$TAF_{II}55$ binding to $TAF_{II}250$ inhibits its acetyltransferase activity

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The general transcription factor, TFIID, consists of the TATA-binding protein (TBP) associated with a series of TBP-associated factors (TAFs) that together participate in the assembly of the transcription preinitiation complex. One of the TAFs, $TAF_{II}250$, has acetyltransferase (AT) activity that is necessary for transcription of MHC class I genes: inhibition of the AT activity represses transcription. To identify potential cellular factors that might regulate the AT activity of $TAF_{II}250$, a yeast two-hybrid library was screened with a $TAF_{II}250$ segment (amino acids 848-1279) that spanned part of its AT domain and it's the domain that binds to the protein, RAP74. The TFIID component, $TAF_{II}55$, was isolated and found to interact predominantly with the RAP74-binding domain. $TAF_{II}55$ binding to $TAF_{II}250$ inhibits its AT activity. Importantly, the addition of recombinant $TAF_{II}55$ to *in vitro* transcription assays inhibits $TAF_{II}250$-dependent MHC class I transcription. Thus, $TAF_{II}55$ is capable of regulating $TAF_{II}250$ function by modulating its AT activity.

Transcription mediated by RNA polymerase II (RNAP) requires the assembly of a preinitiation complex at the promoter. Assembly is initiated by the association of the general transcription factor (GTF), TFIID, with the promoter. Subsequently, the GTFs TFIIB, TFIIE, TFIIF, and TFIIH enter the complex; interactions between the GTFs and RNAP result in transcription initiation and elongation (1). Recruitment of RNAP to the promoter results in the phosphorylation of its carboxyl terminal domain (CTD) by TFIIH, which is required for initiation. Reinitiation depends on the prior dephosphorylation of the CTD by a phosphatase that is activated by TFIIF (2). More recently, it has been demonstrated that the function of the TFIID complex itself is regulated. TFIID consists of the TATA-binding protein (TBP) associated with a series of TBP-associated factors (TAFs) that, together, participate in the assembly of the transcription preinitiation complex. Binding of TBP to its site on DNA is inhibited by the interaction of TBP with N-terminal polypeptide of the TFIID component, $TAF_{II}250$ (3). Thus, binding of TFIID to DNA seems to be regulated by allosteric changes in $TAF_{II}250$ conformation that expose the TBP-binding site.

In addition to preinitiation complex assembly, transcription depends on a cascade of enzymatic activities. Included among these is the acetyltransferase (AT) activity of $TAF_{II}250$ (4, 5). As we have shown, $TAF_{II}250$ AT is essential for MHC class I promoter activity (4). In a temperature-sensitive $TAF_{II}250$ mutant-cell line, the class I promoter is active at the permissive temperature, but inactive at the restrictive temperature where $TAF_{II}250$ loses its AT activity. Furthermore, the HIV transactivator (Tat) represses transcription from the MHC class I promoter by binding to the AT domain of $TAF_{II}250$ and inhibiting its AT activity (4). These findings suggested that the AT activity of $TAF_{II}250$ might be a normal cellular target for regulation of transcription.

In the present study, we report that $TAF_{II}250$ AT activity is regulated by $TAF_{II}55$, which inhibits the AT activity upon binding to $TAF_{II}250$ and represses class I transcription. Interestingly, $TAF_{II}55$ binds more efficiently to the $TAF_{II}250$ domain that binds the TFIIF component, RAP74 (RA4iD) than to the AT domain. We speculate that $TAF_{II}55$ is a normal, cellular regulator of transcription through this interaction with $TAF_{II}250$.

Materials and Methods

Constructs. The (Gal4) DNA-binding domain $TAF_{II}250$ vector was constructed by inserting the mouse $TAF_{II}250$ fragment (amino acids 2541–3839) (4) into the *Nco*I/*Bam*HI sites of the pAS1-CYH2 vector. The Gal4 activation domain-HeLa cell cDNA library was obtained from CLONTECH, and the mouse-thymus library kindly provided by A. Singer (National Institutes of Health, Bethesda, MD).

The Gal4 BD-AT and GAL4 BD-RAP74 interacting domain (RAPiD) vectors were generated by cloning into the *Nco*I and *Bam*HI sites of pAS1-CYH2, the *Nco*I and *Bam*HI linker-containing fragments of $TAF_{II}250$ extending from nucleotides 2551 to 3361 and from 3363 to 3843, respectively. The subclones of the $TAF_{II}250$ fragment, pCR3.1-$TAF_{II}250$-AT(amino acids 848-1120) and pCR3.1-$TAF_{II}250$-RAP74 (amino acids 1120–1279) were generated as described (6). The control SNAP23-expression plasmid was as described (7).

The pF:55–11d expressing the Flag-tagged $TAF_{II}55$ was kindly provided by R. Roeder (Rockefeller Univ., New York). The ADTA$F_{II}55$ clone was generated by PCR amplification of $TAF_{II}55$ between the *Nde*I and *Bam*HI sites, to generate a DNA fragment spanning the first 325 nucleotides of $TAF_{II}55$. The MHC class I promoter construct, −313 chloramphenicol acetyltransferase (CAT), consisting of 313 bp of 5′ flanking sequences derived from the swine class I gene *PD1* ligated to the CAT reporter gene, have been described (8). Cytomegalovirus (CMV)-promoter construct was provided with HeLa nuclear extract *in vitro* transcription system kit (Promega).

Yeast Two-Hybrid Screening. *Saccharomyces cerevisiae* strain Y190 was sequentially transformed with the pAS1-$TAF_{II}250$ bait vector and either the HeLa cDNA library or the mouse-thymus cDNA library, according to the Matchmaker yeast two-hybrid protocol (CLONTECH). Approximately 5×10^5 and 1.6×10^6 of each library were transformed into Y190 cells carrying the GAL BD-$TAF_{II}250$ construct and were plated on selection medium lacking Trp, Leu, and His and supplemented with 50 mM 3-aminotriazole. Clones expressing His3 and β-galactosidase activity were isolated, and plasmid DNA was recovered and sequenced. The specificity of each clone was tested further by cotransformation into Y190 cells of the different clones with either Gal4 BD-$TAF_{II}250$ or Gal4 BD-SNAP23 (as negative control), with reselection on medium lacking Trp and Leu.

Abbreviations: TBP, TATA binding protein; TAF, TBP-associated factors; AT, acetyltransferase; Tat, an HIV transactivator; RAP74, 74-kd component of TFIIF; BD, binding domain; AD, activation domain; CMV, cytomegalovirus; GTF, general transcription factor; CTD, carboxyl terminal domain; RAPiD, RAP74 interacting domain; HAT, histone acetyltransferase.

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Clones were retested for His3 expression and β-galactosidase activity.

Isolation of Recombinant $TAF_{II}55$ and AD$TAF_{II}55$ Proteins. Purification of both Flag-tagged proteins was as described (9). Briefly, bacterial cultures were induced with isopropyl β-D-thiogalactoside (IPTG) but without rifampicin. Bacterial pellets from 1-liter cultures were resuspended in 30 ml of lysis buffer (20 mM Tris, pH 7.9/20% (vol/vol) glycerol/500 mM NaCl/0.2 mM EDTA/10 mM 2-mercapto-ethanol/0.2 mM PMSF and Roche protease inhibitors tablets) and lysed by sonication. After centrifugation, the supernatant was incubated for 1 h at 4°C with 600 μl of M2 agarose beads (Sigma). The M2 agarose with the bound protein was washed four times in BC300 buffer (20 mM Tris, pH 7.9/20% (vol/vol) glycerol/300 mM KCl/0.2 mM EDTA/1 mM DTT/0.1% Nonidet/0.2 mM PMSF and Roche protease inhibitors tablets) and then incubated with 600 μl of Flag peptide (0.2 mg/ml in BC300 buffer) for 20 min. The elution was repeated four times. The eluate then was loaded at 4°C on a G25 Sephadex column to eliminate the Flag peptide. Fractions containing the purified protein were combined and dialyzed at 4°C with 20 mM Tris, pH 7.9/10% (vol/vol) glycerol/150 mM KCl/0.1 mM EDTA/1 mM DTT/0.2 mM PMSF.

Purification of $dTAF_{II}250$. $dTAF_{II}250$ baculovirus stock was kindly provided by R. Tjian (Univ. of California, Berkeley). Insect SF9 cells infected by recombinant Flag-tagged $dTAF_{II}250$ baculovirus were resuspended in 0.4 M KCl-HEMG buffer (including 0.1% Nonidet P-40, 1 mM DTT, 0.2 mM PMSF, and Roche inhibitors) and lysed with a tight Dounce. After centrifugation, the supernatant was incubated for 2 h at 4°C with 500 μl of M2 agarose beads, washed four times with 0.3 M KCl-HEMG, and the bound protein was eluted four times with 500 μl of Flag peptide (0.2 mg/ml in 0.3 M KCl-HEMG). The Flag peptide was eliminated either by gel filtration or dialysis with 20 mM Tris, pH 7.9/10% (vol/vol) glycerol/0.15 M KCl/0.1 mM EDTA/1 mM DTT/0.2 mM PMSF.

***In Vitro* Translation and Immunoprecipitations.** pCR3.1-$TAF_{II}250$-AT and pCR3.1-$TAF_{II}250$-RAP74 and SNAP23 were translated *in vitro* in the TnT-Coupled Reticulocyte Lysate System (Promega) from the T7 promoter with [^{35}S]methionine (ICN). M2 agarose beads (Sigma) were prewashed in cold buffer B (20 mM Hepes, pH 7.9/100 mM KCl/12.5 mM MgCl2/0.1 mM DTT/0.2% Nonidet P-40/17% (vol/vol) glycerol) with 0.5 mg/ml of BSA, incubated for 2 h at 4°C with either buffer (control beads) or 2 μg of $TAF_{II}55$ in buffer B. Either control or $TAF_{II}55$/M2 beads were further incubated for 30 min in buffer B with 0.5 mg/ml BSA and washed. Aliquots were incubated for 1 h at 4°C with *in vitro* translated $TAF_{II}250$ AT, $TAF_{II}250$ RAPiD, or SNAP23 fragments. The complexes were washed four times in wash buffer (50 mM Tris, pH 7.9/150 mM NaCl/0.2% Nonidet P-40), and samples were resolved on a reducing SDS/15% PAGE gel and quantified by phosphorimaging (Amersham Pharmacia).

Histone Acetyltransferase (HAT) Assay. Histones H3 and H4 were prepared as described (10). The acetylation assays were a modification of the procedure described (11). $dTAF_{II}250$ (250 ng) was incubated with 2 μg of histones H3/H4, 70 nCi (1 Ci = 37 GBq) of [^{14}C]acetyl-CoA (60 mCi/mmol; Amersham Pharmacia) and 15 nCi of [^{3}H]acetyl-Co (4.5 Ci/mmol; NEN). Reactions were performed at 30°C for 30 min in HAT buffer (25 mM Tris, pH 8/0.1 mM EDTA/1 mM DTT/10 mM butyric acid/10% (vol/vol) glycerol/0.2% PMSF). Where indicated, $dTAF_{II}250$ was first preincubated with either 250 ng of control protein or 250 ng of $TAF_{II}55$. Control protein in these assays was a fragment of PB1, a $TAF_{II}250$ interacting protein that does not affect AT activity (A.G., *et al.*, unpublished work). Yeast HAT1 protein was a generous gift of P. A. Wade (National Institutes of Health, Bethesda). The reactions were resolved on a reducing SDS/15% PAGE gel, processed, and quantified by phosphorimaging. In the work presented in Fig. 2, $TAF_{II}55$ acetylation is performed as above, but in the absence of histones.

***In Vitro* Transcription Reactions.** *In vitro* transcription reactions contained 90 μg of HeLa nuclear extract and 0.8 mM rNTPs and were performed for 1 h at 20°C. $TAF_{II}55$ or AD $TAF_{II}55$ (250 ng, unless otherwise specified) were preincubated with the HeLa nuclear extract for 15 min at 20°C. The reaction was initiated by the addition of the promoter DNA. The conditions of the transcription reactions were optimized for each promoter. For MHC class I transcripts, 2 μg of −313 DNA was used in the presence of 0.8 mM $MgCl_2$. For CMV transcripts, 1 μg of CMV DNA (Promega) was used in the presence of 0.3 mM $MgCl_2$. The analysis of −313 MHC transcripts was by primer extension (4, 6). The analysis of CMV transcripts was by direct labeling of the transcripts by using 1 μl of [α-^{32}P]UTP (NEN) in the *in vitro* transcription reaction according to the manufacturer's instructions.

Results

$TAF_{II}55$ Binds to the $TAF_{II}250$ Domain Spanning the RAP74-Binding Site. We have shown that the HIV Tat protein binds to the AT domain of $TAF_{II}250$ and inhibits its activity, resulting in the repression of $TAF_{II}250$-dependent MHC class I promoter activity (4). This finding raised the possibility that $TAF_{II}250$ AT activity normally might be regulated by interactions with cellular proteins. To identify any such cellular proteins, we performed a yeast two-hybrid screen by using as bait the Tat-interacting domain of $TAF_{II}250$ (amino acids 848-1279) fused to the Gal4 DNA-BD (Fig. 1*A*). Two-yeast cDNA libraries were screened: a human-HeLa library and a mouse-thymus library. Three clones were isolated from the human-HeLa library and two from the mouse-thymus library that reproducibly and specifically interacted with the $TAF_{II}250$ bait and whose sequencing revealed that they encoded the $TAF_{II}55$ gene. Previous studies reported that $TAF_{II}55$ binds $TAF_{II}250$ *in vitro*, but did not map the site of $TAF_{II}250$ interaction (12, 13). By this screening, we show that this interaction occurs *in vivo* and, further, that $TAF_{II}55$ binds $TAF_{II}250$ in the 848-1279 region of $TAF_{II}250$.

The human $TAF_{II}55$ is a protein of 349 amino acids (12, 13); mouse $TAF_{II}55$ consists of 341 amino acids (14). $TAF_{II}55$ homologues are found also in *Drosophila* and yeast (15, 16, 17). Of the three human $TAF_{II}55$ clones isolated in the two-hybrid screen with the $TAF_{II}250$ bait, one started at amino acid 10 (Fig. 1*B*), and the other two started at amino acids 125 and 136. Of the two mouse $TAF_{II}55$ clones isolated, one began at amino acid 106 and the other at amino acid 132. All of the clones extended to the 3′ end of the coding sequence. This analysis maps a $TAF_{II}55$ region of interaction with $TAF_{II}250$ between amino acids 136 and 341, which is consistent with the earlier mapping of the binding of the central core of $TAF_{II}55$ to $TAF_{II}250$ (12). Because both the human and mouse clones bind to $TAF_{II}250$, the $TAF_{II}55$ segment between amino acids 233 and 242 in the human (which is missing in the mouse) is not necessary for the interaction.

The $TAF_{II}250$ segment used as bait in the two-hybrid screen spans both part of the AT domain (amino acids 848-1120) and the domain RAPiD (amino acids 1120–1279). (In Fig. 1, the $TAF_{II}250$ AT domain is located between amino acids 640 and 1093, corresponding to the *Drosophila* AT domain of amino acids 612-1140; ref. 18). To determine which of these $TAF_{II}250$ domains interacted with $TAF_{II}55$, we analyzed the ability of isolated $TAF_{II}250$ AT and RAPiD domains to bind full-length human $TAF_{II}55$ (12). In an *in vitro* pull-down assay, a bacterially expressed Flag-tagged $TAF_{II}55$ protein bound stably to the

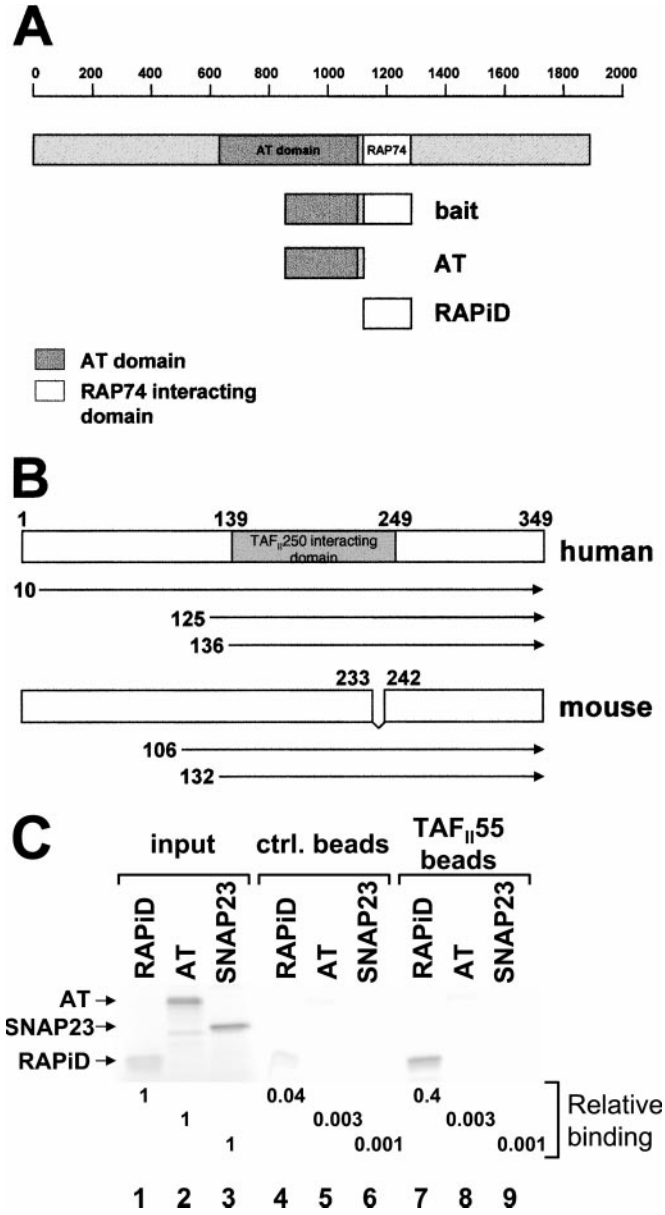

Fig. 1. $TAF_{II}55$ binds $TAF_{II}250$. (*A*) The $TAF_{II}250$ fragment (amino acids 848-1279), spanning the AT and RAPiDs, was used as bait in a yeast two-hybrid screen to isolate $TAF_{II}55$ clones. The location of the bait fragment, relative to the full-length molecule, is shown. $TAF_{II}250$ AT domain (shaded box) is located approximately between amino acids 640 and 1093, which corresponds to the *Drosophila* AT domain (amino acids 612-1140) (18). (*B*) $TAF_{II}55$ clones isolated in yeast two-hybrid screens. The locations of the five isolated $TAF_{II}55$ clones are shown relative to the full-length human and mouse proteins. Although the clones differed in their 5′ termini, they shared a common 3′ end that extended to encode the carboxyl terminus of the $TAF_{II}55$ protein. The mouse $TAF_{II}55$ differs from the human in a deletion spanning amino acids 233–242. (*C*) $TAF_{II}55$ binds to the RAPiD of $TAF_{II}250$. Flag-tagged $TAF_{II}55$ was used to assess its ability to bind to either [^{35}S]methionine labeled, *in vitro* translated AT, or RAPiD domains, as shown in *A* (AT, lanes 2, 5, 8; RAPiD, lanes 1, 4, 7). *In vitro* translated SNAP23 protein was used as a control for nonspecific binding (lanes 3, 6, 9). The relative binding of the $TAF_{II}250$ fragments to $TAF_{II}55$ was quantitated and calculated relative to input, as shown beneath the lanes. Arrowheads mark the positions of input proteins.

RAPiD domain of $TAF_{II}250$; binding to the AT domain was not detected above background under these conditions (Fig. 1*C*). In a direct *in vivo* yeast two-hybrid assay, $TAF_{II}55$ (fused to the Gal4-activation domain) also interacted with the $TAF_{II}250$ RAPiD domain fused to the Gal4 DNA-BD (data not shown). These results demonstrate that $TAF_{II}55$ binds to the $TAF_{II}250$ RAPiD.

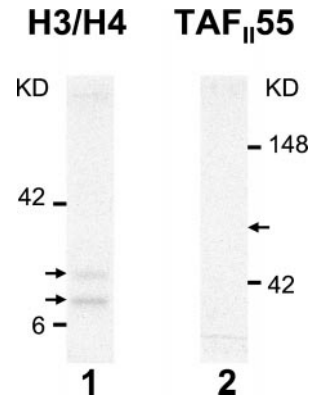

Fig. 2. $TAF_{II}55$ is not acetylated by $TAF_{II}250$. An AT assay was performed with 250 ng $TAF_{II}250$ incubated in the presence of either 2 μg of histones H3/H4 (lane 1) or 0.5 μg of $TAF_{II}55$ (lane 2). Arrowheads mark the positions of H3/H4 (lane 1) and $TAF_{II}55$ (lane 2).

In the functional studies reported below, the same full-length human $TAF_{II}55$ protein is used (12).

$TAF_{II}55$ Is Not Acetylated by $TAF_{II}250$. Although most ATs have been characterized by their ability to acetylate histones, there is an increasing recognition that non-histone proteins are also substrates for AT activity. For example, P300, a well characterized AT, has been shown to acetylate the transcription factor p53, thereby increasing its DNA-binding activity (19, 20). Similarly, acetylation of the transcription factor HNF-4 by CBP results in its enhanced retention in the nucleus and increased DNA binding (21). The finding that $TAF_{II}55$ binds to $TAF_{II}250$ raised the possibility that $TAF_{II}55$ is a substrate for acetylation by $TAF_{II}250$. However, under conditions in which purified $TAF_{II}250$ acetylated histones H3 and H4, no acetylation of purified full-length $TAF_{II}55$ protein could be detected (Fig. 2). (Increasing the amount of $TAF_{II}55$ in the reaction did not reveal any acetylation; data not shown.) Therefore, $TAF_{II}55$ is not a detectable substrate for the $TAF_{II}250$ AT activity *in vitro*.

$TAF_{II}55$ Inhibits $TAF_{II}250$ HAT Activity. We next considered the possibility that $TAF_{II}55$ binding could alter $TAF_{II}250$ AT activity. To test this possibility, we measured the AT activity of $TAF_{II}250$ in the presence of either $TAF_{II}55$ or control protein by using histones H3/H4 as substrate. As shown in Fig. 3*A*, the presence of $TAF_{II}55$ in the HAT assay reduced $TAF_{II}250$ AT activity, whereas the control protein had no effect (Fig. 3*A*, compare lanes 3 and 4 and histogram). To extend this observation, we analyzed the dose–response of $TAF_{II}250$ AT activity to $TAF_{II}55$ (Fig. 3*B*). Inhibition of $TAF_{II}250$ AT activity increased in the presence of increasing amounts of $TAF_{II}55$; the control protein had no effect. (It is important to note that the histone substrate was present in a 15- to 20-fold molar excess relative to $TAF_{II}55$. Therefore, it is unlikely that $TAF_{II}55$ inhibited acetylation through an interaction with histones.)

In contrast to $TAF_{II}250$, the AT activity of yeast HAT1 is not affected by $TAF_{II}55$, indicating that $TAF_{II}55$ is not a histone deacetylase, nor is it a nonspecific inhibitor (A.G., data not shown). These findings are consistent with the conclusion that $TAF_{II}55$ is a specific inhibitor of $TAF_{II}250$ AT activity.

To map the domain of $TAF_{II}55$ responsible for inhibition of AT activity, the ability of a $TAF_{II}55$ fragment extending from amino acids 1 to 109 ($\Delta TAF_{II}55$) to inhibit AT activity was examined. As shown in Fig. 3*B*, the amino terminal fragment of

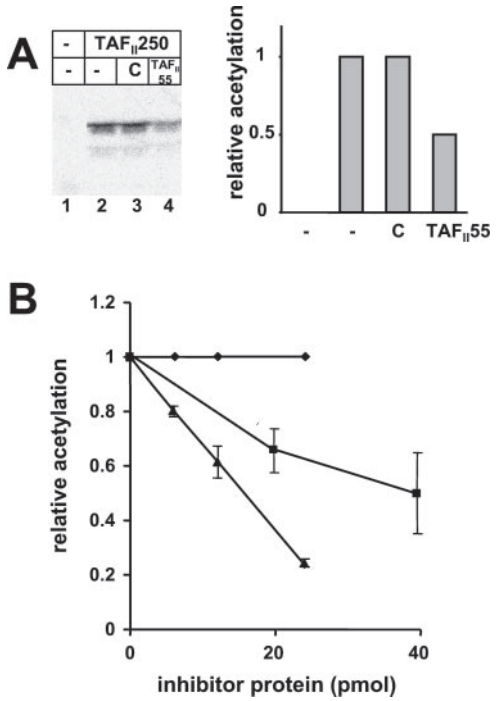

Fig. 3. $TAF_{II}55$ inhibits $TAF_{II}250$ AT activity. (*A*) The AT activity of $TAF_{II}250$ (250 ng) was assayed in the presence of 2 μg of histones H3/H4 with buffer (lane 2), 250 ng control protein (lane 3), or 250 ng $TAF_{II}55$ (lane 4). Lane 1 contains histones incubated without $TAF_{II}250$. Acetylation of histones was quantitated by PhosphorImager (Molecular Dynamics) and is plotted in the histogram relative to the level of acetylation observed in lane 2. (*B*) Increasing amounts of $TAF_{II}55$ or the amino terminal fragment of $TAF_{II}55$ (amino acids 1–109) result in increasing inhibition of $TAF_{II}250AT$ activity. $TAF_{II}250$ AT activity was assayed on 2 μg of histones H3/H4 by using 250 ng $TAF_{II}250$ in the presence of increasing amounts of $TAF_{II}55$ (▲), the amino terminal fragment of $TAF_{II}55$ ($ADTAF_{II}55$, ■), or control protein (♦). The diagram represents the relative acetylation of histones in the presence of the respective proteins and summarizes seven different experiments performed in the presence of $TAF_{II}55$ and four experiments performed in presence of AD $TAF_{II}55$. Error bars show the standard deviation.

$TAF_{II}55$, like the full-length molecule, was able to inhibit $TAF_{II}250$ AT activity. However, approximately a 3-fold molar equivalent of $ADTAF_{II}55$ was required to achieve the same level of inhibition as the full-length molecule. These findings demonstrate that $TAF_{II}55$ modulates $TAF_{II}250$ AT activity and maps the $TAF_{II}55$ inhibitory domain to its amino terminus. Interestingly, although the amino terminus of $TAF_{II}55$ contains the inhibitory activity of the molecule, and thus must interact with $TAF_{II}250$, we have not been able to detect stable binding of the isolated domain to $TAF_{II}250$ in pull-down assays. Rather, the major interaction of $TAF_{II}55$ with $TAF_{II}250$ is to the RAPiD and is mediated by the segment of $TAF_{II}55$ between amino acids 139 and 249 (Fig. 1; ref. 12).

$TAF_{II}55$ Represses $TAF_{II}250$-Dependent MHC Class I Expression. Because $TAF_{II}250$ AT activity is necessary for transcription from the MHC class I promoter (4), we next examined the effect of $TAF_{II}55$ on *in vitro* transcription of an MHC class I promoter (Fig. 4). Whereas HeLa nuclear extract alone supported *in vitro* transcription from the MHC class I promoter, the addition of increasing amounts of exogenous $TAF_{II}55$ to the reaction decreased levels of transcription (Fig. 4*A*, compare lanes 1, 2, and 3; Fig. 4 *C* and *D*). To demonstrate that this observation reflects a specific effect of $TAF_{II}55$ on $TAF_{II}250$-dependent gene transcription and not a general repression of all transcription, we assessed the effect of $TAF_{II}55$ on a promoter known to be $TAF_{II}250$-independent, namely, the CMV promoter (22). As predicted, transcription from the CMV promoter was not affected by $TAF_{II}55$ (Fig. 4*A*, lanes 4 and 5; ref. 4). Consistent with its ability to inhibit $TAF_{II}250$ AT activity, the $ADTAF_{II}55$ construct, containing only the amino terminal of amino acid 109, also represses transcription of the MHC class I promoter (Fig. 4 *B–D*). Transcription, like $TAF_{II}250$ AT activity, is less efficiently inhibited by $ADTAF_{II}55$ than by the full-length $TAF_{II}55$ on a molar basis (Fig. 4*C*).

Taken together, these results indicate that repression of MHC class I transcription in the presence of $TAF_{II}55$ is caused by the inhibition of $TAF_{II}250$ AT activity by $TAF_{II}55$.

Discussion

$TAF_{II}55$ is a component of the TFIID complex that nucleates the preinitiation complexes associated with a large number of core promoters. In TFIID, $TAF_{II}55$ interacts with other components: $TAF_{II}250$, $TAF_{II}100$, $TAF_{II}28$, $TAF_{II}20$, and $TAF_{II}18$ (12, 13). *In vitro*, $TAF_{II}55$ also interacts with a number of cellular transcription factors including USF, Sp1, and YY1, as well as viral transcription factors such as HIV Tat and E1A (12). Consistent with its association with TFIID, which is found in all cell types, $TAF_{II}55$ is ubiquitously expressed (13). Despite the previous characterization of $TAF_{II}55$ as a member of the TFIID complex, nothing was known about its function in transcription initiation.

In the present studies, we have identified functional activities of $TAF_{II}55$. We have demonstrated that the binding of $TAF_{II}55$ to $TAF_{II}250$ results in the inhibition of the intrinsic AT activity of $TAF_{II}250$. Most significantly, the binding of $TAF_{II}55$ to $TAF_{II}250$ results in the repression of the activity of an MHC class I promoter that is $TAF_{II}250$-dependent. This repression is not seen with a $TAF_{II}250$-independent promoter. Finally, we have mapped the regions of interaction of $TAF_{II}55$ and $TAF_{II}250$. Both inhibition of $TAF_{II}250$ AT activity and transcription are mediated by the N-terminal of amino acids 1–109 of $TAF_{II}55$. This segment of the protein contains two cysteines that could potentially form disulfide bonds and two regions capable of forming α-helices.

The mechanism by which $TAF_{II}55$ regulates $TAF_{II}250$ AT activity remains to be determined. Although there are multiple lysine residues in $TAF_{II}55$, it is not acetylated as a result of its interaction with $TAF_{II}250$. Because $TAF_{II}55$ is not acetylated by $TAF_{II}250$ AT activity, it is not simply competing with histone substrates; it may be acting as a competitive inhibitor. The precise mechanism by which $TAF_{II}55$ represses transcription and the role of the N-terminal structure also remain to be established. We propose that repression of transcription by $TAF_{II}55$ is a direct consequence of its inhibition of $TAF_{II}250$ AT activity.

In previous studies, we have reported that Tat binds to $TAF_{II}250$. As in the case of $TAF_{II}55$, the binding of Tat results in the inhibition of the $TAF_{II}250$ AT activity and the consequent repression of transcription of $TAF_{II}250$-dependent promoters (4). Tat mediates repression through its C-terminal domain, which binds only weakly to the AT domain of $TAF_{II}250$. It is the core of Tat that binds strongly to the RAPiD of $TAF_{II}250$ that anchors Tat to $TAF_{II}250$ (6). In the present study, we have found that the core of $TAF_{II}55$, like the core of Tat, binds strongly to the RAPiD of $TAF_{II}250$. Like Tat, the functionally active N-terminal segment of $TAF_{II}55$, which inhibits the AT activity of $TAF_{II}250$, must bind only weakly because binding is not detected in standard pull-down assays (data not shown). Given the striking parallels between HIV Tat and $TAF_{II}55$, as shown here, we speculate that $TAF_{II}55$ is a cellular analog of Tat that modulates $TAF_{II}250$ function *in vivo*. Despite the apparent similarities in their interactions and inhibition of $TAF_{II}250$ AT activity, $TAF_{II}55$ and Tat have no primary amino acid sequence

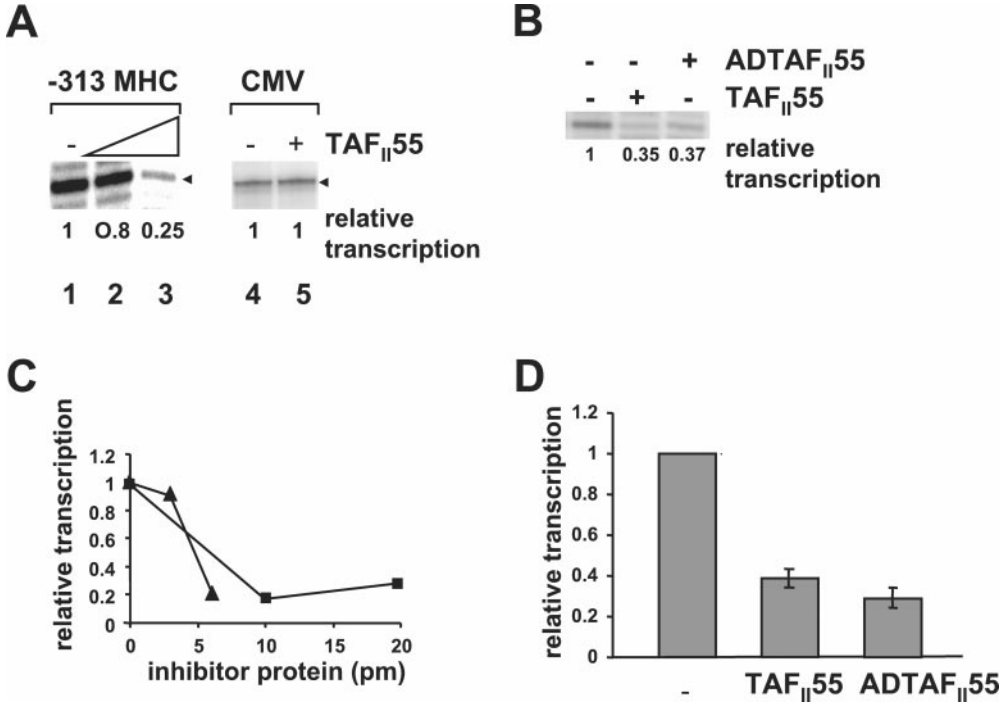

Fig. 4. TAF$_{II}$55 represses *in vitro* transcription from the MHC class I promoter, but not the viral CMV promoter. (*A*) *In vitro* transcription reactions were performed by using either an MHC class I promoter (−313 MHC, lanes 1–3) or the viral CMV early promoter (lanes 4–5) in the absence (lanes 1, 4) or in the presence of TAF$_{II}$55 (150 ng, lane 2; 250 ng, lane 3; 250 ng, lane 5). The MHC class I transcripts are detected by primer extension. The CMV transcripts are directly synthesized in the presence of [α-^{32}P]UTP. They have been aligned for the purposes of presentation. The relative transcription is quantitated and reported below each lane. (*B*) Repression of transcription is mediated by the N-terminal domain of TAF$_{II}$55. *In vitro* transcription reactions were performed by using the MHC class I promoter (−313 MHC) in the presence of buffer, TAF$_{II}$55 (250 ng), or AD TAF$_{II}$55 (250 ng). The relative transcription is quantitated and reported below each lane. (*C*) Titration of TAF$_{II}$55 and ADTAF$_{II}$55 inhibition of *in vitro* transcription of the MHC class I promoter. Increasing amounts of either TAF$_{II}$55 (0, 3, or 6 pmol, ▲) or ADTAF$_{II}$55 (0, 10, or 20 pmol, ■) were added to a standard *in vitro* transcription reaction with the −313 MHC class I promoter; the relative levels of transcription were quantitated by PhosphorImager analysis. (*D*) Summary of the TAF$_{II}$55 and ADTAF$_{II}$55 inhibition of *in vitro* transcription. The relative levels of transcription of −313 MHC class I promoter, in the presence or absence of 6 pmol of TAF$_{II}$55 from seven different experiments or 20 pmol of ADTAF$_{II}$55 from two experiments are compiled. Error bars represent standard deviation.

similarity. However, like Tat, the ability of TAF$_{II}$55 to inhibit TAF$_{II}$250 AT activity suggests that it plays a pivotal role in regulating normal cellular transcription from TAF$_{II}$250-dependent promoters through its modulation of the AT activity.

What purpose would such regulation of TAF$_{II}$250 function serve? Although basal transcription of MHC class I genes requires TAF$_{II}$250, this dependence is context-dependent. Thus, it can be overcome by a variety of activators associated with upstream regulatory elements. For example, ligation of the simian virus (SV)40 viral 72-bp enhancer upstream of the class I promoter relieves it of its requirement for TAF$_{II}$250 (22). As an aside, it is interesting to note that, whereas the viral SV40 promoter does not require TAF$_{II}$250 in the presence of its strong viral 72-bp enhancer, removal of the enhancer renders the promoter TAF$_{II}$250-dependent. Even more striking, activation of the native class I promoter by the coactivator CIITA occurs independently of the AT activity of TAF$_{II}$250 (22). Thus, normal cellular mechanisms modulate the requirement for TAF$_{II}$250, and TAF$_{II}$55 may help to regulate this requirement.

Two distinct mechanisms by which TAF$_{II}$55 could regulate transcription can be considered. In the first model, TAF$_{II}$55 is constitutively associated with the TFIID complex during basal transcription but displaced by upstream activators that function through TAF$_{II}$250; the displacement of TAF$_{II}$55 would increase AT activity and transcription initiation. The *in vitro* association of TAF$_{II}$55 with known activators, such as USF, is consistent with this model (12). Alternatively, in a second model, activators that bypass the requirement for TAF$_{II}$250, such as CIITA, might recruit TAF$_{II}$55 to the TFIID complex, thereby inhibiting TAF$_{II}$250 AT activity and refocusing activity to alternative initiation complexes. In another system, Roeder and coworkers (23) have demonstrated that TFIID is not necessary for transcription in the presence of the liganded thyroid hormone receptor/TRAP coactivator complex. These findings raise the intriguing possibility of initiation complex selectivity by coactivator complexes, in which TAF$_{II}$55 would block the function of TAF$_{II}$250-containing complexes. Currently, we cannot distinguish between these two models. In either case, we propose that the function of TAF$_{II}$55 is to regulate the activity of the TFIID complex through its inhibition of TAF$_{II}$250 AT activity.

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