Targeting of p300 to the Interleukin-2 Promoter via CREB-Rel Cross-talk during Mitogen and Oncogenic Molecular Signaling in Activated T-cells*

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In this report, we explore the mechanisms of targeting of p300 to the interleukin-2 (IL-2) promoter in response to mitogenic and oncogenic molecular signals. Recruitment of p300 by cAMP-responsive element-binding protein-Rel cross-talk at the composite CD28 response element (CD28RE)-TRE element of the IL-2 promoter is essential for promoter inducibility during T-cell activation, and CD28RE-TRE is the exclusive target of the human T-cell lymphotropic virus type I oncoprotein Tax. The intrinsic histone acetyltransferase activity of p300 is dispensable for activation of the IL-2 promoter, and the N-terminal 743 residues contain the minimal structural requirements for synergistic transactivation of the CD28RE-TRE, the IL-2 promoter, and endogenous IL-2 gene expression. Mutational analysis of p300 reveals differential structural requirements for the N-terminal p300 module by individual cis-elements within the IL-2 promoter. These findings provide evidence that p300 assembles at the IL-2 promoter to form an enhancer-like signal transduction target that is centrally integrated at the CD28RE-TRE element of the IL-2 promoter through specific protein module-targeted associations in activated T-cells.

The role of nuclear transcription factors and coactivators as primary targets of molecular signaling in eukaryotic cells has emerged as a fundamental concept in signal transduction biology (1–3). Detailed functional characterization of the molecular correlates that underlie the control of these events has been essential to our understanding of their importance in cellular homeostasis and has provided key insights into the manner in which they become deranged during oncogenic transformation. The study of molecular signaling events involved in the control of cytokine gene expression in activated T-cells has led to several important paradigms with relevant application to the understanding of signal transduction and gene regulation in various cell types (4–7).

The interleukin-2 (IL-2)1 gene is a key molecular signaling target in activated T-cells and plays a pivotal role in the control of the immune response. As such it has become a model system through which the fundamental mechanisms of stimulus-evoked responses can be studied. Although we are far from a precise understanding of the “fine tuned” regulation of IL-2, there is general agreement that many of the molecular signaling events, set in motion during T-cell activation, converge on a 300-bp pair region just upstream of the transcription start site (4, 8, 9). This region is often referred to as the interleukin-2 proximal promoter. A common theme that has emerged from the study of the IL-2 promoter and other Ras-controlled genes is that they are modulated by the activity of one or more composite gene regulatory elements. These composite sites are targeted cooperatively by the interaction of two or more separate transcription factors, in a linked fashion, to provide both increased specificity and stability.

Recently it has been shown that the CD28 response element (CD28RE), one of the major Rel-κB-controlled sites in the IL-2 promoter, functions as a composite site in conjunction with a 3′-sequence that interacts with members of the basic leucine zipper (B-Zip) family of transcription factors (10, 11). This composite site, termed the CD28RE-TRE (CD28RE-AP1), is located at position −174 to −146 within the proximal IL-2 promoter and provides a sequence-specific regulatory interface where Rel and CREB-targeted molecular signaling events converge in synergy with p300 (10–12).

Human T-cell lymphotropic virus type I is the etiologic agent of adult T-cell leukemia/lymphoma and the demyelinating syndrome, tropical spastic paraparesis (13, 14). The genome of human T-cell lymphotropic virus type I encodes a 40-kDa transactivator protein, Tax, which drives oncogenic transformation of human T-lymphocytes and can act, in conjunction with Ras, to promote the transformation of rodent fibroblasts (15). In infected cells, Tax can act to increase the activation of target cellular genes containing nuclear factor (NF)-κB and CREB/ATF sites by up-regulating the activity of both CREB/ATF and Rel/NF-κB transcription factors (16, 17). IL-2 and the IL-2 receptor-α are well known targets of Tax activation in T-cells (15, 18–20). Although the ability of Tax to up-regulate Rel/κB function is thought to be the sole underlying mechanism for its action at the IL-2 promoter, many details of its true mode of regulation at the CD28RE-TRE remain debated (20, 21). As a result, a precise accounting of the role of Tax at the IL-2 promoter continues to be elusive.

In this report we analyze the mechanism through which cross-talk between the Rel/κB and ATF/CREB signaling pathways together with p300 control the transactivation of the IL-2 factor one; UAS, upstream activating sequence; CBP, CREB-binding protein.

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3 The abbreviations used are: IL-2, interleukin-2; CD28RE, CD28 response element; B-Zip, basic leucine zipper; CREB, cAMP-responsive element-binding protein; NF, nuclear factor; CAT, chloramphenicol acetyltransferase; wt, wild type; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRE, TPA response element; ATF-1 activating transcription factor one; UAS, upstream activating sequence; CBP, CREB-binding protein.
promoter at its central composite enhancer when challenged by both mitogens and the T-cell oncogene tax. We present a model predicting that a key target for the signal transduction pathways generated during T-cell activation is the assembly of a CD28RE-TRE directed “enhanceosome-like” complex at the IL-2 proximal promoter. This assembly is centrally linked to distinct domains of p300 through multiple, yet specific, combinatorial interactions with factors coordinately bound to its composite gene regulatory elements.

**MATERIALS AND METHODS**

**Plasmids**

CD28RE-TRE-CAT, IL-2 CAT, NF-AT-CAT, HIV-B CAT, AP1-CAT, and Δ56-CAT parental control reporter plasmids have been described (10, 22). The NFI-2A CAT reporter was constructed by inserting two copies of the sequence AGCTTCAGGTTTTCATACATCAATATCATCTTTCAAAAAG into the HindIII site upstream of the minimal fse promoter of Δ56-CAT. The CRE-CAT was a generous gift from Dr. Maria Laura Avantaggiati. The UAS-CAT reporter plasmids contains five tandem copies of the UAS Gal4 recognition site linked to an E1B promoter of Drosophila.

**300 Protein-DNA Binding Assays**

A simple screen for functional domains within the N-terminal 743 amino acids of p300 in the Gal4-p300 fusion constructs was designed by mutating 4 of 10 N-terminal sequences that were predicted to assume an α-helical structure by the method of Chou and Fasman. The targeted regions were arbitrarily referred to as: helix I, residues 21–32; helix II, residues 72–85; helix VIII, residues 600–616; and helix IX, residues 633–663 (see Fig. 5B). The α-helical structure was disrupted in either single or double mutants by the substitution of three proline residues for three consecutive residues in the center of each of the putative α-helical segments described above. The mutations were introduced by site-directed mutagenesis using the Quickchange site-directed mutagenesis kit (Stratagene, La Jolla CA). The IL-2 mutant CAT reporter plasmid contains the IL-2 flanking region from –575 to +47 with a 3-base substitution at the TRE portions of the CD28RE-TRE composite site (see Fig. 2A). This reporter mutant was constructed by site-directed mutagenesis of the IL-2-CAT plasmid using the primers CTGTTAAGAAATTTCCAAAGCAACATCAAGAAGAGAAATTG and CATTTCCTCTCCTGTAGGTGTTGGTAAATTCCAAAC and was provided by Dr. V. Doseeva.2

**Cell Culture and Transfection Assays**

Jurkat T-cells were cultured in RPMI medium (Life Technologies, Inc.), 10% fetal calf serum, and 100 units/ml penicillin/streptomycin at 37 °C in 5% CO2. Cells (1–2 × 106) were incubated in 250 μl of ice-cold phosphate buffer with 2.5–5 μg of reporter plasmid and 1–5 μg of the indicated expression vectors followed by electroporation (200 V, 1180 microfarads). The cells were then resuspended in 5 ml of complete RPMI at 37 °C. After transfection and resuspension, the cells were either left unstimulated or were stimulated with the indicated combinations of 1 μg ionomycin (Calbiochem), 50 ng/ml phorbol 12-myristate 13-acetate (Sigma Chemical Co.), 2 μg/ml phytohemagglutinin (Murex), a 1:100 dilution of monoclonal antibody 9.3 ascites (anti-CD28) (Bristol-Myers Squibb Research Institute, Seattle), and a 1:1000 dilution of monoclonal antibody OKT3 ascites (ATCC). The construction of Jurkat cells showing stable expression of the Gal4-p300 (amino acids 1–743) was established by selection of cells transfected with a pcDNA3 vector carrying the Gal4-p300 cDNA and a neomycin resistance gene. Cells were selected by growth in medium containing 1.6 mg/ml G418. Resistant pools of cells were cloned for stimulation of the G4-p300 expression by transient transfection with UAS-CAT and subsequent CAT assay. Positive pools of cells were cloned by limiting dilution in G418 and screened a second time by UAS-CAT reporter assay. All transient transfections studies were performed in duplicate or triplicate and, in all cases, are representative of at least two independent experiments. CAT activity in cell extracts was determined as described (10). Quantitation of the CAT activity was performed using a Molecular Dynamics PhosphorImager. Results are expressed as the mean ± S.E.

**300 Protein-DNA Binding Assays**

0.3–1.5 μg of affinity-purified ATF-1/CREB and c-Rel (10) was incubated for 15 min at 25 °C in binding buffer containing 10 mM Hepes, pH 7.5, 0.1 mM EDTA, 0.1 mM EGTA, 10% fetal calf serum, and 100 units/ml penicillin/streptomycin with 500 ng of biotinylated duplex oligonucleotides encoding the CD28RE-TRE sequence 5′-TGCGGTTTAAAAAGATCCAGAAGAGGGGTTTTGGAATTTCTTTAAAC and was provided by Dr. V. Doseeva. This reporter mutant was constructed by site-directed mutagenesis of the IL-2-CAT plasmid using the primers

**300 Protein-DNA Binding Assays**

**Antibodies and Western Blots and Nuclear Extracts**

Western blots were performed as described previously using anti-Gal4-DNA binding domain (CLONTECH and Santa Cruz) (10, 29). Nuclear extracts were prepared from resting or stimulated Ficoll-purified Jurkat T-cells following electrophoresis as described previously (30).

**Determination of IL-2**

IL-2 levels were determined by enzyme-linked immunosorbent analysis using the Quantikine immunoassay kit (R&D Systems) according to the manufacturer’s instructions.

2 C. M. Haggerty, V. Doseeva, W. G. Butcher, S. Chaudhry, W. Freehern, J. I. Smith, and K. Gardner, manuscript in preparation.
**RESULTS**

Mitogen Induction of CREB-Rel Targeting Pathways Is an Essential Component of Molecular Signaling at the IL-2 Proximal Promoter and Acts in Synergy with p300—As reported previously (10), overexpression of p300 leads to synergistic increases in the transactivation of the IL-2 CD28RE-TRE gene regulatory element (Fig. 1A) in response to the well-characterized T-cell mitogens phorbol ester and ionomycin (31, 32). The dependence of this synergy on intact Ras-responsive CREB and kB molecular signaling pathways is demonstrated by the disruption of the p300-controlled CD28RE-TRE transactivation by the activity of three previously described pathway-specific inhibitors: a dominant negative CREB/ATF (CREB leucine zipper with acidic extension) expression vector, a superdominant I-kB (hydrolysis-resistant I-kB-a mutant, Rel/kB inhibitor) expression vector, and a dominant negative vector for Ras (Asn-17 substitution; see “Materials and Methods”). As shown in Fig. 1, B–D, blockade of Ras, Rel/kB, or CREB/ATF effector signaling abrogates mitogen-induced transactivation of the CD28RE-TRE, in both the presence and absence of exogenous p300.

Tax Targets both kB and CREB-dependent of Elements of CD28RE-TRE to Transactivate the IL-2 Promoter in Synergy with p300—The Tax oncoprotein has pleiotropic effects on a variety of immediate early and cytokine genes in activated T-cells. Several earlier studies have shown that the IL-2 promoter is a major target of the human T-cell lymphotropic virus type I Tax oncoprotein (18, 20). Although it has been generally reported that the kB pathway is the exclusive target of Tax action at the IL-2 reporter through its ability to increase the phosphorylation-dependent degradation of the I-kB inhibitor, other laboratories have suggested roles for factors distinct from NF-kB (20, 21). Our observation that the TRE half of the IL-2 CD28RE-TRE element associates with ATF/CREB B-Zip factors (10) suggests that CREB signaling at this site may be a major mechanism through which Tax acts at the IL-2 promoter. To test this possibility, the action of Tax at an IL-2-CAT reporter construct, in which the TRE portion of the CD28RE-TRE composite element within the promoter was altered by site-directed mutagenesis (IL2-mutTRE), was compared with that of the wild type promoter sequence (wt IL2). As demonstrated in Fig. 2A, induction of the IL-2 promoter by tax in either

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**Fig. 1.** Tax synergizes with p300 to transactivate the CD28RE-TRE. Panel A and B, Jurkat T-cells were cotransfected with 4 µg of CD28RE-TRE-CAT reporter plasmid in the presence (right) or absence (left) of 3 µg of the p300 expression vector and either 3 µg of Ras dominant negative expression vector (N17Ras) or 3 µg of control cytomegalovirus expression vector. The cells were then costimulated with 50 ng of phorbol 12-myristate 13-acetate/ionomycin and 1 µM ionomycin plus anti-CD28 monoclonal antibody (1:1,000 dilution; Co-stim). Panel C cells were transfected with 4 µg of CD28RE-TRE-CAT and 8 µg of either control expression vector or a Rel/kB dominant negative expression vector (dominant interfering non-phosphorylated I-kB-a) in the presence or absence of 3 µg of p300 expression vector. Panel D cells were cotransfected with 4 µg of CD28RE-TRE-CAT and 3 µg of control vector or 3 µg of CREB dominant negative expression vector.
FIG. 2. Tax targeting of the IL-2 CD28RE-TRE through CREB/ATF and Rel/kB. Panel A, top, shows a schematic of the IL-2 promoter mutated at the CREB/ATF recognition site of the CD28RE-TRE (IL2-mutTRE) compared with wild type (wt IL2-CAT). Bottom, wild type and mutant IL-2 reporter plasmids (4 μg) were cotransfected in the presence or absence of 156 ng of Tax expression vector. Cells were left untreated or stimulated with either phorbol ester and ionomycin (P/I) or phorbol ester/ionomycin plus anti-CD28 antibodies as indicated. Panel B, cells were cotransfected with 4 μg of CD28RE-TRE-CAT with either no additions, 156 ng of Tax expression vector, 3 μg of p300 expression vector, or both Tax and p300 expression vectors in the presence or absence of either dominant (dominant interfering or superdominant) expression vectors for 3 μg of Ras, 8 μg of Rel/kB, or 3 μg of CREB. After transfection, cells were stimulated with ionomycin and phorbol ester and assayed for CAT activity. Panel C, Jurkat cells were cotransfected with 4 μg of the CD28RE-TRE-CAT reporter with or without 3 μg of expression vector for either wild type Tax or Tax mutants defective in CREB-dependent (M47) or Rel/kB-dependent (M22) pathways. Cells were then left unstimulated or stimulated with ionomycin and phorbol ester as described above.
stimulated or costimulated cells is completely dependent on the integrity of the IL-2 CD28RE-TRE 3′-site. Reporter studies utilizing a single copy of the CD28RE-TRE show that Tax directly up-regulates the CD28RE-TRE, and this response is increased dramatically in the presence of exogenous p300 (Fig. 2B). Tax-dependent transactivation is repressed effectively by dominant negative Ras, the superdominant I-κB, and the dominant negative CREB expression vectors (Fig. 2B), thus demonstrating that Tax targeting of the CD28RE-TRE and its synergy with p300 are dependent on both an intact I-κB and CREB/ATF signaling pathway. This point is demonstrated further by using mutants of Tax which are deficient in transactivating either CREB/ATF or Rel/κB-dependent genes (Fig. 2C). The Tax mutant M47 can activate Rel/κB pathways but not the CREB/ATF pathways. Similarly, the M22 Tax mutant is capable of activating the CREB/ATF pathways but not the Rel/κB pathways (16). As demonstrated in Fig. 2C, coexpression of either pathway-defective Tax mutant produces negligible activation of CD28RE-TRE in the presence of p300. This evidence, in combination with the dramatic effects of both the CREB and Rel/κB-specific dominant negative vectors on Tax induction of CD28RE-TRE, shows that the primary mechanism of action of Tax at this element involves a dual targeting of CREB-Rel cross-talk in activated T-cells.

**T-cell Signaling via p300-Mediated CREB-Rel Cross-talk**

**FIG. 3.** Tax selectively targets the CD28RE-TRE and represses AP-1-dependent enhancers. Panel A, Jurkat cells were transfected with 4 μg of the CD28RE-TRE reporter plasmid in the absence or presence of 156 ng of wt Tax expression vectors. Cells were either untreated or stimulated with phorbol ester and ionomycin (P/I), harvested, and assayed for CAT activity. Panel B, Jurkat cells were transfected with 4 μg of the NF-AT reporter plasmid in the absence or presence of 156 ng of the wt Tax expression vectors. Cells were either untreated or stimulated and assayed for reporter activity as described above. Panel C, Jurkat cells were transfected with 4 μg of the NFIL-2A reporter plasmid in the absence or presence of 156 ng of wt Tax expression vectors. Cells were either untreated or stimulated and assayed for reporter activity as described above. Panel D, Jurkat cells were transfected with 4 μg of the 3X AP-1 site reporter plasmid in the absence or presence of 156 ng of wt Tax expression vectors. Cells were either untreated or stimulated and assayed for reporter activity as described above. Panel E, Jurkat cells were transfected with 2 μg of UAS-CAT reporter in the presence or absence of 3 μg of Gal4-CREB expression vectors alone or with 156 ng of cotransfected Tax expression vector. Cells were unstimulated or stimulated with P/I alone or in the presence of 1:1,000 a-CD28 antibodies and processed for reporter activity as described above.
Fig. 4. The N-terminal 743 amino acids of p300 are necessary and sufficient for recruitment to and transactivation of the CD28RE-TRE and IL-2 promoter via CREB/ATF and Rel kB. Panel A, schematic diagram of [35S]methionine-labeled in vitro translated T-cell Signaling via p300-Mediated CREB-Rel Cross-talk.
that CREB molecular signaling is targeted effectively by Tax in mitogen-activated T-cells. In this experiment, low levels of the transfected Gal4-CREB fusion are synergistically transactivated when coexpressed with Tax. Notably, the level of expression is maximal with phorbol ester and ionomycin alone, as it shows little increase by CD28 costimulation.

The N-terminal 743 amino acids of p300 Are Necessary and Sufficient for the Mediation of CREB-Rel Cross-talk at the CD28RE-TRE and Potently Transactivates the CD28RE-TRE, the IL-2 Promoter, and Endogenous IL-2 Expression—The transcriptional activity of p300 at individual promoters occurs in the absence of any intrinsic DNA binding activity. The main mechanism of p300 action occurs through its recruitment to individual cis-elements and promoters through protein-protein interactions with specific transcription factors and components of the basal transcriptional apparatus (34). The CREB/ATF binding domain has been localized previously to the KIX domain of p300/CBP, which encompasses amino acids 566–647 in p300 (35, 36). Two separate Rel/kB binding domains have been identified recently: one in the N-terminal 594 amino acids of p300, and a portion spanning residues 1572–2414 of the C terminus (37, 38). To test which portions of p300 are necessary for effective recruitment to the CD28RE-TRE, we determined the in vitro domain requirements of p300 to be effectively recruited to the CD28RE-TRE. In this assay, biotinylated duplex oligonucleotides encoding a single copy of the CD28RE-TRE were incubated with in vitro translated Gal4 fusion constructs containing different portions of p300 (see upper panel Fig. 4A). Incubations were carried out in the presence or absence of affinity-purified CRE-B complex isolated from phorbol ester- and ionomycin-activated Jurkat T-cells (10). DNA-bound versus free complexes were then separated by centrifugation with avidin-coated beads. In Fig. 4B, the portion of p300 containing the N-terminal 743 amino acids (and thus both Rel and CREB/ATF-1 binding domains) was effectively recruited to the CD28RE-TRE with increasing concentrations of purified CREB-Rel but did not associate with CD28RE-TRE in their absence.

A screen of the CREB-Rel-dependent recruitment of different p300 truncation mutants (described in Fig. 4A) to the CD28RE-TRE shows that the p300 mutant encompassing residues 1–743 is the most efficiently recruited (see Fig. 4C, bottom panel). Thus the portion of p300 which contains both the CREB/ATF-1 binding KIX domain and the N-terminal Rel binding domain is the most efficiently targeted to the CD28RE-TRE by purified CREB-Rel complexes. In addition, this portion of p300 (residues 1–743) is the most effective at inducing transcriptional activation of the CD28RE-TRE-CAT reporter when stimulated by phorbol ester and ionomycin (see Fig. 4D). The small amount of recruitment and transactivation potential demonstrated by the C-terminal 1945–2414 residues of p300 (see Fig. 4, C and D) is consistent with a single Rel-interacting domain identified previously in that region (38). The N-terminal 743 amino acids of p300 is also sufficient to superinduce transactivation of the entire proximal IL-2 promoter (Fig. 4E).

Cis-element Recruitment and the Transactivation Potential of p300 Show Differential Dependence on Distinct Structural Domains within p300—To determine the domain dependence of p300 for its ability to interact with the transcriptional activators and other cofactors that regulate the IL-2 promoter, a series of domain specific mutants was generated within the N-terminal 743 residues of p300 (see “Materials and Methods”). One class of mutations was a 1-amino acid substitution of alanine for tyrosine at position 638 within the KIX domain of p300 (Y638A p300). This mutation has been characterized previously to be critical for the interaction of CREB/ATF with the KIX domain (35). As shown in Fig. 5A, the N-terminal 743 amino acid residues of p300 are competent to activate the NF-AT and NFIL-2A sites of the IL-2 promoter, in addition to consensus kB and AP-1 sites. In contrast, the KIX mutation impairs the ability of the p300 N terminus to induce the CD28RE-TRE, kB, AP-1, and the NF-AT sites (Fig. 5, A and C top). Interestingly, the induction of the NFIL-2A element by the KIX mutant is equivalent to wild type (Fig. 5A).

To identify other candidate domains of p300 which contribute to the regulation of the CD28RE-TRE and the IL-2 promoter, a set of p300 N-terminal mutants was derived by substituting 3 consecutive proline residues in the center of 9 predicted α-helical regions within the p300 743-amino acid N terminus (see Fig. 5B). With the exception of the double mutant II/IX (not shown), each of the p300 mutants showed expression comparable to that of the wild type fusion protein in activated Jurkat T-cells (see Fig. 5B, inset). The activity of these mutants, including the p300 Y638A substitution, was assayed for their function as Gal4 fusions when cotransfected with either the recruitment-dependent CD28RE-TRE or the recruitment-independent (receptor bypass) UAS-CAT reporters (Fig. 5C, top and bottom, respectively). As shown in Fig. 5C, although the KIX Y638A mutant is deficient in transactivating the CD28RE-TRE, it has near wild type activity in the recruitment bypass assay. This functional dissociation between the intrinsic transactivation potential and recruitment is also observed to a lesser extent with the helix IX mutants. Interestingly, both of these mutants contain substitutions in the α-helical regions of the KIX domain as determined by its solution structure. Of note is that mutation of the helix II domain of p300 results in a gain-of-function mutant that has above wild type inducibility in both the CD28RE-TRE recruitment assay and the recruitment bypass experiments, even in the absence of stimulation. This observation could partially reflect a slight increased stability of this protein over the wild type as suggested by the Western blot analysis (Fig. 5B, inset); however,
the helix II domain is also known to contain a nuclear hormone receptor binding motif, (LXXX), which is a suspected target for steroid hormone-mediated repression of p300/CBP-controlled genes (39, 40).

DISCUSSION

p300 plays a quintessential role as an adaptor by providing the necessary scaffolding to link diverse factors in an active transcriptional complex at the IL-2 promoter (Fig. 6). A prominent feature of the IL-2 enhancerosome which distinguishes it from others, such as the prototypical interferon-β promoter (41), is the capacity of the N-terminal module of p300 (743 amino acids) alone to assemble the enhanceosome and activate the IL-2 promoter. This difference is particularly notable because the N-terminal module not only lacks histone acetyltransferase activity but is missing interaction domains for polymerase II, TFIIB, and other basal factors (1, 2). These observation suggests that the interaction of p300 with polymerase II (as shown in Fig. 6) may be a redundant or sequential feature of the enhanceosome which can be compensated for by interactions with other coactivator complexes such as mediator (for discussions, see Ref. 50). It is also possible that p300 may interact with promoters as a dimer, although no evidence of p300 oligomerization has ever been reported. Prior studies with isolated enhancer elements have shown differential requirements for the intrinsic histone acetyl-transferase activity of p300/CBP (47). However, few of these studies have extended this observation to whole functional promoters in vitro or in vivo, and none has shown the selective modular requirement of p300 domains as demonstrated in this work. To the contrary, some promoters, such as the interferon-β gene, are repressed by the N terminus of the p300/CBP module (48). Understanding the differential requirements of promoters for specific regulatory and enzymatic modules of p300 will be an important challenge in the future for determining how they are targeted selectively by molecular signaling events in activated T-cells.

This is particularly true given that the N terminus of p300 is the region with least homology to CBP. In this regard, the use of the proline mutations to dissect the functional domains within the p300 N-terminal module (see Fig. 5) will be an effective tool in determining the structural requirements for...
and consensus AP-1 enhancers (44). In another study, the CD28RE-TRE was shown to be repressed in T-cells by activation of ICER, an endogenous CREB dominant negative induced by elevations in intracellular cAMP (40). Finally, consistent with recent observations that Tax expression represses AP-1 transactivation by competing for AP-1 binding to p300/CBP (45), work in this report confirms that both a consensus AP-1 enhancer and NF-AT are repressed rather than stimulated by Tax. An explanation for the discrepancy between these findings and those published by Sun and co-workers (21), who suggest that Tax up-regulates NF-AT transactivation, may lie in the fact that the element in their study contained multiple tandem copies of only the CD28RE half of the CD28RE-TRE site in binding and transactivation studies with overexpressed NF-AT. Our findings do not exclude a role for AP-1 family members at the IL-2 promoter because there is overwhelming evidence that Fos/Jun plays a significant role as a congener at the distal NF-AT site. In fact, the B-Zip component at the CD28RE-TRE is not clear. An AP-1/CREB heterodimer (e.g., Jun/ATF-2 or CREB2/Fra) could be the true active B-Zip dimer at the CD28RE-TRE. In all likelihood, there is probably a stochastic distribution of B-Zip heterodimers that target the CD28RE-TRE in any given activated T-cell. Certainly the current evidence presented in this paper does more to rule in a role for CREB/ATF rather than rule out one for fos/jun at the CD28RE-TRE.

It was suggested previously that AP-1 may be a molecular target for T-cell anergy. In this prior study, a reporter plasmid driven by six tandem copies of the entire CD28RE-TRE sequence was shown to be down-regulated in anergic cells (46). Although the site was referred to as the IL-2 AP-1 element, this current work provides dramatic evidence in support of an expanded role for CREB family members and p300/CBP modules in T-cell anergy via the CD28RE-TRE.

Finally, the difference shown by the p300 N-terminal mutants in the recruitment and bypass transcriptional assays (Fig. 5C) suggests a prominent role for protein-protein interaction between p300 and other non-DNA-binding regulatory complexes. This is likely to be a promoter-specific process that distinguishes among various coregulator complexes serving as coactivators or corepressors at the IL-2 gene through a dynamic exchange linked to different modules of p300 (see 51 and references cited therein). A major imperative in the future will be to define the molecular rules of interaction which determine what type of p300-containing regulatory complexes will assemble at various target genes during T-cell activation and how these complexes are influenced by upstream signaling cascades.

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**REFERENCES**

1. Goldman, P. S., Tran, V. K., and Goodman, R. H. (1997) *Recent Prog. Horm. Res.* 52, 103–119
2. Eckner, R. (1996) *Biol. Chem.* 17, 685–688
3. Karin, M., and Hunter, T. (1995) *Curr. Biol.* 5, 747–757
4. Avots, A., Kocher, C., Muller-Deubert, S., Neumann, M., and Serfling, E. (1995) *Immunobiology* 193, 254–258
5. Hentsch, B., Mouzaki, A., Pfeuffer, I., Rungger, D., and Serfling, E. (1997) *Biochim. Biophys. Acta* 1363, 181–200
6. Jain, J., Loh, C., and Rao, A. (1995) *Curr. Opin. Immunol.* 7, 259–274
7. Serfling, E., Avots, A., and Neumann, M. (1995) *Biochem. Biophys. Acta* 1263, 181–200
8. Bannister, A. J., Oehler, T., Wilhelm, D., Angel, P., and Kouzarides, T. (1995)
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13. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 7415–7419
14. Yoshida, M., Seiki, M., Yamaguchi, K., and Takatsuki, K. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 5389–5393
15. Bex, F., and Gaynor, R. B. (1998) *Methods* 16, 83–94
16. Smith, M. R., and Greene, W. C. (1990) *Genes Dev.* 4, 1875–1885
17. Munoz, E., and Israel, A. (1995) *Immunobiology* 193, 128–136
18. Bex, F., and Gaynor, R. B. (1998) *Methods* 16, 83–94
19. Ballard, D. W., Bohnlein, E., Lowenthal, J. W., Wano, Y., Franza, B. R., and Greene, W. C. (1988) *Science* 241, 1652–1655
20. Himes, S. R., Katsikeros, R., and Shannon, M. F. (1996) *J. Virol.* 70, 4001–4008
21. Good, L., Maggiwarg, S. B., and Sun, S. C. (1996) *EMBO J.* 15, 9730–9738
22. Arany, Z., Newsome, D., Oldread, E., Livingston, D. M., and Eckner, R. (1995) *Nature* 374, 81–84
23. Yuan, W., Condorelli, G., Caruso, M., Felsani, A., and Giordano, A. (1996) *J. Biol. Chem.* 271, 9009–9013
24. Chou, P. Y., and Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol.* 47, 45–148
25. Weiss, A., and Imboden, J. B. (1987) *Adv. Immunol.* 41, 1–38
26. Arany, Z., Newsome, D., Oldread, E., Livingston, D. M., and Eckner, R. (1995) *Nature* 374, 81–84
27. Semmes, O. J., Bohnlein, E., Lowenthal, J. W., Wano, Y., Franza, B. R., and Greene, W. C. (1988) *Science* 241, 1652–1655
28. Deleted in proof
29. Gardiner, K., Moore, T. C., Davis-Smyth, T., Krutzsch, H., and Levens, D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 1134–1138
30. Deleted in proof
31. Deleted in proof
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33. Deleted in proof
34. Deleted in proof
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