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The $TAF_{II}250$ Subunit of TFIID Has Histone Acetyltransferase Activity

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Summary

The transcription initiation factor TFIID is a multimeric protein complex composed of TATA box–binding protein (TBP) and many TBP-associated factors (TAF_{II}s). TAF_{II}s are important cofactors that mediate activated transcription by providing interaction sites for distinct activators. Here, we present evidence that human $TAF_{II}250$ and its homologs in Drosophila and yeast have histone acetyltransferase (HAT) activity in vitro. HAT activity maps to the central, most conserved portion of $dTAF_{II}230$ and $yTAF_{II}130$. The HAT activity of $dTAF_{II}230$ resembles that of yeast and human GCN5 in that it is specific for histones H3 and H4 in vitro. Our findings suggest that targeted histone acetylation at specific promoters by $TAF_{II}250$ may be involved in mechanisms by which TFIID gains access to transcriptionally repressed chromatin.

Introduction

Transcription of protein-coding genes in eukaryotes requires the orchestrated assembly of a large preinitiation complex containing a well-studied collection of general transcription factors, TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH and RNA polymerase II, at promoters (reviewed by Burley and Roeder, 1996; Orphanides et al., 1996; Roeder, 1996). Reconstitution of transcription has shown that functional preinitiation complexes can be assembled in a step-wise fashion. The first step is TFIID binding, a process that may be facilitated by TFIIA. The subsequent binding of TFIIB creates a platform that is in turn recognized by a complex containing RNA polymerase II and TFIIF. Further incorporation of TFIIE and TFIIH completes preinitiation complex formation. Recent studies have revealed that complexes of RNA polymerase II, general initiation factors, and cofactors may enter the preinitiation complex as a preassembled unit (reviewed by Koleske and Young, 1995; Orphanides et al., 1996; Pugh, 1996). Although the assembly pathway most relevant to the in vivo situation remains unclear, TFIID binding to the promoter could be an important step for promoter activation in different pathways since TFIID is the only component of the preinitiation complex that is capable of binding specifically to core promoters.

Control of promoter recognition by TFIID appears to represent an important pathway for transcriptional regulation. TFIID is a target for a number of transcriptional activators whose interactions with TFIID may enhance the rate of promoter binding by TFIID or stabilize TFIID–promoter complexes (reviewed by Kingston and Green, 1994; Struhl, 1996). TFIID itself is a multimeric protein complex consisting of TBP and TBP-associated factors (TAF_{II}s) whose sizes range from $M_r \sim 10{,}000$ to >200,000 (for review, see Burley and Roeder, 1996). To date, cDNAs encoding nine TAF_{II} subunits of Drosophila TFIID ($dTAF_{II}230$, 150, 110, 85, 62, 42, 28a, 28β, and 22) have been cloned. Although TBP alone can bind core promoters containing TATA elements and support basal transcription in conjunction with other general transcription factors and RNA polymerase II, TAF_{II}s are required for activated transcription. Several TAF_{II}s have been shown to provide interaction sites for distinct activators and transcription-initiation factors. These interactions could serve either to facilitate TFIID recruitment per se or to induce conformational alterations that affect recruitment or function of downstream factors (reviewed in Orphanides et al., 1996; Roeder, 1996; Verrijzer and Tjian, 1996).

Recent studies showing capabilities for transcriptional activation in yeast depleted of the TAF subunits of TFIID (Moqtaderi et al., 1996; Walker et al., 1996) suggest redundant pathways for activator responses. Consistent with this, direct linkage of enhancer-binding domains and either TBP (Chatterjee and Struhl, 1995; Klages and Strubin, 1995), TAFs (reviewed in Moqtaderi et al., 1996), or subunits within the RNA polymerase II holoenzyme (Barberis et al., 1995) can bypass the requirement for activation domains. These lines of evidence suggest that recruitment of either factor may lead to at least some transcriptional activation, although multiple interactions could be required for full activation.

Central to eukaryotic transcriptional regulation are how TFIID gains access to a chromatin template and how a stable association is maintained within the chromosomal enviroment. A potentially relevant finding is the presence of a histone octamer-like substructure in TFIID (Hoffmann et al., 1996; Nakatani et al., 1996; Xie

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et al., 1996). The N-terminal regions of the dTAF$_{II}$42 and dTAF$_{II}$62 proteins have sequence similarities with the C-terminal core domains, but not the N-terminal tails, of histones H3 and H4, respectively (Kokubo et al., 1994a). The crystal structure of the N-terminal portions of dTAF$_{II}$42/dTAF$_{II}$62 reveals that they adopt the canonical histone fold, consisting of two short α helices flanking a long, central α helix (Xie et al., 1996). Moreover, the dTAF$_{II}$42/dTAF$_{II}$62 complex exists as a heterotetramer, resembling the $(H3/H4)_2$ heterotetrameric core of the histone octamer. In addition, biochemical studies with human TAF$_{II}$s (hTAF$_{II}$s) suggest that TFIID contains a histone octamer-like structure composed of two dimers of the histone H2B-like TAF$_{II}$ (hTAF$_{II}$15/20 or dTAF$_{II}$28α) attached to a tetramer of histones H3/H4-like TAF$_{II}$s (Hoffmann et al., 1996). These similarities to histone complexes have prompted the suggestion that TAF$_{II}$ complexes may wrap and interact with promoter DNA in a nucleosome-like structure (Hoffmann et al., 1996).

Biochemical studies have demonstrated that physical interactions of activators with general transcription factors play an important role in activated transcription from naked DNA templates (reviewed by Burley and Roeder, 1996; Orphanides et al., 1996; Roeder, 1996). Moreover, the recent discovery that dTAF$_{II}$230/hTAF$_{II}$250 possesses kinase activity that phosphorylates the RAP74 subunit of TFIIF in vitro (Dikstein et al., 1996) suggests that enzymatic mechanisms are also involved, although the role of phosphorylation has not been demonstrated. Additionally, various enzymatic activities, such as histone acetyltransferases (HATs), histone kinases, and DNA helicases, may be required for transcriptional regulation within the context of nucleosomal and higher-order chromatin structure that represses transcription in general (reviewed by Owen-Hughes and Workman, 1994; Felsenfeld, 1996).

A large number of studies have established a correlation between chromatin transcriptional activation, i.e., derepression, and acetylation of highly conserved lysine residues situated in the amino-terminal tails of the core histones (reviewed by Loidl, 1994; Turner and O'Neill, 1995; Brownell and Allis, 1996). Although the mechanistic details underlying acetylation-mediated activation from chromatin templates remain unclear, it is generally thought that neutralization of the positive charge of lysine residues upon acetylation perturbs histone-DNA contacts within nucleosomal and higher-order structure and influences histone interactions with specific nonhistone regulatory proteins (reviewed by Wolffe, 1994; Brownell and Allis, 1996). The recent findings that the yeast transcriptional adaptor-protein GCN5 (Brownell et al., 1996) and related human proteins (hGCN5, Wang et al., 1997; hP/CAF, Yang et al., 1996) possess HAT activity in vitro provide strong evidence that histone acetylation is linked to transcriptional activation and suggest that histone acetylation may be a targeted phenomenon (reviewed by Brownell and Allis, 1996; Wolffe and Pruss, 1996).

In this report, we demonstrate that, like the GCN5 protein family, TAF$_{II}$250 and its homologs in Drosophila and yeast (hereafter referred to collectively as TAF$_{II}$250) have HAT activity in vitro. This finding suggests that acetylation of histone amino termini at or near core promoters by TAF$_{II}$250 could be involved in facilitating transcription from natural chromatin templates by increasing the accessibility of TFIID to promoters. Our data provide additional support for the notion that targeted histone acetylation represents an important pathway in gene activation. We favor the view that eukaryotic cells have evolved a scheme of targeted histone acetylation wherein HAT activities, and potentially other chromatin-modifying activities (for example, Roest et al., 1996; reviewed by Kaiser and Meisterernst, 1996), are recruited to specific promoters through selective interactions with activator proteins.

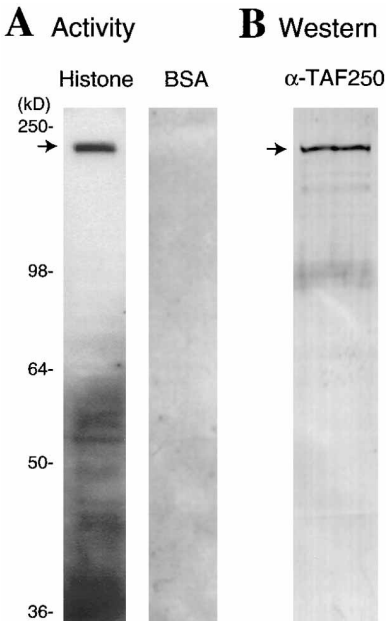

Figure 1. HeLa Nuclear Extracts Contain a High-Molecular-Weight Polypeptide with HAT Activity

(A) Aliquots of HeLa cell crude nuclear extract were resolved on 8% SDS–PAGE gels containing histone or BSA as indicated and processed to detect acetyltransferase activity. The arrow denotes an activity band in the activity gel containing histone with an apparent molecular weight similar to that of hTAF$_{II}$250, as shown by immunoblotting in (B).

(B) An aliquot of HeLa-cell crude nuclear extract was resolved on an 8% SDS–PAGE gel and analyzed by Western blotting using antisera specific for hTAF$_{II}$250. The arrow denotes hTAF$_{II}$250. The activity gels and the immunoblot were aligned using prestained molecular weight markers, as indicated on the left with molecular weight given in kilodaltons.

Results

A Large Molecular-Weight, TBP-Associated Polypeptide Has HAT Activity

We recently demonstrated the utility of an activity gel assay to detect proteins with HAT activity in complex samples (Brownell and Allis, 1995; Brownell et al., 1996). In this technique, samples are resolved in SDS–PAGE gels containing histones prior to detection of HAT activity, and thus the molecular weight estimates of active polypeptides can be determined. Using this assay to characterize a crude HeLa nuclear extract, we identified a polypeptide with a molecular weight of approximately 200 kDa that possessed strong HAT activity (Figure 1A).

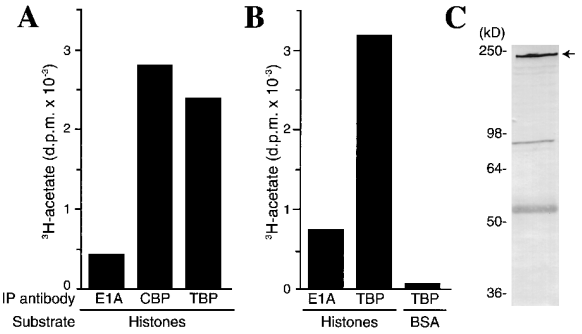

Figure 2. Anti-TBP Antibodies Immunoprecipitate HAT Activity

(A and B) Immunoprecipitations were performed from COS whole-cell extracts (A) and HeLa-cell nuclear extracts (B) with the indicated antibodies. After extensive washing, the resulting immune complexes were assayed for their ability to acetylate either free histones or BSA. The anti-CBP antibody served as a positive control since both CBP and the associated P/CAF have HAT activity (see text). The anti-E1A antibody served as a negative control since E1A is not detectable in these cell lines.

(C) The anti-TBP immunoprecipitate was resolved on an 8% SDS–PAGE gel and analyzed by Western blotting using antisera specific for hTAF$_{II}$250. The arrow denotes hTAF$_{II}$250.

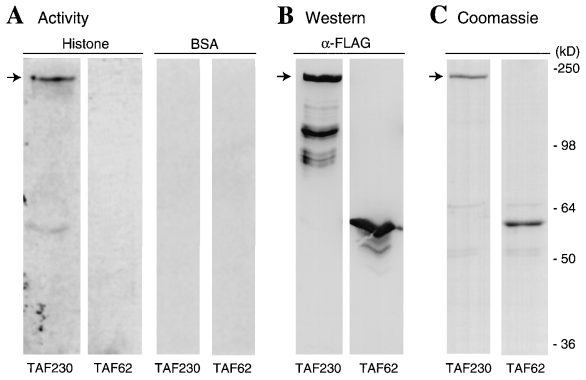

Figure 3. Recombinant Drosophila TAF$_{II}$230 Possesses HAT Activity

(A) FLAG epitope–tagged, affinity-purified recombinant dTAF$_{II}$230 and FLAG–dTAF$_{II}$62 were resolved on 8% SDS gels containing histone or BSA as indicated and processed to detect acetyltransferase activity.

(B) Identical dTAF$_{II}$230 and dTAF$_{II}$62 samples were electrophoresed in a parallel SDS–PAGE gel and immunoblotted with antisera to the FLAG epitope.

(C) Identical dTAF$_{II}$230 and dTAF$_{II}$62 samples were electrophoresed in a parallel SDS–PAGE gel and stained with Coomassie blue. Arrows in (A), (B), and (C) indicate the position of full-length dTAF$_{II}$230.

This activity appeared to be specific for histone and was not due to autoacetylation, since activity was not detected when a sample was analyzed on a gel containing BSA. Interestingly, we did not detect activities resembling hGCN5 (apparent molecular weight 60 kDa) or hP/CAF (apparent molecular weight 95 kDa) in crude HeLa nuclear extract, suggesting that the total activities of these known HATs in this sample are lower or that these proteins are not renatured as readily in the assay procedure. Due to the similarity in molecular weight, we speculated that the active species might be hTAF$_{II}$250, the 250 kDa (nominal) subunit of human TFIID (Hisatake et al., 1993; Ruppert et al., 1993). Immunoblot analysis of the nuclear extract using an hTAF$_{II}$250-specific antisera (Figure 1B) demonstrated that the immunoreactive band possessed electrophoretic mobility similar to that of the detected HAT, further supporting the hypothesis that hTAF$_{II}$250 has HAT activity in this assay.

To further test this hypothesis and to determine if HAT activity could be detected in TAFs, we used antibodies to TBP to immunoprecipitate TBP–TAF complexes from COS whole-cell extracts and HeLa-cell nuclear extracts and assayed the immunoprecipitates for HAT activity. To monitor the specificity of this procedure, two controls were employed. First, an E1A-specific antibody was used as a negative control. Second, a CBP-specific antibody was used as a positive control, since CBP per se (Ogryzko et al., 1996) and its associated hP/CAF (Yang et al., 1996) have HAT activity.

The results of these immunoprecipitation-HAT assays are shown in Figure 2. A low level of HAT activity was associated with anti-E1A immunoprecipitates prepared from whole-cell extract (Figure 2A) and nuclear extract (Figure 2B). This represents background activity since we found several other antibodies against nuclear proteins precipitated similar amounts of activity (data not shown). In contrast, the amount of HAT activity associated with anti-CBP immunoprecipitates from whole-cell extracts was routinely more than 6-fold greater than background (Figure 2A).

HAT activity associated with anti-TBP immunoprecipitates was approximately 5-fold greater than background when immunoprecipitates were prepared from whole-cell extracts (Figure 2A) and from nuclear extracts (Figure 2B). Moreover, the HAT activity associated with TBP was specific to histones, since BSA was not acetylated by anti-TBP immunoprecipitates under similar conditions (Figure 2B). As expected, Western blot analysis confirmed that hTAF$_{II}$250 was present in the anti-TBP immunoprecipitate (Figure 2C). Together, these results suggest that HAT activity may be attributable to hTAF$_{II}$250 and further suggest that this activity is preserved in TFIID.

Recombinant dTAF$_{II}$230 Has HAT Activity

To rigorously test the hypothesis that TAF$_{II}$250 has HAT activity, we expressed dTAF$_{II}$230 as a FLAG epitope–tagged protein in Sf9 cells using baculovirus-mediated transfection and assayed the affinity-purified recombinant protein for HAT activity. Recombinant dTAF$_{II}$230 displayed acetyltransferase activity when assayed with a histone-containing gel but not when the gel contained BSA (Figure 3A). The absence of HAT activity in a nonhomologous recombinant protein, FLAG–dTAF$_{II}$62, prepared by identical procedures indicated that HAT activity was a property of dTAF$_{II}$230 rather than contaminants from baculovirus-infected Sf9 cells. Similar amounts of dTAF$_{II}$62 and dTAF$_{II}$230 were detected at their respective molecular weights by both immunoblot analysis with antisera to the FLAG epitope (Figure 3B) and Coomassie blue staining (Figure 3C). Furthermore, Coomassie blue staining revealed that the recombinant proteins were highly purified; only low levels of a small set of extraneous proteins were detected. These data demonstrate that HAT activity is associated with recombinant dTAF$_{II}$230 under the conditions employed.

Substrate Specificity of Recombinant dTAF$_{II}$230 HAT Activity

To characterize the recombinant dTAF$_{II}$230 HAT activity further, we performed HAT assays in solution with purified protein. Acetylation of a HeLa core histone mixture and individual HeLa core histones, purified by reverse-phase high pressure liquid chromatography (HPLC), was measured by a filter-binding assay and by fluorography (Figure 4). As a positive control, we assayed a crude extract of recombinant hGCN5 expressed in bacteria. Extracts of uninduced bacteria and purified dTAF$_{II}$62 were used as negative controls for hGCN5 and dTAF$_{II}$230, respectively. Liquid-scintillation counting of reactants retained by P81 filters (Figure 4A) showed that both hGCN5 and dTAF$_{II}$230, in contrast to uninduced bacterial extract or dTAF$_{II}$62, catalyzed efficient [^{3}H]-acetate transfer to the HeLa core histone mixture. In agreement with the activity gel assay (Figure 3), acetate incorporation into BSA was not observed with either hGCN5 or dTAF$_{II}$230, nor was acetylation of proteins endogenous to the enzyme preparations detected. Given that the amino-terminal portions of dTAF$_{II}$42 and dTAF$_{II}$62 adopt structures similar to the histone fold and can form a (dTAF$_{II}$42/dTAF$_{II}$62)$_2$ heterotetramer resembling the (H3/H4)$_2$ tetramer of nucleosomes (Xie et al., 1996), we tested these TAFs as substrates for acetylation. Consistent with the lack of sequences related to the histone amino-terminal tails in dTAF$_{II}$42/dTAF$_{II}$62, they were not acetylated by dTAF$_{II}$230 or hGCN5 (data not shown).

Histone substrates acetylated by dTAF$_{II}$230 and hGCN5 in vitro were identified by fluorography. As shown in the upper portion of Figure 4B, H3 and H4 were the major acetate acceptors when the HeLa core histone mixture (lane 1) was incubated with dTAF$_{II}$230. H3 and H4 were acetylated by dTAF$_{II}$230 regardless of whether they were presented individually (lanes 4 and 5) or as part of the mixture. Although H2A was acetylated by dTAF$_{II}$230 when presented individually (lane 2), little or no H2A acetylation was detected in the core histone mixture. H2B was not acetylated by dTAF$_{II}$230 when presented individually (lane 3) or as part of the mixture. No acetylation was detected in a control reaction where H4 (or H3, data not shown) and [^{3}H]-acetyl-CoA were incubated without enzyme (lane 6). When an equimolar mixture of H2A, H2B, H3, and H4, reconstituted from purified fractions (lane 7), was employed as substrate, acetylation of H3 and H4 was indistinguishable from that seen in the histone mixture prior to purification.

The dual H3 and H4 specificity of dTAF$_{II}$230 differed from a strong preference for only H3 displayed by hGCN5. As shown in the lower panels of Figure 4B, H3 was the preferred substrate for hGCN5 under all conditions tested (see also Wang et al., 1997). Acetylation of H2A, H2B, and H4 by hGCN5 was not detected in the histone mixture (lane 1) or when presented individually (lanes 2, 3, and 5, respectively). Based on approximate measurements, we estimate that the specific activity of hGCN5 (dpm ^{3}H-acetate transferred/pmol enzyme) is two to four times that of dTAF$_{II}$230 (data not shown).

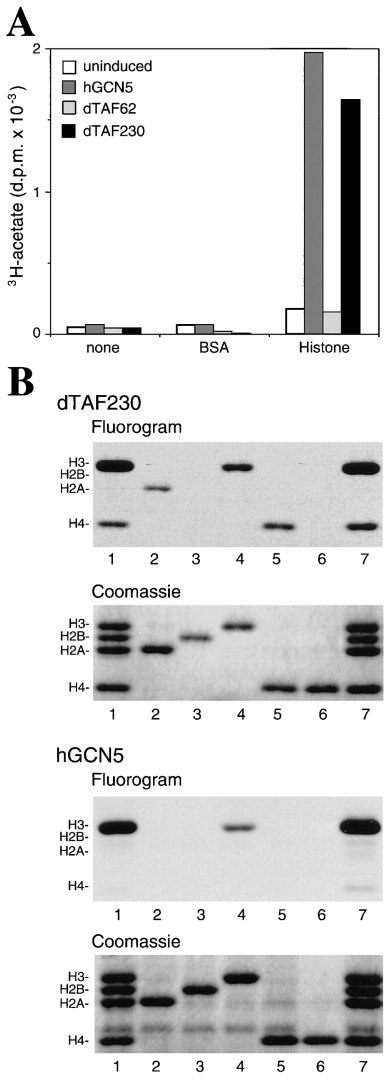

Figure 4. Substrate Specificities of Drosophila TAF$_{II}$230 and Human GCN5

(A) Acetylation of proteins endogenous to enzyme preparations (no substrate) BSA and HeLa core histones by recombinant dTAF$_{II}$230 and hGCN5 in vitro was assessed by measuring ^{3}H-acetate incorporation using a filter-binding assay. Uninduced bacterial extract (uninduced) and recombinant dTAF$_{II}$62 were used as negative controls for hGCN5 and dTAF$_{II}$230, respectively.

(B) Specificity of HeLa core histone acetylation by dTAF$_{II}$230 (two upper panels) and hGCN5 (two lower panels) was determined by fluorography of acetylated reaction products resolved on SDS–PAGE gels. Fluorograms are shown aligned with the corresponding Coomassie blue–stained gels as indicated. Lane 1 shows unfractionated HeLa core histone mixture; lanes 2–5, reverse-phase HPLC-purified HeLa histones H2A, H2B, H3, and H4, respectively; lane 6, reverse-phase HPLC-purified HeLa H4 incubated with [^{3}H]-acetyl-CoA but without added enzyme; and lane 7, equimolar mixture of HeLa core histones reconstituted from RP-HPLC–purified fractions. The positions of the individual histones in the gels are indicated at the left of the figure. The lack of H4 acetylation by hGCN5 compared to that described by Yang et al. (1996) may be related to differences in the recombinant proteins employed.

Site Specificity of Recombinant dTAF$_{II}$230 HAT Activity

Utilization of specific acetylation sites within the core histone amino termini is remarkably nonrandom and closely correlated with distinct biological processes (see Sobel et al., 1995; Turner and O'Neill, 1995). To determine whether specific residues modified by dTAF$_{II}$230

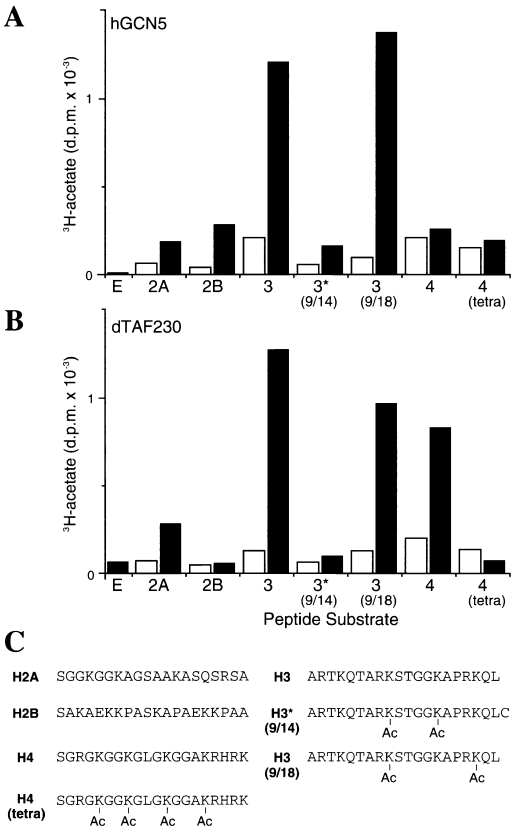

Figure 5. Site Specificity of Drosophila TAF$_{II}$230 and Human GCN5

(A and B) Acetylation of histone amino-terminal peptides by hGCN5 (A) and dTAF$_{II}$230 (B) was assessed by measuring ^{3}H-acetate incorporation using the filter-binding assay. For each peptide substrate, incubations without (open bars) and with (closed bars) enzyme were performed in parallel. Acetylation of proteins endogenous to the enzyme preparations was assessed by incubations without peptides and is shown in the columns marked (E).

(C) The structures of the peptides used in (A) and (B) are shown. Sites where ϵ-N-acetyllysine was incorporated during peptide synthesis in order to mimic sites that are acetylated in vivo are indicated by (Ac). All peptides were MAP reagents except the diacetyl-(9/14)-H3 peptide (denoted by the asterisk), which was synthesized with a C-terminal cysteine.

are the same as those modified by GCN5 (Kuo et al., 1996), we assessed the ability of dTAF$_{II}$230 and hGCN5 to acetylate histone amino-terminal peptides synthesized with and without acetyl groups on the ϵ-amino groups of specific lysines. The peptide sequences and positions of acetate groups incorporated during synthesis are depicted in Figure 5C.

Acetylation of these peptides by hGCN5 and dTAF$_{II}$230 are shown in Figures 5A and 5B, respectively. Two columns, representing assays performed without and with enzyme addition, are presented for each peptide assayed. In agreement with the results described above for intact histone, significant levels of acetylation by hGCN5 and dTAF$_{II}$230 were observed for the unacetylated H3 peptide. Note that although the diacetyl-(9/18)-H3 peptide was also a good substrate for both hGCN5 and dTAF$_{II}$230, neither enzyme acetylated the diacetyl-(9/14)-H3 peptide significantly. Comparison of the acetylation of the diacetyl-(9/14)-H3 and diacetyl-(9/18)-H3 peptides suggests that Lys14 is a preferred site for both hGCN5 and dTAF$_{II}$230. Interestingly, this same residue has recently been shown to be the preferred site of H3 acetylation by yeast GCN5 (yGCN5) in vitro (Kuo et al., 1996). While little acetylation of the unacetylated H4 peptide was seen with hGCN5, this peptide was a good substrate for dTAF$_{II}$230, in agreement with the results obtained with intact histones (Figure 4B).

Putative Catalytic Domain of dTAF$_{II}$230 and yTAF$_{II}$130

To determine the portion of the dTAF$_{II}$230 molecule responsible for HAT activity, we expressed a series of C-terminal deletion mutants as depicted in Figure 6A. A HAT-activity gel analysis employing equimolar amounts of these proteins (Figure 6B) revealed that the three mutants bearing the largest deletions, N545, N596, and N885, did not possess detectable HAT activity. In contrast, mutant proteins N1140 and N1480 and also full-length dTAF$_{II}$230 displayed HAT activity. These results suggest that sequences involved in histone acetylation by dTAF$_{II}$230 are situated between residues 1 and 1140. Furthermore, sequences between residues 885 and 1140 are critical for HAT activity, owing to possible roles in either catalysis or protein folding.

To further define the HAT catalytic domain, we analyzed the yeast homolog. yTAF$_{II}$130 has 52% sequence similarity with the N-terminal region of dTAF$_{II}$230 (aa 1–1370) and lacks the region corresponding to the C-terminus, including the bromodomains (see Figure 6A). The full-length recombinant yTAF$_{II}$130 displays HAT activity both in the activity gel and liquid assays (data not shown). For mapping the HAT domain, yTAF$_{II}$130 was divided into the three overlapping fragments, namely N (aa 1–450), M (aa 354–817), and C (aa 690–1066) (Figure 7A), each of which was expressed in and purified from E. coli. HAT-activity gel analysis revealed that only the M region, representing the central portion of yTAF$_{II}$130, had HAT activity (Figure 7B). Significantly, this region is highly conserved in dTAF$_{II}$230 and hTAF$_{II}$250, aligning to residues 499–1003 of dTAF$_{II}$230, which are included in the active mutant protein N1140 (see Figure 6B).

Among various acetyltransferases including the GCN5 protein family, cytoplasmic HAT1, and α-N-acetyltransferases, putative acetyl-CoA binding sites (Lu et al., 1996) are conserved (Kleff et al., 1995; Borrow et al., 1996; Parthun et al., 1996; Reifsnyder et al., 1996). However, multiple-alignment analysis (Lawrence et al., 1993) showed no significant sequence similarity within residues 1 to 1140 of dTAF$_{II}$230 (Figure 6) and 354 to 817 of yTAF$_{II}$130 (Figure 7) to the putative acetyl-CoA binding sites. Moreover, comparison of these regions with protein-sequence databases (Altschul et al., 1990) showed no obvious sequence similarity to any other proteins. These results lead us to suggest that the HAT catalytic domain of TAF$_{II}$250 may represent a second type of HAT domain.

Discussion

HAT Activity Is Intrinsic to TAF$_{II}$250

In this report, we demonstrate that recombinant dTAF$_{II}$230 and yTAF$_{II}$130 and natural hTAF$_{II}$250 possess

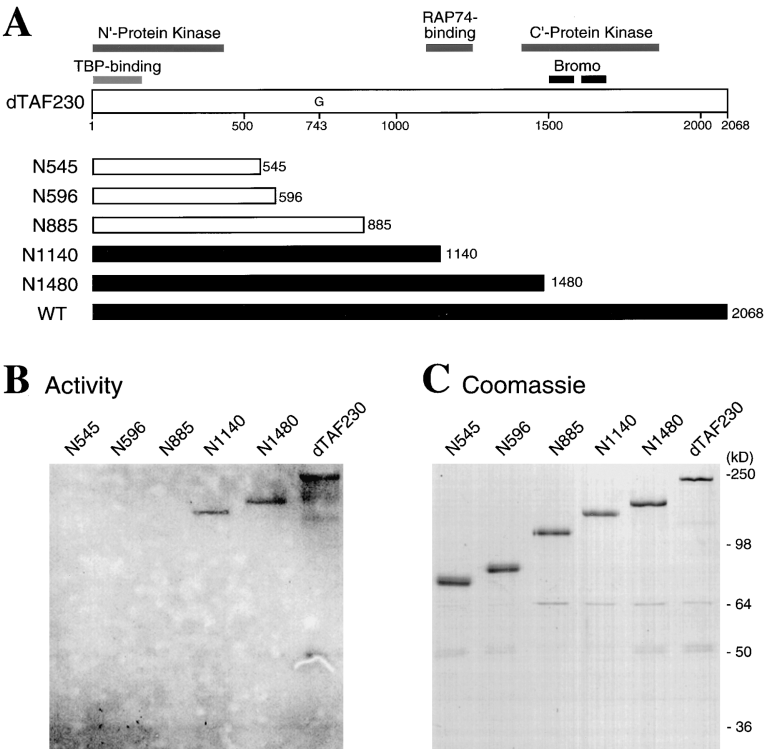

Figure 6. HAT Activity Maps to the N-terminal Portion of dTAF$_{II}$230

(A) The uppermost diagram depicts structural and functional domains found in dTAF$_{II}$230. The TBP-binding domain (Kokubo et al., 1994b; Nishikawa et al., 1997), the two protein kinase domains (Dikstein et al., 1996), the RAP74 binding domain (Ruppert and Tjian, 1995), and the two bromodomains (Kokubo et al., 1993; Weinzierl et al., 1993) are indicated. (G) denotes the glycine residue at position 743 equivalent to the site of the ts-13 mutation in hamster TAF$_{II}$250 that causes G1 arrest in cell-cycle progression (Hayashida et al., 1994). C-terminal deletion mutants that were tested for HAT activity in the activity gel assay are depicted in the lower portion. Open bars denote proteins that did not possess HAT activity, and closed bars denote proteins with HAT activity.

(B) Equimolar amounts of the C-terminal–deletion mutants depicted in (A) and full-length dTAF$_{II}$230 were compared using the activity gel assay.

(C) Equivalent amounts of the C-terminal–deletion mutants depicted in (A) and tested for HAT activity in (B) are shown following SDS–PAGE and Coomassie blue staining.

HAT activity and show that the HAT activity of dTAF$_{II}$230 is specific for H3 and H4 in vitro. Several independent lines of evidence suggest that these observations are not artifactual. Recombinant dTAF$_{II}$230 displayed HAT activity in both the liquid and activity gel assays. The activity gel assay, which involves denaturation of samples in 1% SDS (with boiling) prior to fractionation and subsequent renaturation within the gel matrix, strongly suggests that the HAT activity detected is intrinsic to dTAF$_{II}$230. Polypeptides potentially associated with dTAF$_{II}$230 following affinity purification are expected to dissociate and separate from the dTAF$_{II}$230 polypeptide during electrophoresis, and their reassociation during renaturation is prevented by entrapment within the gel matrix.

However, one might argue the possibility that HAT activity is catalyzed by polypeptides tightly associated with dTAF$_{II}$230 in complexes that resist dissociation in 1% SDS. The following results indicate that this is not the case. The use of stronger denaturing conditions (4% SDS or 4% SDS plus 8 M urea) or dTAF$_{II}$230 purified further by size-exclusion chromatography in buffer containing 6 M guanidine hydrochloride gave results similar to those shown in Figure 3 (data not shown). Moreover, the active deletion mutants of dTAF$_{II}$230 (N1140 and N1480 in Figure 6) and of yTAF$_{II}$130 ([M] in Figure 7) show the expected molecular weights on both Coomassie blue–stained gels and HAT-activity gels. Since the mutants of dTAF$_{II}$230 and yTAF$_{II}$130 were expressed in insect cells and bacteria, respectively, and since the yTAF$_{II}$130 mutants were purified using buffer containing 6 M guanidine hydrochloride, it is unlikely that the HAT activity of both sets of mutant proteins is due to contaminating polypeptides. Finally, our analyses of HeLa nuclear extract (Figure 1) and anti-TBP immunoprecipitates (Figure 2) provide independent support that hTAF$_{II}$250 also has HAT activity.

The Bromodomains of dTAF$_{II}$230 Are Dispensable for HAT Activity

Since the HAT activities of GCN5 and dTAF$_{II}$230 both show a strong preference for H3 as a substrate in vitro and since the only significant homology between these proteins is found in their bromodomains (one in hGCN5 and two in dTAF$_{II}$230; see Figure 6A), we tested whether the bromodomain may play a role in histone recognition or binding. Deletion of the C-terminal 588 amino acids of dTAF$_{II}$230 removes both of the bromodomains but does not affect HAT activity in vitro (N1480 in Figure 6) and does not alter the H3/H4 specificity of dTAF$_{II}$230 (data not shown). Thus, it is unlikely that the bromodomain is involved in histone recognition or acetate transfer by dTAF$_{II}$230. This is supported by our finding that yTAF$_{II}$130, which lacks bromodomains, has HAT activity in vitro (Figure 7). Moreover, the bromodomain in yGCN5 is dispensable for HAT activity in vitro (Candau et al., 1997).

A function has not yet been demonstrated for the bromodomain in any protein, but it has been suggested to mediate protein–protein interactions (Haynes et al., 1992; Tamkun et al., 1992). Given that the bromodomain is required for yGCN5 function in yeast (Marcus et al., 1994), a role in targeting the HAT activity of GCN5 to appropriate chromatin loci has been proposed (Brownell and Allis, 1996). However, our finding that both yTAF$_{II}$130 and the dTAF$_{II}$230 mutants lacking the bromodomain possess HAT activity in vitro argues against a role in substrate recognition.

Substrate Specificity of the dTAF$_{II}$230 HAT

The dual H3/H4 substrate specificity of dTAF$_{II}$230 is similar to that described previously for hGCN5 and P/CAF (Yang et al., 1996) and yGCN5 (Kuo et al., 1996). Similarly, we have found that dTAF$_{II}$230, like hGCN5 (Yang et al.,

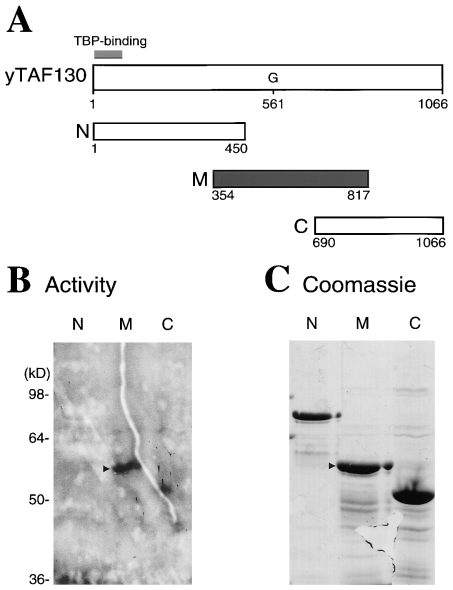

Figure 7. HAT Activity Maps to the Central Portion of yTAF$_{II}$130

(A) The uppermost diagram depicts the structural and functional domains found in yTAF$_{II}$130. The TBP binding domain (T. Kokubo, J. Nishikawa, and Y. N., unpublished data) is indicated. (G) denotes the glycine residue at position 561 equivalent to the site of the ts-13 mutation in hamster TAF$_{II}$250 that causes G1-arrest in cell-cycle progression (Hayashida et al., 1994). The three deletion mutants that were tested for HAT activity in the activity gel assay are depicted in the lower portion. Open bars denote proteins without HAT activity, and closed bars denote proteins with HAT activity.

(B) Equimolar amounts of the deletion mutants depicted in (A) were compared using the activity gel assay. Repeated analyses confirmed that the minor density visible in lane (C) is not an activity band but is an artifact related to the crease apparent in the dried gel. The arrowhead indicates the position of the mutant protein M.

(C) Equivalent amounts of the deletion mutants depicted in (A) and tested for HAT activity in (B) are shown following SDS–PAGE and Coomassie blue staining. The N-terminal fragment (aa 1–450) migrates with an apparent molecular weight larger than expected, owing to physical properties of its amino acid sequence. The arrowhead indicates the position of the mutant protein (M).

1996) and yGCN5 (J. B. and C. D. A., unpublished data), acetylates nucleosomal histones weakly in vitro. This is in contrast to the ability of P/CAF to acetylate H3 in core particles (Yang et al., 1996).

Acetylation-site utilization determined by assays with synthetic histone amino-terminal peptides revealed overall similarity in site utilization by hGCN5 and dTAF$_{II}$230. Significantly, the experiments with H3 peptides containing ϵ-N-acetyllysine at distinct positions revealed that Lys-14 but not Lys-9 of H3 is a preferred site of acetylation by both hGCN5 (Kuo et al, 1996) and dTAF$_{II}$230 (Figure 5). In lower eukaryotes, acetylation at Lys-9 is associated with deposition of newly synthesized H3 in vivo (Sobel et al., 1995), whereas Lys-14 of H3 is preferentially acetylated by yGCN5 in vitro (Kuo et al., 1996). Thus, preferential modification of Lys-14 by dTAF$_{II}$230 is consistent with a role in transcription-associated acetylation, as suggested previously for yGCN5 (Brownell et al., 1996).

The basis for the observed site and substrate specificity of dTAF$_{II}$230 (or any other known HAT) with free histones is not understood. Recently, it has been proposed that efficient acetylation of chromatin substrates by HATs like GCN5 may require multimeric HAT complexes containing subunits capable of transiently exchanging with nucleosomal histones (Roth and Allis, 1996). It is an intriguing possibility that the histone-like features of TAF$_{II}$s described above may play a role in facilitating chromatin acetylation by TAF$_{II}$250.

Possible Significance of TAF$_{II}$250 HAT Activity

Several lines of evidence suggest that acetylation or removal of core histone amino termini enhances transcription-factor binding to nucleosomal DNA (Lee et al., 1993; Vettesse-Dadey et al., 1994). Nucleosome assembly in vitro represses transcription of plasmids in vitro unless TBP-promoter or TFIID–promoter complexes are formed prior to nucleosome assembly (Workman and Roeder, 1987; Meisterernst et al., 1990), suggesting that nucleosomes inhibit binding of TBP or TFIID to natural (chromatin) templates. Moreover, experimental evidence suggests that the amino termini of the core histones mediate this inhibition. Imbalzano et al. (1994) reported that TBP binding to a TATA box in nucleosomal DNA is inhibited unless human SWI/SNF and ATP are present. Significantly, SWI/SNF and ATP are not required for TBP binding when nucleosomes contain hyperacetylated histones. Similarly, Godde et al. (1995) demonstrated that TBP binding occurs only when the TATA box is positioned within linker DNA at the edge of nucleosomes in which the core histone amino termini are removed by proteolysis (presumably mimicking acetylation). These authors found that TBP does not bind nucleosomes containing intact core histones under any of the conditions tested. Taken together, these studies demonstrate that core histone amino termini regulate the accessibility of the TATA box for binding by TBP.

Our data suggest that acetylation of nucleosomes at promoters by TAF$_{II}$250 may be part of a process acting to enhance the exposure of promoter elements for binding by TFIID. Acetylation could positively regulate promoter binding by TFIID directly by alleviating masking of binding sites by histone amino-terminal tails or indirectly by increasing promoter exposure through acetylation-induced changes in nucleosome conformation (Norton et al., 1990; Bauer et al., 1994) or effects on higher-order chromatin structure (Garcia-Ramirez et al., 1992; 1995). This proposed role is not necessarily limited to promoters containing TATA elements, since TFIID plays a role in the recognition of other initiation elements (e.g., the initiator) in TATA-less promoters (reviewed by Orphanides et al., 1996; Roeder, 1996; Verrijzer and Tjian, 1996). The possible existence of histone octamer-like TAF$_{II}$ complexes in TFIID (Hoffmann et al., 1996; Xie et al., 1996) suggests the possibility that acetylation of nucleosomes by TAF$_{II}$250 may facilitate nucleosome displacement by TAF$_{II}$ complexes or facilitate exchange of histone and TAF$_{II}$ proteins.

It seems likely that knowledge of any enzymatic activities associated with TAF$_{II}$s, such as the kinase activity (Dikstein et al., 1996) or the HAT activity we describe here for TAF$_{II}$250, will factor significantly into understanding TAF$_{II}$ function in the native context. Our finding that HAT activity is associated with a component of the preinitiation complex suggests that chromatin-modifying activities associated with other components of the

transcriptional apparatus are likely to have important roles in transcriptional activation from chromatin templates.

Experimental Procedures

Preparation of Recombinant Proteins

Drosophila TAF_II230 (Kokubo et al., 1993) and TAF_II62 (Kokubo et al., 1994a) were expressed as FLAG-tagged proteins in Sf9 cells via baculovirus FLAG fusions. FLAG fusions were affinity purified with M2-agarose (Kodak-IBI) according to the protocols of the manufacturers. Buffer B (20 mM Tris-HCl [pH 8.0], 0.5 mM $MgCl_2$, 10% glycerol, and 0.1% NP-40) supplemented with 10 mg/ml aprotinin, 10 mg/ml leupeptin, 1 mg/ml pepstatin A, 1 mM DTT, and 0.5 M KCl (or, in some cases, 0.15 M KCl) was used for extraction and column binding and washing. Fusion proteins were eluted with buffer B, supplemented as above and containing 0.1 mg/ml FLAG peptide.

For $yTAF_II130$ expression, cDNAs corresponding to the $yTAF_II130$ portions shown in Figure 7A were amplified by PCR and subcloned into E. coli expression plasmid pET28a or 6His-pET5a. Recombinant proteins were affinity purified with Ni^{2+}-NTA-agarose (Qiagen) according to the protocol of the manufacturer, except that Buffer B/0.5M KCl/6M guanidine was used for chromatography.

hGCN5 was expressed in E. coli strain JM109 as the 6 × His fusion of the 5′-BglII-3′–EcoRI fragment from the original phage clone (Candau et al., 1996) inserted into the pRSET vector (Invitrogen) at BamHI–EcoRI sites. Crude hGCN5 was prepared by sonicating IPTG-induced bacterial pellets in 20% sucrose, 50 mM Tris [pH 8.0], 0.3 M NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, and the supernatant prepared by centrifugation at 12,000 × g for 30 min used without further purification.

Preparation of Substrates for HAT Assays

Crude core histones were prepared from isolated chicken erythrocyte nuclei (Olins et al., 1976) or HeLa-cell nuclei (Annunziato and Seale, 1983) by 0.4 N H_2SO_4 acid extraction and 5% perchloric acid precipitation. Purified HeLa core histone fractions were prepared by reverse-phase HPLC of acid extracts on a 4.6 × 200 mm Brownlee Aquapore RP-300 column (Applied Biosystems) eluted with an acetonitrile gradient in 0.1% TFA. Carboxy-terminal cysteine histone amino-terminal peptides and histone amino-terminal MAP peptides (Tam, 1988) were obtained from the protein core facility at Baylor College of Medicine (Houston, TX).

HAT Assays

HAT-activity gel assays were performed as described previously (Brownell and Allis, 1995) except that polyacrylamide gels and cathode reservoir buffers were made 0.1 mg/ml in calf thymus histone or BSA (Sigma) prior to use. Liquid assays for HAT activity were performed essentially as described (Brownell and Allis, 1995). Crude core histones (8 μg) or purified HeLa histones (2 μg) and enzyme samples were incubated for 10 min at 30°C in a final volume of 50 μl of buffer A (50 mM Tris-HCl [pH 8.0], 10% (v/v) glycerol, 1 mM DTT, 1mM PMSF, and 0.1 mM EDTA). Reactions were initiated by the addition of [^{3}H]-acetyl-CoA (100 nCi, 6.1 Ci/mmol; ICN) to a final concentration of 0.328 μM. HAT activity was determined by liquid-scintillation counting of aliquots of reactions spotted on P-81 filters (Whatman) and processed as described (Brownell and Allis, 1995). Aliquots of reaction mixtures were resolved on 12% SDS–PAGE gels, stained with Coomassie blue, and fluorographed to identify acetylated proteins. Acetylation of synthetic histone amino-terminal peptides was performed as above employing 300 ng peptide per reaction and an incubation time of 20 min.

Immunoprecipitation-HAT Assays

COS cells were grown in 14 cm culture dishes. Cells were harvested by scraping off in 1 ml ice-cold PBS and gently pelleted by centrifugation. The PBS was aspirated, and the cells were resuspended into 1 ml Lysis Buffer IPH (50 mM Tris-HCl [pH 8.0]; 150 mM NaCl; 5 mM EDTA; 0.5% (v/v) NP-40; and 0.1 mM PMSF, aprotinin, leupeptin, and pepstatin). The lysis mixture was incubated on ice for 20 min and then cleared by centrifugation at 12,000 × g for 10 min at 4°C. The supernatant constituted whole-cell extract. HeLa-cell nuclear extracts were prepared according to Dignam et al., 1983.

Antibodies were added to 1 ml of whole-cell extract or 100 μl of nuclear extract and incubated at 4°C for 2 hr. A 50:50 mix of Protein A–Sepharose/Protein G–Sepharose (15 μl each) was added, and the mixture rotated slowly overnight at 4°C. Immune complexes were pelleted by gentle centrifugation and washed three times with 1 ml Lysis Buffer IPH. After the final wash, the buffer was aspirated down to 30 μl. 1.25 μl of 20 mg/ml histones and 1 μl of ^{3}H-acetyl-CoA were added, and a HAT assay was performed at 30°C. Histone acetylation was measured using the P-81 filter assay described above.

Antibodies against CBP (PharMingen), $hTAF_II250$, and E1A (Santa Cruz Biotechnology) are commercially available. Antisera to TBP have been described previously (Pruzan et al., 1992). Other antisera tested were directed against Sp1 (S. Jackson), MDM2 (A. Levine), myc (J. Pines), DNA-PK catalytic subunit (S. Jackson), hBRM (C. Muchardt), and E7 (Santa Cruz).

Other Procedures

Concentrations of wild-type and mutant TAFs employed in assays were normalized according to Coomassie blue staining of 8% SDS–PAGE minigels. FLAG epitope–tagged proteins were also characterized by immunoblotting with M2 monoclonal antisera to the FLAG tag (Kodak). Western blots were visualized using goat anti-mouse secondary antibodies conjugated to alkaline phosphatase following reaction with NBT and BCIP.

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