The Histone Acetyltransferase Domains of CREB-binding Protein (CBP) and p300/CBP-associated Factor Are Not Necessary for Cooperativity with the Class II Transactivator*

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The class II transactivator (CIITA) is a transcriptional co-activator regulating the constitutive and interferon-γ-inducible expression of class II major histocompatibility complex (MHC) and related genes. Promoter remodeling occurs following CIITA induction, suggesting the involvement of chromatin remodeling factors. Transcription of numerous genes requires the histone acetyltransferase (HAT) activities of CREB-binding protein (CBP), p300, and/or p300/CBP-associated factor (pCAF). These co-activators cooperate with CIITA and are hypothesized to promote class II major histocompatibility complex transcription through their HAT activity. To directly test this, we used HAT-defective CBP and pCAF. We demonstrate that cooperation between CIITA and CBP is independent of CBP HAT activity. Further, although pCAF enhances CIITA-mediated transcription, pCAF HAT domain dependence appears contingent upon the concentration of available CIITA. When HAT-defective CBP and pCAF are both present, cooperativity with CIITA is maintained. Consistent with a recent report, we show that nuclear localization of CIITA is enhanced by lysine 144, an *in vitro* target of pCAF-mediated HAT. Yet we find that neither mutation of lysine 144 nor deletion of residues 132–209 affects transcriptional cooperation with CBP or pCAF. Thus, acetylation of this residue may not be the primary mechanism for pCAF/CBP cooperation with CIITA. In conclusion, the HAT activities of the co-activators are not necessary for cooperation with CIITA.

Expression of class II major histocompatibility complex (MHC)[1] molecules is a critical feature of the normal immune response playing a central role in inflammatory, T cell-mediated, and humoral responses through presentation of exogenous, processed antigens to CD4+ T cells. Constitutive class II MHC expression is tissue-specific and restricted to B cells, monocytes/macrophages, and dendritic cells (reviewed in Refs.

[1] The abbreviations used are: MHC, major histocompatibility complex; CIITA, class II transactivator; NLS, nuclear localization sequence; LRR, leucine-rich repeat; HAT, histone acetyltransferase; CBP, CREB-binding protein; pCAF, p300/CBP-associated factor.

1 and 2), although inducible class II MHC is seen on most class II negative tissues/cells in response to interferon-γ. Both constitutive and inducible class II MHC expression is globally regulated at the transcriptional level by the transcriptional co-activator class II transactivator (CIITA) (reviewed in Ref. 3). All known class II MHC-related genes containing the classical W, X, and Y promoter motifs are regulated by CIITA (4–8).

The molecular mechanism by which CIITA activates and regulates the transcription of class II MHC genes has been an area of intense interest. CIITA is regulated by up to four promoters that allow for the observed patterns of constitutive and inducible expression (9, 10). CIITA is expressed in both the cytoplasm and the nucleus (11). Nuclear import of CIITA depends upon the presence of a defined nuclear localization sequence (NLS) (11), requires GTP-binding by CIITA (12), and is contingent upon leucine-rich repeats (LRRs) in the carboxyl-terminal portion of CIITA (3, 13).[2] Recent studies have shown that CIITA self-associates through its LRR, sequences within its GTP-binding region, and the amino terminus (15–17), although how this impacts nuclear translocation and transcriptional activation by CIITA is not well understood. Once inside the nucleus, CIITA interacts with both the required class II MHC transcription factors (RFX, CREB, and NF-Y) and basal transcription components (TFIIB, TBP, and TAFs) (18–23). *In vivo* footprinting studies of the human leukocyte antigen DR α gene (HLA-DRA) and invariant chain promoters in non-B cells revealed that these promoters are usually "closed" in the absence of CIITA (24, 25) with little if any detectable binding by X and Y box binding factors. The observation that promoters can be "opened" by CIITA is indicative of chromatin remodeling that could be mediated directly by CIITA or through recruitment of chromatin remodeling co-activators that may rely upon histone acetyltransferase (HAT) activity. Some examples include CREB-binding protein (CBP), p300, and p300/CBP-associated factor (pCAF). Interestingly, recent work shows that histones H3 and H4 are acetylated at the HLA-DRA promoter in the presence of CIITA (26).

CBP interacts and synergizes with CIITA in the activation of class II MHC transcription in transient transfection experiments through an interaction with the amino terminus of CIITA (21, 27). This interaction has recently been mapped and shown to occur between residues 68 and 103 of CIITA (28). When overexpressed, CIITA sequesters CBP (thus down-regulating other CBP-dependent genes) (28), and a dominant negative form of CBP can inhibit class II MHC expression (21). CIITA also interacts and cooperates with p300 (29) and pCAF (30). Although these observations suggest that CBP and other

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co-activators are important for class II MHC transcription, another critical issue is whether the HAT domains of CBP/pCAF are required for the observed effects. Likewise, it is not clear whether acetylation of CIITA affects its transactivator function. The answers to these issues would greatly affect our view of how CIITA transactivates its target promoters.

In this report, we demonstrate that the HAT activity of CBP is not required for the synergistic cooperation between CIITA and CBP. Further we demonstrate that the CBP-associated factor pCAF, which also possesses a HAT domain, similarly cooperates with CIITA and can also function in a HAT-independent fashion. Complementing these findings, we also show that a lysine residue that can be acetylated is not required for cooperativity with pCAF. These findings have important implications regarding the mode of action of CIITA.

MATERIALS AND METHODS

Tissue Culture Cells and Conditions—The African green monkey kidney cell line COS-7 was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and streptomycin-penicillin. All cells were grown at 37 °C with 5% CO_2.

Plasmids—The plasmid expression vectors encoding CIITA, Δ132–209, CBP, pCAF, and the DRA300Luc reporter have been described previously (5, 11, 31, 32). pCAFΔHATA (Δ579–608) and pCAFΔHATB (Δ609–624) lack HAT activity (32) and fail to enhance p300/EBNA2-mediated LMP1 transcription (33). CBPHAT(−) was produced by introducing two amino acid substitutions at residues 1690 and 1691 of wild-type CBP by Quick Change™ (Stratagene) mutagenesis. This mutation has been previously shown to completely abolish histone acetyltransferase activity, resulting in a CBP molecule that fails to activate a CRE-containing promoter (34), and demonstrates diminished transcription activation in cooperation with the Epstein-Barr virus Z protein (35).

Transfection and Promoter Assays—Cells (2×10^4 to 2×10^5) were plated in 6-well tissue culture plates 18–24 h prior to transfection of plasmid DNAs using FuGene6 (Roche Molecular Biochemicals) transfection reagent per the manufacturers instructions. Following transfection (24–48 h), the cells were lysed in $1\times$ reporter lysis buffer (Promega), and luciferase assays were performed as described previously (36).

Immunofluorescence Microscopy—Immunofluorescent staining of transiently transfected COS-7 cells was performed as described previously (16). Briefly, 8×10^4 cells were grown overnight and transfected with 1.5 µg of DNA using the FuGene6 transfection reagent (Roche Molecular Biochemicals). Following fixation with 40% acetone in phosphate-buffered saline, the cells were blocked with 1% bovine serum albumin in phosphate-buffered saline and stained with anti-FLAG M5 (Sigma) and goat anti-mouse IgG-fluorescein isothiocyanate. Photomicrographs were acquired using Scion Series 7 video capture hardware and an Olympus BX40 fluorescence microscope.

RESULTS

Cooperation between CIITA and CBP and/or pCAF Does Not Require CBP or pCAF Histone Acetyltransferase Activity—Transcriptional cooperation of the co-activators CBP and pCAF with CIITA has been previously reported (21, 27, 30), implicating the importance of HAT activity in transactivation by CIITA. A variety of transfection methods using differing ratios of co-activator to CIITA have been reported (21, 27). To reduce the risk of missing the effects of cooperation by overexpressing CIITA, we transfected various quantities of CIITA with a fixed quantity of the DRA reporter (Fig. 1A). In COS-7 cells, activation of DRA transcription was maximal with 100 ng of transfected CIITA DNA. Significant transcriptional activation occurs with 20 ng as well. Transfecting 1 µg of CIITA DNA gives a level of activation similar to 20 ng, suggesting some form of inhibition at this high concentration. The relationship observed between the amount of DNA transfected and the degree of activation is consistent for a variety of cell lines (data not shown). A previous analysis of the dose response of class II MHC transcription to CIITA demonstrated a linear relationship in various tissues (37). This disagreement is likely due to
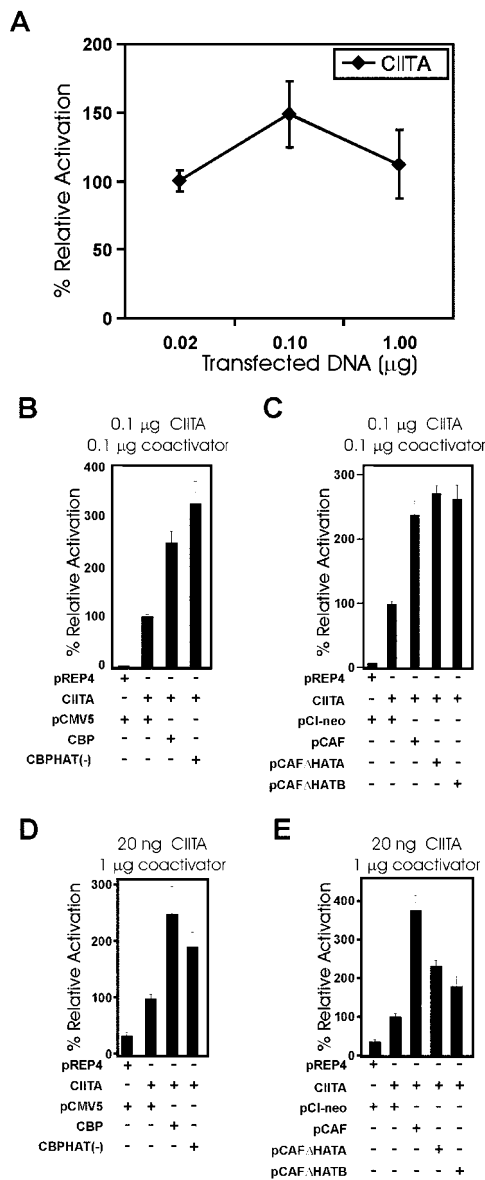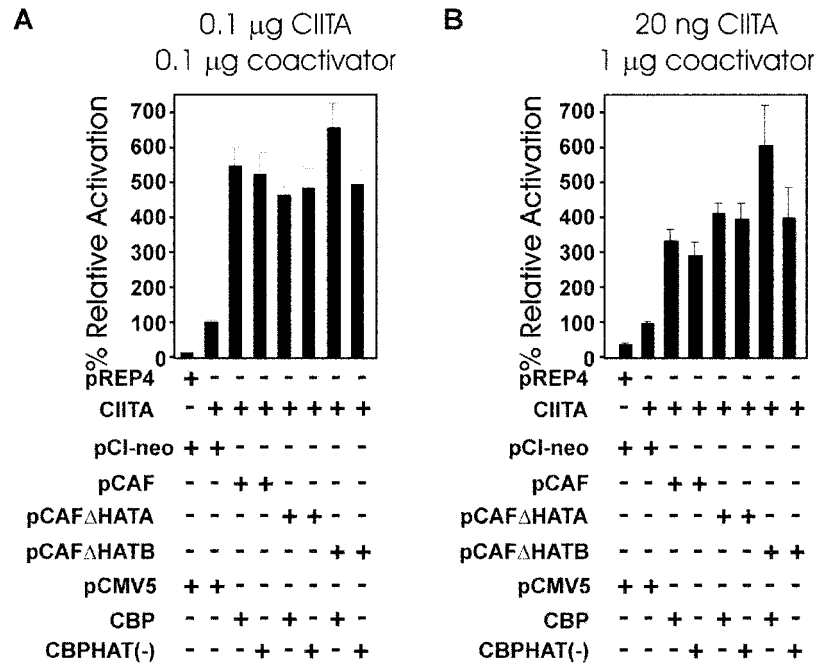

FIG. 1. **Activation of the DRA promoter by CIITA does not require the HAT domain of either CBP or pCAF.** *A*, dose response of the DRA300Luc reporter with transfected CIITA. COS-7 cells were co-transfected with the indicated amounts of CIITA DNA and 1 µg of DRA reporter. Sufficient empty vector (pcDNA3) was added to bring the total quantity of transfected DNA to 2 µg. Luciferase activity was normalized to the 20-ng CIITA transfectant (100%), which displayed 10–15-fold activation compared with vector alone. *B* and *C*, effect of equivalent concentrations of co-activators on transcriptional activation mediated by 0.1 µg of CIITA. COS-7 cells were co-transfected with the indicated amounts of CIITA or vector alone and CBP or a CBP HAT-defective mutant (*B*) and pCAF or pCAF HAT-deficient mutants (*C*). *D* and *E*, effect of overexpressed CBP (*D*) or pCAF (*E*) and the respective HAT-defective mutants on activation by 20 ng of CIITA. COS-7 cells were co-transfected with equal amounts of the indicated constructs. The values are shown as the mean percentages of relative luciferase activity ± S.E. for three experiments, each of which was repeated in triplicate. pREP4, pCMV5, and pCI-neo are empty vector controls for CIITA, CBP, and pCAF respectively.

the high transfection efficiency (60–90%) using the FuGene reagent in this report as compared with the other transfection method. For the purposes of this study only the 20- and 100-ng quantities of CIITA are used.

To investigate the importance of CBP HAT activity in transcriptional activation of class II MHC genes by CIITA, we tested the ability of a HAT-defective form of CBP to cooperate with CIITA in transient transfection experiments. This same

A 0.1 µg CIITA 0.1 µg coactivator

B 20 ng CIITA 1 µg coactivator

FIG. 2. **Co-expression of CIITA with both CBP and pCAF reveals no requirement for CBP or pCAF HAT domains.** *A*, effect of CBP and pCAF co-expression on activation of DRA reporter by 0.1 µg of CIITA. COS-7 cells were co-transfected with 0.1 µg each of vector alone or CIITA and the indicated co-activator constructs along with the DRA reporter. *B*, effect of CBP and pCAF co-expression on activation of DRA reporter by 20 ng of CIITA. COS-7 cells were co-transfected with CIITA or vector alone, 1 µg of the indicated co-activator constructs and 500 ng of DRA reporter. The values shown represent the mean data from three experiments. See Fig. 1 legend for additional details.

A (% Relative Activation)

pREP4	+	-	-	-	-	-	-	-
CIITA	-	+	+	+	+	+	+	+
pCI-neo	+	+	-	-	-	-	-	-
pCAF	-	-	+	+	-	-	-	-
pCAFΔHATA	-	-	-	-	+	+	-	-
pCAFΔHATB	-	-	-	-	-	-	+	+
pCMV5	+	+	-	-	-	-	-	-
CBP	-	-	+	-	+	-	+	-
CBPHAT(-)	-	-	-	+	-	+	-	+

B (% Relative Activation)

pREP4	+	-	-	-	-	-	-	-
CIITA	-	+	+	+	+	+	+	+
pCI-neo	+	+	-	-	-	-	-	-
pCAF	-	-	+	+	-	-	-	-
pCAFΔHATA	-	-	-	-	+	+	-	-
pCAFΔHATB	-	-	-	-	-	-	+	+
pCMV5	+	+	-	-	-	-	-	-
CBP	-	-	+	-	+	-	+	-
CBPHAT(-)	-	-	-	+	-	+	-	+

construct has been previously tested as being HAT-deficient in other systems (34, 35). The HAT-defective form of CBP and two HAT-defective forms of pCAF (32) enhanced CIITA activity as well as wild-type CBP and pCAF, respectively (Fig. 1, *B* and *C*). Co-transfection of 0.1 µg of CIITA with equivalent concentrations of pCAF or CBP constructs reveals HAT-independent cooperation of CIITA with either CBP or pCAF (Fig. 1, *B* and *C*). When CBP is overexpressed (50-fold relative to CIITA; Fig. 1*D*), only a small difference is observed between cooperation with wild-type and HAT-defective CBP. However, under similar conditions, wild-type pCAF enhances the activity of CIITA, whereas the two pCAF HAT-defective mutants (pCAFΔHATA and pCAFΔHATB) display a reduced, but still detectable, capacity to enhance CIITA-mediated transcription (Fig. 1*E*). Taken together these data suggest that cooperation of CIITA with CBP is mostly HAT-independent. Cooperation of pCAF with CIITA is partially HAT-dependent when reporter activation by CIITA is suboptimal (20 ng of transfected CIITA plasmid) (Fig. 1*E*). When CIITA activity is optimal (0.1 µg), cooperation occurs but is HAT-independent. No cooperation is observed when an excess of CIITA plasmid (1 µg) is used (data not shown).

CBP often recruits pCAF, and it is very likely that CBP and pCAF act together (38, 39). Thus, we further examined the role of the HAT domains of CBP and pCAF using equivalent amounts (0.1 µg) of the DNAs in co-transfection experiments. Under these conditions, CBP and pCAF together gave a greater than 5-fold enhancement of HLA-DRA activation compared with CIITA alone (Fig. 2*A*). No significant change in enhancement is observed when the HAT-defective forms of CBP, pCAF, or both are used. Similar results are obtained using 50-fold overexpression of the co-activators relative to CIITA (Fig. 2*B*). This cooperation between CIITA/CBP/pCAF, maintained by HAT-defective co-activators, strongly suggests that the HAT activities of CBP and pCAF are not required for transcriptional cooperativity with CIITA.

Cooperation between CIITA and CBP or pCAF Is Independent of Residues 132–209 of CIITA—Our previous analysis has shown that residues 132–209 of CIITA are not required for activation of DR transcription (5). However, it has been recently shown that mutation of lysines 141 and 144 or lysines 156 and 159 to arginine within this region has an effect on

nuclear localization, consistent with the existence of a putative bipartite NLS acetylated by both CBP and pCAF (30). Fig. 3*A* shows the sequence of CIITA from 132 to 209, the positions of the bipartite NLS, and lysines in this region. The CIITA deletion mutant Δ132–209 activates DRA transcription and is comparable with wild-type CIITA using 1.0 µg of DNA (Fig. 3*B*). This is consistent with our previous report using this mutant at a high concentration (5). At 0.1 and 0.02 µg, the transactivation ability of Δ132–209 is substantially lower than wild type. Reduced transactivation by Δ132–209 is consistent with a defect in nuclear localization (Fig. 3*C*) and thus the presence of the bipartite NLS. This paradoxical defect of nuclear localization and successful activation of DR transcription at a higher concentration of CIITA has been recently observed using LRR mutants of CIITA.[2] These LRR mutations reduce the import rate of CIITA without diminishing its ability to activate transcription once nuclear CIITA levels are adequate, which is achieved with a higher concentration of CIITA. Contrary to the earlier observation that deleting residues 132–209 of CIITA does not abrogate transactivator function, a recent paper suggests that acetylation of CIITA at residues 141 and 144 could be instrumental to CBP/pCAF-enhanced transcription by CIITA (30). More specifically, nuclear localization was shown to be dependent upon lysines 141 and 144 and was positively affected by the HAT activity of pCAF. However, the contribution of HAT activity to transcriptional activation and the role of each individual lysine residue was not investigated. If CBP and/or pCAF acetylation of CIITA at lysines 141 and/or 144 reflects the mechanism of cooperativity, CBP and pCAF should fail to cooperate with CIITAΔ132–209. To examine this possibility, we transfected cells with different forms of CIITA and either CBP, CBPHAT(−), pCAF, or pCAFΔHATA/B under conditions where the HAT domain of pCAF is necessary for full cooperativity. In these experiments the level of transactivation by Δ132–209 is reduced to 30% of wild type. However, cooperation with CIITA, as measured by the capacity of CBP or pCAF to enhance transactivation, is preserved (Fig. 3, *D* and *E*). Furthermore, this cooperation mirrors that seen with wild-type CIITA with respect to a partial dependence on the pCAF HAT domain (Fig. 3*D*) and independence from CBP HAT activity (Fig. 3*E*). Also, like wild type, when equivalent amounts (0.1 µg) of CIITA and pCAF (or CBP) are used, the dependence on
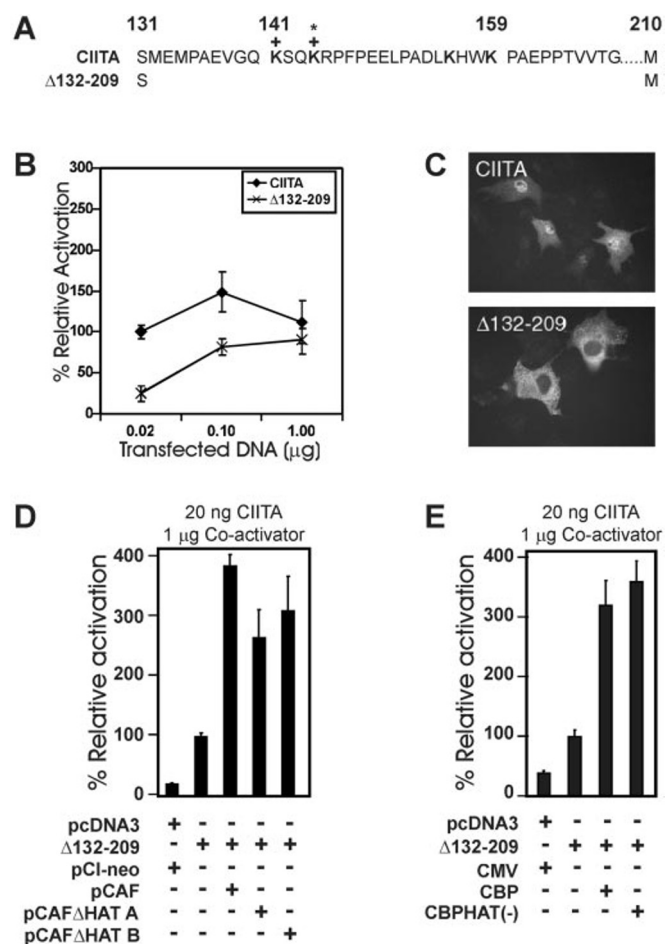
CBP/pCAF HAT Domain-independent Transactivation by CIITA

A

131   141   *   159   210

                 +   +

CIITA SMEMPAEVGQ **K**SQ**K**RPFPEELPADL**K**HW**K** PAEPPTVVTG.....M

Δ132-209 S   M

FIG. 3. **Residues 132–209 of CIITA that contain a bipartite NLS are not necessary for cooperation with CBP or pCAF.** *A*, protein sequence of wild-type human CIITA between residues 131 and 210. The depicted region is missing from the CIITA deletion construct Δ132–209. Lysines comprising the bipartite NLS are shown in *bold type*. Also indicated are the lysine residues that are acetylated by pCAF (*) and likely acetylated by CBP (+) (see text) (30). *B*, dose response of the DRA300Luc reporter with transfected CIITA or Δ132–209. COS-7 cells were co-transfected with the indicated amounts of CIITA or Δ132–209 DNA and 1 μg of DRA reporter. Sufficient empty vector (pcDNA3) was added to bring the total quantity of transfected DNA to 2 μg. Luciferase activity was normalized and expressed as in Fig. 1*A*. *C*, nuclear localization of CIITA and Δ132–209. *D* and *E*, effect of co-activators and the respective HAT-defective mutants on activation by Δ132–209. COS-7 cells were co-transfected with 20 ng of Δ132–209 or vector alone and 1 μg of pCAF (*D*) or CBP (*E*).

pCAF HAT activity disappears (data not shown). These observations demonstrate that deleting residues 132–209 did not affect cooperation with CBP or pCAF. The slight dependence on the pCAF HAT domain is still present despite the absence of the lysine(s) in the bipartite NLS, suggesting that this modest enhancement does not require acetylation in this region of CIITA. The following experiment using site-specific mutagenesis was performed to address this issue further.

Acetylation at CIITA Residue 144 Is Not Necessary for Transcriptional Activation or Cooperation with CBP and pCAF—A previous study demonstrated that lysine 144 of CIITA is acetylated by pCAF and suggested that either lysines 144 and/or 141 are acetylated by CBP (30). The same report also shows that mutation of both of these residues to arginine had a negative effect on pCAF-enhanced CIITA nuclear localization. However, the transactivation function of K144R and K141R individually has not been tested prior to this report. To this end, single point mutagenesis was performed. Single point mutation of lysine 141 to arginine (K141R) has no effect on activity (Fig. 4*B*) or

localization (Fig. 4*C*, *middle panel*). K144R displays no defect in transactivation assays, even when as little as 20 ng of DNA is transfected (Fig. 4*B*). Remarkably, K144R also displayed nearly complete cytoplasmic localization (Fig. 4*B*), consistent with the prior report. This demonstrates that the localization effects previously tested result from mutation of Lys[144], not Lys[141] or the combination of both mutations, and suggests that on a per molecule basis, K144R is likely more active than wild-type CIITA, because K144R exhibits defective nuclear accumulation (Fig. 4*C*). This represents a rare gain-of-function mutation of CIITA.

To assess the effect of the K144R mutation on cooperation with CBP and pCAF, several experiments were performed using this mutant. If acetylation of K144 is responsible for cooperation between pCAF (or CBP) and CIITA, a failure of cooperation would be expected. Using both the 50-fold overexpression and "equivalent" expression systems, pCAF cooperated with K144R (Fig. 5). The ability of CBP and CBPHAT(−) to cooperate with K144R was essentially identical using the 50-fold overexpression conditions (data not shown). These data demonstrate that acetylation of CIITA at lysine 144 by pCAF is not necessary for cooperation with pCAF. The same is true of CBP (data not shown).

DISCUSSION

The ability of CIITA to activate class II MHC transcription can be enhanced by the presence of the co-activators CBP (21, 27), pCAF (30), or p300 (29). Similar observations have been made for CBP using class I MHC reporters consistent with the role of CIITA in class I MHC transcription (8, 40). Acetylation of lysine 144 of CIITA by pCAF and lysines 141 and/or 144 by CBP, which comprise a bipartite nuclear localization sequence, has been demonstrated recently to play a role in enhancing the nuclear import of CIITA (30). These previous studies implicate the importance of HAT activities in promoting the transcription of class II MHC genes. Here we directly test an aspect of this hypothesis. This report explores the implied requirement for HAT activity of CBP and pCAF for enhancing CIITA transactivation of class II MHC genes and finds that the HAT domains of CBP and pCAF are frequently not required. Further, a lysine residue within 132–209 that is acetylated by the pCAF HAT is dispensable for cooperativity with the co-activators. This particular residue is important for nuclear accumulation, as described previously (30).

This report confirms previous findings that CBP and pCAF cooperate with CIITA; however, we find that this cooperation is completely independent of HAT activity contributed by CBP. Further, this cooperation can also be independent of pCAF HAT activity depending on the conditions. Although some dependence for pCAF HAT activity was observed when pCAF was transfected in excess of CIITA, this was not observed when equivalent amounts of either wild-type CBP or HAT-defective CBP and CIITA were co-expressed. HAT independence is also observed when HAT-defective pCAF and CBP are used in combination. HAT-independent cooperation between co-activators and other transcription factors has been well documented (32, 41–43) and is thought to occur through either recruitment of additional co-factors or directly through the non-HAT domains of the co-activators themselves. We consistently observe a lack of HAT dependence for CBP cooperation with CIITA at various concentrations of CIITA, using different amounts of transfected DNA and using different expression vectors (Fig. 1 and data not shown). As the concentration of CIITA increases, we observe a decreased enhancement with CBP (data not shown). We interpret this to indicate a decreasing reliance upon CBP, and perhaps co-activators in general, as the amount of available CIITA increases. These results are consistent with the hypoth-

FIG. 4. **Mutation of pCAF/CBP-acetylated lysine 144 reveals a gain of CIITA function with reduced nuclear import.** *A*, protein sequence of CIITA between residues 131 and 210. Lysines 141 and 144, which are targeted by the HAT domains of pCAF and/or CBP (see text and Fig. 3*A*), were mutated to arginines. *B*, dose response of the DRA300Luc reporter with transfected CIITA or the single-point mutants K141R and K144R. COS-7 cells were co-transfected with the indicated amounts of CIITA, K141R, or K144R and 1 μg of DRA reporter, as in Fig. 1*A*. *C*, nuclear localization of CIITA, K141R, and K144R.
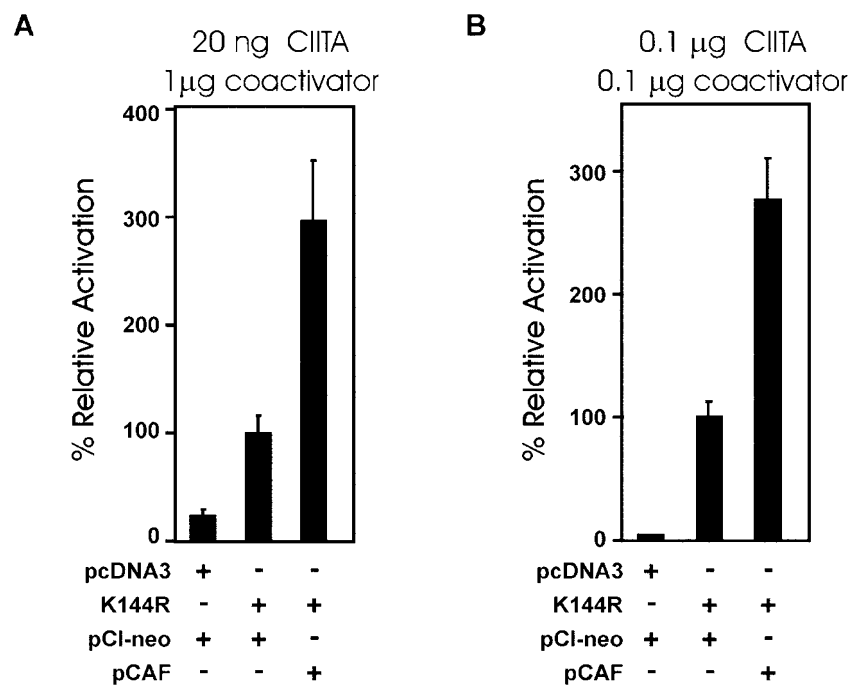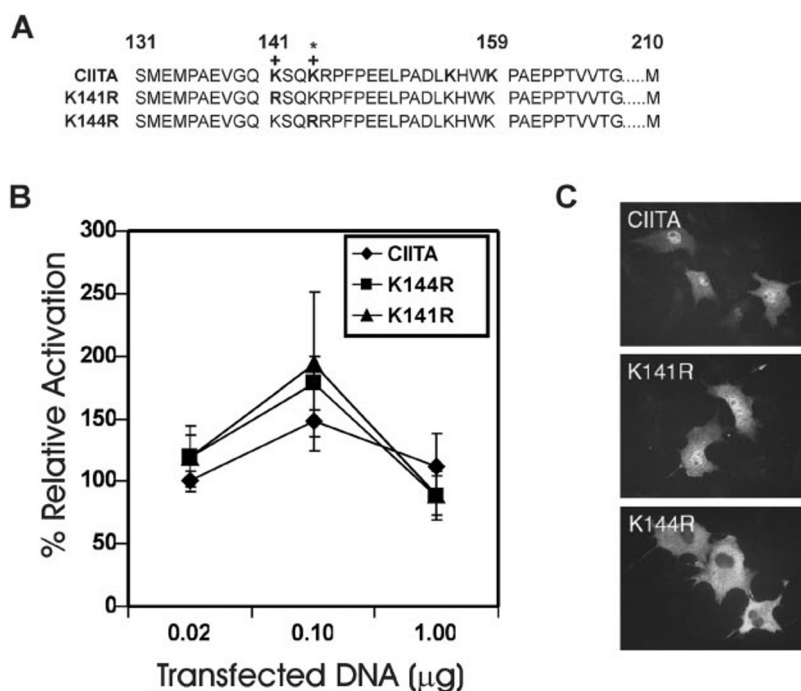

FIG. 5. **Cooperation of CIITA with CBP and pCAF does not require lysine residue 144.** *A*, effect of pCAF or a pCAF HAT-defective mutant on activation by 20 ng of the K144R mutant. COS-7 cells were co-transfected with 20 ng of vector alone or K144R and 1 μg of the indicated co-activator constructs along with 0.5 μg of DRA reporter. *B*, effect of pCAF or a pCAF HAT-defective mutant on activation by 0.1 μg of K144R. COS-7 cells were co-transfected with 0.1 μg of each of the indicated constructs along with 0.5 μg of DRA reporter.

esis that at some defined concentration of CIITA, CBP is less important for activation of class II transcription.

Cooperative activation of class II MHC by CIITA and pCAF or CBP does not require sequences between 132 and 209 of CIITA, despite the presence of pCAF/CBP acetylation sites in this region. In a similar fashion, point mutation of the lysines identified as acetylation targets fail to impair the activity of CIITA or its ability to cooperate with co-activators. Together, these observations suggest that although CIITA interacts and cooperates with CBP and pCAF, the HAT activities of these factors, even in combination (Fig. 2), are not an absolute requirement for cooperative transactivation. In addition, the putative acetylation sites for these HATs in CIITA are also not required for cooperation with pCAF.

These data are consistent with several interpretations: 1) An essential HAT activity is supplied by another co-activator (*e.g.*

p300, GCN5, SRC-1, or $TAF_{II}250$). The interaction of CIITA with p300 has been reported (29), and indirect association with $TAF_{II}250$ has been suggested (23). In light of this report, the role of the HAT activities of these factors in class II MHC transcription should be investigated. 2) CIITA may possess its own HAT activity. This possibility is supported by a recent report that CIITA has acetyltransferase activity (44). 3) Other features of the co-activators, independent of the HATs are important. 4) HAT recruitment is important for acetylation of other factors involved in class II MHC transcription (*e.g.* NF-Y; Ref. 45), and this requirement is sensitive to available CIITA. (5) Lastly, the assay systems currently employed to address the role of HATs in class II MHC transcription may be inadequate.

The characterization of K144R is intriguing because although its nuclear import is clearly impaired, its transactivation function is not (Fig. 4*C*). The nuclear localization of Δ132–

209 is also reduced, although this mutant has impaired function using smaller amounts of transfected DNA (Fig. 3). We have recently observed that decreases in the rate of CIITA import exhibited by the LRR mutants correlate with an activation profile similar to that seen for Δ132–209 over a range of 0.1–1.0 μg, in that the transactivation function is comparable with the wild-type CIITA at a high concentration but is reduced at a lower concentration.[2] A likely explanation is that for Δ132–209 and the LRR mutants, although functional, fewer of these molecules accumulate in the nucleus. K144R exhibits a different pattern: defective nuclear localization, yet transactivation comparable with wild type. These observations suggest that K144R is potentially more active on a per molecule basis than wild type and may represent the first described gain-of-function CIITA mutant. Combined with a previous report indicating that lysine 144 is an acetylation site, we speculate that this acetylation may have the unexpected result of reducing transactivation function on a per molecule basis. Clearly, more detailed analysis is necessary to explore this intriguing possibility.

In summary, our observations regarding co-activator function and CIITA are consistent with reports demonstrating HAT-independent cooperation in other systems (32, 41) and changes in co-activator cooperativity with altered levels of transcription factor expression (14). Interestingly, transcriptional activation by CIITA shows a requirement for the HAT domain of pCAF only when CIITA is less abundant. It seems clear that acetylation by pCAF promotes nuclear import of CIITA (30), and this requires Lys[144]. Once inside the nucleus, CIITA causes specific gene activation assisted by CBP and pCAF. The function of CBP is HAT-independent, whereas the role of pCAF can be HAT-dependent. As CIITA availability increases, even the HAT activity of pCAF is no longer required. Co-activator involvement in the CIITA/class II MHC system is thus complex and underscores the need for a more complete understanding of the role of HATs in CIITA-mediated transcriptional control.

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