Molecular Cell, Vol. 7, 105–115, January, 2001, Copyright ©2001 by Cell Press

Transcriptional Coactivator, CIITA, Is an Acetyltransferase that Bypasses a Promoter Requirement for $TAF_{II}250$

Aparna Raval,* T. Kevin Howcroft,* Jocelyn D. Weissman,* Susan Kirshner,* Xin-Sheng Zhu,† Kazunari Yokoyama,‡ Jenny Ting,† and Dinah S. Singer*§
*Experimental Immunology Branch
National Cancer Institute
Building 10, Room 4B-36
National Institutes of Health
Bethesda, Maryland 20892
†Lineberger Cancer Center
University of North Carolina
Chapel Hill, North Carolina 27599
‡Riken Life Sciences
Tsukuba
Japan

Summary

The CIITA coactivator is essential for transcriptional activation of MHC class II genes and mediates enhanced MHC class I transcription. We now report that CIITA contains an intrinsic acetyltransferase (AT) activity that maps to a region within the N-terminal segment of CIITA, between amino acids 94 and 132. The AT activity is regulated by the C-terminal GTP-binding domain and is stimulated by GTP. CIITA-mediated transactivation depends on the AT activity. Further, we report that, although constitutive MHC class I transcription depends on $TAF_{II}250$, CIITA activates the promoter in the absence of functional $TAF_{II}250$.

Introduction

Transcription of RNA polymerase II-dependent promoters is mediated by sequence-specific transcription factors that bind to upstream regulatory elements and recruit general transcription factors (GTFs) to the downstream core promoter (Sauer et al., 1995; Orphanides et al., 1996; Wang et al., 1997; Carey, 1998; Li et al., 1999). In many cases, this interaction is facilitated by non-DNA binding coactivators (Merika et al., 1998; Hampsey and Reinberg, 1999; Xu et al., 1999). Coactivators are generally large proteins with multiple binding sites for both DNA binding transcriptional activators and GTFs (Barlev et al., 1995; Cho et al., 1998). Their function is, in part, to link upstream activators and repressors with downstream basal transcription machinery, integrating regulatory signals (Chen et al., 1994; Harrod et al., 2000). Coactivators often possess one or more activities, which contribute to transcriptional activation (Krumm et al., 1998). One common feature of many coactivators is the presence of acetyltransferase (AT) activity (Davie, 1998). Two functions have been ascribed to the AT activity of coactivators based upon their target substrates. One set of AT substrates is nucleosomal histones, whose acetylation results in relaxation of chromatin structure, allowing easier access to the promoter by the transcriptional machinery (Ogryzko et al., 1996; Yang et al., 1996; Mizzen et al., 1996). The other set of substrates consists of various transcription factors, such as p53, HMG-1, and GATA-1 (Gu and Roeder, 1997; Boyes et al., 1998), whose acetylation can alter their DNA binding affinity, and HNF-4, which has enhanced nuclear localization upon acetylation (Soutoglou et al., 2000).

Many of the best-characterized coactivators studied are constitutively expressed in most cell types. In contrast, the coactivator CIITA is tissue-specific and inducible (Fontes et al., 1996). CIITA is constitutively expressed in B lymphocytes and other professional antigen presenting cells, but can be induced in other cell types in response to γ-IFN (Chin et al., 1994; Steimle et al., 1994). CIITA forms part of the transcription complex that is necessary for de novo expression of MHC class II genes (Riley et al., 1995; Zhou and Glimcher, 1995) and to enhance constitutive expression of MHC class I genes (Gobin et al., 1997; Martin et al., 1997). The absence of CIITA leads to complete loss of MHC class II and markedly reduced MHC class I expression. CIITA restores expression of both sets of molecules (Chang et al., 1994; Martin et al., 1997). Because the products of both MHC class I and class II genes are involved in the initiation and propagation of immune responses, defects in CIITA expression result in profound immunodeficiencies (Steimle et al., 1993; Chang et al., 1996).

CIITA is a protein of 135 kDa, consisting of 1220 amino acids. Molecular characterization of CIITA has identified a series of regulatory domains: an α-helical activation domain, and a proline/serine/threonine (PST) activation domain in the first 300 N-terminal amino acids (Chin et al., 1997). A GTP-binding domain, located between amino acids 421 and 561, regulates nuclear localization of CIITA (Harton et al., 1999). Typical of coactivators, CIITA does not bind DNA directly (Steimle et al., 1993). Rather, it interacts with DNA binding transcription factors such as RFX (Scholl et al., 1997), basal transcription factors such as $hTAF_{II}32$, $hTAF_{II}70$, TFIIB, (Fontes et al., 1997; Mahanta et al., 1997; Scholl et al., 1997), and the coactivator CBP (Kretsovali et al., 1998; Fontes et al., 1999).

Transcription initiation requires the assembly of a complex consisting of general transcription factors and RNA polymerase II (Roeder, 1996; Verrijzer and Tjian, 1996). It is generally thought that the initiating event is the binding of the general transcription factor TFIID to the core promoter (Klages and Strubin, 1995; Komarnitsky et al., 1998). TFIID is a multi-protein structure consisting of the TATA binding protein (TBP) and a series of TBP-associated factors (TAFs), including $TAF_{II}250$ (Hisatake et al., 1993; Weinzierl et al., 1993). Although early studies suggested that a fully assembled TFIID was necessary for proper transcription initiation (Chen et al., 1994), recent evidence suggests that the TAF requirements vary among different promoters (Moqtaderi et al.,

§To whom correspondence should be addressed (email: dinah.singer@nih.gov).

1996; Walker et al., 1996; Metzger et al., 1999; Kuras et al., 2000; Li et al., 2000). Indeed, in previous studies, we have demonstrated that transcription of MHC class I genes requires $TAF_{II}250$ (Weissman et al., 1998). Further, regulation of class I expression was correlated with $TAF_{II}250$ AT activity. Thus, temperature sensitive ablation of $TAF_{II}250$ AT activity prevented class I transcription (Weissman et al., 1998). However, class I expression was restored when a strong viral enhancer was inserted upstream of the class I promoter (Weissman et al., 2000). This led to the suggestion that upstream activators could modulate the dependence on TAFs of a given promoter.

We now report that the coactivator CIITA contains acetyltransferase activity, which maps to its N terminus and which is modulated by the C-terminal GTP-binding domain and enhanced by GTP. The AT domain is necessary for activation of both class I and class II transcription. Furthermore, and of particular interest, although constitutive MHC class I transcription requires $TAF_{II}250$, CIITA-mediated activation does not; the AT domain of CIITA is necessary for this activity. We propose a model for dynamic modulation of core promoter requirements mediated by CIITA.

Results

An Acetyltransferase Activity Is Associated with CIITA

The coactivator properties of CIITA led us to speculate that CIITA might function as an acetyltransferase, analogous to other coactivators. CIITA immunoprecipitated from transfected HeLa cells was assayed for AT activity using histones H3/H4 as substrates for acetylation. As shown in Figure 1, immunoprecipitates from CIITA transfected cells, but not from control cells, acetylated histones. No autoacetylation of CIITA was detected. Western blot analysis identified a protein with a molecular weight corresponding to that of CIITA (135 KD), demonstrating the presence of CIITA (Figure 1A). These data demonstrate that an AT activity is associated with CIITA. Other known AT-containing proteins, such as $TAF_{II}250$, p300 and PCAF, did not coimmunoprecipitate with CIITA, as detected by Western blot analysis (data not shown). Furthermore, recombinant CIITA (rCIITA) purified from Sf9 cells displayed AT activity (Figure 1), whereas control extract did not. Taken together, these data indicate that the AT activity is intrinsic to the CIITA protein.

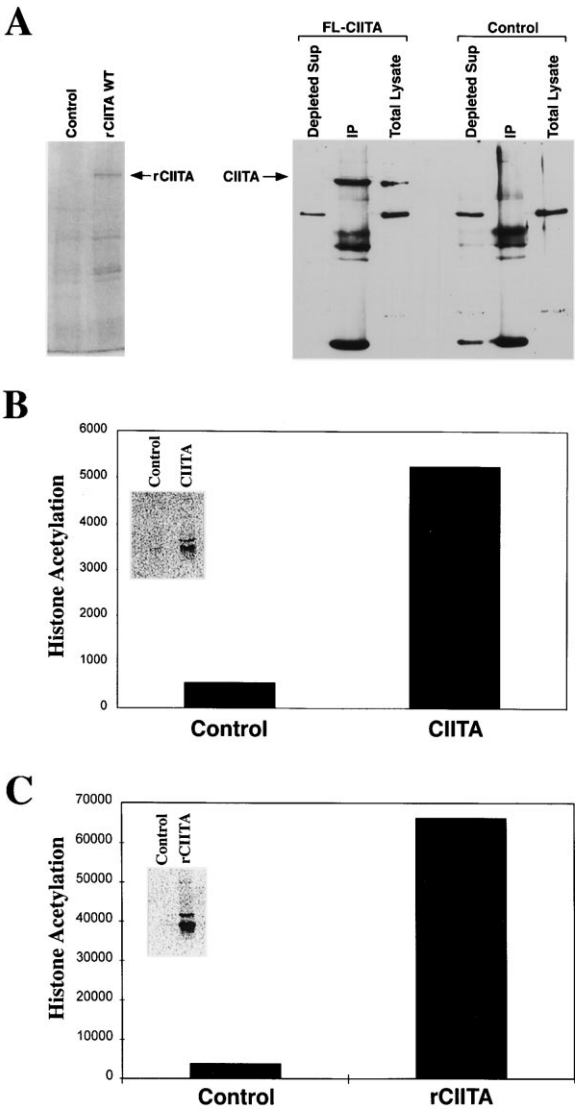

Figure 1. AT Activity Coimmunoprecipitates with CIITA

(A) Western blot of CIITA immunoprecipitated from transfected HeLa cells (right), or rCIITA purified from Sf9 cells (left). Immunoblot of total lysate, anti-Flag immunoprecipitate (IP), and the depleted supernatant of CIITA or control vector transfected cells.

(B and C) CIITA immunoprecipitate contains AT activity. Anti-Flag-CIITA immunoprecipitates from CIITA, or control transfected HeLa cells (B), or purified recombinant CIITA (50 ng) (C) were assayed for AT activity, which was quantitated by phosphorimager and corrected for protein. The insets show phosphorimager profile of acetylated histones.

Mapping of AT Domain in CIITA

To map the AT domain, we generated a C-terminal truncation mutant of CIITA, which terminated at 802 aa (CIITAΔC, Figure 2A). This mutant was expressed in transfected HeLa cells at levels comparable to wild-type CIITA (data not shown). Importantly, it retained full AT activity (Figure 2B), demonstrating that the AT domain resides within the N terminus of CIITA. Previous analyses of CIITA activation domains have identified a proline, serine, threonine rich region (PST domain) between amino acids 132 and 301; removal of the entire PST domain (CIITAΔPST) eliminated CIITA transactivation, but deletion of either the proline domain 132–209 (CIITAΔP) or the serine/threonine domain 209–301 (CIITAΔST) did not (Chin et al., 1997). All three of these deletion mutants retained AT activity (Figure 2 and data not shown); thus, the region 132–301 does not encompass the entire AT domain. However, the deletion spanning the P domain, (CIITAΔP) reduced the AT activity (Figure 2A). Immunoblots confirmed that the deletion mutants are expressed at equivalent levels and are of the expected molecular weight (data not shown). Taken together, these data indicate that the AT domain resides N terminal to the proline domain, but may partially overlap it.

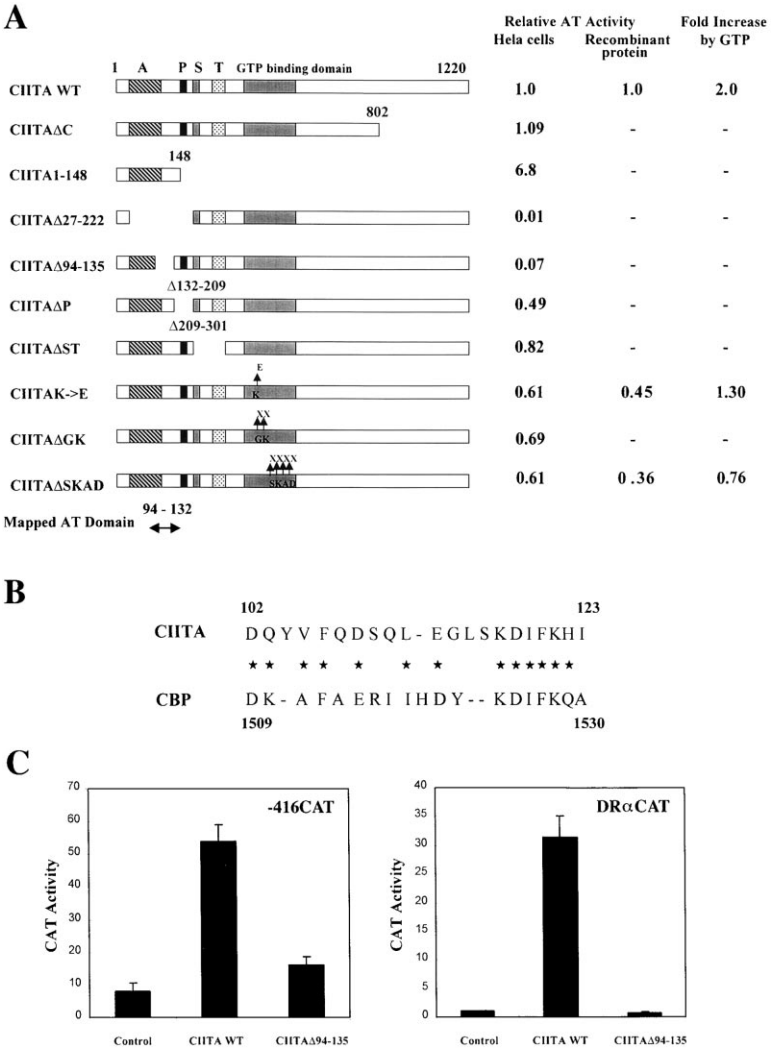

Figure 2. Mapping of the AT Domain of CIITA

(A) Flag-tagged CIITA constructs, as schematized, were immunoprecipitated from HeLa cells or Sf9 cells and assayed for AT activity; AT activity of CIITA mutants relative to CIITA WT is shown. The effect of 1.25 mM GTP on relative activity of rCIITA is shown in the third column.

(B) Sequence alignment of CIITA AT domain with CBP HAT domain. Star indicates same or related amino acid residues.

(C) CIITAΔ94−135 does not activate MHC class I or class II promoters. HeLa cells were cotransfected with 2 μg of CIITA WT, mutant CIITA or control vector, and 5 μg of either −416CAT class I reporter construct (left) or DRα CAT class II reporter construct (right). Data are normalized to transfection control pSV2LUC.

To further map the location of the AT domain, we examined the AT activities of additional mutants. The first mutant contained an internal deletion of the region between 27 to 222 aa (CIITAΔ27–222) (Figure 2A). This mutant was expressed at levels similar to wild-type CIITA and readily immunoprecipitated (data not shown). However, no AT activity was associated with CIITAΔ27–222 (Figure 2). Since the CIITAΔP mutant (132–209) retains AT activity, these results taken together map the AT domain between amino acids 27 and 132. A mutant with a small deletion, extending between amino acids 94 and 135, also had no AT activity, further refining the essential AT domain to a segment between 94–132 amino acids (Figure 2).

To directly demonstrate the presence of AT activity within the N terminus of CIITA, we examined a C-terminal truncation construct encoding amino acids 1 to 148 (CIITA 1–148). CIITA 1–148 displayed significantly higher AT activity than wild-type CIITA (Figure 2), suggesting that there are certain regions in the full-length CIITA that negatively regulate the AT activity. These data confirm the presence of an AT domain within the first 148 amino acids, and, taken together with the deletion mapping, localizes it to the segment 94–132 aa.

CIITA AT Domain Is Homologous to the CBP AT Domain

A homology search of the CIITA AT domain revealed a striking similarity to the known AT domain of CBP (Figure 2B). Between residues 116 and 120 of CIITA there is complete identity with the CBP AT domain. Further short stretches of homology with CBP are observed between aa 102 and 123. No homologies with other known coactivators with AT activity, i.e., $TAF_{II}250$, P/CAF, GCN5 and p300, were found. The AT domain of CIITA does not correspond to any previously defined structural or functional domain. In particular, it is distinct from the three predicted α helices identified by Peterlin (Fontes et al., 1997). From this analysis, we propose that the segment 102–123 of CIITA is contained within the AT domain.

CIITA AT Activity Is Modulated by Its GTP-Binding Domain

The above studies demonstrated that the N-terminal region of CIITA is necessary for AT activity, but did not exclude the participation of other domains in regulating the AT activity. One region that potentially could affect CIITA AT activity is the tri-partite GTP-binding domain

between amino acids 421 and 561 that has been shown to play a role both in transactivation and nuclear localization (Chin et al., 1997; Harton et al., 1999). Therefore, we next asked whether CIITA mutants with crippled GTP-binding domains retain AT activity. Three CIITA mutant proteins, with substitutions in either the phosphate (K→E, ΔKG) or guanine-binding domains (ΔSKAD) were immunoprecipitated from transfected HeLa cells and tested for AT activity (Figure 2). All three retained AT activity. Thus, AT activity is not completely dependent on the GTP-binding domain. However, each of the mutants displayed reduced AT activity relative to wild-type CIITA; recombinant K→E and ΔSKAD mutant proteins purified from Sf9 cells showed a similar reduction in activity (Figure 2). Furthermore, addition of GTP to the AT assay reproducibly and significantly stimulated AT activity of wild-type CIITA (2.1 ± 0.05 fold), whereas it had no effect on the ΔSKAD mutant. These findings indicate that the C-terminal GTP domain modulates the N-terminal AT activity of CIITA.

CIITA AT Domain Is Required for Coactivation

Since CIITA is a coactivator of MHC class I and II gene transcription, the functional requirement for its AT domain was assessed. A CIITA expression plasmid was cotransfected into HeLa cells with a class I promoter construct consisting of 416 bp of upstream promoter sequences, ligated to the CAT reporter. CIITA activation was determined by comparison with a control transfection using an empty expression vector. As previously described, class I transcription was increased ~3 fold in the presence of wild-type CIITA (Figure 2C). In contrast, the CIITAΔ94–135 construct, which has no AT activity but retains the PST activation domain, was unable to transactivate the class I promoter (Figure 2C, left-hand panel). However, the CIITAΔP construct, which retains AT activity, was capable of fully transactivating the class I promoter (data not shown and Figure 5). Thus, a CIITA segment necessary for transactivation maps to the region 94–132 aa, coincident with the AT domain. Deletion of CIITA AT domain also abrogated MHC class II DRα promoter activity (Figure 2C, right-hand panel). These experiments indicate that the CIITA-associated AT activity is necessary for transactivation of both the class I and class II promoters.

CIITA Activity Does Not Require Participation of CBP

CIITA is known to bind to CBP; this interaction results in synergistic activation of MHC class II genes (Kretsovali et al., 1998; Fontes et al., 1999). However, CBP alone does not contribute to constitutive class I promoter activity (Kirshner, Weissman and Singer, unpublished; Fontes et al., 1999). Therefore, we next examined whether CBP participates in CIITA-mediated activation of class I promoter activity. The effect of CIITA on class I promoter activity was assessed in cells deficient in CBP. A CBP-deficient cell line was generated by stably transforming mouse L cells with a ribozyme vector that inactivates CBP transcripts (Kawasaki et al., 1996; Kawasaki et al., 1998). As shown in Figure 3A (inset), levels of CBP in these cell lines were 20% or less than in L cells stably transformed with a mutated CBP ribozyme construct. Nevertheless, the level of AT activity of CIITA isolated from CBP-deficient cells following transfection was indistinguishable from the control (Figure 3A), demonstrating that the AT activity of CIITA is not dependent on CBP. Importantly, cotransfection of CIITA and the class I promoter into the CBP-deficient cells resulted in activation of the promoter (Figure 3B). Taken together, the above experiments demonstrate that CBP is not necessary for CIITA-mediated activation of the class I promoter or for its AT activity.

CIITA Activates MHC Class I Transcription through a Series of Upstream Regulatory Elements

MHC class I transcription is regulated by a series of regulatory elements that extend 1 kB upstream of transcription initiation (Maguire et al., 1992). To determine which elements are targeted by CIITA, activation of a series of promoter deletion mutants by CIITA was examined. As shown in Figure 4A, CIITA stimulated the activity of the longest (−1100 bp) promoter construct by ~15-fold. Successive 5′ truncation of upstream sequences resulted in reduced, but significant levels of CIITA-mediated activation. 2- to 3-fold activation was still observed in a promoter construct containing only 135 bp of flanking sequences. However, further truncation to 68 bp generated a construct that retained substantial constitutive promoter activity but completely failed to be transactivated by CIITA. These data demonstrate that an element located between −135 bp and −68 bp is necessary for CIITA transactivation, and that a series of upstream elements influences the extent of activation by CIITA.

We next examined the target element(s) for CIITA in the −68 to −135 segment of the class I promoter. The interval between −135 bp and −68 bp contains a CRE, which has been shown to be the target of cAMP-mediated repression of class I transcription (Saji et al., 1997; Kirshner et al., 2000). Therefore, we determined the response to CIITA of class I promoter constructs consisting of 209 bp of upstream sequences that had been deleted of either the CRE element (−100 to −107) or an upstream 30 bp enhancer element (−127 to −168) (Figure 4B). Whereas the wild-type −209 bp-construct was activated ~5-fold by CIITA, CIITA had no effect on the basal activity of the −209 construct deleted of the CRE (Figure 4B, −209ΔCRE). Similarly, a promoter construct in which the CRE was mutated, rather than deleted, was also refractory to CIITA transactivation (Figure 4B, −209NPCRE). In contrast, deletion of the 30-mer element reduced, but did not eliminate CIITA-mediated activation (Figure 4B, −209Δ30). Since this latter deletion also eliminates the interferon response element (IRE), CIITA activation of class I does not require the IRE. In support of these conclusions, the isolated CRE element but not the IRE element, was able to confer CIITA responsiveness on a heterologous viral promoter which is not normally activated by CIITA (Figure 4C). Thus, the class I CRE is necessary for CIITA-mediated activation.

CIITA Activation of the Class I Promoter Does Not Depend upon a Specific Core Promoter Element

The MHC class I core promoter spans 50 bp and contains a variant TATAA box (TCTAA), an Inr and an essen-

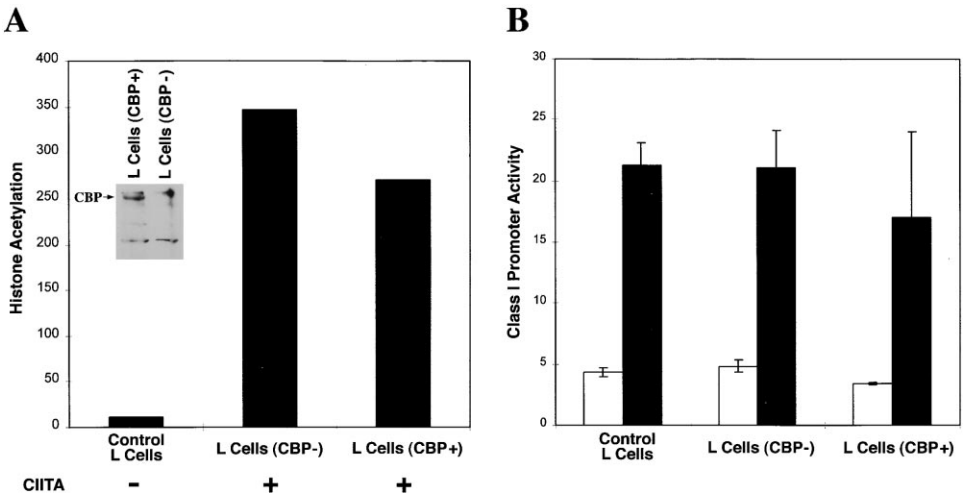

Figure 3. CBP Is Not Required for CIITA-Mediated Activation of the Class I Promoter

(A) L cells (CBP−) and (CBP+), stably transformed with CBP ribozyme or mutant CBP ribozyme respectively, and control L cells were transfected with 10 μg of Flag-CIITA. Flag-CIITA was captured from lysates on anti-Flag M2 agarose beads and assayed for AT activity. Histone acetylation was corrected for input protein concentration. The inset shows anti-CBP Western blot of stably transformed L cell lysate. (B) L cells (CBP−) and (CBP+), stably transformed with CBP ribozyme or mutant CBP ribozyme, respectively, and control L cells were cotransfected with 5 μg of −416 CAT and 2 μg of either CIITA (closed bar) or control vector pcDNA3 (open bar). CAT activity was assayed after 48 hr. Data are normalized to transfection control, pSV2LUC.

tial S box element located between the TCTAA and Inr sequences (Howcroft et al., 1995). The question of whether CIITA requires a specific core promoter element has not been addressed previously. Therefore, we examined the ability of CIITA to activate class I reporter constructs containing mutations within the core promoter (Figure 4D). We have shown previously that mutations of either the TCTAA element (416TM1) or the Inr (416M3) reduced, but did not eliminate, constitutive promoter activity in the absence of CIITA (Howcroft et al., 1995). Indeed, a promoter construct (416M5) in which both promoter elements have been mutated was constitutively active (Howcroft et al., 1995). All three promoter mutants were activated by CIITA; the extent of activation was similar to wild-type promoter (Figure 4D). However, CIITA did not activate a mutant in which the entire core promoter region was deleted (416ΔKH). These data demonstrate that CIITA activation requires a promoter, but does not depend upon a specific promoter element.

Surprisingly, CIITA was able to activate transcription from a promoter mutant that displays no constitutive activity. Mutation of the 17 bp S box located between the TCTAA and Inr elements (416M6) results in a promoter inactive in constitutive expression (Table 1). However, in the presence of CIITA, 416M6 was active. In contrast, the promoter-less control, pSV0, displayed neither constitutive activity nor response to CIITA. Thus, the promoter element requirements for CIITA-mediated transcription are distinct from those of constitutive transcription.

CIITA Bypasses the Promoter Requirement for $TAF_{II}250$

The above data demonstrate that CIITA has AT activity and that the AT domain is necessary for transcriptional activation. We have shown previously that constitutive class I transcription requires the AT activity of the TFIID component, $TAF_{II}250$ (Weissman et al., 1998). Since CIITA interacts with the TFIID component $TAF_{II}32$ (Fontes et al., 1997) and appears to target the transcription initiation complex (Mahanta et al., 1997), we considered the possibility that CIITA activation could bypass the $TAF_{II}250$ requirement of constitutive class I transcription.

This possibility was tested in the hamster cell line tsBN462, which contains a temperature-sensitive mutation in $TAF_{II}250$ (Hayashida et al., 1994). In tsBN462 cells at the permissive temperature (32°C), $TAF_{II}250$ is functional and the class I promoter is active; at the restrictive temperature (39°C), $TAF_{II}250$ loses AT activity and is inactive (Dunphy et al., 2000); basal transcription of the class I promoter is impaired (Weissman et al., 1998). Cotransfection of CIITA with the class I promoter construct, −416CAT, into tsBN462 cells at the permissive temperature resulted in activation of the promoter (Table 2A). Surprisingly, when the transfected cells were shifted to the restrictive temperature (39°C), CIITA restored class I promoter activity (Table 2A). Thus, CIITA could bypass the $TAF_{II}250$ requirement. The MHC class II reporter construct DRαCAT was also activated by CIITA at both restrictive (32°C) and permissive (39°C) temperatures in tsBN462 cells (Table 2B), further demonstrating that CIITA-mediated activation is independent of $TAF_{II}250$.

As shown above, a CRE element in the class I promoter was critical for CIITA activation. We next examined whether the requirements for CIITA-mediated activation are similar in the presence and absence of $TAF_{II}250$. The −416CAT and −135CAT constructs, which contain the CRE, were both activated by CIITA at both the permissive and restrictive temperatures. However, −68CAT, which does not contain the CRE, no

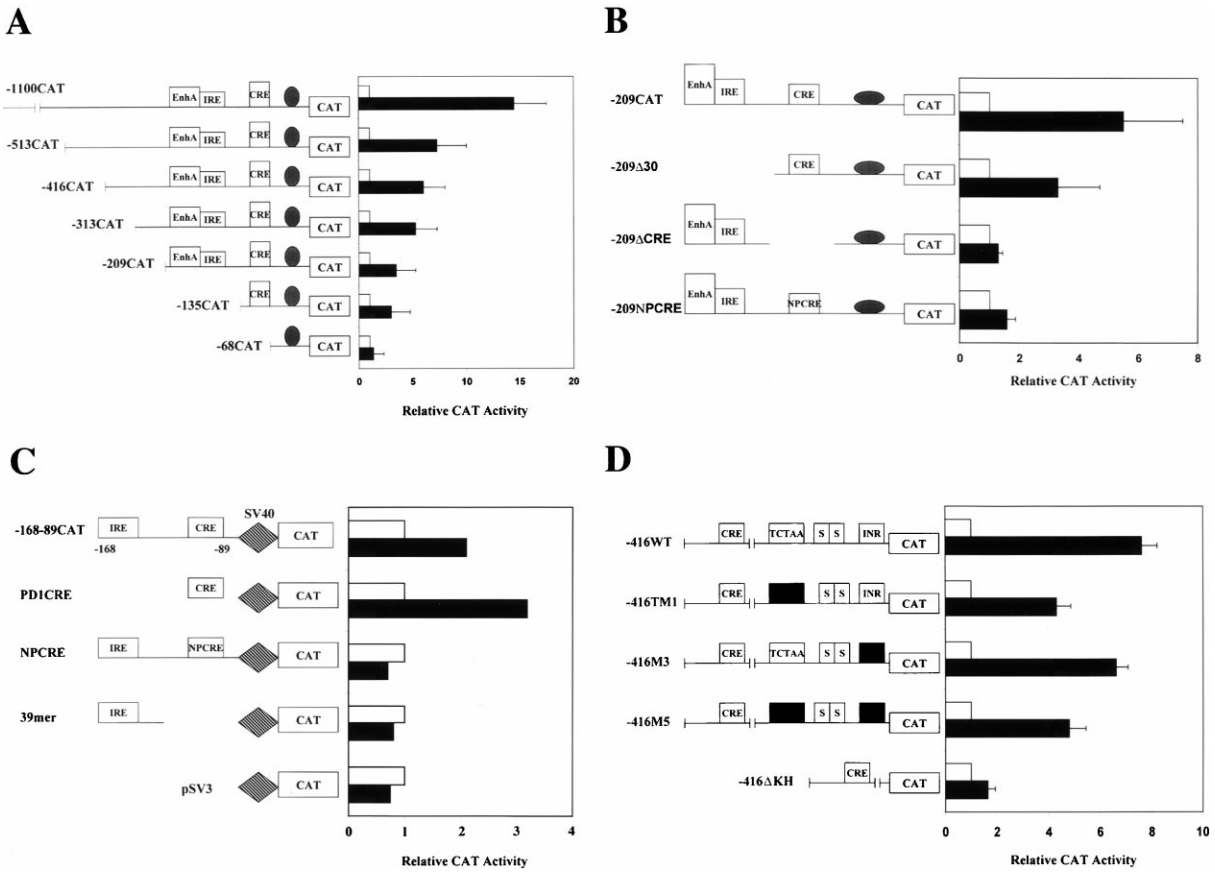

Figure 4. MHC Class I Core and Upstream Regulatory Elements Required for CIITA-Activated Transactivation

HeLa cells were cotransfected with 5 μg of different truncation deletions of MHC class I extended promoter, as shown on left side of each panel, and 2 μg of CIITA (closed bar) or the control vector (open bar). After 48 hr, the promoter activity was studied by CAT assay, normalized to transfection control pSV2LUC. Shown is CAT activity relative to the control vector.

(A) CIITA activates MHC class I transcription through a series of upstream regulatory elements. The −68CAT construct, in the absence of CIITA, is 19.8 ± 2.5 more active than a negative control. The relative activities of the deletion series, in the absence of CIITA, are: −1100CAT, 1.32 ± 0.4; −513CAT, 3.1 ± 1.7; −416CAT, 10.3 ± 6.3; −313CAT, 7.1 ± 5.4; −209CAT, 4.3 ± 1.9; −135CAT, 3.8 ± 1.9; −68CAT, 1. The data presented are the compilation of 5 separate experiments. EnhA, enhancer A; IRE, interferon response element; CRE, cAMP response element.

(B) CIITA activates MHC class I transcription through the class I CRE. NPCRE, non-palindromic mutant CRE.

(C) CIITA activates the heterologous SV40 promoter in the presence of an upstream class I CRE.

(D) Target of CIITA activation of class I does not depend on a single promoter element. Mutated promoter elements are indicated by a solid rectangle. All data are presented as activity in the presence of CIITA, relative to the absence of CIITA.

longer responded to CIITA activation at either temperature (Table 2A and B). Activation of class I promoter at the restrictive temperature was found to be specific for CIITA, since neither p300 nor CBP, two coactivators known to have acetyltransferase activity, activated (Table 2C).

Table 1. CIITA Overcomes a Class I Promoter Defect

Construct	Control	CIITA	Fold Activation
−416WT	55.3 ± 3.2	381.2 ± 17.4	6.7
−416M6	6.1 ± 0.2	144.2 ± 4.0	23.6
pSV0	8.3 ± 0.1	12.0 ± 0.7	1.4

Hela cells were cotransfected with 5 μg of MHC class I reporter constructs −416 WT, −416M6, or the promoterless pSV0 control and 2 μg of either CIITA or the control vector. CAT activity was assessed after 48 hrs, and normalized to transfection control, pSV2LUC. Shown is CAT activity in absence and presence of CIITA and the fold activation.

CIITA Activation in the Absence of $TAF_{II}250$ Depends on Its AT Activity

We next determined which CIITA domain(s) were required to activate class I expression in tsBN462 cells at the restrictive temperature. CIITA deletion mutants lacking AT activity (Δ95–135 and Δ27–222), unlike wild-type CIITA, were unable to restore class I promoter activity at the restrictive temperature (Table 2D). Thus, AT activity is necessary for promoter activation by CIITA in the presence and absence of $TAF_{II}250$ activity. In tsBN462 cells at 32°C, partial deletions of the PST domains (CIITAΔP or CIITAΔST′) partially reduced activation of the class I promoter, whereas a complete deletion (CIITAΔPST) completely abrogated the coactivation function (Figure 5A); none of these deletions abrogated AT activity. Promoter activation by CIITA at 39.5°C shows parallel requirements for the PST domain: partial deletions provided only partial rescue, and the complete deletion was unable to rescue at all (Figure 5A). These

Table 2. CIITA Activation of the Class 1 Promoter Does Not Require $TAF_{II}250$

A. CIITA Activates class I and class II transcription at the restrictive temperature in tsBN462 cells

Construct	Temp.	Control	CIITA
−416CAT	32°C	11.6 ± 0.6	34.4 ± 1.4
	39°C	3.6 ± 0.2	18.3 ± 1.2
DRα CAT	32°C	6.6 ± 0.4	62.0 ± 2.9
	39°C	3.0	58.3 ± 3.2

B. CIITA activation of the class I promoter in tsBN462 cells requires the CRE

Construct	Temp.	Control	CIITA
−135CAT	32°C	21.7 ± 1.8	44.5 ± 2.1
	39°C	1.9 ± 0.2	16.7 ± 0.5
−68CAT	32°C	33.9 ± 1.4	23.5 ± 1.1
	39°C	3.0 ± 0.7	1.8 ± 0.2

C. CBP and p300 do not activate the class I promoter

			Relative Activity
Construct	32°C	39.5°C	(39°C/32°C)
Control	6.3	0.84	0.13
p300	9.1	0.66	0.07
CBP	7.5	1.34	0.18

D. CIITA activation of the class I promoter in the absence of $TAF_{II}250$ depends on its AT domain

		Relative Activity
Promoter construct	CIITA Construct	(39°C/32°C)
−313CAT(class I)	Control	0.18 ± 0.02
	CIITA WT	0.50 ± 0.03
	CIITAΔ94–135	0.11 ± 0.01
	CIITAΔ27–222	0.12 ± 0.01

2 μg of CIITA WT or CIITAΔ94–135 and CIITAΔ27–222 (A, B and D) or 3 μg of p300 or CBP (C) with appropriate vector controls were cotransfected with 5 μg of class I CAT or pSV2 CAT reporter construct, in tsBN462 cells at 32°C. After 24 hrs cells were shifted to 39°C or left at 32°C for an additional 24 hrs before harvesting and performing CAT assay. In A and B data are expressed as percentage of acetylation at permissive (39°C) or restrictive temperature (32°C) normalized to transfection control. Data in C and D are expressed as relative promoter activity which is the ratio of the activity of a given promoter at the two temperatures.

experiments demonstrate that the PST activation domain is necessary for promoter activation in the absence of functional $TAF_{II}250$. Thus, although activation requires the AT domain of CIITA, other domains are also necessary.

Interestingly, mutations within the GTP-binding domain reveal that the requirements for CIITA coactivation in the presence and absence of $TAF_{II}250$ are separable activities within the CIITA molecule. Thus, mutation of the phosphate-binding region (K→E, ΔKG) did not markedly affect CIITA activation at 32°C, but completely eliminated it at 39.5°C (Figure 5B). Although these mutants have reduced nuclear transport, this cannot account for the differential effect (Harton et al., 1999). In contrast, mutation of the guanine-binding segment (ΔSKAD) eliminated both activities. Taken together, these data suggest that activation in the presence and absence of $TAF_{II}250$ largely depends on the same molecular domains, but the activities are separable and have distinct requirements.

Discussion

CIITA is a coactivator necessary for both the induction of de novo MHC class II gene transcription and the enhanced expression of the constitutively transcribed MHC class I genes (Martin et al., 1997; van den Elsen et al., 1998a, 1998b). In the present study, we have demonstrated that CIITA has intrinsic acetyltransferase (AT) activity and have mapped the AT domain to the N terminus of CIITA between 94–132 aa. Deletion of this segment of CIITA results in a loss both of AT activity and of transactivation of class I and class II promoters.

Although the AT domain is necessary for CIITA transactivation, it alone is not sufficient. GTP stimulates AT activity and this stimulation is dependent upon the GTP-binding domain. The GTP-binding domain has multiple functions: it modulates the AT activity and is also required for nuclear localization and dimerization of CIITA (Ting et al., in preparation). Dimerization may require GTP and be necessary to achieve maximal levels of acetylation. Other AT containing coactivators are known to dimerize. PCAF, which has intrinsic AT activity, was originally isolated in a complex with CBP/p300 (Yang et al., 1996). It is interesting to speculate that the extent of acetylation, and pattern of acetylation of the substrate, is determined by the interactions of different AT proteins. Previous analyses of CIITA structure have identified additional activation domains: an N-terminal acidic domain, a ProSerThr domain, a GTP-binding domain, and a C-terminal domain (Riley et al., 1995; Zhou and Glimcher, 1995; Chin et al., 1997). The N-terminal

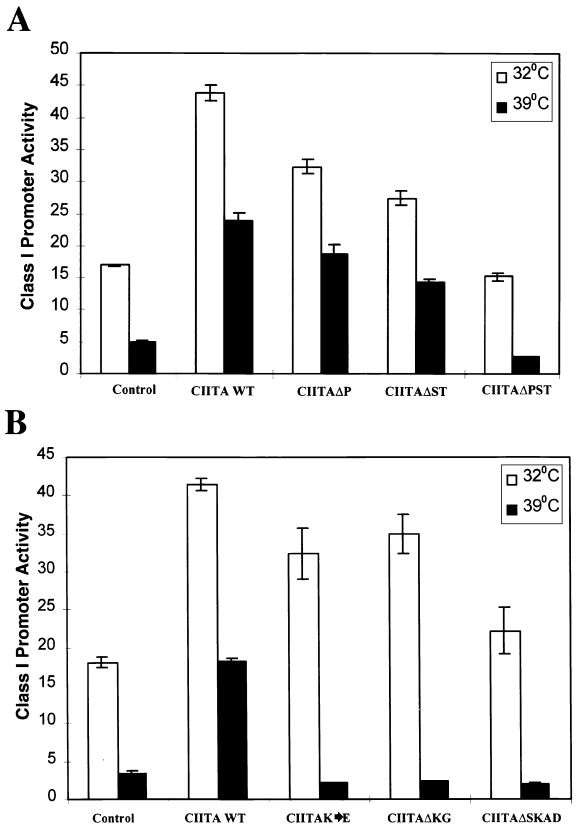

Figure 5. CIITA Activation Domain Deletions Fail to Rescue a $TAF_{II}250$ Defect

(A and B) 2 μg of CIITA WT, mutants (closed bar) or control vector (open bar) were cotransfected with 5 μg −416CAT class I reporter contruct in tsBN462 cells at 32°C. After 24 hr cells were shifted to 39°C or left at 32°C for an additional 24 hr before harvesting and performing CAT assay. Data are expressed as percentage of acetylation normalized to transfection control pSV2LUC.

acidic domain overlaps the CIITA AT domain described in the present study, which may account for its reported activity. The mechanisms of action of the ProSerThr domain and the C-terminal domain are not known. While the Pro subdomain may overlap the CIITA AT domain, the SerThr region is necessary for function, but not for AT activity. Taken together, these data indicate that CIITA is a complex transactivator with multiple functional domains, among which the AT domain plays a critical role.

Class I promoter activation by CIITA requires both downstream core promoter sequences and a CRE element, located between −100 and −107 bp. Activation is augmented by a series of upstream sequence elements; deletion of the upstream modulators reduces the magnitude of activation. We previously identified and characterized the class I CRE because it mediates TSH/cAMP-regulated repression of transcription (Saji et al., 1997; Kirshner et al., 2000). Here, we demonstrate that deletion of the CRE element or the core promoter eliminates CIITA-mediated activation. We further show that the class I CRE is sufficient to confer CIITA responsiveness to a heterologous promoter. Previous studies described an upstream promoter sequence site-α (site-α corresponds to the CRE.) that is required for MHC class I activation by CIITA (Gobin et al., 1997; Martin et al., 1997). Taken together, our findings demonstrate that the class I CRE functions as a context-dependent transcriptional regulatory element.

An important new observation to emerge from the present studies is that the DNA sequence requirements for CIITA-mediated activation of transcription are distinct from those of constitutive transcription. Constitutive transcription, in the absence of CIITA, depends upon a promoter element located between the TATAA and Inr elements of the class I promoter, the S box sequence (Howcroft et al., 1995). A promoter construct mutated in this region (416M6) is completely inactive in constitutive transcription (Howcroft et al., 1995). However, in the presence of CIITA, the same promoter construct is fully active. Furthermore, although CIITA-mediated activation requires a core promoter, no specific sequence element is targeted. This suggests that CIITA activates transcription by a mechanism distinct from that of constitutive transcription.

This interpretation is supported by the observation that the transcription factor requirements for CIITA-mediated activation are distinct from those of constitutive transcription. Constitutive class I transcription, in the absence of CIITA, depends upon the $TAF_{II}250$ component of the general transcription factor, TFIID. In the temperature-sensitive $TAF_{II}250$ mutant, tsBN462, constitutive class I transcription is impaired at the restrictive temperature where $TAF_{II}250$ loses AT activity and is inactive (Weissman et al., 1998; Dunphy et al., 2000). Surprisingly, CIITA-activated transcription does not require $TAF_{II}250$, but does require functional AT activity. In addition to the AT domain, the remaining CIITA activation domains are also necessary to effect promoter rescue. However, the requirements are different in the presence and absence of $TAF_{II}250$. Whereas the GTP-binding domain is not necessary for activation in the presence of functional $TAF_{II}250$, it is necessary in the absence of $TAF_{II}250$. Thus, CIITA does not simply enhance constitutive transcription, but appears to function through a distinct transcriptional pathway.

The role of $TAF_{II}250$ in transcription initiation remains to be fully elucidated. Although $TAF_{II}250$ is clearly required for transcription of a large number of genes, notably those involved in cell cycle regulation, it is not required for many others in both yeast and mammalian cells (Wang and Tjian, 1994; Suzuki-Yagawa et al., 1997; Wang et al., 1997; O'Brien and Tjian, 2000; Fondell et al., 1999). The present findings with CIITA extend these observations, by demonstrating that transcriptional activators can modulate a single promoter's requirement for $TAF_{II}250$ in vivo. Although similar observations have been reported for viral transcription factors, such as the SV40 Tag, HBV pX and HPV16E7 proteins (Sekiguchi et al., 1999), this is the first example of a cellular coactivator with AT activity able to bypass a promoter's $TAF_{II}250$ dependence in vivo.

The present findings suggest CIITA has multiple functions in transcriptional regulation. On one hand, it serves as a nucleation site for the formation of a class II enhanceosome containing the class II X1, X2 and Y box binding factors, providing a platform which integrates upstream regulatory information with the basal tran-

scription machinery (Masternak et al., 2000). In this respect, CIITA is similar to other coactivators. On the other hand, unlike other coactivators which are ubiquitously expressed and have extensive interactions with multiple transcription factors, CIITA is tissue specific and interacts with those transcription factors limited largely to MHC class II transcription. CIITA also interacts with a spectrum of basal transcription factors, including $TAF_{II}32$, $TAF_{II}70$, TFIIB, TBP (Mahanta et al., 1997; Scholl et al., 1997; Fontes et al., 1999). This suggests that its role in transcriptional regulation is distinct from previously described coactivators. Consistent with this hypothesis is the observation that CIITA does not depend on $TAF_{II}250$.

The multifunctionality of CIITA is further evidenced by the differential requirement for CBP in CIITA-mediated activation of class I transcription compared with induction of class II transcription. Whereas optimal activation of the MHC class II promoter by CIITA requires the presence of CBP (Kretsovali et al., 1998; Fontes et al., 1999), CBP has no detectable effect on the ability of CIITA to enhance class I promoter activity. The duality of CIITA function has been proposed previously, based on the observation that CIITA is required both for assembling transcription factors on the class II promoter and for activation in interferon-inducible cells (Kara and Glimcher, 1991; Wright et al., 1998; Villard et al., 1999; O'Brien and Tjian, 2000).

The studies reported here suggest that activation of the class I promoter involves CIITA-mediated triggering of the assembly of a novel array of transcription factors (distinct from those constitutively assembled on the promoter), which enables CIITA-mediated activation. We propose the following model for CIITA activation of class I transcription. In the absence of CIITA, constitutive transcription of class I requires functional $TAF_{II}250$ in a TFIID complex. Nucleation of the TFIID complex on the core promoter requires the presence of sequences that have been mutated in the −416M6 promoter construct, leading to its inactivity. In contrast, the preinitiation complex (PIC) formed in the presence of CIITA no longer requires $TAF_{II}250$, but rather incorporates CIITA into a novel TFIID-like complex, which is likely to contain $TAF_{II}32$. This pre-initiation complex does not require the M6 sequences, but, rather, is anchored by other core promoter sequences. For this reason, CIITA is able to activate the promoter construct M6, which is inactive in its absence. Unlike $TAF_{II}250$, CIITA also requires the presence of an upstream CRE element to mediate activation. This model suggests that the formation of a transcription initiation complex on a given promoter is a dynamic event, whose composition and sequence requirements are determined by both upstream sequences and coactivators.

Experimental Procedures

Cell Line and Plasmids

tsBN462 cells, HeLa cells, and murine L cells were grown as described previously (Weissman et al., 2000). The MHC class I promoter truncation series consists of 5′ flanking sequences derived from swine class I gene PD1 ligated to the CAT reporter gene (Ehrlich et al., 1988). The mammalian expression vector Flag-CIITA WT, Proline/Serine/Threonine deletion mutant and GST-binding domain mutants, and CIITA truncation mutant 1–148 have been described previously (Chin et al., 1997). The CIITAΔC was generated by cloning the Kpn I fragment of Flag-CIITA WT into the Kpn I site of pcDNA3. CIITAΔAT (Δ27–222) was generated by XcmI/Bsu36I digestion, removing amino acids 27–222. The CBP ribozyme control and mutant plasmids have been described previously (Kawasaki et al., 1996; Kawasaki et al., 1998). CIITA WT and mutant variants were cloned into the baculovirus transfer vector PVL1293 at the Eco RI site.

Stable and Transient Transfections

For stable transfection, L cells were cotransfected with 2 μg of either CBP ribozyme or mutant CBP ribozyme plasmid and 200 ng of tk^+ plasmid and selected in HAT medium for 3 weeks. Transient transfections were done by CaPO4 precipitation method, as described previously (Howcroft et al., 1995), with 5 μg of CAT reporter constructs and 2 μg of CIITA wild type mutants or control plasmid. tsBN462 cells were left at 32°C for 24 hr after transfection and then shifted to 39°C (restrictive temperature) or left at 32°C (permissive temperature) for an additional 24 hr. HeLa cells and L cells were maintained at 37°C for 48 hr after transfection. Chloramphenicol acetyltransferase (CAT) activity was normalized to luciferase activity by cotransfecting an internal plasmid control pSV2LUC or RSV LUC.

Immunoprecipitation and Western Blot

Cell lysates of HeLa, L or Sf9 cells transfected with 10 μg of Flag-CIITA WT or mutants were made in 2x PCV lysis buffer (50 mM Tris-HCl [pH 8.0], 5 mM MgCl2, 150 mM KCl, 0.1% NP40, 10% glycerol and protease inhibitors). The anti-Flag M2 agarose beads were activated according to the manufacturer's protocol (Sigma). For immunoprecipitation, the cell lysate was incubated with the activated M2 agarose beads at 4°C overnight; protein was eluted with SDS sample buffer. For immunoblotting, eluted protein was resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose. The primary antibodies were mouse anti-Flag M2 antibody (10 μg/ml) (Sigma) or rabbit anti-CBP (0.7 μg/ml) (Santa Cruz Biotechnology). Secondary antibodies were HRPO conjugated anti-mouse or anti-rabbit antibodies. Proteins were detected by chemiluminescence with SuperSignal substrate (Pierce).

Preparation of Recombinant Protein

Flag-CIITA WT or the mutants were expressed in Sf9 cells using baculovirus-mediated transfection according to the manufacturer's protocol (Pharmingen kit). Recombinant Flag-CIITA was immunoprecipitated using anti-Flag M2 agarose beads (Sigma) and eluted with 100 μg/ml of flag peptide.

HAT Assay

The HAT assay was a modification of a previously described procedure (Mizzen et al., 1996). CIITA was incubated with 1 μg of H3 and H4 histones and 70 nCi of [^{3}H]acetyl-CoA (10 Ci/pmol) and 15 nCi of [^{14}C]acetyl-CoA (60 mCi/pmol) in presence of 10 mM butyric acid, 50 mM Tris pH 8.0, 1 mM DTT, and 0.1 mM EDTA at 30°C for 30 min. Where indicated, 1.25 mM GTP was included in the reaction mix. The reaction was stopped with SDS sample buffer and applied to 16% SDS-polyacrylamide gels, which were fixed overnight; histone acetylation was analyzed using Storm PhosphoImager with the ImageQuant software.

Received June 21, 2000; revised November 8, 2000.

References

Barlev, N.A., Candau, R., Wang, L., Darpino, P., Silverman, N., and Berger, S.L. (1995). Characterization of physical interactions of the putative transcriptional adaptor, ADA2, with acidic activation domains and TATA-binding protein. J. Biol. Chem. *270*, 19337–19344.

Boyes, J., Byfield, P., Nakatani, Y., and Ogryzko, V. (1998). Regulation of activity of the transcription factor GATA-1 by acetylation. Nature *396*, 594–598.

Carey, M. (1998). The enhanceosome and transcriptional synergy. Cell *92*, 5–8.

Chang, C.H., Fontes, J.D., Peterlin, M., and Flavell, R.A. (1994). Class II transactivator (CIITA) is sufficient for the inducible expression of

major histocompatibility complex class II genes. J. Exp. Med. *180*, 1367–1374.

Chang, C.H., Guerder, S., Hong, S.C., van Ewijk, W., and Flavell, R.A. (1996). Mice lacking the MHC class II transactivator (CIITA) show tissue-specific impairment of MHC class II expression. Immunity *4*, 167–178.

Chen, J.L., Attardi, L.D., Verrijzer, C.P., Yokomori, K., and Tjian, R. (1994). Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. Cell *79*, 93–105.

Chin, K.C., Li, G.G., and Ting, J.P. (1997). Importance of acidic, proline/serine/threonine-rich, and GTP-binding regions in the major histocompatibility complex class II transactivator: generation of transdominant-negative mutants. Proc. Natl. Acad. Sci. USA *94*, 2501–2506.

Chin, K.C., Mao, C., Skinner, C., Riley, J.L., Wright, K.L., Moreno, C.S., Stark, G.R., Boss, J.M., and Ting, J.P. (1994). Molecular analysis of G1B and G3A IFN gamma mutants reveals that defects in CIITA or RFX result in defective class II MHC and Ii gene induction. Immunity *1*, 687–697.

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

Davie, J.R. (1998). Covalent modifications of histones: expression from chromatin templates. Curr. Opin. Genet. Dev. *8*, 173–178.

Dunphy, E., Johnson, T., Auerbach, S., and Wang, E. (2000). Requirement for TAFII250 AT activity in cell cycle progression. Mol. Cell. Biol. *20*, 1134–1139.

Ehrlich, R., Maguire, J.E., and Singer, D.S. (1988). Identification of negative and positive regulatory elements associated with a class I major histocompatibility complex gene. Mol. Cell. Biol. *8*, 695–703.

Fondell, J.D., Guermah, M., Malik, S., and Roeder, R.G. (1999). Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID. Proc. Natl. Acad. Sci. USA *96*, 1959–1964.

Fontes, J.D., Jabrane-Ferrat, N., Toth, C.R., and Peterlin, B.M. (1996). Binding and cooperative interactions between two B cell-specific transcriptional co-activators. J. Exp. Med. *183*, 2517–2521.

Fontes, J.D., Jiang, B., and Peterlin, B.M. (1997). The class II transactivator CIITA interacts with the TBP-associated factor TAFII32. Nucleic Acids Res. *25*, 2522–2528.

Fontes, J.D., Kanazawa, S., Jean, D., and Peterlin, B.M. (1999). Interactions between the class II transactivator and CREB binding protein increase transcription of major histocompatibility complex class II genes. Mol. Cell. Biol. *19*, 941–947.

Gobin, S.J., Peijnenburg, A., Keijsers, V., and van den Elsen, P.J. (1997). Site alpha is crucial for two routes of IFN gamma-induced MHC class I transactivation: the ISRE-mediated route and a novel pathway involving CIITA. Immunity *6*, 601–611.

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

Hampsey, M., and Reinberg, D. (1999). RNA polymerase II as a control panel for multiple co-activator complexes. Curr. Opin. Genet. Dev. *9*, 132–139.

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

Harton, J.A., Cressman, D.E., Chin, K.C., Der, C.J., and Ting, J.P. (1999). GTP binding by class II transactivator: role in nuclear import [see comments]. Science *285*, 1402–1405.

Hayashida, T., Sekiguchi, T., Noguchi, E., Sunamoto, H., Ohba, T., and Nishimoto, T. (1994). The CCG1/TAFII250 gene is mutated in thermosensitive G1 mutants of the BHK21 cell line derived from golden hamster. Gene *141*, 267–270.

Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M., and Roeder, R.G. (1993). The p250 subunit of native TATA box-binding factor TFIID is the cell-cycle regulatory protein CCG1. Nature *362*, 179–181.

Howcroft, T.K., Palmer, L.A., Brown, J., Rellahan, B., Kashanchi, F., Brady, J.N., and Singer, D.S. (1995). HIV Tat represses transcription through Sp1-like elements in the basal promoter. Immunity *3*, 127–138.

Kara, C.J., and Glimcher, L.H. (1991). In vivo footprinting of MHC class II genes: bare promoters in the bare lymphocyte syndrome. Science *252*, 709–712.

Kawasaki, H., Eckner, R., Yao, T.P., Taira, K., Chiu, R., Livingston, D.M., and Yokoyama, K.K. (1998). Distinct roles of the co-activators p300 and CBP in retinoic-acid-induced F9-cell differentiation. Nature *393*, 284–289.

Kawasaki, H., Ohkawa, J., Tanishige, N., Yoshinari, K., Murata, T., Yokoyama, K.K., and Taira, K. (1996). Selection of the best target site for ribozyme-mediated cleavage within a fusion gene for adenovirus E1A-associated 300 kDa protein (p300) and luciferase. Nucleic Acids Res. *24*, 3010–3016.

Kirshner, S., Palmer, L., Bodor, J., Saji, M., Kohn, L.D., and Singer, D.S. (2000). Major histocompatibility class I gene transcription in thyrocytes: a series of interacting regulatory DNA sequence elements mediate thyrotropin/cyclic adenosine 3′,5′-monophosphate repression. Mol. Endocrinol. *14*, 82–98.

Klages, N., and Strubin, M. (1995). Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo. Nature *374*, 822–823.

Komarnitsky, P.B., Klebanow, E.R., Weil, P.A., and Denis, C.L. (1998). ADR1-mediated transcriptional activation requires the presence of an intact TFIID complex. Mol. Cell. Biol. *18*, 5861–5867.

Kretsovali, A., Agalioti, T., Spilianakis, C., Tzortzakaki, E., Merika, M., and Papamatheakis, J. (1998). Involvement of CREB binding protein in expression of major histocompatibility complex class II genes via interaction with the class II transactivator. Mol. Cell. Biol. *18*, 6777–6783.

Krumm, A., Madisen, L., Yang, X.J., Goodman, R., Nakatani, Y., and Groudine, M. (1998). Long-distance transcriptional enhancement by the histone acetyltransferase PCAF. Proc. Natl. Acad. Sci. USA *95*, 13501–13506.

Kuras, L., Kosa, P., Mencia, M., and Struhl, K. (2000). TAF-Containing and TAF-independent forms of transcriptionally active TBP in vivo. Science *288*, 1244–1248.

Li, X.Y., Bhaumik, S.R., and Green, M.R. (2000). Distinct classes of yeast promoters revealed by differential TAF recruitment . Science *288*, 1242–1244.

Li, X.Y., Virbasius, A., Zhu, X., and Green, M.R. (1999). Enhancement of TBP binding by activators and general transcription factors. Nature *399*, 605–609.

Maguire, J.E., Frels, W.I., Richardson, J.C., Weissman, J.D., and Singer, D.S. (1992). In vivo function of regulatory DNA sequence elements of a major histocompatibility complex class I gene. Mol. Cell. Biol. *12*, 3078–3086.

Mahanta, S.K., Scholl, T., Yang, F.C., and Strominger, J.L. (1997). Transactivation by CIITA, the type II bare lymphocyte syndrome-associated factor, requires participation of multiple regions of the TATA box binding protein. Proc. Natl. Acad. Sci. USA *94*, 6324–6329.

Martin, B.K., Chin, K.C., Olsen, J.C., Skinner, C.A., Dey, A., Ozato, K., and Ting, J.P. (1997). Induction of MHC class I expression by the MHC class II transactivator CIITA. Immunity *6*, 591–600.

Masternak, K., Muhlethaler-Mottet, A., Villard, J., Zufferey, M., Steimle, V., and Reith, W. (2000). CIITA is a transcriptional co-activator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex . Genes Dev. *14*, 1156–1166.

Merika, M., Williams, A.J., Chen, G., Collins, T., and Thanos, D. (1998). Recruitment of CBP/p300 by the IFN beta enhanceosome is required for synergistic activation of transcription. Mol. Cell *1*, 277–287.

Metzger, D., Scheer, E., Soldatov, A., and Tora, L. (1999). Mammalian TAFII30 is required for cell cycle progression and specific cellular differentiation programmes. EMBO J. *18*, 4823–4834.

Mizzen, C.A., Yang, X.J., Kokubo, T., Brownell, J.E., Bannister, A.J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S.L., Kouzarides, T., et al. (1996). The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. Cell *87*, 1261–1270.

Moqtaderi, Z., Bai, Y., Poon, D., Weil, P., and Struhl, K. (1996). TBP-associated factors are not generally required for transcriptional activation in yeast. Nature *383*, 188–191.

O'Brien, T., and Tjian, R. (2000). Different functional domains of $TAF_{II}250$ modulate expression of distinct subsets of mammalian genes. Proc. Natl. Acad. Sci. USA *97*, 2456–2461.

Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., and Nakatani, Y. (1996). The transcriptional co-activators p300 and CBP are histone acetyltransferases. Cell *87*, 953–959.

Orphanides, G., Lagrange, T., and Reinberg, D. (1996). The general transcription factors of RNA polymerase II. Genes Dev. *10*, 2657–2683.

Riley, J.L., Westerheide, S.D., Price, J.A., Brown, J.A., and Boss, J.M. (1995). Activation of class II MHC genes requires both the X box region and the class II transactivator (CIITA). Immunity *2*, 533–543.

Roeder, R.G. (1996). The role of general initiation factors in transcription by RNA polymerase II. Trends Became Sci *21*, 327–335.

Saji, M., Sheng, M., Napolitano, G., Palmer, L.A., Taniguchi, S.I., Ohmori, M., Ohta, M., Suzuki, K., Kirshner, S.L., Giuliani, C., et al. (1997). Regulation of major histocompatibility complex class I gene expression in thyroid cells. Role of the cAMP response element-like sequence. J. Biol. Chem. *272*, 20096–20107.

Sauer, F., Hansen, S.K., and Tjian, R. (1995). Multiple TAFIIs directing synergistic activation of transcription. Science *270*, 1783–1788.

Scholl, T., Mahanta, S.K., and Strominger, J.L. (1997). Specific complex formation between the type II bare lymphocyte syndrome-associated transactivators CIITA and RFX5. Proc. Natl. Acad. Sci. USA *94*, 6330–6334.

Sekiguchi, T., Nishimoto, T., and Hunter, T. (1999). Overexpression of D-type cyclins, E2F 1, SV40 large T antigen and HPV16 E7 rescue cell cycle arrest of tsBN462 cells caused by the CCG1/TAF(II)250 mutation. Oncogene *18*, 1797–1806.

Soutoglou, E., Katrakili, N., and Talianidis, I. (2000). Acetylation regulates transcription factor activity at multiple levels. Mol. Cell. Biol. *5*, 745–751.

Steimle, V., Otten, L.A., Zufferey, M., and Mach, B. (1993). Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell *75*, 135–146.

Steimle, V., Siegrist, C.A., Mottet, A., Lisowska-Grospierre, B., and Mach, B. (1994). Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. Science *265*, 106–109.

Suzuki-Yagawa, Y., Guermah, M., and Roeder, R.G. (1997). The ts13 mutation in the TAF(II)250 subunit (CCG1) of TFIID directly affects transcription of D-type cyclin genes in cells arrested in G1 at the nonpermissive temperature. Mol. Cell. Biol. *17*, 3284–3294.

van den Elsen, P.J., Gobin, S.J.P., van Eggermond, M.C., and Peijnenburg, A. (1998a). Regulation of MHC class I and II gene transcription: differences and similarities. Immunogenetics *48*, 208–221.

van den Elsen, P.J., Peijnenburg, A., van Eggermond, M.C., and Gobin, S.J. (1998b). Shared regulatory elements in the promoters of MHC class I and class II genes. Immunol. Today *19*, 308–312.

Verrijzer, C.P., and Tjian, R. (1996). TAFs mediate transcriptional activation and promoter selectivity. Trends Biochem. Sci. *21*, 338–342.

Villard, J., Muhlethaler-Mottet, A., Bontron, S., Mach, B., and Reith, W. (1999). CIITA-induced occupation of MHC class II promoters is independent of the cooperative stabilization of the promoter-bound multi-protein complexes. Int. Immunol. *11*, 461–469.

Walker, S., Reese, J., Apone, L., and Green, M. (1996). Transcription activation in cells lacking TAFIIs. Nature *383*, 185–188.

Wang, E.H., and Tjian, R. (1994). Promoter-selective transcriptional defect in cell cycle mutant ts13 rescued by $hTAF_{II}250$. Science *263*, 811–814.

Wang, E.H., Zou, S., and Tjian, R. (1997). $TAF_{II}250$-dependent transcription of cyclin A is directed by ATF activator proteins. Genes Dev. *11*, 2658–2669.

Weinzierl, R.O., Dynlacht, B.D., and Tjian, R. (1993). Largest subunit of Drosophila transcription factor IID directs assembly of a complex containing TBP and a co-activator. Nature *362*, 511–517.

Weissman, J.D., Brown, J.A., Howcroft, T.K., Hwang, J., Chawla, A., Roche, P.A., Schiltz, L., Nakatani, Y., and Singer, D.S. (1998). HIV-1 tat binds $TAF_{II}250$ and represses $TAF_{II}250$-dependent transcription of major histocompatibility class I genes. Proc. Natl. Acad. Sci. USA *95*, 11601–11606.

Weissman, J.D., Howcroft, T.K., and Singer, D.S. (2000). TAF(II)250-independent transcription can be conferred on a TAF(II)250-dependent basal promoter by upstream activators. J. Biol. Chem. *275*, 10160–10167.

Wright, K.L., Chin, K.C., Linhoff, M., Skinner, C., Brown, J.A., Boss, J.M., Stark, G.R., and Ting, J.P. (1998). CIITA stimulation of transcription factor binding to major histocompatibility complex class II and associated promoters in vivo. Proc. Natl. Acad. Sci. USA *95*, 6267–6272.

Xu, L., Glass, C.K., and Rosenfeld, M.G. (1999). Co-activator and corepressor complexes in nuclear receptor function. Curr. Opin. Genet. Dev. *9*, 140–147.

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

Zhou, H., and Glimcher, L.H. (1995). Human MHC class II gene transcription directed by the carboxyl terminus of CIITA, one of the defective genes in type II MHC combined immune deficiency. Immunity *2*, 545–553.