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Interactions between the Class II Transactivator and CREB Binding Protein Increase Transcription of Major Histocompatibility Complex Class II Genes

JOSEPH D. FONTES, SATOSHI KANAZAWA, DICKSON JEAN, AND B. MATIJA PETERLIN*

Howard Hughes Medical Institute, Departments of Medicine, Immunology, and Microbiology, University of California San Francisco, San Francisco, California 94143-0703.

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Class II major histocompatibility (class II) genes are regulated in a B-cell-specific and gamma interferon-inducible fashion. The master switch for the expression of these genes is the class II transactivator (CIITA). In this report, we demonstrate that one of the functions of CIITA is to recruit the CREB binding protein (CBP) to class II promoters. Not only functional but also specific binding interactions between CIITA and CBP were demonstrated. Moreover, a dominant negative form of CBP decreased the activity of class II promoters and levels of class II determinants on the surface of cells. Finally, the inhibition of class II gene expression by the glucocorticoid hormone could be attributed to the squelching of CBP by the glucocorticoid receptor. We conclude that CBP, a histone acetyltransferase, plays an important role in the transcription of class II genes.

The expression of major histocompatibility (MHC) complex class II (class II) genes is controlled at the level of transcription. Their coordinate B-cell-specific and gamma interferon-inducible expression is dictated by shared *cis*-acting elements in their promoters. At least four conserved sequences, called the S, X, X2, and Y boxes, have been identified (reviewed in references 2 and 33). With the help of the heterotrimeric nuclear factor Y complex, which binds to the Y box (29, 42, 52), the multimeric regulatory factor X (RFX) complex binds specifically to S and X boxes (15, 21). The X2 binding protein also facilitates the binding of RFX to these conserved upstream sequences (CUS) (30, 34, 41).

The occupancy of these CUS is necessary but not sufficient for the transcription of class II genes (25); expression of the class II transactivator (CIITA) is also required (48–50). CIITA is a 125-kDa transcriptional coactivator which does not bind to DNA directly (49) but interacts with protein complexes on CUS. Its C-terminal 800 amino acids interact with these DNA-bound proteins (43, 55). Indeed, weak interactions between CIITA and RFX5, which is the largest subunit of RFX, have been described previously (45). The N terminus of CIITA contains a transcriptional activation domain of 143 amino acids, which is rich in acidic amino acids (43, 55). Mutational analyses of this region identified two putative α helices which are required for the activation of transcription by CIITA (18). They bind to the 32-kDa subunit of TFIID, $TAF_{II}32$, and presumably activate transcription by recruiting TFIID to the promoter (18). CIITA also recruits the B-cell-specific coactivator Bob-1 (also known as OBF-1 or OCA-B), thereby increasing the expression of class II genes in B cells (17).

The CREB binding protein (CBP), which interacts directly with the phosphorylated form of CREB, was identified as a coactivator for the transcription of cyclic AMP-responsive genes (13, 27). Subsequently, many other transcription factors were found to interact with CBP (reviewed in reference 19). Direct binding of CBP to these transcription factors was also demonstrated (reviewed in reference 22). CBP activates transcription through its histone acetyltransferase (HAT) activity (7, 38) and its ability to recruit additional proteins p/CAF (53) and ACTR (11), which also possess HAT activities. Additionally, CBP can acetylate general transcription factors (20), which may contribute to its transcriptional effects. Finally, CBP binds to RNA helicase A and thereby RNA polymerase II holoenzyme (26, 36) as well as other general transcription factors, which include TATA-binding protein (3, 14) and TFIIB (27). Thus, it appears that CBP activates transcription by more than one mechanism.

In this study, we identified CBP as a cofactor for the transcription of class II genes. We found that CIITA and CBP cooperate synergistically in the activation of transcription from the DRA promoter. These two proteins also bound to each other directly, and these interactions were highly specific. Moreover, a dominant negative form of CBP, (DN-CBP) blocked the increased expression of class II genes by CBP. Finally, the inhibition of class II transcription by dexamethasone, which is a glucocorticoid hormone, was reversed by the overexpression of CBP, indicating that it could have resulted from the squelching of CBP by the glucocorticoid receptor (GR). From this and previous studies, it appears that CIITA organizes multiple transcriptional activities, integrating inputs from sequence-specific DNA-bound proteins with various effector pathways to regulate the expression of class II genes.

MATERIALS AND METHODS

Cell culture and transfection. The human kidney fibroblast cell line 293T and African green monkey kidney cell line COS were maintained in Dulbecco modified Eagle medium with 10% fetal calf serum and streptomycin-penicillin. The human B-lymphoblastoid cell line RM3 was maintained in RPMI with 10% fetal calf serum and streptomycin-penicillin. All cells were grown at 37°C with 5% CO_2. Transfection of 293T and COS cells was carried out with 1 or 2 μg of plasmid DNA (including 10 ng of a plasmid expressing hCG as a transfection control) and Lipofectamine (Life Technologies, Gaithersburg, Md.) according to the manufacturer's instructions. RM3 cells were transfected by electroporation (300 V, 975 μF) with 20 μg of plasmid DNA including 1 μg of pEGFP (Clontech, Palo Alto, Calif.).

Plasmid DNA construction. pSVCIITA, pSGCIITA, and pDRASCAT (17) and pCMVCBP (a gift from M. G. Rosenfeld) (24) were previously described. Plasmids expressing glutathione *S*-transferase (GST)–CBP fusion proteins were constructed as follows. The appropriate region of CBP was amplified by PCR

* Corresponding author. Mailing address: Howard Hughes Medical Institute, Departments of Medicine, Immunology, and Microbiology, University of California San Francisco, San Francisco, CA 94143-0703. Phone: (415) 502-1902. Fax: (415) 502-1901. E-mail: matija@itsa.ucsf.edu.

using primers containing *Bam*HI (5′ end) and *Xho*I (3′ end) sites. These amplified DNAs were digested with *Bam*HI and *Xho*I and subcloned into plasmid pGEX4T3 (Promega Inc., Madison, Wis.) cut with the same enzymes. Each CBP insert was sequenced to verify identity. The primers for PCR were as follows: GST-CBP(1-101), 5′-CGCGGATCCATGGCTGAGAACTTGC and 5′-GCAC TCGAGCTAGCCACCCAGGCCCTG; GST-CBP(461-661), 5′-CGCGGATCC GGCACAGGGCAACAG and 5′-GCACCGGAGCTATATCTTGTAGATTT TCTC; GST-CBP(1621-1891), 5′- CGCGGATCCTGTATGCCACCATGG and 5′-GCACTGGAGCTAGCCTCCCATCGCCTGC; and GST-CBP(2058-2163), 5′-CGCGGATCCCCACCCAGGAGGATCTCACC and 5′-GCACTCGAGCT ATGTCTTGCGATTATAG. (Numbers in parentheses denote amino acids positions spanned by the CBP constructs.) Plasmid pCRDN-CBP was constructed by amplifying CBP cDNA sequences from nucleotides 5709 to 7323, adding an ATG start codon and consensus sequence immediately preceding amino acid 1903 of CBP, and subcloning the amplified product directly into plasmid pCR3.1 (InVitrogen, Carlsbad, Calif.). The CBP was sequenced to verify its identity. The primers for PCR were 5′-GCCGCCACCATGCCGCAGCCCCCTGCCCAG and 5′-CTACAAGCCCTCCACAAACTTC.

Reverse transcriptase-mediated PCR (RT-PCR). Total RNA was isolated from transfected 293T cells by the guanidinium acid-phenol chloroform method (12). Reverse transcription was performed with 1 μg of total RNA, 1 μg of oligo (dT) (Boehringer Mannheim, Indianapolis, Ind.), and murine leukemia virus reverse transcriptase (Boehringer Mannheim) according to the manufacturer's instructions. One microliter of the reverse transcriptase reaction was used as the template in a PCR with 1 U of *Taq* polymerase (Boehringer Mannheim) in a 50-μl reaction according to the manufacturer's instructions. The reaction conditions were 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s for 25 cycles. Then 10 μl of each reaction product was run on a 1.5% agarose gel. The primers for CIITA were previously described (10). The primers for human TAP1 were 5′-AGGTAGACGAGGCTGGGAGCC and 5′-CATTCTGGAGCATCTGCA GG.

CAT assay and FACS analysis. The chloramphenicol acetyltransferase (CAT) assay was performed exactly as previously described (18). Forty-eight hours after the transfection, RM3 cells were stained for human class II and class I determinants with monoclonal antibodies raised against HLA-DP (Becton Dickinson, San Jose, Calif.) and HLA-A, -B, and -C (Pharmingen, San Diego, Calif.) determinants, respectively. After washing, cells were incubated with a phycoerythrin-conjugated rat anti-mouse antibody (Zymed, Los Angeles, Calif.). Fluorescence-activated cell sorting (FACS) analyses were performed on a FACScalibur apparatus (Becton Dickinson).

Immunoprecipitation and Western blotting. Forty-eight hours after transfection, COS cells were washed with phosphate-buffered saline and lysed in 1 ml of lysis buffer containing 1% Nonidet P40, 10 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, and protease and phosphatase inhibitors for 20 min at 4°C. Cell lysates were then cleared at 12,000 × *g* for 10 min at 4°C, and supernatants were incubated with 10 μl of anti-CBP antibody (Santa Cruz Biotechnology, Santa Cruz, Calif.) for 1 h at 4°C. Samples were then mixed with 15 μl of protein A-conjugated agarose beads for another hour, and immunoprecipitates were washed four times and resuspended in sodium dodecyl sulfate (SDS) sample buffer. Protein samples were then resolved by SDS-polyacrylamide gel electrophoresis (PAGE) on an 8% gel and transferred to nitrocellulose, using a Bio-Rad (Hercules, Calif.) Western blotting apparatus. One-quarter of the lysate used for the immunoprecipitation was also examine for the presence of CBP and CIITA in transfected cells. Membranes were blocked with phosphate-buffered saline containing 5% nonfat milk overnight and incubated with primary antibodies (anti-hemagglutinin epitope [12CA5] and anti-CBP) for at least 1 h. After three washes with Tris-buffered saline (pH 7.4) containing 5% nonfat milk, membranes were incubated with a secondary antibody conjugated with horseradish peroxidase for 1 h, washed with the same buffer, and analyzed by enhanced chemiluminescence assay (ECL kit; Amersham, Arlington Heights, Ill.).

GST pull-down assays. GST-CBP fusion proteins were produced and purified, and GST pull-down assays between GST-CBP and in vitro-transcribed and -translated CIITA and CIITA deletions were performed exactly as described elsewhere (18).

RESULTS

CBP and CIITA increase expression from the DRA promoter synergistically. Previous studies demonstrated that CBP facilitates the transcriptional activation of a wide variety of genes via its HAT domain (reviewed in reference 22) and its ability to interact with general transcription factors (3, 14, 26, 27, 36). To determine if CBP could also activate class II genes and if it cooperated with CIITA, transient expression assays were performed with the human kidney fibroblast cell line 293T. Plasmid effectors coding for CIITA (pSVCIITA) and CBP (pCMVCBP) were cotransfected with the plasmid target pDRASCAT. CAT enzymatic assays were performed 48 h later.

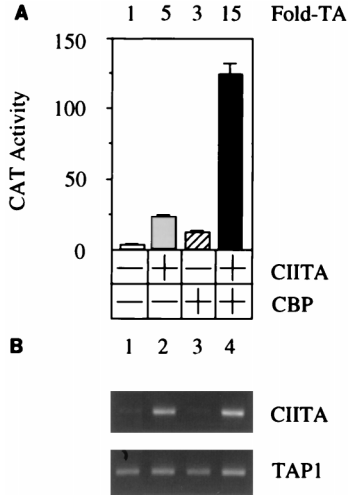

FIG. 1. CIITA and CBP act synergistically to increase expression from the DRA promoter. The plasmid target (pDRASCAT) was coexpressed with CIITA and/or CBP in 293T cells (+ denotes protein expression). Amounts of cotransfected plasmid DNA were held constant (0.25 μg of each plasmid effector and target, balanced to a total of to 1.0 μg with the empty plasmid vector DNA). pDRASCAT was coexpressed with empty plasmid vector (white bar), CIITA (gray bar), CBP (striped bar), or CIITA and CBP (black bar). Fold transactivation (Fold-TA) was calculated from the bar graph and represents values of top bars over the value obtained with pDRASCAT alone. CAT activities represent mean values of three independent experiments performed in triplicate with indicated standard errors of the mean. RT-PCR was performed on total RNA isolated from 293T cells transfected as noted above. Primers for CIITA mRNA (B, top; from nucleotide positions 3309 to 3507) or human TAP1 (bottom; from nucleotide positions 1904 to 2247) were used in the PCR.

CIITA increased the expression from the DRA promoter fivefold over baseline levels (Fig. 1A, column 2). CBP had a smaller effect, transactivating the DRA promoter only threefold (column 3). However, when coexpressed, CIITA and CBP transactivated the DRA promoter 15-fold (column 4). To rule out that CBP increased the transcription of CIITA in 293T cells, total RNA was isolated from the cotransfected 293T cells and subjected to RT-PCR. CIITA mRNA was found to be present in equal amounts when plasmid pSVCIITA was transfected into 293T cells, regardless of the presence of pCMVCBP (Fig. 1B, top, lanes 2 to 4). Control RT-PCR was also performed with primers complementary to the ubiquitously expressed human TAP1 gene (Fig. 1B, bottom). These controls indicated that CBP did not affect expression from the DRA promoter due to increased transcription of CIITA. We conclude that both CBP and CIITA transactivate the DRA promoter and do so in a synergistic manner.

CIITA interacts directly with CBP in cells. Since functional interactions between CIITA and CBP could reflect physical interactions between the two proteins, we performed a modified one-hybrid binding assay in 293T cells. An effector plasmid directing expression of the fusion protein between CIITA and the DNA binding domain of the yeast Gal4 protein (pSGCIITA) was cotransfected with a reporter plasmid containing a single Gal4 binding site (UASg) linked to the CAT reporter gene (pG1bCAT) (28). The hybrid Gal4-CIITA protein increased the expression from pG1bCAT threefold over baseline levels (Fig. 2A, column 2). However, inclusion of pCMVCBP in the cotransfection transactivated pG1bCAT 18-fold, indicating that CBP interacted directly with CIITA (column 3). pCMVCBP without pSGCIITA had no effect on the transcription form pG1bCAT (column 4). Direct interactions between CIITA and CBP could also be demonstrated in COS cells,

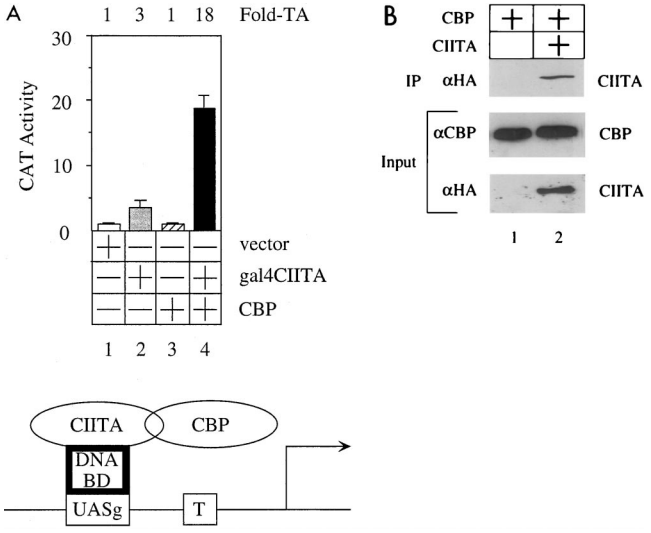

FIG. 2. CBP interacts with CIITA in cells. (A) CBP interacts with CIITA in a modified one-hybrid assay in vivo. The plasmid target (pG1bCAT) was coexpressed with Gal4-CIITA and/or CBP in 293T cells (+ denotes protein expression). Amounts of cotransfected plasmid DNA were held constant (0.25 μg of each plasmid effector and target, balanced to a total of to 1.0 μg with the empty plasmid vector DNA). pG1bCAT was coexpressed with empty plasmid vector (white bar), Gal4-CIITA (grey bar), CBP (striped bar), or Gal4-CIITA and CBP (black bar). Fold transactivation (Fold-TA) was calculated from the bar graph and represents values of top bars over the value obtained with pG1bCAT alone. CAT activities represent mean values of three independent experiments performed in triplicate with indicated standard errors of the mean. BD, binding domain; T, TATA box. (B) CIITA can be immunoprecipitated with CBP in cells. COS cells were transfected with pCMVCBP (1.0 μg) alone (lane 1) or with pCMVCBP (1.0 μg) and pSVCIITA (1.0 μg) (lane 2). The empty plasmid vector was included to maintain the total amount of transfected plasmid at 2.0 μg. One-quarter of the cellular lysate used for the immunoprecipitation (IP) was examined for the presence of expressed proteins (Input). Anti-CBP (αCBP) immunoprecipitates were also probed for the presence of CIITA by Western blotting, αHA, anti-hemagglutinin epitope antibody.

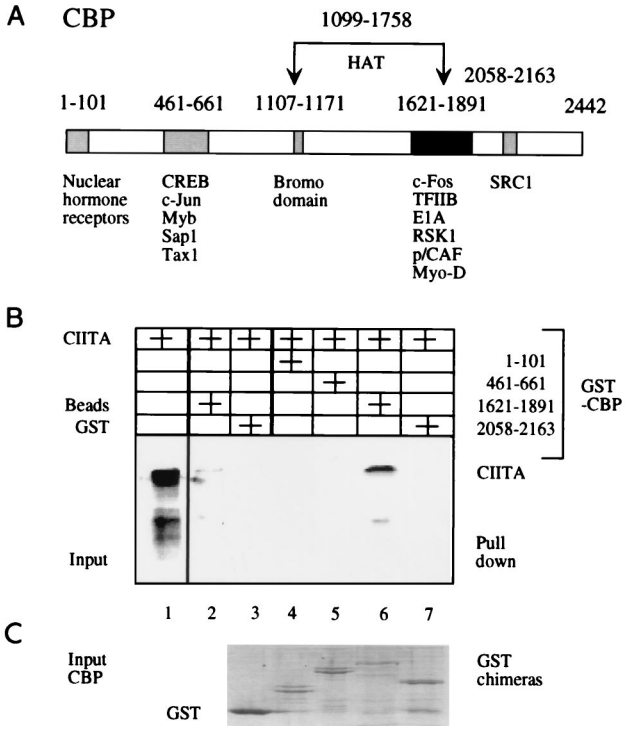

FIG. 3. CIITA binds to CBP via residues from positions 1621 to 1891. (A) Schematic representation of CBP. Domains which interact with other proteins that regulate transcription of various genes are depicted as gray and black rectangles (22). (B) In vitro binding assays were carried out with the transcribed and translated CIITA and GST-CBP fusion proteins containing amino acids from positions 1 to 101 (lane 4), 461 to 661 (lane 5), 1621 to 1891 (lane 6), and 2058 to 2163 (lane 7). Glutathione-Sepharose beads (lane 2) and GST alone (lane 3) did not bind to CIITA. Lane 1 contains 10% of the input CIITA protein. (C) Coomassie blue-stained SDS-polyacrylamide gel demonstrating that equivalent amounts of GST-CBP fusion proteins were included in the binding reactions.

which were cotransfected with pCMVCBP and pSVCIITA (Fig. 2B). Cellular lysates were incubated with an anti-CPB antibody and then examined for the presence of CIITA in these immunoprecipitates by Western blotting. Only when both CBP and CIITA were coexpressed was CIITA detected in our CBP complexes (Fig. 2B, lane 2). We conclude that CBP increases the transcription of class II genes by interacting with CIITA.

CIITA binds to a specific domain of CBP in vitro. Previous studies have produced a long list of transcription factors that interact directly with one of the four domains of CBP (22). These sequences contain amino acids from positions 1 to 101, 461 to 661, 1621 to 1891, and 2058 and 2163 in CBP (Fig. 3A) (22). To determine if CIITA bound specifically to CBP, we created fusion proteins between these four domains of CBP and GST. These chimeras were used in a binding assay with CIITA, which was transcribed and translated with [^{35}S]methionine in vitro. Hybrid GST-CBP proteins, along with any bound CIITA, were resolved by SDS-PAGE and subjected to autoradiography.

One-tenth of the CIITA protein used for the binding reactions is presented in Fig. 3B, lane 1. When CIITA was combined with glutathione-Sepharose beads, alone (Fig. 3B, lane 2) or complexed with GST (lane 3), essentially no CIITA bound to our beads. Similarly, CIITA did not bind to GST-CBP(1-101) (lane 4), GST-CBP(461-661) (lane 5), or GST-CBP(2058-2163) (lane 7). However, CIITA bound efficiently to GST-CBP(1621-1891) (lane 6). As indicated by the staining of the autoradiographed gel with the Coomassie blue dye, amounts of GST and GST-CBP fusion proteins were equivalent in these binding reactions (Fig. 3C). Thus, CIITA binds to specific sequences of CBP which are found in the C-terminal part of the HAT domain. This region was previously demonstrated to bind c-Fos (8), TFIIB (27), E1A (4, 32), RSK1 (35), and MyoD (44) (Fig. 3A).

The N-terminal activation domain of CIITA binds to CBP. Next, we wanted to determine which domain of CIITA was required for these interactions with CBP. The N-terminal 143 amino acids of CIITA have been identified as an independent transcriptional activation domain (Fig. 4A) (43, 55). This region is rich in acidic amino acids and also interacts with $TAF_{II}32$ (18). To map the interaction between CBP and CIITA, two partial CIITA proteins, one spanning the activation domain from positions 1 to 143 [CIITA(1-143)] and the other containing amino acids from positions 144 to 1103 of [CIITA(143-1103)], were transcribed and translated in vitro (Fig. 4A). These two truncated CIITA proteins were allowed to interact with GST-CBP(1621-1891).

As previously demonstrated, the full-length CIITA bound to GST-CBP(1621-1891) (Fig. 4B, lane 1). However, CIITA(143-1103), which lacks the N-terminal activation domain and is unable to transactivate class II genes, did not bind significantly to GST-CBP(1621-1891) (lane 2). In sharp contrast, CIITA(1-143), which contains the transcriptional activation domain of CIITA, bound to GST-CBP(1621-1891) (lane 3). As demon-

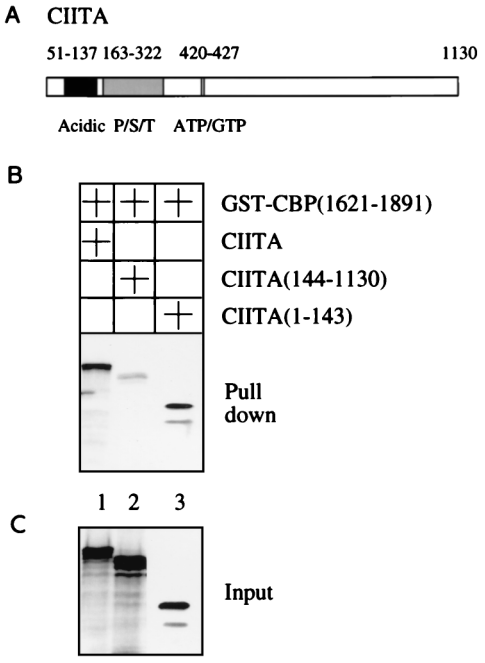

FIG. 4. CBP binds to the N-terminal transcriptional activation domain of CIITA. (A) Schematic representation of CIITA (33). Structural domains: Acidic, region rich in acidic amino acids; P/S/T, region rich in proline, serine, and threonine residues; ATP/GTP, putative purine ribonucleotide binding site. (B and C) In vitro binding assays were carried out with the transcribed and translated full-length CIITA (lane 1), CIITA(144-1103) (lane 2), and CIITA(1-143) (lane 3). (B) GST-CBP(1621-1891) pull-down assay; (C) input CIITA and truncated CIITA proteins used in the binding reaction.

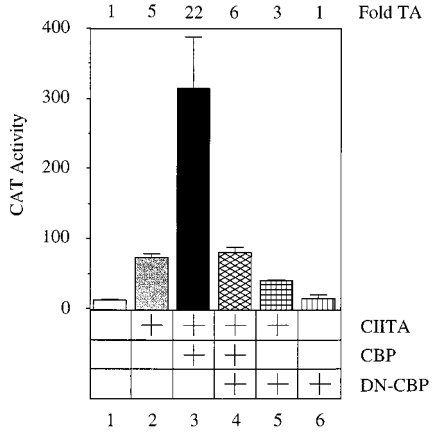

FIG. 5. DN-CBP inhibits the transcriptional synergy between CIITA and CBP. pDRASCAT was coexpressed with CIITA individually and in combination with CBP in 293T cells. In addition, DN-CBP, which contains amino acids from positions 1903 to 2441 in CBP, was coexpressed in these cells. Amounts of cotransfected plasmids were kept constant (0.5 μg of pDRASCAT and pCRDN-DBP; 0.25 μg of pCMVCBP and pSVCIITA; empty plasmid vector was included to maintain the total amount of transfected plasmid at 2.0 μg). pDRASCAT was coexpressed with empty plasmid vector (white bar), CIITA (gray bar), CIITA and CBP (black bar), CIITA, CBP, and DN-CIITA (cross-hatched bar), CIITA and DN-CBP (small-squares bar), or DN-CBP (striped bar). Fold transactivation (TA) was calculated from the bar graph and represents values of top bars over the value obtained with pDRASCAT alone. CAT activities represent mean values of three different experiments performed in triplicate with indicated standard errors of the mean.

strated in Fig. 4C, equal amounts of these CIITA proteins were included in our binding reactions. We conclude that CBP interacts with CIITA via its N-terminal transcriptional activation domain.

DN-CBP inhibits the transcriptional synergy between CIITA and CBP. To characterize further transcriptional synergy between CIITA and CBP, we created a dominant negative form of CBP that should inhibit the function of the wild-type protein. Two dominant negative forms of p300, a factor that is highly related to CBP, have been identified (6). We transposed the amino acid coordinates of one of these dominant negative p300 proteins onto the sequence of CBP to create DN-CBP, containing amino acids from positions 1903 to 2441 in CBP.

A plasmid effector was constructed to express DN-CBP under the control of the cytomegalovirus promoter (pCRDN-CBP). pSVCIITA and pCMVCBP were cotransfected with pDRASCAT into 293T cells, resulting in 22-fold-increased CAT enzymatic activity over baseline levels (Fig. 5, column 3). Including pCRDN-CBP in a similar cotransfection transactivated pDRASCAT only sixfold (column 4), indicating that DN-CBP inhibited the function of its wild-type counterpart. DN-CBP also had a smaller but reproducible effect on the ability of CIITA to activate the DRA promoter in the absence of exogenous CBP. In this instance, the cotransfection of pSVCIITA and pCRDN-CBP transactivated pDRASCAT only threefold (column 5). Cotransfecting pCRDN-CBP with pDRASCAT has no effect on the already low baseline levels of expression form the DRA promoter (column 6). Thus, not only does CBP promote the transcription of class II genes, but DN-CBP can block this effect.

DN-CBP reduces the expression of class II determinants on the surface of B-lymphoblastoid cells. To determine if CBP played a similar role in the transcription of class II genes in B cells, where these genes and CIITA are expressed at high levels, we coexpressed DN-CBP and CIITA in human B-lymphoblastoid RM3 cells (33). RM3 cells are mutant Raji cells, where CIITA is no longer functional. However, the introduction of CIITA into these cells leads to the expression of class II determinants at levels observed in parental Raji cells. RM3 cells were chosen because they synchronized the expression of DN-CBP and CIITA proteins and avoided the issues of preexisting high levels and slow turnover of class II determinants in wild-type B-lymphoblastoid cells. Identical results were also obtained with Raji cells, which expressed DN-CBP constitutively (data not presented). Forty-eight hours after cotransfection, RM3 cells were stained for class II and class I determinants (Fig. 6). As the green fluorescence protein was coexpressed in these cells, only 10% of the brightest cells were analyzed by FACS. The expression of CIITA alone led to very high levels of expression of class II determinants (Fig. 6A, dark gray peak). At a ration of 3 to 1, DN-CBP protein led to a 60% reduction in levels of class II determinants (HLA-DP) on these cells (Fig. 6A, black peak). In sharp contrast, no reduction in levels of class I determinants was observed with DN-CBP in these cells (Fig. 6B, black peak). These data indicate that CBP plays a role in the expression of class II genes in B cells where their transcription is high and constitutive.

Increased expression of CBP can reverse the inhibition of class II transcription by the glucocorticoid hormone. Next, we wanted to determine if CBP had a role in the inhibition of class II transcription by the glucocorticoid hormone. Previous studies demonstrated that dexamethasone (9, 47) and ursodeoxycholic acid (51) can inhibit the transcription of class II genes and that this inhibition requires the translocation of GR from the cytoplasm to the nucleus. Similarly, glucocorticoid hor-

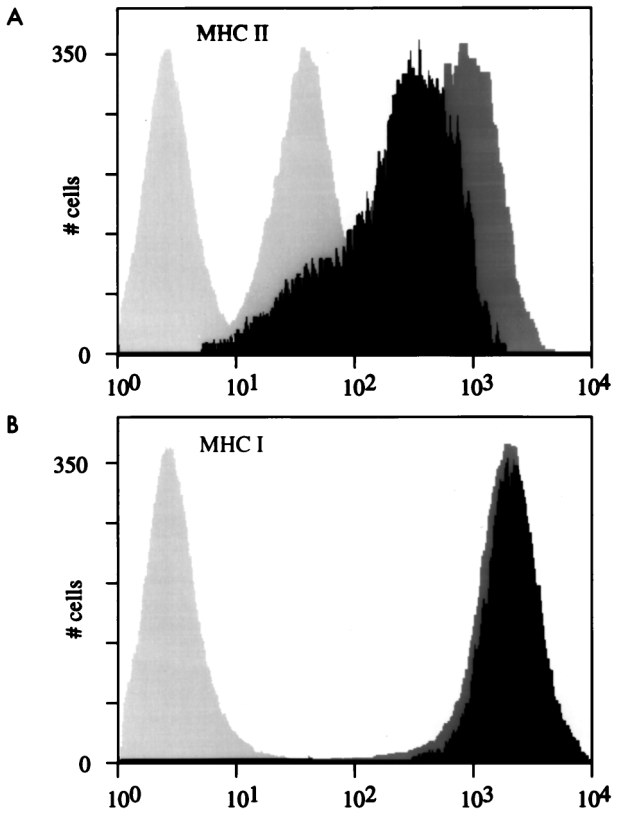

FIG. 6. DN-CBP inhibits the expression of class II determinants on the surface of B-lymphoblastoid RM3 cells. RM3 cells were transfected with the pSVCIITA (5 and 15 μg of empty plasmid vector) (dark gray peaks), with pSVCIITA (5 μg) and pCRDN-CBP (15 μg) (black peaks), or with the empty plasmid vector (20 μg) (light gray peaks). Cells were incubated with the anti-HLA-DP (MHC II) or anti-HLA-A, -B, and -C (MHC I) antibodies and subsequently visualized with a phycoerythrin-conjugated rat anti-mouse antibody. As pEGFP (1 μg) was cotransfected into these cells, only 10% of the brightest cells were analyzed by FACS. In panel A, two light gray peaks on the left represent unstained and anti-HLA-DP-stained RM3 cells that were transfected with the plasmid vector alone; the light gray peak in panel B represents unstained RM3 cells.

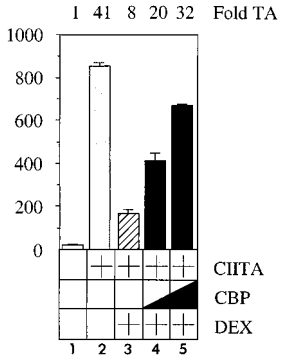

FIG. 7. Increasing amounts of CBP relieve the inhibition of class II transcription by dexamethasone. pDRASCAT was coexpressed with CIITA, and transfected cells were treated with 1 μM dexamethasone (DEX; striped bar) or dimethyl sulfoxide vehicle (gray bar). Increasing amounts of CBP were coexpressed in these cells (0.25 and 0.5 μg of pCMVCBP; black bars). Amounts of cotransfected plasmids were kept constant (0.5 μg of pDRASCAT and 0.75 of pSVCIITA; 0.25 μg and 0.5 μg of pCMVCBP; empty plasmid vector was included to maintain the total amount of transfected plasmid at 2.0 μg). pDRASCAT was coexpressed with empty plasmid vector (white bar), CIITA (gray bar), CIITA and dexamethasone (striped bar), or CIITA and different amounts of CBP (0.25 and 0.5 μg) in the presence of dexamethasone (black bar). Fold transactivation (TA) was calculated from the bar graph and represents values of top bars over the value obtained with pDRASCAT alone. CAT activities represent mean values of three independent experiments performed in triplicate with indicated standard errors of the mean.

mone can inhibit the ability of AP-1 to activate the transcription of promoters containing an AP-1 binding site (23, 31, 46, 54). Kamei et al. (24) demonstrated that one mechanism by which the inhibition of AP-1 might occur is by the squelching of a limited amount of CBP by the ligand-bound GR in the nucleus (5). Since we determined that CBP plays an important role in the expression of class II genes, we wanted to test if a similar mechanism of squelching was responsible for the inhibition of class II transcription by the glucocorticoid hormone.

pSVCIITA was transfected into 293T cells, which transactivated pDRASCAT 41-fold (Fig. 7, column 2). When these cells were subsequently treated with 1 μM dexamethasone, transcriptional activation fell sevenfold to sixfold over baseline levels, even though identical amounts of pSVCIITA and pDRASCAT were used in these transfections (column 3). However, when pCMVCBP was cotransfected with pSVCIITA and pDRASCAT, this inhibition by dexamethasone was reversed in a dose-dependent fashion (columns 4 and 5, respectively). With higher amounts of pCMVCBP, levels of expression increased 32-fold despite the presence of dexamethasone, approaching those observed in the absence of dexamethasone (compare columns 2 and 5). We conclude that this glucocorticoid hormone inhibits the transcription of class II genes by causing the GR to translocate to the nucleus and compete with CIITA for a limited amount of CBP.

DISCUSSION

CIITA is the key regulatory protein for the transcription of class II genes (48–50). In this study, we identified CBP as an additional coactivator. CBP and CIITA not only synergized in the transcription from the DRA promoter but physically interacted with each other. CIITA bound to CBP at residues from positions 1621 to 1891, where CBP also interacts with c-Fos (8), TFIIB (27), E1A (4, 32), RSK1 (35), and MyoD (44) (Fig. 3A). Additionally, the binding of CBP to CIITA occurred via the N-terminal activation domain of CIITA. DN-CBP specifically inhibited the transcriptional synergy between CBP and CIITA and reduced the expression of class II genes on the surface of B-lymphoblastoid cells. Our study also provides a mechanistic insight into the inhibition of class II transcription by the glucocorticoid hormone. Not only did dexamethasone inhibit the transcription of class II genes, but the overexpression of CBP was able to reverse this effect.

It was reported previously that the treatment of cells with dexamethasone inhibited the transcription of class II genes (9, 47). This inhibition required the translocation of GR to the nucleus (47). In vitro protein-DNA binding assays suggested that GR might be able to inhibit the binding of an unidentified complex to the X1 box of the DRB1 promoter (9). However, using an in vivo binding assay (16), we found that dexamethasone treatment had little, if any, effect on the interaction of RFX with the X box (data not presented). Moreover, Kamei et al. demonstrated that the inhibition of AP-1 by dexamethasone was due to the squelching of a limited amount of CBP by GR in the nucleus (24). In these studies, the overexpression of CBP resulted in the reversal of the inhibition by dexamethasone of transcription which depended on a tetradecanoyl phorbol acetate-responsive element. Moreover, the inhibition of NF-κB by STAT1 (40) and the inhibition of AP-1 and NF-κB by the

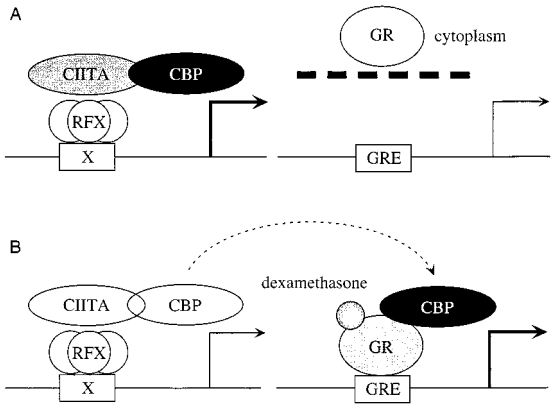

FIG. 8. Diagrammatic representation of how dexamethasone inhibits the transcription of class II genes. (A) In the absence of dexamethasone, CBP binds to CIITA. In turn, CIITA is attracted to class II promoters via RFX, which binds to the X box. (B) The administration of dexamethasone results in the translocation of the GR to the nucleus, where it binds to CBP. In this manner, GR sequesters a limited amount of CBP in the nucleus, leaving little to no free CBP to interact with CIITA on class II promoters. GRE, glucocortoid response element.

androgen receptor are also due to the competition for limiting amounts of CBP (1). Similarly, we determined that the decreased transcription of class II genes following the administration of dexamethasone could be reversed by the overexpression of CBP. We conclude that the primary mechanism for the inhibition of class II transcription by the glucocorticoid hormone is the squelching of a limited amount of CBP, not the blocking of proteins that interact with the X box. This mechanism is presented diagrammatically in Fig. 8.

Direct interactions between CIITA and CBP were mapped to the N-terminal transcriptional activation domain of CIITA. This domain is rich in acidic amino acids. Mutageneses of this region identified two putative α helices which are required not only for transcriptional activation but also for the binding of CIITA to $TAF_{II}32$ (18). The interaction of CBP with CREB occurs via the so-called KIX domain, in which serine 133 of CREB is phosphorylated (13, 27, 39). In addition, CREB has a glutamine-rich activation domain which also interacts with human $TAF_{II}130$ (37). It appears that CIITA may also function in a similar manner, not only contacting a component of TFIID but also recruiting CBP and its associate HAT activity. Although experiments have not ruled out the formation of a tripartite complex composed of $TAF_{II}32$, CBP and CIITA, it seems unlikely that this would occur due to steric considerations. Rather, CIITA probably interacts with these two complexes at different times in the transcription cycle. CIITA could initiate transcription via its interactions with TFIID and subsequently promote chromatin remodeling by recruiting CBP.

CBP is the second coactivator which has been identified as a binding partner for CIITA, which is itself a transcriptional coactivator. The B-cell-specific coactivator Bob-1 binds to CIITA and synergizes with it in the transcription of class II genes (17). Thus, the transcription of class II genes depends not only on the assembly of a complex protein-DNA structure but also on the formation of a multipartite coactivator network. CIITA can therefore be viewed as a transcriptional integrator, coordinating the communication between sequence-specific DNA binding proteins with activities that promote transcriptional initiation and promoter clearance.

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