THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 285, NO. 50, pp. 38772–38780, December 10, 2010
Printed in the U.S.A.

Novel Functions for TAF7, a Regulator of TAF1-independent Transcription[S]

Received for publication, August 10, 2010, and in revised form, September 16, 2010 Published, JBC Papers in Press, October 11, 2010, DOI 10.1074/jbc.M110.173864

Ballachanda N. Devaiah[‡], Hanxin Lu[‡], Anne Gegonne[‡], Zeynep Sercan[‡], Hongen Zhang[§], Robert J. Clifford[§], Maxwell P. Lee[§], and Dinah S. Singer[‡1]

From the [‡]Experimental Immunology Branch and [§]Laboratory of Population Genetics, NCI, National Institutes of Health, Bethesda, Maryland 20892

The transcription factor TFIID components TAF7 and TAF1 regulate eukaryotic transcription initiation. TAF7 regulates transcription initiation of TAF1-dependent genes by binding to the acetyltransferase (AT) domain of TAF1 and inhibiting the enzymatic activity that is essential for transcription. TAF7 is released from the TAF1-TFIID complex upon completion of preinitiation complex assembly, allowing transcription to initiate. However, not all transcription is TAF1-dependent, and the role of TAF7 in regulating TAF1-independent transcription has not been defined. The IFNγ-induced transcriptional co-activator CIITA activates MHC class I and II genes, which are vital for immune responses, in a TAF1-independent manner. Activation by CIITA depends on its intrinsic AT activity. We now show that TAF7 binds to CIITA and inhibits its AT activity, thereby repressing activated transcription. Consistent with this TAF7 function, siRNA-mediated depletion of TAF7 resulted in increased CIITA-dependent transcription. A more global role for TAF7 as a regulator of transcription was revealed by expression profiling analysis: expression of 30–40% of genes affected by TAF7 depletion was independent of either TAF1 or CIITA. Surprisingly, although TAF1-dependent transcripts were largely down-regulated by TAF7 depletion, TAF1-independent transcripts were predominantly up-regulated. We conclude that TAF7, until now considered only a TFIID component and regulator of TAF1-dependent transcription, also regulates TAF1-independent transcription.

Transcription depends on the temporal and spatial integration of preinitiation complex assembly, initiation, elongation, and termination. Precise regulation of the transition from preinitiation complex (PIC)[2] assembly to initiation and then from initiation to elongation is necessary to assure proper rates of productive transcription. The transcription factor component TAF7 plays a critical role in this regulation. PIC assembly is nucleated by TFIID, a complex composed of the TATA-binding protein (TBP) and a series of TBP-associated factors (TAFs). TAF1, the largest TAF, has an intrinsic acetyltransferase (AT) activity that is required for transcription (1, 2). TAF7 regulates TAF1 by inhibiting the TAF1 AT activity necessary for transcription initiation until PIC assembly is complete (3). Release of TAF7 from TFIID results in transcription initiation (4). Thus, TAF7 plays a critical role in regulating TFIID-dependent transcription initiation by preventing premature transcription initiation (4).

Importantly, TAF7 also functions independently of the TFIID complex. It binds to and inhibits the kinase activities of the general transcription factor TFIIH and the elongation factor P-TEFb, which are required for Ser-5 and Ser-2 phosphorylation, respectively, of the polymerase II C-terminal domain, thereby regulating promoter clearance and elongation (5). Consistent with TFIID-independent functions, TAF7 is found in both TFIID and non-TFIID complexes in both B and T cell lines (5). Because not all transcription is TAF1-dependent, the occurrence of TAF7 complexes without TAF1 raised the question of whether TAF7 regulates transcription initiation that does not require the AT activity of TAF1.

MHC class I and II molecules are cell-surface glycoproteins that play critical but distinct roles in the development and function of immunity. Both MHC class I and II molecules serve as receptors for processed antigens. However, MHC class I molecules bind peptides derived from processed intracellular antigens, thereby providing immune surveillance against intracellular pathogens such as viruses. In contrast, MHC class II molecules present peptides derived from extracellular antigens, triggering immune responses to extracellular pathogens such as bacteria. Consistent with their distinct roles in immune surveillance, class I proteins are constitutively expressed on all somatic tissues, whereas expression of class II molecules is normally restricted to professional antigen-presenting cells (6–8). Aberrant expression of MHC genes is associated with tumorigenesis (9) and autoimmune diseases (10).

In the absence of infection or inflammation, constitutive transcription of MHC class I genes is dependent on the TFIID component TAF1 and its AT activity (2, 3). In response to inflammatory signals, the inflammatory mediator IFNγ induces the transcriptional co-activator CIITA. CIITA mediates increases class I transcription and induction of *de novo* transcription of MHC class II genes. In contrast to constitutive MHC class I transcription, IFNγ-activated transcription of class I and II genes is independent of TAF1 (11, 12). Instead, the intrinsic AT activity of CIITA allows it to bypass the requirement for TAF1 AT activity (12). CIITA AT activity is

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental "Methods," Figs. S1–S5, and Tables S1–S3.

[1] To whom correspondence should be addressed: Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892. Fax: 301-480-0977; E-mail: dinah.singer@nih.gov.

[2] The abbreviations used are: PIC, preinitiation complex; TF, transcription factor; TBP, TATA-binding protein; TAF, TBP-associated factor; AT, acetyltransferase; HAT, histone acetyltransferase; CAT, chloramphenicol acetyltransferase.

This is an Open Access article under the CC BY license.

necessary for transcription initiation because inactivation of the AT activity renders CIITA incapable of inducing transcription of MHC class II genes (12). Thus, in contrast to constitutive transcription, CIITA-activated MHC transcription is independent of TAF1 AT function.

The parallels between CIITA and TAF1 (both have intrinsic AT activities that are required for MHC gene transcription) led us to predict that like TAF1, CIITA may be regulated by TAF7. Here, we report that TAF7 binds to CIITA and inhibits its AT activity. We show that TAF7 regulates CIITA-mediated activation of MHC class I and II transcription and that this regulation is independent of functional TAF1. These findings demonstrate that TAF7 regulates both CIITA-activated and constitutive transcription of MHC class I genes. Importantly, TAF7 regulates a larger set of TAF1-independent promoters as evidenced by our finding that ~40% of transcripts affected by the silencing of TAF7 are not affected by TAF1 silencing. Thus, TAF7 has a broad role in regulating transcription that extends beyond its role as a TFIID component, with functions independently of TAF1.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture—HeLa cells, HeLa/CIITA, CHO, and tsBN462 cells were grown as described previously (4, 13). *Drosophila* Sf9 cells were grown in TNM-FH insect medium (Pharmingen) and maintained at 27 °C.

Plasmid Constructs—The MHC class I promoter used in these studies was derived from the swine class I gene *PD1* (14). The MHC class I core promoter construct (−313CAT) was described previously (15). TAF7 in the pcDNA3 vector (3) and GST- and FLAG-tagged full-length TAF7 and fragments of TAF7 for recombinant protein expression (4) have been described previously. Full-length FLAG-WT CIITA, proline/serine/threonine domain (positions 132–301) and AT domain (positions 27–222) deletion mutants, and CIITA truncation mutant 1–148 in the baculovirus transfer vector pVL1393 have also been described previously (12).

Preparation of Recombinant Proteins—Recombinant CIITA, TAF7, and their respective mutants were prepared as described before (4, 12). FLAG-WT CIITA and mutant proteins were expressed in Sf9 cells using baculovirus-mediated transfection according to the manufacturer's protocol (BD Pharmingen). Recombinant FLAG-tagged protein was immunoprecipitated using anti-FLAG M2-agarose beads (Sigma) and eluted with 100 μg/ml FLAG peptide. GST-TAF7 protein was expressed in a BL21 bacterial expression system and purified using a GST column according to the manufacturer's protocol (Thermo Scientific).

Immunoprecipitation and Immunoblotting—Cell lysates of HeLa or Sf9 cells transfected with the appropriate plasmid construct were made in 2× lysis buffer (50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 150 mM KCl, 0.1% Nonidet P-40, 10% glycerol, and protease inhibitors). Anti-FLAG M2-agarose beads were activated according to the manufacturer's protocol. 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 antibody used was mouse anti-FLAG antibody M2 (10 μg/ml; Sigma). The secondary antibody used was horseradish peroxidase-conjugated anti-mouse antibody. Proteins were detected by chemiluminescence with SuperSignal substrate (Pierce).

Histone Acetyltransferase Assay—The histone acetyltransferase (HAT) assay was done as described previously with minor changes (12). Purified CIITA or control TAF1 was incubated with 1 μg of histone H4 (New England Biolabs), 70 nCi of [^{3}H]acetyl-CoA (10 Ci/pmol), and 15 nCi of [^{14}C]acetyl-CoA (60 mCi/pmol) in the presence of HAT buffer (10 mM butyric acid, 50 mM Tris (pH 8.0), 1 mM DTT, and 0.1 mM EDTA) at 30 °C for 30 min. TAF7 was included in the reaction mixture where indicated. The reaction was stopped with SDS sample buffer, and the samples were run on 15% SDS-polyacrylamide gels, which were fixed overnight, dried, and exposed to a tritium phosphorimaging screen. Histone acetylation was analyzed using a Storm PhosphorImager with ImageQuant software.

Transient Transfections—Transient transfections were done using the $CaPO_4$ technique described (14). Briefly, CHO cells were transfected with the MHC promoter-reporter construct, TAF7, or CIITA expression vectors as detailed in individual experiments. The tsBN462 cells were transfected in a similar manner but in two sets. After 24 h, one set was moved from 32 to 39 °C (restrictive temperature), whereas the other was left at 32 °C (permissive temperature). All cells were harvested 48 h after transfection and assayed for chloramphenicol acetyltransferase (CAT) activity as described previously (15). CAT activity was normalized to total protein concentration.

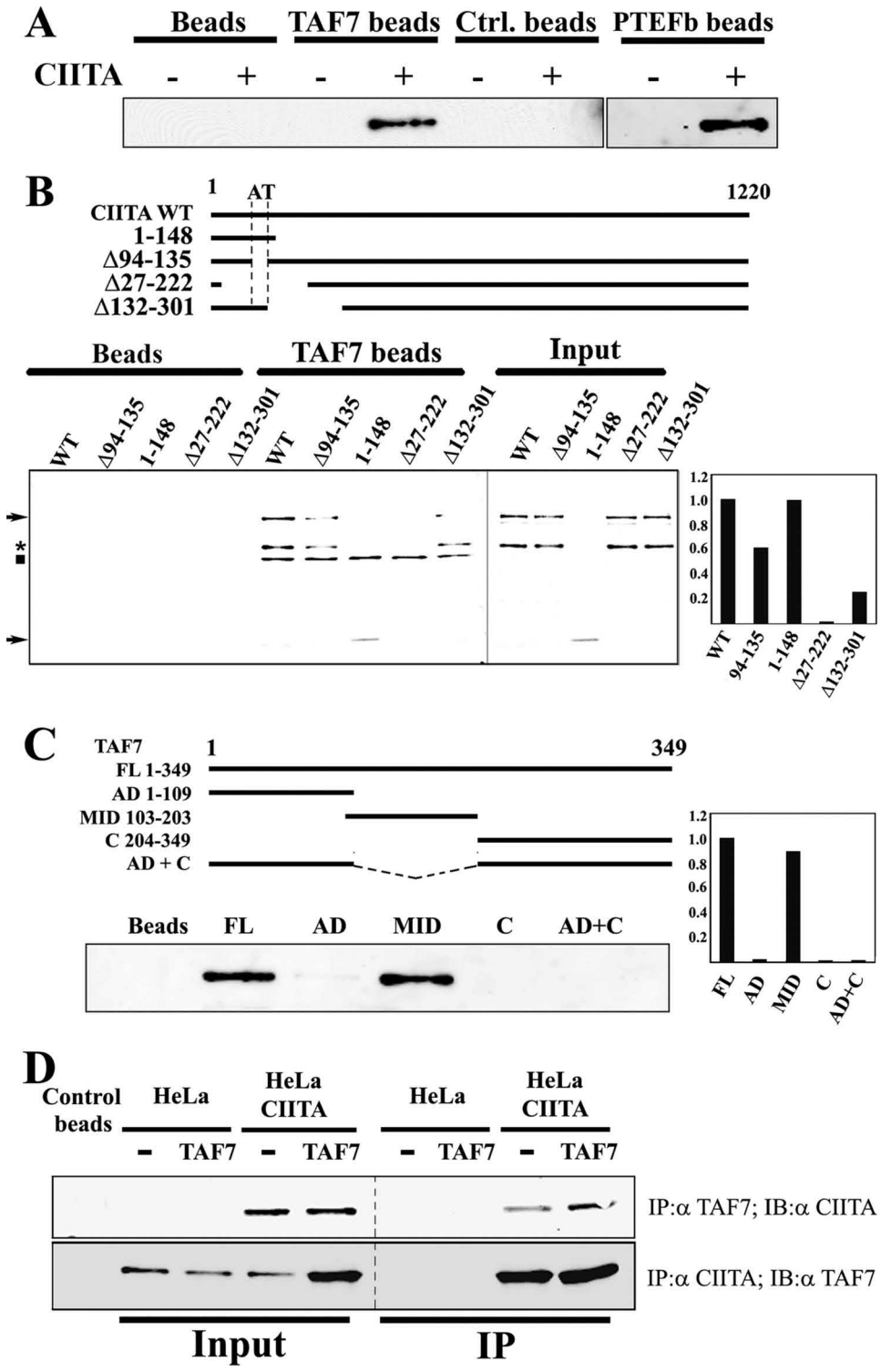
In Vitro Transcription—*In vitro* transcription reactions were done by incubating 100 μg of nuclear extract from HeLa (Promega) or stable HeLa/CIITA cells with varying amounts of TAF7 as indicated in the figure legends. The samples were preincubated at 23 °C for 15 min in the presence of 0.8 mM rNTPs, 6 mM $MgCl_2$ and 250 μM trichostatin A. The reaction was initiated by the addition of 2 μg of −313CAT DNA and then incubated for 1 h. Transcripts were revealed by primer extension of −313CAT.

Gene Expression Profiling of TAF7 and TAF1 Knockdown-affected Transcripts—Transfections with siRNA, RNA purification, and array analysis are described in detail in the supplemental data. Briefly, RNA was isolated from 293T cells transfected with control scrambled siRNA or TAF7 or TAF1 siRNA (20 nM; Dharmacon). Total RNA extracted was used for microarray analysis with the oligonucleotide microarray HG-U133 Plus 2.0 (Affymetrix, Santa Clara, CA) according to the manufacturer's recommendations. A minimum of five replicates were analyzed for each condition at each time point. Data were analyzed using the Genomatix ChipInspector program; up- and down-regulated genes were defined by significance analysis of microarray (SAM) analysis with false discovery rate (FDR) = 0.7% and a minimal ratio of 0.2. The data were also analyzed by MAS5 analysis; probe sets with $p > 0.01$ were excluded. The microarray results were validated by RT-PCR of both MHC class I genes and randomly selected genes that were observed to be either up- or down-regulated on the microarrays (supplemental Table S3). The results were concordant.

RESULTS

TAF7 Interacts with CIITA in Vitro and in Vivo—We have shown previously that TAF7 binds to the TFIID component TAF1 and inhibits its essential AT activity (3). Because the IFNγ-induced co-activator CIITA bypasses the requirement for TAF1, we considered the possibility that TAF7 also interacts with and regulates CIITA. To test this hypothesis, we first assessed the ability of TAF7 to interact with CIITA in a pulldown assay using purified recombinant proteins. As shown in Fig. 1*A*, immobilized TAF7, like P-TEFb but not the control p58 protein, specifically and efficiently pulled down CIITA. Thus, recombinant CIITA forms a complex with TAF7 that is sufficiently stable to be pulled down *in vitro*.

CIITA is a 135-kDa protein composed of a series of structural and functional domains. Its AT domain is located between amino acids 94 and 135 (12). To identify the TAF7-



interacting domain, CIITA proteins with various truncations or deletions were examined for their ability to interact with TAF7. In pulldown assays with immobilized TAF7, a CIITA fragment spanning amino acids 1–148 bound most efficiently, whereas a CIITA mutant with a deletion between amino acids 27 and 222 did not bind at all (Fig. 1*B*). CIITA mutants with deletions between amino acids 94 and 135 or between amino acids 132 and 301 bound only weakly to TAF7. Taken together, these data map the TAF7 interaction site on CIITA between amino acids 27 and 148, a region that completely overlaps the AT domain located between amino acids 94–135 but also extends both N- and C-terminal to it. Thus, TAF7 binding encompasses, but is not restricted to, the AT domain of CIITA.

TAF7 is a 55-kDa protein consisting of 349 amino acids. To map the CIITA-interacting domain of TAF7, we performed pulldown assays of full-length CIITA protein with immobilized GST-tagged TAF7 peptide fragments. CIITA bound most efficiently to a TAF7 fragment spanning the mid-domain (amino acids 103–203) (Fig. 1*C*, *MID*). In contrast, neither a TAF7 N-terminal fragment consisting of amino acids 1–109 (Fig. 1*C*, *AD*) nor a TAF7 fragment derived from the C-terminal end (amino acids 204–349) (Fig. 1*C*, *C*) interacted with CIITA. Additionally, a mutant TAF7 protein lacking the mid-domain was also incapable of interacting with CIITA (Fig. 1*C*). A pulldown assay of TAF7 fragments with immobilized CIITA showed complementary results (supplemental Fig. S1) Thus, the mid-domain segment of TAF7 spanning amino acids 103–203 is responsible for binding CIITA. Interestingly, the mid-domain region of TAF7 that binds to CIITA is the same region that binds TAF1, encompassing their respective AT domains (3).

To determine whether CIITA interacts with TAF7 *in vivo*, native HeLa cells or HeLa cells stably expressing CIITA were transfected with either an expression vector for TAF7 or a control empty vector. Nuclear extracts were immunoprecipitated with either anti-TAF7 or anti-CIITA antibody and immunoblotted with the reciprocal antibody. Anti-TAF7 antibody co-immunoprecipitated CIITA from TAF7-transfected and control nuclear extracts of HeLa/CIITA cells but not native HeLa cells, reflecting the ability of both exogenous and endogenous TAF7 to specifically interact with CIITA (Fig. 1*D*). Similarly, anti-CIITA antibody co-immunoprecipitated TAF7 from HeLa/CIITA (but not native HeLa) cell nuclear extracts. Taken together, these results demonstrate that TAF7 interacts with CIITA both *in vivo* and *in vitro*.

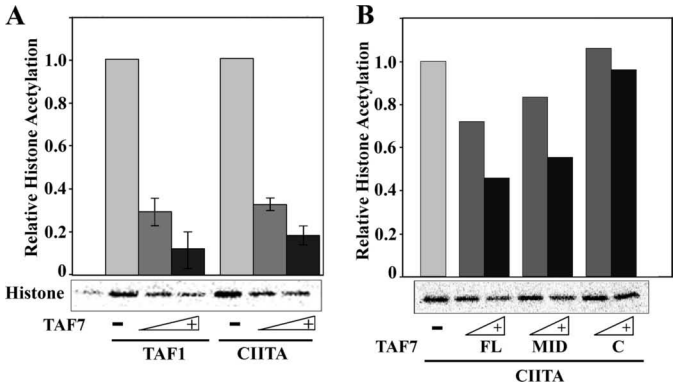


FIGURE 2. *A*, TAF7 inhibits the AT activity of CIITA and TAF1. AT assays of CIITA and TAF1 were performed in the presence or absence of recombinant TAF7 using histone H4 as a substrate. Approximately molar equivalents of TAF1 (250 ng) and CIITA (150 ng) were used in the reactions. The effect of increasing levels of TAF7 (250 and 500 ng) on the acetylation of histone H4 was quantitated by densitometric analysis of the autoradiograms. These results are representative of two independent experiments. The results are graphically represented with all the values relative to acetylation in the absence of TAF7. *Error bars* represent S.E. ($n = 3$). *B*, the TAF7 mid-domain inhibits CIITA AT activity. AT assays were done with CIITA as described above either alone or without increasing amounts of full-length (*FL*) TAF7 (250 and 500 ng), the TAF7 mid-domain (*MID*), and the C-terminal end fragment (*C*). Molar equivalents of full-length TAF7 and TAF7 fragments were used in the assay. The results are expressed relative to the level of histone H4 acetylation in the absence of TAF7 or TAF7 fragments.

TAF7 Inhibits the AT Activity of CIITA—Our finding that TAF7 binding spans the AT domain of CIITA led us to examine the effect of TAF7 on CIITA AT activity. As shown in Fig. 2*A*, the addition of increasing amounts of TAF7 in a HAT assay resulted in a substantial reduction in the ability of CIITA to acetylate a histone H4 substrate. The magnitude of inhibition was similar to the previously documented inhibition of TAF1 AT activity by TAF7. We have previously reported that the AT activity of yeast HAT1 is not affected by TAF7 (3), indicating that TAF7 is neither a histone deacetylase nor a nonspecific inhibitor. The effect of TAF7 was not due to competitive inhibition because TAF7 itself was not acetylated (data not shown), and the histone substrate was in molar excess relative to TAF7. The inhibitory domain of TAF7 mapped to the mid-domain (amino acids 103–203), the segment that binds to CIITA, and was as efficient as full-length TAF7 in inhibiting CIITA AT activity (Fig. 2*B*). In con-

FIGURE 1. *A*, TAF7 binds to CIITA *in vitro*. FLAG-CIITA (1 μg) was incubated with either GST-TAF7 (1 μg) or the negative control GST-p58 (1 μg) immobilized on beads or on beads alone (*left panel*) or with His-tagged P-TEFb (1 μg), as a positive control, immobilized on beads (*right panel*). Bound CIITA was resolved by gel electrophoresis and subjected to immunoblotting with anti-FLAG antibody. *B*, TAF7 binding overlaps the AT domain of CIITA. Full-length FLAG-tagged CIITA (2 μg) and equimolar amounts of mutant CIITA proteins (*upper panel*) were incubated with or without FLAG-tagged TAF7 protein (2 μg) immobilized on protein G beads with monoclonal anti-TAF7 antibody. Bound protein was resolved on a 15% acrylamide gel, which resolves CIITA from TAF7 but does not resolve the different CIITA deletion proteins from each other or from native CIITA. Following transfer, the filter was subjected to immunoblotting with anti-FLAG antiserum, which reveals both CIITA proteins and TAF7. CIITA proteins are identified by *arrows*; a degradation product of CIITA is denoted with an *asterisk* (*lower left panels*). In the TAF7-bead pulldown lanes, the anti-FLAG antiserum revealed the TAF7 band common to all the lanes (■) in addition to the CIITA proteins. The results are representative of two independent experiments. The graph (*right panel*) quantitates the relative binding efficiencies of the various CIITA proteins corrected for the inputs. *C*, CIITA binds to the mid-domain of TAF7. Recombinant FLAG-tagged CIITA protein (1 μg) was incubated with GST-tagged full-length (*FL*) TAF7 (1 μg) and equimolar amounts of truncated TAF7 proteins (*upper panel*) immobilized on GST-Sepharose beads. Bound protein was detected with anti-CIITA antibody as described above. The relative binding efficiencies of CIITA for the TAF7 proteins are graphically represented corrected for input (*right panel*). *AD*, activation domain; *MID*, mid-domain; *C*, C-terminal end. *D*, TAF7 binds to CIITA *in vivo*. Nuclear extracts were prepared from HeLa cells and HeLa cells stably expressing CIITA either directly or following transfection with a TAF7 expression vector. Reciprocal immunoprecipitations (*IP*) and immunoblotting (*IB*) were performed with anti-TAF7 and anti-CIITA antibodies using these nuclear extracts, as indicated. Control beads were coated with normal IgG. It is important to note that the input of the HeLa/TAF7 extract was half of the HeLa-alone extract; all other inputs were equivalent.

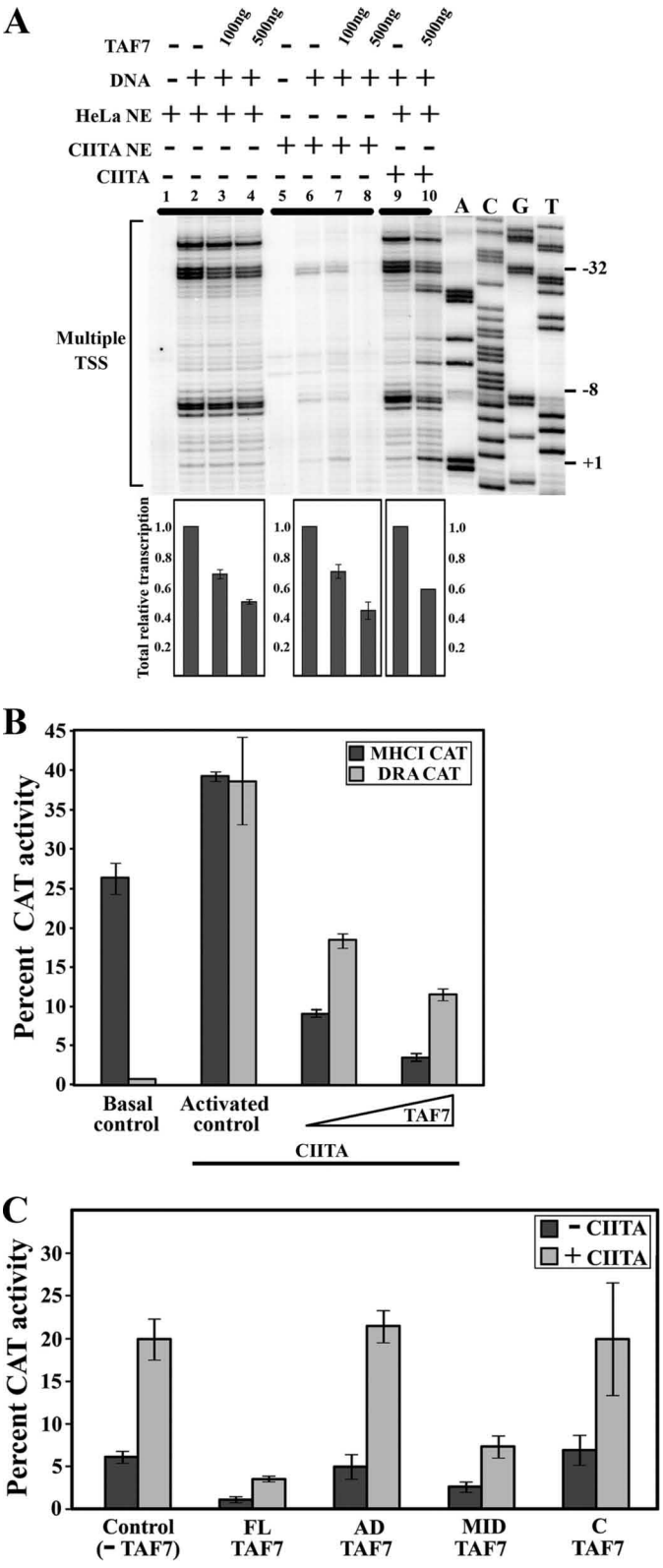


FIGURE 3. *A*, TAF7 represses transcription *in vitro* in the presence of CIITA. *In vitro* transcription reactions utilized either commercially prepared HeLa nuclear extracts (*NE*; Promega) alone (*upper panel*, *lanes 1–4*) or supplemented with 150 ng of purified recombinant CIITA (*lanes 9* and *10*) or nuclear extracts from HeLa cells stably transfected with CIITA (*lanes 5–8*). The *in vitro* transcription reactions were done in the absence or presence of increasing amounts of TAF7 as indicated. Total transcriptional activity was assessed from band intensities of transcripts initiating between positions +1 and −40 in each sample (multiple transcription start sites (*TSS*)). The graph in the *lower panel* represents results from four independent experiments, and *error bars* represent S.E. It should be noted that the HeLa/CIITA cells express cell-surface MHC class II molecules, indicative of functional CIITA; nevertheless, the *in vitro* activity of the HeLa/CIITA nuclear extracts prepared in the laboratory was consistently lower than that of the commercially prepared extract. *B*, TAF7 represses CIITA-mediated transcriptional activation *in vivo*. CHO cells were transfected with 5 μg of an MHC class I (*MHCI*) core promoter construct or an MHC class II DRα promoter ligated to CAT reporters either alone (basal control) or with a CIITA expression vector (activated control) or CIITA (25 ng) in combination with 2.5 and 5.0 μg of TAF7 expression vector, respectively. The total amount of DNA in all transfections was kept constant, adjusting with the empty vector. CAT activity is expressed normalized to total protein concentration. *Error bars* represent S.E. ($n = 3$). *C*, the TAF7 mid-domain is sufficient to repress CIITA-mediated transcriptional activation. CHO cells were transfected as described above with the MHC class I core promoter construct either alone or with CIITA in combination with vectors expressing full-length (*FL*) TAF7, the TAF7 activation domain (*AD*), the TAF7 mid-domain (*MID*), or the C-terminal end fragment (*C*). CAT activity is expressed normalized to total protein concentration. *Error bars* represent S.E. ($n = 3$).

trast, the C-terminal domain of TAF7 neither bound stably to CIITA nor inhibited its AT activity. Taken together, these results clearly demonstrate that TAF7 binds to the AT domain of CIITA and inhibits its AT activity.

TAF7 Represses Transcriptional Activation by CIITA in Vitro and in Vivo—CIITA-mediated transactivation of MHC genes depends on the AT activity of CIITA (12). In light of our finding that TAF7 inhibited the AT activity of CIITA (Fig. 2*A*), we investigated the effect of TAF7 on CIITA-mediated activation of both MHC class I and II transcription. We have previously demonstrated that class II transcription is CIITA-dependent and TAF1-independent, whereas class I transcription is regulated by both, but independently (11, 16, 17). Furthermore, overexpression of TAF7 represses TAF1-dependent constitutive transcription (3).

To examine the effect of TAF7 on CIITA-activated transcription, its effect on *in vitro* transcription of the MHC class I promoter was examined in nuclear extracts either from native HeLa cells or from HeLa cells stably transfected with CIITA (Fig. 3*A*). Under both constitutive and activated conditions, MHC class I transcription initiates at multiple sites both *in vitro* (11) and *in vivo* (18). The addition of increasing amounts of TAF7 to native HeLa extract reduced the overall extent of *in vitro* transcription from the class I promoter (Fig. 3*A*), as reported previously. Importantly, TAF7 also reduced transcription of the class I promoter in extracts from HeLa cells stably transfected with CIITA and in native HeLa nuclear extract supplemented with recombinant CIITA protein.

The effect of TAF7 overexpression on CIITA activation of transcription *in vivo* was assessed by transfection of CHO cells, which express no endogenous CIITA. CHO cells were cotransfected with a CIITA expression vector, increasing concentrations of a TAF7 expression vector, and either MHC class I or II DRA promoters/reporters. In the absence of TAF7, CIITA induced *de novo* expression of the class II promoter and enhanced transcription of the class I promoter above the control level of its constitutive TAF1-dependent transcription (Fig. 3*B*). Importantly, increasing amounts of TAF7 increasingly repressed the CIITA-activated transcription of both promoters. (Exogenous TAF7 did not affect the level of *de novo* CIITA protein expressed, even at the highest

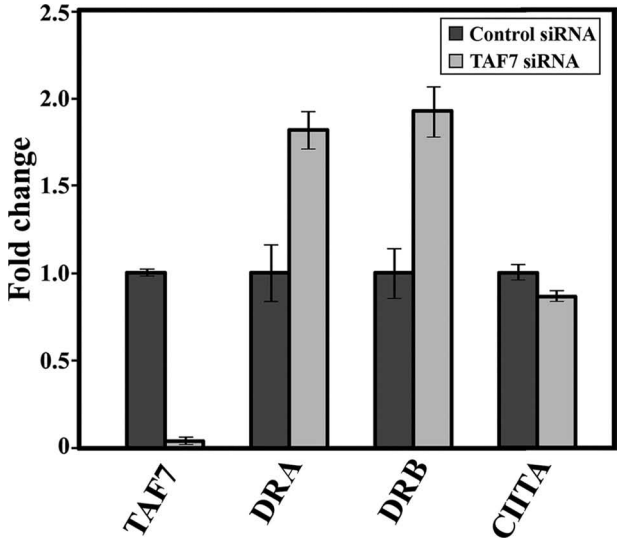


FIGURE 4. **CIITA-dependent MHC class II gene expression is affected by TAF7 knockdown.** HeLa cells stably expressing CIITA were transfected with either control scrambled or TAF7-specific siRNA (20 nM). Expression of TAF7, CIITA, and MHC class II genes *DRA* and *DRB* was quantified by real-time PCR at day 5. Gene expression in TAF7 siRNA-transfected samples is graphically represented as -fold change compared with expression in cells transfected with control siRNA, which is expressed as 1. *Error bars* represent S.E. ($n = 4$). The graph is representative of two independent experiments, each performed in duplicate.

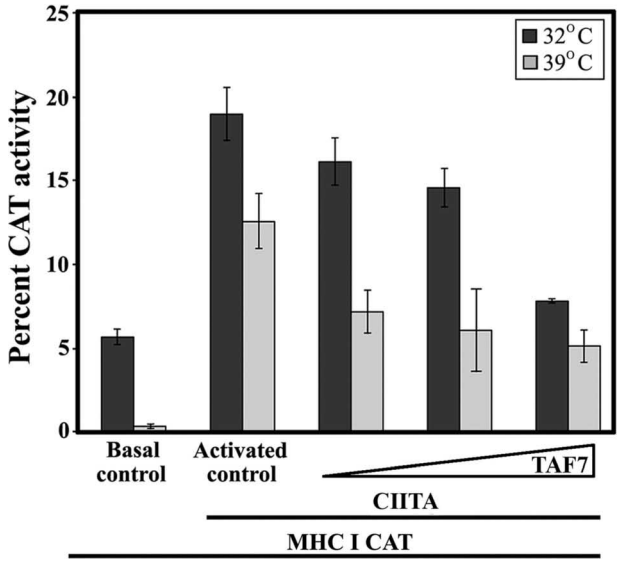


FIGURE 5. **TAF7 represses CIITA-activated transcription in the absence of functional TAF1.** The MHC class I core promoter-CAT reporter construct (5 μg) was cotransfected with CIITA alone (75 ng) or with TAF7 (1.0, 2.5, and 5.0 μg). The total amount of transfected DNA was held constant by adjusting with empty vector. Replicate transfections were incubated at the permissive temperature (32 °C) or at the nonpermissive temperature (39 °C). CAT activity is as described in Figure 3. *Error bars* represent S.E. ($n = 3$).

concentration.) Thus, TAF7-mediated repression of MHC class I and II transcription was not due to nonspecific repression of transcription or to decreased CIITA protein levels (supplemental Fig. S2). Interestingly, TAF7 repressed transcription of the class I promoter to a level below the basal TFIID-dependent level, consistent with the interpretation that TAF7 repressed both CIITA-activated transcription and TAF1-dependent constitutive expression.

To map the functional domain(s) of TAF7 responsible for regulating CIITA activity *in vivo*, expression vectors with fragments of TAF7 comprising the activation domain, mid-domain, and C-terminal domain of TAF7 were tested for their ability to repress MHC class I promoter/reporter activity. The mid-domain of TAF7 was almost as efficient as full-length TAF7 in repressing transcription, whereas the activation and C-terminal domains had no effect (Fig. 3*C*). These results are consistent with the *in vitro* results showing that the TAF7 mid-domain is responsible for binding to CIITA (Fig. 1*C*) and regulating its AT activity (Fig. 2*B*).

To further confirm the effect of TAF7 on CIITA-mediated transcription *in vivo*, HeLa/CIITA cells, which express endogenous MHC class II genes, were depleted of TAF7 by siRNA knockdown. As shown in Fig. 4, the steady-state levels of two CIITA-dependent class II genes, *DRA* and *DRB*, were increased in the absence of TAF7. However, the expression of CIITA remained constant. Thus, the increased expression of *DRA* and *DRB* resulted from the derepression of CIITA AT activity by the removal of TAF7. (Endogenous levels of TAF7 and CIITA were comparable in untreated HeLa/CIITA cells (supplemental Fig. S3).) Thus, TAF7 inhibits CIITA-mediated transcriptional activation *in vivo*.

Repression of CIITA-activated Transcription by TAF7 Does Not Depend on Functional TAF1—The above results suggested that TAF7-negative regulation of CIITA-mediated activation of transcription occurs through its repression of the essential CIITA AT activity. Although it has been shown previously that CIITA-activated transcription bypasses the requirement for functional TAF1 (12), the possibility remained that TAF7 repression of CIITA activation might require TAF1 AT activity. Therefore, we next examined whether TAF7 repression of CIITA-activated transcription depends on functional TAF1.

The hamster cell line tsBN462 (19) has a temperature-sensitive mutation in TAF1: AT activity is abrogated at the restrictive temperature (39 °C). Using tsBN462 cells, we have shown previously that CIITA activates MHC class I transcription at both permissive and restrictive temperatures, demonstrating that CIITA-mediated activation is TAF1-independent (13). Therefore, we examined the effect of TAF7 on CIITA activation of the class I promoter construct in tsBN462 cells (Fig. 5). As observed previously, constitutive class I promoter activity depended on functional TAF1. In contrast, CIITA activated class I promoter function at both the permissive and restrictive temperatures. At the permissive temperature, TAF7 repressed class I transcription, consistent with its inhibition of both TAF1- and CIITA-dependent transcription. Importantly, TAF7 repressed CIITA-activated transcription at the restrictive temperature, at which TAF1 is inactive (Fig. 5). Therefore, TAF7 repression of CIITA-activated transcription is independent of TAF1 function, suggesting that TAF7 independently regulates constitutive and activated expression of MHC class I genes.

TAF7 Regulates a Set of Transcripts Distinct from That of TAF1—The above findings show that TAF7 independently regulates CIITA and TAF1 in the context of MHC promoters.

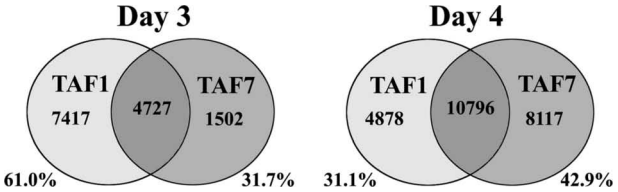


FIGURE 6. **TAF7 and TAF1 depletions affect distinct subpopulations of transcripts.** 293T cells were transfected with siRNA specific for TAF7 or TAF1 or with control scrambled siRNA (20 nM). Cells were harvested after days 3 and 4; the relative abundance of transcripts was assessed by hybridization to an Affymetrix array. Data were analyzed using the Genomatix program, and the *numbers* represent total genes affected. The effects of selected transcripts were verified by RT-PCR (supplemental Table S2).

TABLE 1
TAF7 depletion differentially affects TAF1-dependent and TAF1-independent transcripts

	Effect of TAF7 depletion[a]			
	Up-regulated	Down-regulated	Total	Up-regulated
	n	*n*	*n*	%
TAF1-independent	4712	3405	8117	58
TAF1-dependent	1958	8838	10,796	18.1

[a] Day 4 p value was $<2.2e{-}16$ by Fisher's exact test.

This raised the possibility that TAF7 may regulate other promoters independently of either CIITA or TAF1. To assess this possibility, the spectrum of transcripts affected by knockdown of TAF7 was compared with transcripts affected by TAF1 knockdown in 293T cells, which do not express CIITA. In this way, we would be able to identify any transcripts affected by TAF7 but not by either TAF1 or CIITA. siRNAs specific for either TAF1 or TAF7 were transfected into 293T cells; total RNA and protein were isolated 3, 4, and 5 days post-transfection and analyzed for levels of TAF7 and TAF1. Both genes were knocked down substantially by 3 days and continued to decrease through day 4 (supplemental Fig. S4). Unlike the HeLa/CIITA cells, 293 cells transfected with either siRNA showed evidence of significant cell death by day 5. Therefore, although the TAF7 and TAF1 RNA levels continued to decrease at day 5, transcript analysis was limited to days 3 and 4. Knockdown of TAF7 or TAF1 affected expression of 65.2 and 54.4% of all transcripts, respectively, after 4 days (supplemental Fig. S5 and Table S1). Among the transcripts most affected by TAF7 depletion were those involved in cell growth and proliferation (supplemental Table S2). This is consistent with our previous finding that TAF7 depletion inhibits cell growth (5). Importantly, a comparison of the transcripts affected by TAF7 and TAF1 knockdown after 4 days revealed that 42.9% of the transcripts affected by TAF7 knockdown were not affected by TAF1 knockdown; similarly, 31.1% of TAF1-dependent transcripts were not affected by TAF7 (Fig. 6). Surprisingly, TAF7 knockdown differentially affected TAF1-independent and TAF1-dependent transcripts. Thus, similar to class II genes, TAF1-independent genes were predominantly up-regulated, whereas like class I genes, TAF1-dependent genes were largely down-regulated (Table 1). In addition, the molecular functions of transcripts affected by the knockdown of the two factors, although similar, were distinct (supplemental Table S2). Interestingly, the effects of TAF7 or TAF1 knockdown on expression were not correlated with whether the promoters were TATA or non-TATA. Taken together, these results indicate that TAF7 regulates the transcription of a subset of genes that are not affected by either CIITA or TAF1.

DISCUSSION

The TBP-associated factor TAF7 was originally isolated as an integral (and presumed structural) component of TFIID. TAF7 was shown subsequently to function as a "checkpoint regulator" for each of the steps in constitutive TFIID-dependent transcription initiation (4). Within TFIID, TAF7 binds to and inhibits the essential AT activity of TAF1, which is required for the formation of the first phosphodiester bond (3). In addition, TAF7 also inhibits the critical kinase activities of TFIIH and P-TEFb, which are necessary for the recruitment of capping and splicing complexes, respectively, and are critical for proper transcription initiation and the transition to elongation (5).

Remarkably, TAF7 also interacts with the IFNγ-inducible transcriptional co-activator CIITA, binding to its AT domain and inhibiting its intrinsic AT activity. Overexpression of TAF7 inhibits CIITA-activated transcription, and this inhibition is independent of TAF1. Thus, TAF7 regulation of transcription initiation is not restricted to TAF1-dependent transcription.

Furthermore, we found that 10–30% of transcripts in 293 cells are TAF1-independent, which is within the range reported in yeast (20, 27). Of the TAF1-independent transcripts, between 30 and 40% are TAF7-dependent. These findings fundamentally alter our understanding of the roles of TAFs in transcription by demonstrating that TAF7 plays a critical role in regulating TAF1-independent transcription initiation.

The finding that TAF7 functions independently of a TAF1-TFIID complex significantly extends the growing body of evidence that TAFs contribute to transcriptional regulation outside of their assembly in the intact TFIID complex. For example, we have observed TAF7 in non-TFIID complexes *in vivo* (5). Also, during myogenesis, TBP and all TAFs are lost with the exception of TAF3, which, together with TRF3, supports transcription (21). TAF1 has been reported to interact with and ubiquitinate the androgen receptor during prostate cancer (22). TAFs are also associated with a variety of non-TFIID transcription complexes. A number of TAFs are found in SAGA/PCAF complexes (23, 24). The TFTC transcription initiation complex consists of a subset of TAFs but lacks TAF1 and TBP (25). Furthermore, it has been reported that robust transcription initiation occurs even with a severe disruption of the TFIID complex (26), providing further evidence that TAFs can function independently.

We propose that TAF7 performs the same function in TAF1-independent CIITA-activated transcription as it does in constitutive transcription. Because TAF7 inhibits the AT activity of CIITA during CIITA-activated transcription, it appears to function as a checkpoint regulator of activated transcription, ensuring that initiation does not occur until the PIC recruited by CIITA is completely assembled. Because CIITA recruits TFIIH and P-TEFb to the transcription machinery (27, 28), we speculate that TAF7 functions to regulate their activities in the context of both activated and constitu-

tive transcription. Because transcriptional events are extremely transient and frequently overlapping, it is not possible to clearly distinguish between the effects of TAF7 on CIITA, P-TEFb, and TFIIH. Therefore, the possibility that transcriptional inhibition caused by TAF7 might be an overlapping response of its interactions with all three factors cannot be excluded.

Although TAF7 inhibits the AT activity of both CIITA and TAF1, the mechanisms involved appear to differ. Whereas TAF7 interacts with the AT domain of both proteins, the mid-domain of TAF7 is sufficient to bind CIITA and to inhibit its AT activity, but both the mid-domain and N-terminal inhibitory domain of TAF7 are required to suppress TAF1 AT activity. This is consistent with our earlier finding that the CIITA AT domain is homologous to the AT domain of CBP (cAMP-response element-binding protein-binding protein) but has no homology to the TAF1 AT domain (12).

Earlier studies have shown that CIITA nucleates an enhanceosome consisting of a series of DNA-binding transcription factors such as RFX5, cAMP-response element-binding protein, and NF-Y and associated chromatin-modifying enzymes that target upstream enhancer elements, including a cyclic AMP-response element (12, 29–34). The interaction of TAF7 with CIITA raises the intriguing possibility that CIITA also assembles a TFIID-like complex that is capable of substituting for TAF1 function. Indeed, previous studies have demonstrated that CIITA binds TAF6, TAF9, TBP, TFIIH, and P-TEFb (27, 35, 36). In this way, the functions of CIITA would parallel those of TFIID, integrating the effects of the upstream elements on the promoter. The existence of a CIITA-anchored TFIID-like complex will be the focus of future studies.

The possibility that CIITA may assemble a TFIID-like complex further suggests that there may be a variety of TFIID-like complexes responsible for mediating transcription initiation. Our finding that in 293T cells, which do not contain CIITA, TAF7 depletion affects the expression of a subset of genes that are not affected by TAF1 depletion supports the model that TAF7 regulates TAF1-independent transcription complexes. This conclusion is supported further by earlier studies of essential TAFs in yeast, where only 9–14% of genes depend on TAF1, whereas 24% depend on TAF7 (20). Our analysis of the effects of depletion of TAF7 and TAF1 on gene expression also revealed that distinct gene ontology functions were targeted, further suggesting that TAF7 plays a regulatory role in transcription that is distinct from TAF1.

Surprisingly, depletion of TAF7 differentially affected TAF1-dependent and TAF1-independent transcription: TAF1-dependent transcripts decreased, whereas TAF1-independent transcripts increased. We speculate that this difference may be related to interactions downstream of PIC assembly. We have proposed that in TAF1-dependent transcription, TAF7 acts as a checkpoint regulator, ensuring an orderly progression through the different steps of transcription initiation, pausing, and transition to efficient elongation (5). In this context, TAF7 prevents premature transcription initiation by inhibiting the TAF1 AT activity until the PIC is fully assembled. Depletion of TAF7 could relieve the inhibition of TAF1 AT activity, resulting in premature initiation. As in the case of HIV Tat, this could lead to abortive transcripts. In contrast to TAF1, which is not known to interact with other PIC components, CIITA functionally interacts with P-TEFb (27). We speculate that this interaction might stabilize initiated transcripts and support productive elongation. In the absence of TAF7, both the AT activity of CIITA and the kinase activity of P-TEFb would be derepressed, leading to an increase in the steady-state levels of RNA. This difference in the interactions of TAF1 and CIITA with downstream factors could explain the different effects of TAF7 depletion. Similar explanations might be adduced for other mechanisms of TAF1-independent transcription. However, the mechanism(s) responsible for the differential effect remains to be determined.

In conclusion, this study demonstrates that the role of TAF7 extends beyond canonical TFIID-dependent transcription because it also plays a critical role in regulating CIITA-dependent transcription. Importantly, in defining the regulation of CIITA-dependent transcription by TAF7, this study elucidates a critical regulatory mechanism of the immunological repertoire in terms of MHC I and II gene expression. This first report of a TFIID component regulating a key non-TFIID transcriptional activator emphasizes the importance of TAF7 as a critical regulator of transcription initiation.

Acknowledgments—We thank Mike Radonovich for help during preparation of the microarrays and Aaron Rosenberg for assistance in confirming the in vivo binding of TAF7 and CIITA. We also thank all members of the laboratory for helpful discussions throughout these studies and Drs. Brian Lewis, David Levens, Ranjan Sen, Stoney Simons, Al Singer, and Aparna Kotekar for helpful discussions and critical review of the manuscript.

REFERENCES

1. 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., Nakatani, Y., and Allis, C. D. (1996) *Cell* **87,** 1261–1270
2. 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) *Proc. Natl. Acad. Sci. U.S.A.* **95,** 11601–11606
3. Gegonne, A., Weissman, J. D., and Singer, D. S. (2001) *Proc. Natl. Acad. Sci. U.S.A.* **98,** 12432–12437
4. Gegonne, A., Weissman, J. D., Zhou, M., Brady, J. N., and Singer, D. S. (2006) *Proc. Natl. Acad. Sci. U.S.A.* **103,** 602–607
5. Gegonne, A., Weissman, J. D., Lu, H., Zhou, M., Dasgupta, A., Ribble, R., Brady, J. N., and Singer, D. S. (2008) *Proc. Natl. Acad. Sci. U.S.A.* **105,** 5367–5372
6. Singer, D. S., and Maguire, J. E. (1990) *Crit. Rev. Immunol.* **10,** 235–257
7. van den Elsen, P. J., and Gobin, S. J. (1999) *Microbes Infect.* **1,** 887–892
8. van den Elsen, P. J., Holling, T. M., Kuipers, H. F., and van der Stoep, N. (2004) *Curr. Opin. Immunol.* **16,** 67–75
9. Vora, A. R., Rodgers, S., Parker, A. J., Start, R., Rees, R. C., and Murray, A. K. (1997) *Br. J. Cancer.* **76,** 836–844
10. Mozes, E., Lovchik, J., Zinger, H., and Singer, D. S. (2005) *Lupus* **14,** 308–314
11. Howcroft, T. K., Raval, A., Weissman, J. D., Gegonne, A., and Singer, D. S. (2003) *Mol. Cell. Biol.* **23,** 3377–3391
12. Raval, A., Howcroft, T. K., Weissman, J. D., Kirshner, S., Zhu, X. S., Yokoyama, K., Ting, J., and Singer, D. S. (2001) *Mol. Cell* **7,** 105–115
13. Weissman, J. D., Howcroft, T. K., and Singer, D. S. (2000) *J. Biol. Chem.* **275,** 10160–10167
14. Howcroft, T. K., Palmer, L. A., Brown, J., Rellahan, B., Kashanchi, F.,

Brady, J. N., and Singer, D. S. (1995) *Immunity* **3,** 127–138
15. Howcroft, T. K., Strebel, K., Martin, M. A., and Singer, D. S. (1993) *Science* **260,** 1320–1322
16. Riley, J. L., Westerheide, S. D., Price, J. A., Brown, J. A., and Boss, J. M. (1995) *Immunity* **2,** 533–543
17. Zhou, H., and Glimcher, L. H. (1995) *Immunity* **2,** 545–553
18. Lee, M. P., Howcroft, K., Kotekar, A., Yang, H. H., Buetow, K. H., and Singer, D. S. (2005) *Genome Res.* **15,** 1189–1197
19. Hayashida, T., Sekiguchi, T., Noguchi, E., Sunamoto, H., Ohba, T., and Nishimoto, T. (1994) *Gene* **141,** 267–270
20. Shen, W. C., Bhaumik, S. R., Causton, H. C., Simon, I., Zhu, X., Jennings, E. G., Wang, T. H., Young, R. A., and Green, M. R. (2003) *EMBO J.* **22,** 3395–3402
21. Deato, M. D., and Tjian, R. (2007) *Genes Dev.* **21,** 2137–2149
22. Tavassoli, P., Wafa, L. A., Cheng, H., Zoubeidi, A., Fazli, L., Gleave, M., Snoek, R., and Rennie, P. S. (2010) *Mol. Endocrinol.* **24,** 696–708
23. Grant, P. A., Schieltz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R., 3rd, and Workman, J. L. (1998) *Cell* **94,** 45–53
24. Ogryzko, V. V., Kotani, T., Zhang, X., Schiltz, R. L., Howard, T., Yang, X. J., Howard, B. H., Qin, J., and Nakatani, Y. (1998) *Cell* **94,** 35–44
25. Wieczorek, E., Brand, M., Jacq, X., and Tora, L. (1998) *Nature* **393,** 187–191
26. Chen, Z., and Manley, J. L. (2003) *J. Biol. Chem.* **278,** 35172–35183
27. Kanazawa, S., Okamoto, T., and Peterlin, B. M. (2000) *Immunity* **12,** 61–70
28. Spilianakis, C., Kretsovali, A., Agalioti, T., Makatounakis, T., Thanos, D., and Papamatheakis, J. (2003) *EMBO J.* **22,** 5125–5136
29. Fontes, J. D., Kanazawa, S., Nekrep, N., and Peterlin, B. M. (1999) *Microbes Infect.* **1,** 863–869
30. Harton, J. A., and Ting, J. P. (2000) *Mol. Cell. Biol.* **20,** 6185–6194
31. Kirshner, S., Palmer, L., Bodor, J., Saji, M., Kohn, L. D., and Singer, D. S. (2000) *Mol. Endocrinol.* **14,** 82–98
32. Masternak, K., Muhlethaler-Mottet, A., Villard, J., Zufferey, M., Steimle, V., and Reith, W. (2000) *Genes Dev.* **14,** 1156–1166
33. Ting, J. P., and Trowsdale, J. (2002) *Cell* **109,** (suppl.) S21–S33
34. Zika, E., Greer, S. F., Zhu, X. S., and Ting, J. P. (2003) *Mol. Cell. Biol.* **23,** 3091–3102
35. Fontes, J. D., Jiang, B., and Peterlin, B. M. (1997) *Nucleic Acids Res.* **25,** 2522–2528
36. Mahanta, S. K., Scholl, T., Yang, F. C., and Strominger, J. L. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94,** 6324–6329