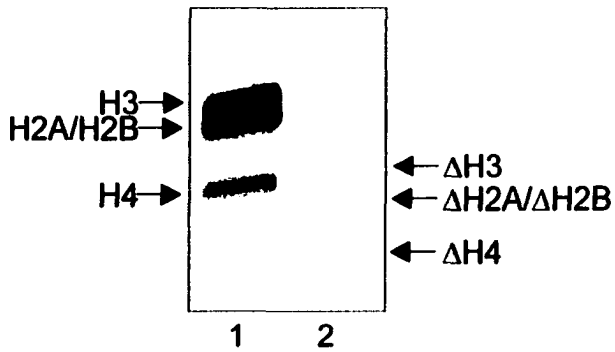


Figure 4.1. **Tailless histones are weakly acetylated by p300.** In vitro acetylation assay. Equivalent amounts of wild type (lane 1) and tailless (lane 2) free histones were acetylated in vitro in the presence of p300 and ^{14}C-acetyl CoA.

the tailless recombinant histones and have seen apparent acetylation by p300, although the relative level of acetylation is substantially lower compared to wild type histones (see Fig 2.5B and Fig. 4.1). In addition, preliminary mass spectrometry analysis of free tailless histones incubated with p300 and acetyl CoA reveals chemical shifts consistent with acetylation on histones H2A, H2B and H4. It is not known, however, which residues might be acetylated on tailless histones, and whether this acetylation has consequences for HTLV-I transcriptional regulation. Future work on this question will include mass spectrometry analysis of tailless chromatin, which may identify whether one or more histones are acetylated in that context. If acetylation is found, then experiments will be conducted to determine amino acid sites of acetylation, to mutate the acetylated residues, and to test whether the resulting mutant chromatin can support Tax-transactivation.

It is also possible that the acetylation of a nonhistone protein by CBP/p300 is required for activation from chromatin templates. This protein may be a factor involved in the remodeling of nucleosomes, or a component of the general transcription machinery that must be acetylated in order to facilitate derepression of chromatin-assembled genes. The identification of such a target would be considerably difficult, since many there are many CBP/p300 acetylation substrates endogenous to the nuclear extract used in our transcription studies, and these targets may not be involved in Tax-transactivation. One initial approach might be to assemble a fragment of the HTLV-I promoter into chromatin, immobilize the template on a bead, incubate this template with ^{14}C-

acetyl CoA, Tax/CREB, p300 and nuclear extract, and analyze bound proteins for acetylation by CBP/p300. This strategy might enable us to identify a novel CBP/p300 acetylation substrate involved in HTLV-I transcriptional regulation.

4.2 DO HISTONE TAILS DIRECT THE ASSEMBLY OF TRANSCRIPTIONAL INITIATION COMPLEXES?

The deletion of histone amino terminal tails has been shown previously to facilitate the binding of certain transcriptional activators to their target DNA sequences. However, other evidence suggests that histone tails are required for transcription factor function. We have found that tailless chromatin templates are highly responsive to Tax-transactivation. We hypothesize that histone amino terminal tail deletion, and histone acetylation, facilitates binding of the Tax/CREB complex to the viral CREs. We would like to test this hypothesis using in vitro chromatin immunoprecipitation assays to compare factor binding to wild type versus tailless HTLV-I promoter templates. If the Tax/CREB complex can be precipitated from tailless chromatin templates, this will indicate that histone tails are not required for the binding of Tax/CREB to the viral CREs. It would also be interesting to test whether Tax/CREB can bind to unacetylated, wild type chromatin templates, or whether preacetylation of histones enhances access of Tax/CREB to the viral CREs. Additionally, it would be interesting to evaluate the binding of other proteins, such as CBP/p300, TBP, and RNA polymerase II, under the template conditions described above to determine whether the state of

the histone tails directs the assembly of additional factors on the HTLV-I promoter.

In a recent study, it was suggested that the GAL4-VP16 activator required chromatin containing intact, acetylated histone tails in order to promote transcriptional activation (6). Since GAL4-VP16 protein and plasmid DNA containing GAL4- response elements are available in the laboratory, it would be interesting to test the ability of GAL4-VP16 to associate with wild type versus tailless chromatin templates. If GAL4-VP16 is found to have a different mode than Tax/CREB for binding to chromatin templates, this result may illuminate important distinctions in the ways histone tails regulate transcriptional activation from diverse promoters.

4.3 DO CBP AND p300 HAVE DISTINCT ROLES IN MEDIATING TAX-ACTIVATED TRANSCRIPTION?

Although CBP and p300 have both been shown to interact with Tax and activate HTLV-I transcription in vitro and in vivo, it is not clear whether the coactivators have redundant or distinct functions in Tax-transactivation. One interesting possibility is that either CBP or p300 may play a dominant or exclusive role in the transcription of viral genes. Alternatively, the coactivators may have unique functions in the transcription process. For example, a time-course study of transcriptional activation from the cathepsin D promoter suggests that while p300 is involved in transcriptional initiation, CBP is primarily involved in the reinitiation of active genes (194). Although we have found that exogenous

p300 strongly stimulates Tax-transactivation from chromatin templates, previous attempts in the laboratory to use recombinant CBP to activate Tax-dependent transcription have been unsuccessful. However, these experiments have always been carried out using nuclear extract that already contains a substantial amount of endogenous CBP and p300. It is therefore possible that these Tax-activated templates are unresponsive to exogenously added CBP, but utilize endogenous CBP. To more clearly define a role for CBP in transcriptional activation, we would like to deplete nuclear extract of endogenous CBP/p300, and then use this extract to assay transcription in the absence and presence of recombinant CBP. It is possible that depletion of CBP/p300 might also deplete the nuclear extract of basal transcription factors known to associate with the coactivators as part of the RNA polymerase II holoenzyme. To address this concern, we propose to use an amino terminal portion of the E1A oncoprotein, fused to GST, to deplete the extract, since this protein has previously been shown to bind CBP/p300 and disrupt its interactions with the holoenzyme (155). This should produce a CBP/p300-depleted extract that is competent for transcriptional activation. Recombinant CBP, p300, or both coactivators can then be added to this extract to determine whether CBP and p300 play distinct, or redundant, roles as coactivators for Tax-transactivation on chromatin templates.

REFERENCES

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

2. **Adya, N., L. J. Zhao, W. Huang, I. Boros, and C. Z. Giam.** 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 of CREB. Proc. Natl. Acad. Sci. USA **91:**5642-5646.

3. **Agalioti, T., S. Lomvardas, B. Parekh, J. Yie, T. Maniatis, and D. Thanos.** 2000. Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. Cell **103:**667-678.

4. **Allfrey, V., R. M. Faulkner, and A. E. Mirsky.** 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc. Natl. Acad. Sci. USA **51:**786-794.

5. **Altman, R., D. Harrich, J. A. Garcia and R. B. Gaynor.** 1988. Human T-Cell Leukemia Virus Types I and II exhibit different DNase I protection patterns. J. Virol. **62:**1339-1346.

6. **An, W., V. B. Palhan, M. A. Karymov, S. H. Leuba, and R. G. Roeder.** 2002. Selective requirements for histone H3 and H4 N termini in p300-dependent transcriptional activation from chromatin. Mol. Cell **9:**811-821.

7. **Anderson, M. G., and W. S. Dynan.** 1994. Quantitative studies of the effect of HTLV-I Tax protein on CREB-proteins-DNA binding. Nucleic Acids Res. **22:**3194-3201.

8. **Anderson, M. G., K. E. Scoggin, C. M. Simbulan-Rosenthal, and J. A. Steadman.** 2000. Identification of Poly(ADP-ribose) polymerase as a transcriptional coactivator of the human T-cell leukemia virus type 1 tax protein. J. Virol. **74:**2169-2177.

9. **Annunziato, A. T., and J. C. Hansen.** 2000. Role of histone acetylation in the assembly and modulation of chromatin structures. Gene Expr. **9:**37-61.

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

11. **Arany, Z., W. R. Sellers, D. M. Livingston, and R. Eckner.** 1994. E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. Cell **77:**799-800.

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

13. **Asahara, H., B. Santoso, E. Guzman, K. Du, P. A. Cole, I. Davidson, and M. Montminy.** 2001. Chromatin-dependent cooperativity between constitutive and inducible activation domains in CREB. Mol. Cell. Biol. **21:**7892-7900.

14. **Ausio, J., F. Dong, and K. E. van Holde.** 1989. Use of selectively trypsinized nucleosome core particles to analyze the role of the histone "tails" in the stabilization of the nucleosome. J. Mol. Biol. **206:**451-463.

15. **Ausio, J., and K. E. van Holde.** 1986. Histone hyperacetylation: its effects on nucleosome conformation and stability. Biochemistry **25:**1421-1428.

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

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

18. **Baranger, A. M., C. R. Palmer, M. K. Hamm, H. A. Giebler, A. Brauweiler, J. K. Nyborg, and A. Schepartz.** 1995. Mechanism of DNA binding enhancement by the HTLV-I transactivator Tax. Nature **376:**606-608.

19. **Bazarbachi, A., and O. Hermine.** 2001. Treatment of adult T-cell leukaemia/lymphoma: current strategy and future perspectives. Virus Res. **78:**79-92.

20. **Berger, S. L.** 2002. Histone modifications in transcriptional regulation. Curr. Opin. Genet. Dev. **12:**142-148.

21. **Bex, F., K. Murphy, R. Wattiez, A. Burny, and R. B. Gaynor.** 1999. Phosphorylation of the human T-cell leukemia virus type 1 transactivator

tax on adjacent serine residues is critical for tax activation. J. Virol. **73**:738-745.

22. **Bex, F., M. J. Yin, A. Burny, and R. B. Gaynor.** 1998. Differential transcriptional activation by human T-cell leukemia virus type 1 Tax mutants is mediated by distinct interactions with CREB binding protein and p300. Mol. Cell. Biol. **18**:2392-2405.

23. **Bohm, L., and C. Crane-Robinson.** 1984. Proteases as structural probes for chromatin: the domain structure of histones. Biosci. Rep. **4**:365-386.

24. **Boyer, L. A., C. Logie, E. Bonte, P. B. Becker, P. A. Wade, A. P. Wolffe, C. Wu, A. N. Imbalzano, and C. L. Peterson.** 2000. Functional delineation of three groups of the ATP-dependent family of chromatin remodeling enzymes. J. Biol. Chem. **275**:18864-18870.

25. **Brauweiler, A., P. Garl, A. A. Franklin, H. A. Giebler, and J. K. Nyborg.** 1995. A molecular mechanism for HTLV-I latency and Tax transactivation. J. Biol. Chem. **270**:12814-12822.

26. **Burmeister, T.** 2001. Oncogenic retroviruses in animals and humans. Rev. Med. Virol. **11**:369-380.

27. **Cann, A. J., J. D. Rosenblatt, W. Wachsman, N. P. Shah, and I. S. Chen.** 1985. Identification of the gene responsible for human T-cell leukaemia virus transcriptional regulation. Nature **318**:571-574.

28. **Carruthers, L. M., and J. C. Hansen. 2000.** The core histone N termini function independently of linker histones during chromatin condensation. J. Biol. Chem. **275**:37285-37290.

29. **Champagne, N., N. R. Bertos, N. Pelletier, A. H. Wang, M. Vezmar, Y. Yang, H. H. Heng, and X. J. Yang.** 1999. Identification of a human histone acetyltransferase related to monocytic leukemia zinc finger protein. J. Biol. Chem. **274**:28528-28536.

30. **Chan, H. M., and N. B. La Thangue.** 2001. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J. Cell. Sci. **114**:2363-2373.

31. **Chen, C. J., Z. Deng, A. Y. Kim, G. A. Blobel, and P. M. Lieberman.** 2001. Stimulation of CREB binding protein nucleosomal histone acetyltransferase activity by a class of transcriptional activators. Mol. Cell. Biol. **21**:476-487.

32. **Chen, H., M. Tini, and R. M. Evans.** 2001. HATs on and beyond chromatin. Curr. Opin. Cell Biol. **13**:218-224.

33. **Chen, I. S., D. J. Slamon, J. D. Rosenblatt, N. P. Shah, S. G. Quan, and W. Wachsman.** 1985. The x gene is essential for HTLV replication. Science **229**:54-58.

34. **Chen, I. S. Y., J. McLaughlin, J. C. Gasson, S. C. Clark, and D. W. Golde.** 1983. Molecular characterization of genome of a novel human T-cell leukaemia virus. Nature **305**:502-505.

35. **Chirinos, M., F. Hernandez, and E. Palacian.** 1998. Repressive effect on oligonucleosome transcription of the core histone tail domains. Biochemistry **37**:7251-7259.

36. **Cho, H., G. Orphanides, X. Sun, X. J. Yang, V. Ogryzko, E. Lees, Y. Nakatani, and D. Reinberg.** 1998. A human RNA polymerase II complex containing factors that modify chromatin structure. Mol. Cell. Biol. **18**:5355-5363.

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

38. **Clapier, C. R., G. Langst, D. F. Corona, P. B. Becker, and K. P. Nightingale.** 2001. Critical role for the histone H4 N terminus in nucleosome remodeling by ISWI. Mol. Cell. Biol. **21**:875-883.

39. **Coscoy, L., D. Gonzalez-Dunia, F. Tangy, S. Syan, M. Brahic, and S. Ozden.** 1998. Molecular mechanism of tumorigenesis in mice transgenic for the human T cell leukemia virus Tax gene. Virology **248**:332-341.

40. **Dallas, P. B., P. Yaciuk, and E. Moran.** 1997. Characterization of monoclonal antibodies raised against p300: both p300 and CBP are present in intracellular TBP complexes. J. Virol. **71**:1726-1731.

41. **Dilworth, F. J., C. Fromental-Ramain, K. Yamamoto, and P. Chambon.** 2000. ATP-Driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR In vitro. Mol. Cell **6**:1049-1058.

42. **Dover, J., J. Schneider, M. A. Boateng, A. Wood, K. Dean, M. Johnston, and A. Shilatifard.** 2002. Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by RAD6. J. Biol. Chem. **17**:in press.

43. **Dynan, W. S.** 1987. DNase I footprinting as an assay for mammalian gene regulatory proteins. Genet. Engineer. **9**:75-87.

44. **Eberharter, A., S. Ferrari, G. Langst, T. Straub, A. Imhof, P. Varga-Weisz, M. Wilm, and P. B. Becker.** 2001. Acf1, the largest subunit of CHRAC, regulates ISWI-induced nucleosome remodeling. EMBO J. **20**:3781-3788.

45. **Eckner, R., M. E. Ewen, D. Newsome, M. Gerdes, J. A. DeCaprio, J. B. Lawrence, and D. M. Livingston.** 1994. Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev. **8**:869-884.

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

47. **Fletcher, T. M., and J. C. Hansen.** 1995. Core histone tail domains mediate oligonucleosome folding and nucleosomal DNA organization through distinct molecular mechanisms. J. Biol. Chem. **270**:25359-25362.

48. **Franklin, A. A., M. F. Kubik, M. N. Uittenbogaard, A. Brauweiler, P. Utaisincharoen, M. A. Matthews, W. S. Dynan, J. P. Hoeffler, and J. K. Nyborg.** 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.

49. **Fujisawa, J., M. Seiki, T. Kiyokawa, and M. Yoshida.** 1985. Functional activation of the long terminal repeat of human T-cell leukemia virus type I by a trans-acting factor. Proc. Natl. Acad. Sci. USA **82**:2277-2281.

50. **Fujisawa, J. I., M. Toita, and M. Yoshida.** 1989. A unique enhancer element for the transactivator (p40tax) of human T cell leukemia virus type I that is distinct from cyclic AMP- and 12-O-tetradecanoylphorbal-13-acetate-responsive element. J. Virol. **63**:3234-3239.

51. **Furia, B., L. Deng, K. Wu, S. Baylor, K. Kehn, H. Li, R. Donnelly, T. Coleman, and F. Kashanchi.** 2002. Enhancement of nuclear factor-kappa B acetylation by coactivator p300 and HIV-1 Tat proteins. J. Biol. Chem. **277**:4973-4980.

52. **Galasinski, S. K., T. N. Lively, A. Grebe De Barron, and J. A. Goodrich.** 2000. Acetyl coenzyme A stimulates RNA polymerase II transcription and promoter binding by transcription factor IID in the absence of histones. Mol. Cell. Biol. **20**:1923-1930.

53. **Galvin, K. M., and Y. Shi.** 1997. Multiple mechanisms of transcriptional repression by YY1. Mol. Cell. Biol. **17:**3723-3732.

54. **Garcia-Ramirez, M., F. Dong, and J. Ausio.** 1992. Role of the histone "tails" in the folding of oligonucleosomes depleted of histone H1. J. Biol. Chem. **267:**19587-19595.

55. **Garcia-Ramirez, M., C. Rocchini, and J. Ausio.** 1995. Modulation of chromatin folding by histone acetylation. J. Biol. Chem. **270:**17923-17928.

56. **Georgel, P. T., T. Tsukiyama, and C. Wu.** 1997. Role of histone tails in nucleosome remodeling by Drosophila NURF. EMBO J. **16:**4717-4726.

57. **Georges, S. A., W. L. Kraus, K. Luger, J. K. Nyborg, and P. J. Laybourn. 2002.** p300-Mediated Tax transactivation from recombinant chromatin: Histone tail deletion mimics coactivator function. Mol. Cell. Biol. **22:**127-137.

58. **Giebler, H. A., J. E. Loring, K. Van Orden, M. A. Colgin, J. E. Garrus, K. W. Escudero, A. Brauweiler, and J. K. Nyborg.** 1997. Anchoring of CREB binding protein to the human T-cell leukemia virus type 1 promoter: a molecular mechanism of Tax transactivation. Mol. Cell. Biol. **17:**5156-5164.

59. **Giles, R. H., J. G. Dauwerse, C. Higgins, F. Petrij, J. W. Wessels, G. C. Beverstock, H. Dohner, M. Jotterand-Bellomo, J. H. Falkenburg, R. M. Slater, G. J. van Ommen, A. Hagemeijer, B. A. van der Reijden, and M. H. Breuning.** 1997. Detection of CBP rearrangements in acute myelogenous leukemia with t(8;16). Leukemia **11:**2087-2096.

60. **Giles, R. H., D. J. Peters, and M. H. Breuning.** 1998. Conjunction dysfunction: CBP/p300 in human disease. Trends Genet. **14:**178-183.

61. **Giordano, A., and M. L. Avantaggiati.** 1999. p300 and CBP: partners for life and death. J. Cell. Physiol. **181:**218-230.

62. **Godde, J. S., Y. Nakatani, and A. P. Wolffe.** 1995. The amino-terminal tails of the core histones and the translational position of the TATA box determine TBP/TFIIA association with nucleosomal DNA. Nucleic Acids Res. **23:**4557-4564.

63. **Goll, M. G., and T. H. Bestor.** 2002. Histone modification and replacement in chromatin activation. Genes Dev. **16:**1739-1742.

64. **Goodman, R. H., and S. Smolik.** 2000. CBP/p300 in cell growth, transformation, and development. Genes Dev. **14:**1553-1577.

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

66. **Grant, C., K. Barmak, T. Alefantis, J. Yao, S. Jacobson, and B. Wigdahl.** 2002. Human T cell leukemia virus type I and neurologic disease: events in bone marrow, peripheral blood, and central nervous system during normal immune surveillance and neuroinflammation. J. Cell. Physiol. **190:**133-159.

67. **Grassmann, R., S. Berchtold, I. Radant, M. Alt, B. Fleckenstein, J. G. Sodroski, W. A. Haseltine, and U. Ramstedt.** 1992. Role of human T-cell leukemia virus type 1 X region proteins in immortalization of primary human lymphocytes in culture. J. Virol. **66:**4570-4575.

68. **Grossman, W. J., J. T. Kimata, F. H. Wong, M. Zutter, T. J. Ley, and L. Ratner.** 1995. Development of leukemia in mice transgenic for the tax gene of human T- cell leukemia virus type I. Proc. Natl. Acad. Sci. USA **92:**1057-1061.

69. **Gu, W., and R. G. Roeder.** 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell **90:**595-606.

70. **Guyon, J. R., G. J. Narlikar, S. Sif, and R. E. Kingston.** 1999. Stable remodeling of tailless nucleosomes by the human SWI-SNF complex. Mol. Cell. Biol. **19:**2088-2097.

71. **Hamiche, A., J. G. Kang, C. Dennis, H. Xiao, and C. Wu.** 2001. Histone tails modulate nucleosome mobility and regulate ATP-dependent nucleosome sliding by NURF. Proc. Natl. Acad. Sci. USA **98:**14316-14321.

72. **Hansen, J. C.** 2002. Conformational dynamics of the chromatin fiber in solution: Determinants, mechanisms, and functions. Annu. Rev. Biophys. Biomol. Struct. **31:**361-392.

73. **Hansen, J. C., C. Tse, and A. P. Wolffe.** 1998. Structure and function of the core histone N-termini: more than meets the eye. Biochemistry **37:**17637-17641.

74. **Hansen, J. C., and A. P. Wolffe.** 1992. Influence of chromatin folding on transcription initiation and elongation by RNA polymerase III. Biochemistry **31:**7977-7988.

75. **Hansen, J. C., and A. P. Wolffe.** 1994. A role for histones H2A/H2B in chromatin folding and transcriptional repression. Proc. Natl. Acad. Sci. USA **91**:2339-2343.

76. **Harrod, R., Y. L. Kuo, Y. Tang, Y. Yao, A. Vassilev, Y. Nakatani, and C. Z. Giam.** 2000. p300 and p300/cAMP-responsive element-binding protein associated factor interact with human T-cell lymphotropic virus type-1 Tax in a multi-histone acetyltransferase/activator-enhancer complex. J. Biol. Chem. **275**:11852-11857.

77. **Harrod, R., Y. Tang, C. Nicot, H. S. Lu, A. Vassilev, Y. Nakatani, and C. Z. Giam.** 1998. An exposed KID-like domain in human T-cell lymphotropic virus type 1 Tax is responsible for the recruitment of coactivators CBP/p300. Mol. Cell. Biol. **18**:5052-5061.

78. **Hasan, S., P. O. Hassa, R. Imhof, and M. O. Hottiger.** 2001. Transcription coactivator p300 binds PCNA and may have a role in DNA repair synthesis. Nature **410**:387-391.

79. **Hayes, J. J., D. J. Clark, and A. P. Wolffe.** 1991. Histone contributions to the structure of DNA in the nucleosome. Proc. Natl. Acad. Sci. USA **88**:6829-6833.

80. **Hernandez, F., L. Lopez-Alarcon, C. Puerta, and E. Palacian.** 1998. Transcriptional inhibitory role of the tail domains of histone (H3 x H4)2 tetramers. Arch. Biochem. Biophys. **358**:98-103.

81. **Hinuma, Y., K. Nagata, M. Hanaoka, M. Nakai, T. Matsumoto, K. I. Kinoshita, S. Shirakawa, and I. Miyoshi.** 1981. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc. Natl. Acad. Sci. USA **78**:6476-6480.

82. **Ida, K., I. Kitabayashi, T. Taki, M. Taniwaki, K. Noro, M. Yamamoto, M. Ohki, and Y. Hayashi.** 1997. Adenoviral E1A-associated protein p300 is involved in acute myeloid leukemia with t(11;22)(q23;q13). Blood **90**:4699-4704.

83. **Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston.** 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature **370**:481-485.

84. **Imhof, A., X. J. Yang, V. V. Ogryzko, Y. Nakatani, A. P. Wolffe, and H. Ge.** 1997. Acetylation of general transcription factors by histone acetyltransferases. Curr. Biol. **7**:689-692.

85. **Ito, T., M. Bulger, R. Kobayashi, and J. T. Kadonaga.** 1996. Drosophila NAP-1 is a core histone chaperone that functions in ATP-facilitated assembly of regularly spaced nucleosomal arrays. Mol. Cell. Biol. **16:**3112-3124.

86. **Ito, T., M. Bulger, M. J. Pazin, R. Kobayashi, and J. T. Kadonaga.** 1997. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell **90:**145-155.

87. **Ito, T., T. Ikehara, T. Nakagawa, W. L. Kraus, and M. Muramatsu.** 2000. p300-mediated acetylation facilitates the transfer of histone H2A-H2B dimers from nucleosomes to a histone chaperone. Genes Dev. **14:**1899-1907.

88. **Ito, T., M. E. Levenstein, D. V. Fyodorov, A. K. Kutach, R. Kobayashi, and J. T. Kadonaga.** 1999. ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. Genes Dev. **13:**1529-1539.

89. **Jeang, K. T., I. Boros, J. Brady, M. Radonovich, and G. Khoury.** 1988. Characterization of cellular factors that interact with the human T-cell leukemia virus type I $p40^{x}$-responsive 21-base-pair sequence. J. Virol. **62:**4499-4509.

90. **Jenuwein, T., and C. D. Allis.** 2001. Translating the histone code. Science **293:**1074-1080.

91. **Jiang, H., H. Lu, R. L. Schiltz, C. A. Pise-Masison, V. V. Ogryzko, Y. Nakatani, and J. N. Brady.** 1999. PCAF interacts with tax and stimulates tax transactivation in a histone acetyltransferase-independent manner. Mol. Cell. Biol. **19:**8136-8145.

92. **Jiang, W., S. K. Nordeen, and J. T. Kadonaga.** 2000. Transcriptional analysis of chromatin assembled with purified ACF and dNAP1 reveals that acetyl-CoA is required for preinitiation complex assembly. J. Biol. Chem. **275:**39819-39822.

93. **Jin, D. Y., and K.T. Jeang.** 1997. HTLV-I Tax self-association in optimal trans-activation function. Nucleic Acids Res. **25:**379-388.

94. **Jin, D. Y., and K. T. Jeang.** 1997. Transcriptional activation and self-association in yeast: Protein-protein dimerization as a pleiotropic mechanism of HTLV-I Tax function. Leukemia **11:**3-6.

95. **Josephs, S. F., F. Wong-Staal, V. Manzari, R.C. Gallo, J.G. Sodroski, M.D. Trus, D. Perkins, R. Patarca, and W.A. Haseltine.** 1984. Long

terminal repeat structure of an American isolate of type I human T-cell leukemia virus. Virology **139:**340-345.

96. **Karantza, V., E. Freire, and E. N. Moudrianakis.** 2001. Thermodynamic studies of the core histones: Stability of the octamer subunits is not altered by removal of their terminal domains. Biochemistry **40:**13114-13123.

97. **Kashanchi, F., J. F. Duvall, R. P. Kwok, J. R. Lundblad, R. H. Goodman, and J. N. Brady.** 1998. The coactivator CBP stimulates human T-cell lymphotrophic virus type I Tax transactivation in vitro. J. Biol. Chem. **273:**34646-34652.

98. **Kawano, F., K. Yamaguchi, H. Nishimura, H. Tsuda, and K. Takatsuki.** 1985. Variation in the clinical courses of adult T-cell leukemia. Cancer **55:**851-856.

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

100. **Kelly, T. J., S. Qin, D. E. Gottschling, and M. R. Parthun.** 2000. Type B histone acetyltransferase Hat1p participates in telomeric silencing. Mol. Cell. Biol. **20:**7051-7058.

101. **Kiernan, R. E., C. Vanhulle, L. Schiltz, E. Adam, H. Xiao, F. Maudoux, C. Calomme, A. Burny, Y. Nakatani, K. T. Jeang, M. Benkirane, and C. Van Lint.** 1999. HIV-1 tat transcriptional activity is regulated by acetylation. EMBO J. **18:**6106-6118.

102. **Kim, M. Y., S. J. Hsiao, and W. L. Kraus.** 2001. A role for coactivators and histone acetylation in estrogen receptor alpha-mediated transcription initiation. EMBO J. **20:**6084-6094.

103. **Kim, T. K., T. H. Kim, and T. Maniatis.** 1998. Efficient recruitment of TFIIB and CBP-RNA polymerase II holoenzyme by an interferon-beta enhanceosome in vitro. Proc. Natl. Acad. Sci. USA **95:**12191-12196.

104. **Kimzey, A. L., and W. S. Dynan.** 1999. Identification of a human T-cell leukemia virus type I tax peptide in contact with DNA. J. Biol. Chem. **274:**34226-34232.

105. **Kimzey, A. L., and W. S. Dynan.** 1998. Specific regions of contact between human T-cell leukemia virus type I Tax protein and DNA identified by photocross-linking. J. Biol. Chem. **273:**13768-13775.

106. **Kingston, R. E., and G. J. Narlikar.** 1999. ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. Genes Dev. **13:**2339-2352.

107. **Kitabayashi, I., Y. Aikawa, L. A. Nguyen, A. Yokoyama, and M. Ohki.** 2001. Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. EMBO J. **20:**7184-7196.

108. **Kitabayashi, I., Y. Aikawa, A. Yokoyama, F. Hosoda, M. Nagai, N. Kakazu, T. Abe, and M. Ohki.** 2001. Fusion of MOZ and p300 histone acetyltransferases in acute monocytic leukemia with a t(8;22)(p11;q13) chromosome translocation. Leukemia **15:**89-94.

109. **Kitabayashi, I., A. Yokoyama, K. Shimizu, and M. Ohki.** 1998. Interaction and functional cooperation of the leukemia-associated factors AML1 and p300 in myeloid cell differentiation. EMBO J. **17:**2994-3004.

110. **Kiyokawa, T., M. Seiki, S. Iwashita, K. Imagawa, F. Shimizu, and M. Yoshida.** 1985. p27x-III and p21x-III, proteins encoded by the pX sequence of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA **82:**8359-8363.

111. **Krajewski, W. A., and J. Ausio.** 1996. Modulation of the higher-order folding of chromatin by deletion of histone H3 and H4 terminal domains. Biochem. J. **316:**395-400.

112. **Kraus, W. L., and J. T. Kadonaga.** 1998. p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. Genes Dev. **12:**331-342.

113. **Kraus, W. L., E. T. Manning, and J. T. Kadonaga.** 1999. Biochemical analysis of distinct activation functions in p300 that enhance transcription initiation with chromatin templates. Mol. Cell. Biol. **19:**8123-8135.

114. **Kundu, T. K., V. B. Palhan, Z. Wang, W. An, P. A. Cole, and R. G. Roeder.** 2000. Activator-dependent transcription from chromatin in vitro involving targeted histone acetylation by p300. Mol. Cell **6:**551-561.

115. **Kung, A. L., V. I. Rebel, R. T. Bronson, L. E. Ch'ng, C. A. Sieff, D. M. Livingston, and T. P. Yao.** 2000. Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. Genes Dev. **14:**272-277.

116. **Kuo, Y. L., Y. Tang, R. Harrod, P. Cai, and C. Z. Giam.** 2000. Kinase-inducible domain-like region of HTLV type 1 tax is important for NF-kappaB activation. AIDS Res. Hum. Retroviruses **16:**1607-1612.

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

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

119. **Lau, O. D., T. K. Kundu, R. E. Soccio, S. Ait-Si-Ali, E. M. Khalil, A. Vassilev, A. P. Wolffe, Y. Nakatani, R. G. Roeder, and P. A. Cole.** 2000. HATs off: Selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF. Mol. Cell **5**:589-595.

120. **Lavau, C., C. Du, M. Thirman, and N. Zeleznik-Le.** 2000. Chromatin-related properties of CBP fused to MLL generate a myelodysplastic-like syndrome that evolves into myeloid leukemia. EMBO J. **19**:4655-4664.

121. **Laybourn, P. J., and J. T. Kadonaga.** 1991. Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. Science **254**:238-245.

122. **Lee, D. Y., J. J. Hayes, D. Pruss, and A. P. Wolffe.** 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell **72**:73-84.

123. **Lee, T. H., J. E. Coligan, J. G. Sodroski, W. A. Haseltine, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, and M. Essex.** 1984. Antigens encoded by the 3'-terminal region of human T-cell leukemia virus: evidence for a functional gene. Science **226**:57-61.

124. **Lemasson, I., and J. K. Nyborg.** 2001. Human T-cell leukemia virus type I Tax repression of p73beta is mediated through competition for the C/H1 domain of CBP. J. Biol. Chem. **276**:15720-15727.

125. **Lemasson, I., N. Polakowski, P. J. Laybourn, and J. K. Nyborg.** 2002. Transcription factor binding and histone modifications on the integrated proviral promoter in HTLV-I-infected T-cells. J Biol Chem **16**:in press.

126. **Lenzmeier, B. A., E. E. Baird, P. B. Dervan, and J. K. Nyborg.** 1999. The tax protein-DNA interaction is essential for HTLV-I transactivation in vitro. J. Mol. Biol. **291**:731-744.

127. **Lenzmeier, B. A., H. A. Giebler, and J. K. Nyborg.** 1998. Human T-cell leukemia virus type 1 Tax requires direct access to DNA for recruitment of CREB binding protein to the viral promoter. Mol. Cell. Biol. **18**:721-731.

128. **Lenzmeier, B. A., and J. K. Nyborg.** 1999. Molecular mechanisms of viral transcription and cellular deregulation associated with the HTLV-I Tax protein. Gene Ther. Mol. Biol **3**:327-345.

129. **Levenstein, M. E., and J. T. Kadonaga.** 2002. Biochemical analysis of chromatin containing recombinant Drosophila core histones. J. Biol. Chem. **277**:8749-8754.

130. **Levin, M. C., and S. Jacobson.** 1997. HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP): a chronic progressive neurologic disease associated with immunologically mediated damage to the central nervous system. J. Neurovirol. **3**:126-140.

131. **Levin, M. C., S. M. Lee, F. Kalume, Y. Morcos, F. C. Dohan, Jr., K. A. Hasty, J. C. Callaway, J. Zunt, D. Desiderio, and J. M. Stuart.** 2002. Autoimmunity due to molecular mimicry as a cause of neurological disease. Nat. Med. **8**:509-513.

132. **Li, J., B. W. O'Malley, and J. Wong.** 2000. p300 requires its histone acetyltransferase activity and SRC-1 interaction domain to facilitate thyroid hormone receptor activation in chromatin. Mol. Cell. Biol. **20**:2031-2042.

133. **Li, Q., A. Imhof, T. N. Collingwood, F. D. Urnov, and A. P. Wolffe.** 1999. p300 stimulates transcription instigated by ligand-bound thyroid hormone receptor at a step subsequent to chromatin disruption. EMBO J. **18**:5634-5652.

134. **Libertini, L. J., J. Ausio, K. E. van Holde, and E. W. Small.** 1988. Histone hyperacetylation. Its effects on nucleosome core particle transitions. Biophys. J. **53**:477-487.

135. **Liu, L., D. M. Scolnick, R. C. Trievel, H. B. Zhang, R. Marmorstein, T. D. Halazonetis, and S. L. Berger.** 1999. p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage. Mol. Cell. Biol. **19**:1202-1209.

136. **Loyola, A., G. LeRoy, Y. H. Wang, and D. Reinberg.** 2001. Reconstitution of recombinant chromatin establishes a requirement for histone-tail modifications during chromatin assembly and transcription. Genes Dev. **15**:2837-2851.

137. **Lu, H., C. A. Pise-Masison, T. M. Fletcher, R. L. Schiltz, A. K. Nagaich, M. Radonovich, G. Hager, P. A. Cole, and J. N. Brady.** 2002. Acetylation of nucleosomal histones by p300 facilitates transcription from tax-responsive human T-cell leukemia virus type 1 chromatin template. Mol. Cell. Biol. **22:**4450-4462.

138. **Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent, and T. J. Richmond.** 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature **389:**251-260.

139. **Luger, K., T. J. Rechsteiner, A. J. Flaus, M. M. Waye, and T. J. Richmond.** 1997. Characterization of nucleosome core particles containing histone proteins made in bacteria. J. Mol. Biol. **272:**301-311.

140. **Luger, K., T. J. Rechsteiner, and T. J. Richmond.** 1999. Preparation of nucleosome core particle from recombinant histones. Methods Enzymol. **304:**3-19.

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

142. **Lundblad, J. R., R. P. Kwok, M. E. Laurance, M. S. Huang, J. P. Richards, R. G. Brennan, and R. H. Goodman.** 1998. The human T-cell leukemia virus-1 transcriptional activator Tax enhances cAMP-responsive element-binding protein (CREB) binding activity through interactions with the DNA minor groove. J. Biol. Chem. **273:**19251-19259.

143. **Manning, E. T., T. Ikehara, T. Ito, J. T. Kadonaga, and W. L. Kraus.** 2001. p300 forms a stable, template-committed complex with chromatin: role for the bromodomain. Mol. Cell. Biol. **21:**3876-3887.

144. **Martinez-Balbas, M. A., A. J. Bannister, K. Martin, P. Haus-Seuffert, M. Meisterernst, and T. Kouzarides.** 1998. The acetyltransferase activity of CBP stimulates transcription. EMBO J. **17:**2886-2893.

145. **Mayall, T. P., P. L. Sheridan, M. R. Montminy, and K. A. Jones.** 1997. Distinct roles for P-CREB and LEF-1 in TCR alpha enhancer assembly and activation on chromatin templates in vitro. Genes Dev. **11:**887-899.

146. **McGhee, J. D., J. M. Nickol, G. Felsenfeld, and D. C. Rau.** 1983. Histone hyperacetylation has little effect on the higher order folding of chromatin. Nucleic Acids Res. **11:**4065-4075.

147. **Mizuguchi, G., A. Vassilev, T. Tsukiyama, Y. Nakatani, and C. Wu.** 2001. ATP-dependent nucleosome remodeling and histone

hyperacetylation synergistically facilitate transcription of chromatin. J. Biol. Chem. **276:**14773-14783.

148. **Montagne, J., C. Beraud, I. Crenon, G. Lombard-Platet, L. Gazzolo, A. Sergeant, and P. Jalinot.** 1990. Tax1 induction of the HTLV-I 21 bp enhancer requires cooperation between two cellular DNA-binding proteins. EMBO J. **9:**957-964.

149. **Montminy, M. R., and L. M. Bilezikjian.** 1987. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature **328:**175-178.

150. **Moore, S. C., and J. Ausio.** 1997. Major role of the histones H3-H4 in the folding of the chromatin fiber. Biochem. Biophys. Res. Commun. **230:**136-139.

151. **Morales, V., and H. Richard-Foy.** 2000. Role of histone N-terminal tails and their acetylation in nucleosome dynamics. Mol. Cell. Biol. **20:**7230-7237.

152. **Munshi, N., M. Merika, J. Yie, K. Senger, G. Chen, and D. Thanos.** 1998. Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. Mol. Cell. **2:**457-467.

153. **Murata, T., R. Kurokawa, A. Krones, K. Tatsumi, M. Ishii, T. Taki, M. Masuno, H. Ohashi, M. Yanagisawa, M. G. Rosenfeld, C. K. Glass, and Y. Hayashi.** 2001. Defect of histone acetyltransferase activity of the nuclear transcriptional coactivator CBP in Rubinstein-Taybi syndrome. Hum. Mol. Genet. **10:**1071-1076.

154. **Naar, A. M., P. A. Beaurang, K. M. Robinson, J. D. Oliner, D. Avizonis, S. Scheek, J. Zwicker, J. T. Kadonaga, and R. Tjian.** 1998. Chromatin, TAFs, and a novel multiprotein coactivator are required for synergistic activation by Sp1 and SREBP-1a in vitro. Genes Dev. **12:**3020-3031.

155. **Nakajima, T., C. Uchida, S. F. Anderson, J. D. Parvin, and M. Montminy.** 1997. Analysis of a cAMP-responsive activator reveals a two-component mechanism for transcriptional induction via signal-dependent factors. Genes Dev. **11:**738-747.

156. **Narlikar, G. J., M. L. Phelan, and R. E. Kingston.** 2001. Generation and interconversion of multiple distinct nucleosomal states as a mechanism for catalyzing chromatin fluidity. Mol. Cell **8:**1219-1230.

157. **Neish, A. S., S. F. Anderson, B. P. Schlegel, W. Wei, and J. D. Parvin.** 1998. Factors associated with the mammalian RNA polymerase II holoenzyme. Nucleic Acids Res. **26:**847-853.

158. **Nerenberg, M., S. H. Hinrichs, R. K. Reynolds, G. Khoury, and G. Jay.** 1987. The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. Science **237:**1324-1329.

159. **Ng, H. H., and A. Bird.** 2000. Histone deacetylases: silencers for hire. Trends Biochem. Sci. **25:**121-126.

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

161. **Ogryzko, V. V., R. L. Schiltz, V. Russanova, B. H. Howard, and Y. Nakatani.** 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell **87:**953-959.

162. **Oike, Y., A. Hata, T. Mamiya, T. Kaname, Y. Noda, M. Suzuki, H. Yasue, T. Nabeshima, K. Araki, and K. Yamamura.** 1999. Truncated CBP protein leads to classical Rubinstein-Taybi syndrome phenotypes in mice: implications for a dominant-negative mechanism. Hum. Mol. Genet. **8:**387-396.

163. **Oike, Y., N. Takakura, A. Hata, T. Kaname, M. Akizuki, Y. Yamaguchi, H. Yasue, K. Araki, K. Yamamura, and T. Suda.** 1999. Mice homozygous for a truncated form of CREB-binding protein exhibit defects in hematopoiesis and vasculo-angiogenesis. Blood **93:**2771-2779.

164. **Oliva, R., D. P. Bazett-Jones, L. Locklear, and G. H. Dixon.** 1990. Histone hyperacetylation can induce unfolding of the nucleosome core particle. Nucleic Acids Res. **18:**2739-2747.

165. **O'Neill, T. E., M. Roberge, and E. M. Bradbury.** 1992. Nucleosome arrays inhibit both initiation and elongation of transcripts by bacteriophage T7 RNA polymerase. J. Mol. Biol. **223:**67-78.

166. **Paca-Uccaralertkun, S., L.-J. Zhao, N. Adya, J. V. Cross, B. R. Cullen, I. Boros, and C.-Z. Giam.** 1994. In vitro selection of DNA elements highly responsive to the Human T-Cell Lymphotropic Virus Type I transcriptional activator, Tax. Mol. Cell. Biol. **14:**456-462.

167. **Panagopoulos, I., T. Fioretos, M. Isaksson, U. Samuelsson, R. Billstrom, B. Strombeck, F. Mitelman, and B. Johansson.** 2001. Fusion

of the MORF and CBP genes in acute myeloid leukemia with the t(10;16)(q22;p13). Hum. Mol. Genet. **10**:395-404.

168. **Parekh, B. S., and T. Maniatis.** 1999. Virus infection leads to localized hyperacetylation of histones H3 and H4 at the IFN-beta promoter. Mol. Cell **3**:125-129.

169. **Paskalis, H., B.K. Felber, G.N. Pavlakis.** 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.

170. **Pazin, M. J., P. L. Sheridan, K. Cannon, Z. Cao, J. G. Keck, J. T. Kadonaga, and K. A. Jones.** 1996. NF-kappa B-mediated chromatin reconfiguration and transcriptional activation of the HIV-1 enhancer in vitro. Genes Dev. **10**:37-49.

171. **Pelletier, G., V. Y. Stefanovsky, M. Faubladier, I. I. Hirschler-Laszkiewicz, J. Savard, L. I. Rothblum, J. Cote, and T. Moss.** 2000. Competitive recruitment of CBP and Rb-HDAC regulates UBF acetylation and ribosomal transcription. Mol. Cell **6**:1059-1066.

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

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

174. **Poiesz, B. J., F. W. Ruscetti, M. S. Reitz, V. S. Kalyanaraman, and R. C. Gallo.** 1981. Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sezary T-cell leukaemia. Nature **294**:268-271.

175. **Polach, K. J., P. T. Lowary, and J. Widom.** 2000. Effects of core histone tail domains on the equilibrium constants for dynamic DNA site accessibility in nucleosomes. J. Mol. Biol. **298**:211-223.

176. **Polesskaya, A., I. Naguibneva, A. Duquet, E. Bengal, P. Robin, and A. Harel-Bellan.** 2001. Interaction between acetylated MyoD and the bromodomain of CBP and/or p300. Mol. Cell. Biol. **21**:5312-5320.

177. **Polesskaya, A., I. Naguibneva, L. Fritsch, A. Duquet, S. Ait-Si-Ali, P. Robin, A. Vervisch, L. L. Pritchard, P. Cole, and A. Harel-Bellan.** 2001. CBP/p300 and muscle differentiation: no HAT, no muscle. EMBO J. **20**:6816-6825.

178. **Popovic, M., M.S. Reitz Jr, M.G. Sarngadharan, M. Robert-Guroff, V.S. Kalyanaraman, Y. Nakao, I. Miyoshi, J. Minowada, M. Yoshida, Y. Ito, and R.C. Gallo.** 1982. The virus of Japanese adult T-cell leukaemia is a member of the human T-cell leukaemia virus group. Nature **300**:63-66.

179. **Protacio, R. U., G. Li, P. T. Lowary, and J. Widom.** 2000. Effects of histone tail domains on the rate of transcriptional elongation through a nucleosome. Mol. Cell. Biol. **20**:8866-8878.

180. **Puri, P. L., M. L. Avantaggiati, C. Balsano, N. Sang, A. Graessmann, A. Giordano, and M. Levrero.** 1997. p300 is required for MyoD-dependent cell cycle arrest and muscle-specific gene transcription. EMBO J. **16**:369-383.

181. **Robert-Guroff, M., Y. Nakao, K. Notake, Y. Ito, A. Sliski, and R. C. Gallo.** 1982. Natural antibodies to human retrovirus HTLV in a cluster of Japanese patients with adult T cell leukemia. Science **215**:975-978.

182. **Robzyk, K., J. Recht, and M. A. Osley.** 2000. Rad6-dependent ubiquitination of histone H2B in yeast. Science **287**:501-504.

183. **Roth, S. Y., J. M. Denu, and C. D. Allis.** 2001. Histone acetyltransferases. Annu. Rev. Biochem. **70**:81-120.

184. **Rowley, J. D., S. Reshmi, O. Sobulo, T. Musvee, J. Anastasi, S. Raimondi, N. R. Schneider, J. C. Barredo, E. S. Cantu, B. Schlegelberger, F. Behm, N. A. Doggett, J. Borrow, and N. Zeleznik-Le.** 1997. All patients with the T(11;16)(q23;p13.3) that involves MLL and CBP have treatment-related hematologic disorders. Blood **90**:535-541.

185. **Schiltz, R. L., C. A. Mizzen, A. Vassilev, R. G. Cook, C. D. Allis, and Y. Nakatani.** 1999. Overlapping but distinct patterns of histone acetylation by the human coactivators p300 and PCAF within nucleosomal substrates. J. Biol. Chem. **274**:1189-1192.

186. **Schwarz, P. M., A. Felthauser, T. M. Fletcher, and J. C. Hansen.** 1996. Reversible oligonucleosome self-association: dependence on divalent cations and core histone tail domains. Biochemistry **35**:4009-4015.

187. **Scoggin, K. E., A. Ulloa, and J. K. Nyborg.** 2001. The oncoprotein Tax binds the SRC-1-interacting domain of CBP/p300 to mediate transcriptional activation. Mol. Cell. Biol. **21**:5520-5530.

188. **Seiki, M., S. Hattori, and M. Yoshida.** 1982. Human adult T-cell leukemia virus: molecular cloning of the provirus DNA and the unique terminal structure. Proc. Natl. Acad. Sci. USA **79**:6899-6902.

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

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

191. **Seiki, M., J. Inoue, M. Hidaka, and M. Yoshida.** 1988. Two cis-acting elements responsible for posttranscriptional trans-regulation of gene expression of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA **85**:7124-7128.

192. **Seiki, M., J. Inoue, T. Takeda, and M. Yoshida.** 1986. Direct evidence that p40x of human T-cell leukemia virus type I is a trans-acting transcriptional activator. EMBO J. **5**:561-565.

193. **Sewack, G. F., T. W. Ellis, and U. Hansen.** 2001. Binding of TATA binding protein to a naturally positioned nucleosome is facilitated by histone acetylation. Mol. Cell. Biol. **21**:1404-1415.

194. **Shang, Y., X. Hu, J. DiRenzo, M. A. Lazar, and M. Brown.** 2000. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell **103**:843-852.

195. **Shaywitz, A. J., and M. E. Greenberg.** 1999. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu. Rev. Biochem. **68**:821-861.

196. **Sheridan, P. L., C. T. Sheline, K. Cannon, M. L. Voz, M. J. Pazin, J. T. Kadonaga, and K. A. Jones.** 1995. Activation of the HIV-1 enhancer by the LEF-1 HMG protein on nucleosome-assembled DNA in vitro. Genes Dev. **9**:2090-2104.

197. **Simpson, R. T., J. P. Whitlock, Jr., M. Bina-Stein, and A. Stein.** 1978. Histone-DNA interactions in chromatin core particles. Cold Spring Harb. Symp. Quant. Biol. **42**:127-136.

198. **Slamon, D. J., M. F. Press, L. M. Souza, D. C. Murdock, M. J. Cline, D. W. Golde, J. C. Gasson, and I. S. Chen.** 1985. Studies of the putative transforming protein of the type I human T-cell leukemia virus. Science **228:**1427-1430.

199. **Sobulo, O. M., J. Borrow, R. Tomek, S. Reshmi, A. Harden, B. Schlegelberger, D. Housman, N. A. Doggett, J. D. Rowley, and N. J. Zeleznik-Le.** 1997. MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). Proc. Natl. Acad. Sci. USA **94:**8732-8737.

200. **Sodroski, J., C. Rosen, W. C. Goh, and W. Haseltine.** 1985. A transcriptional activator protein encoded by the x-lor region of the human T-cell leukemia virus. Science **228:**1430-1434.

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

202. **Spencer, T. E., G. Jenster, M. M. Burcin, C. D. Allis, J. Zhou, C. A. Mizzen, N. J. McKenna, S. A. Onate, S. Y. Tsai, M. J. Tsai, and B. W. O'Malley.** 1997. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature **389:**194-198.

203. **Stein, R. W., M. Corrigan, P. Yaciuk, J. Whelan, and E. Moran.** 1990. Analysis of E1A-mediated growth regulation functions: binding of the 300-kilodalton cellular product correlates with E1A enhancer repression function and DNA synthesis-inducing activity. J. Virol. **64:**4421-4427.

204. **Sterner, D. E., and S. L. Berger.** 2000. Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. **64:**435-459.

205. **Strahl, B. D., and C. D. Allis.** 2000. The language of covalent histone modifications. Nature **403:**41-45.

206. **Sun, Z. W., and C. D. Allis.** 2002. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature **418:**104-108.

207. **Tang, Y., F. Tie, I. Boros, R. Harrod, M. Glover, and C. Z. Giam.** 1998. An extended alpha-helix and specific amino acid residues opposite the DNA-binding surface of the cAMP response element binding protein basic domain are important for human T cell lymphotropic retrovirus type I Tax binding. J. Biol. Chem. **273:**27339-27346.

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

209. **Tini, M., A. Benecke, S. J. Um, J. Torchia, R. M. Evans, and P. Chambon.** 2002. Association of CBP/p300 acetylase and thymine DNA glycosylase links DNA repair and transcription. Mol. Cell **9**:265-277.

210. **Tse, C., and J. C. Hansen.** 1997. Hybrid trypsinized nucleosomal arrays: identification of multiple functional roles of the H2A/H2B and H3/H4 N-termini in chromatin fiber compaction. Biochemistry **36**:11381-11388.

211. **Tse, C., T. Sera, A. P. Wolffe, and J. C. Hansen.** 1998. Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. Mol. Cell. Biol. **18**:4629-4638.

212. **Turgeman, H., and M. Aboud.** 1998. Evidence that protein kinase A activity is required for the basal and tax-stimulated transcriptional activity of human T-cell leukemia virus type-I long terminal repeat. FEBS Lett. **428**:183-187.

213. **Ura, K., H. Kurumizaka, S. Dimitrov, G. Almouzni, and A. P. Wolffe.** 1997. Histone acetylation: influence on transcription, nucleosome mobility and positioning, and linker histone-dependent transcriptional repression. EMBO J. **16**:2096-2107.

214. **van Leeuwen, F., P. R. Gafken, and D. E. Gottschling.** 2002. Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell **109**:745-756.

215. **Van Orden, K., and J. K. Nyborg.** 2000. Insight into the tumor suppressor function of CBP through the viral oncoprotein Tax. Gene Expr. **9**:29-36.

216. **Vettese-Dadey, M., P. A. Grant, T. R. Hebbes, C. Crane-Robinson, C. D. Allis, and J. L. Workman.** 1996. Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. EMBO J. **15**:2508-2518.

217. **Vettese-Dadey, M., P. Walter, H. Chen, L. J. Juan, and J. L. Workman.** 1994. Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH, to nucleosome cores. Mol. Cell. Biol. **14**:970-981.

218. **Vitolo, J. M., C. Thiriet, and J. J. Hayes.** 2000. The H3-H4 N-terminal tail domains are the primary mediators of transcription factor IIIA access to 5S DNA within a nucleosome. Mol. Cell. Biol. **20:**2167-2175.

219. **von Mikecz, A., S. Zhang, M. Montminy, E. M. Tan, and P. Hemmerich.** 2000. CREB-binding protein (CBP)/p300 and RNA polymerase II colocalize in transcriptionally active domains in the nucleus. J. Cell. Biol. **150:**265-273.

220. **Wagner, S., and M. R. Green.** 1993. HTLV-I Tax protein stimulation of DNA binding of bZIP proteins by enhancing dimerization. Science **262:**395-399.

221. **Wang, X., C. He, S. C. Moore, and J. Ausio.** 2001. Effects of histone acetylation on the solubility and folding of the chromatin fiber. J. Biol. Chem. **276:**12764-12768.

222. **Whitlock, J. P. J., and A. Stein.** 1978. Folding of DNA by histones which lack their NH2-terminal regions. J. Biol. Chem. **253:**3857-3861.

223. **Wolffe, A. P.** 1998. Chromatin: Structure and Function, Third ed. Academic Press, San Diego.

224. **Xu, L., R. M. Lavinsky, J. S. Dasen, S. E. Flynn, E. M. McInerney, T. M. Mullen, T. Heinzel, D. Szeto, E. Korzus, R. Kurokawa, A. K. Aggarwal, D. W. Rose, C. K. Glass, and M. G. Rosenfeld.** 1998. Signal-specific co-activator domain requirements for Pit-1 activation. Nature **395:**301-306.

225. **Yan, J. P., J. E. Garrus, H. A. Giebler, L. A. Stargell, and J. K. Nyborg.** 1998. Molecular interactions between the coactivator CBP and the human T-cell leukemia virus Tax protein. J. Mol. Biol. **281:**395-400.

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

227. **Yao, T. P., G. Ku, N. Zhou, R. Scully, and D. M. Livingston.** 1996. The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. Proc. Natl. Acad. Sci. USA **93:**10626-10631.

228. **Yao, T. P., S. P. Oh, M. Fuchs, N. D. Zhou, L. E. Ch'ng, D. Newsome, R. T. Bronson, E. Li, D. M. Livingston, and R. Eckner.** 1998. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. Cell **93:**361-372.

229. **Yie, J., K. Senger, and D. Thanos.** 1999. Mechanism by which the IFN-beta enhanceosome activates transcription. Proc. Natl. Acad. Sci. USA **96:**13108-13113.

230. **Yin, M. J., and R. B. Gaynor.** 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. Cell. Biol. **16:**3156-3168.

231. **Yin, M. J., E. Paulssen, J. Seeler, and R. B. Gaynor.** 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. Virol. **69:**6209-6218.

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

233. **Yoshida, M.** 2001. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. Annu. Rev. Immunol. **19:**475-496.

234. **Yoshida, M., I. Miyoshi, and Y. Hinuma.** 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc. Natl. Acad. Sci. USA **79:**2031-2035.

235. **Yoshida, M., M. Seiki, K. Yamaguchi, and K. Takatsuki.** 1984. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. Proc. Natl. Acad. Sci. USA **81:**2534-2537.

236. **Yuan, Z. M., Y. Huang, T. Ishiko, S. Nakada, T. Utsugisawa, H. Shioya, Y. Utsugisawa, K. Yokoyama, R. Weichselbaum, Y. Shi, and D. Kufe.** 1999. Role for p300 in stabilization of p53 in the response to DNA damage. J. Biol. Chem. **274:**1883-1886.

237. **Zhang, W., and J. J. Bieker. 1998.** Acetylation and modulation of erythroid Kruppel-like factor (EKLF) activity by interaction with histone acetyltransferases. Proc. Natl. Acad. Sci. USA **95:**9855-9860.

238. **Zhang, W., S. Kadam, B. M. Emerson, and J. J. Bieker.** 2001. Site-specific acetylation by p300 or CREB binding protein regulates erythroid Kruppel-like factor transcriptional activity via its interaction with the SWI-SNF complex. Mol. Cell. Biol. **21:**2413-2422.

239. **Zhao, L. J., and C. Z. Giam.** 1992. Human T-cell lymphotropic virus type I (HTLV-I) transcriptional activator, Tax, enhances CREB binding to HTLV-I

21-base-pair repeats by protein-protein interaction. Proc. Natl. Acad. Sci. USA **89**:7070-7074.

240. **Zhao, L. J., and C. Z. Giam.** 1991. Interaction of the human T-cell lymphotrophic virus type I (HTLV-I) transcriptional activator Tax with cellular factors that bind specifically to the 21-base-pair repeats in the HTLV-I enhancer. Proc. Natl. Acad. Sci. USA **88**:11445-11449.