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The Histone Folds in Transcription Factor TFIID*

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The transcription factor TFIID is a multimeric protein complex containing the TATA box-binding polypeptide (TBP) and TBP-associated factors. We have previously reported that the N-terminal regions of dTAF$_{II}$62 and dTAF$_{II}$42 have sequence similarities with histones H4 and H3. Here, we demonstrate that the histone-homologous regions of dTAF$_{II}$62 and dTAF$_{II}$42 form a heteromeric complex both *in vitro* and in a yeast two-hybrid system. Neither dTAF$_{II}$62 nor dTAF$_{II}$42 forms a homomeric complex, in agreement with a nucleosomal histone character. Moreover, circular dichroism measurements show that the heteromeric complex is dominated by α-helical secondary structure. These results strongly suggest the existence of a histone-like surface on TFIID.

The transcription factor TFIID plays a central role in the assembly of the basic transcriptional machinery into a preinitiation complex (for review, see Ref. 1). TFIID binds to the core promoter and thus provides a foundation for association of the other initiation factors and RNA polymerase II. Biochemical studies of eukaryotic transcriptional activation demonstrate both physical and functional interactions of activators with TFIID that in turn facilitate preinitiation complex assembly and function (for review, see Ref. 1). TFIID is a multimeric protein complex, comprising the TATA box-binding protein (TBP)[1] and numerous tightly associated factors called TAFs (for review, see Refs. 2 and 3). To date, nine TAF subunits of *Drosophila* TFIID (dTAF$_{II}$230, -150, -110, -85, -62, -42, -28α, -28β, and -22) have been cloned (reviewed in Ref. 3). In addition, several homologs have been isolated from both human (hTAF) and yeast (yTAF), suggesting that TAF-mediated activation pathways are conserved from yeast to man (reviewed in Ref. 3).

A central question in eukaryotic transcriptional regulation is how TFIID gains access to a chromatin template and how stable association is maintained within the chromosome. A potentially relevant finding is that the N-terminal regions of the dTAF$_{II}$62 and dTAF$_{II}$42 proteins have sequence similarities with the C-terminal core domain of histones H4 and H3, respectively (4). More recently, it has been shown that the histone-like regions are well conserved in both the yeast (5)[2] and human (6–9) counterparts to dTAF$_{II}$62 and dTAF$_{II}$42.

The nucleosome core consists of 146 bp of DNA wrapped around an octameric complex of two copies of each of histones H2A, H2B, H3, and H4. Despite a low sequence similarity, all histones contain a long helix flanked on either side by a loop segment and short helix, termed the "histone fold" (reviewed in Refs. 10–12). The histone fold is involved in formation of the stable H2A-H2B and H3-H4 heterodimers. H3-H4 dimers further associate to form the $(H3\text{-}H4)_2$ tetramer. The $(H3\text{-}H4)_2$ tetramer alone is responsible for organizing the core 120 bp of DNA into the arrangement found in the octameric complex (13).

Recently, numerous proteins potentially containing the histone fold have been identified by data base search (14). This group includes TAF$_{II}$62 and TAF$_{II}$42, plus many DNA-binding proteins and multimeric proteins. The data base search also identified transcription initiation factor TFIIB as a protein that has the histone fold. However, both NMR (15) and x-ray crystallography (16) show that TFIIB does not have the histone fold. Thus, it is crucial to confirm the prediction using biochemical and physical techniques. To investigate the significance of the sequence similarities between dTAF$_{II}$62-dTAF$_{II}$42 and histones H4-H3, we have tested the ability of the histone-like regions in dTAF$_{II}$62 and dTAF$_{II}$42 to form a stable heteromeric complex. Here, we demonstrate that the histone-homologous regions of dTAF$_{II}$62 and dTAF$_{II}$42 form a heteromeric complex both *in vitro* and in a yeast two-hybrid system. We further demonstrate that this heteromeric complex, like the H3-H4 complex, is predominantly α-helical. The results described here strongly suggest that TFIID has a histone-like surface.

MATERIALS AND METHODS

Plasmids—To make a glutathione *S*-transferase (GST)-tagged dTAF$_{II}$62 expression plasmid, DNA encoding dTAF$_{II}$62 residues 1–91 (4) was amplified by polymerase chain reaction as an *Eco*RI-*Bam*HI fragment and subcloned into pGEX2 (Pharmacia Biotech Inc.). To create a histidine (His)-tagged dTAF$_{II}$42 expression plasmid, DNA encoding dTAF$_{II}$42 residues 1–100 (4) was amplified by polymerase chain reaction as an *Nde*I-*Eco*RI fragment and subcloned into pET15e (17). For yeast two-hybrid plasmids, both dTAF$_{II}$62 (amino acids 1–91) and dTAF$_{II}$42 (amino acids 1–100) were amplified as *Nco*I-*Bam*HI fragments and subcloned into both pAS1-CYH2 and pACTII.[3] Note that a stop codon was created just after the TAF sequence in all constructs.

In Vitro Interactions—GST-tagged dTAF$_{II}$62 and His-tagged dTAF$_{II}$42 were expressed in *Escherichia coli* BL21(DE3) and BL21 cells, respectively, according to a standard protocol (17). After harvesting the bacteria, all procedures were carried out at 4 °C. Bacterial pellets were resuspended into [1,25] culture volume of buffer B (20 mM Hepes-KOH, pH 7.5, 5 mM $MgCl_2$, 10% glycerol, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride) containing 0.5 M KCl and 6 M guanidine HCl and rotated for 60 min. After centrifugation at 50,000

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[1] The abbreviations used are: TBP, TATA box-binding protein; TAF, TBP-associated factor; bp, base pair(s); GST, glutathione *S*-transferase; NTA, nitriloacetate; aa, amino acids.

[2] M. Swanson and Y. Nakatani, unpublished observations.

[3] S. J. Elledge, unpublished data.

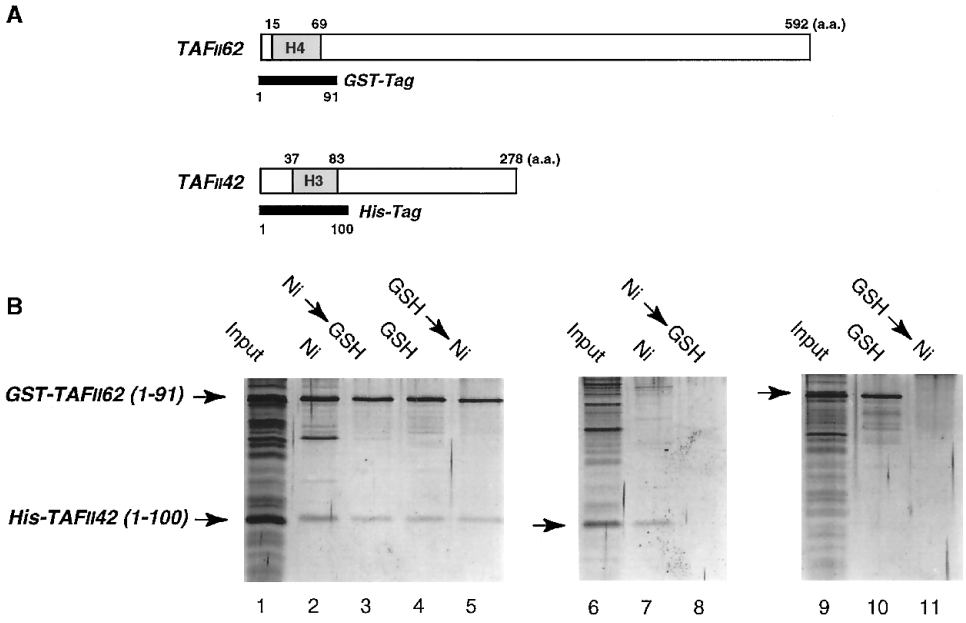

FIG. 1. **Histone-like regions in dTAF$_{II}$62 and dTAF$_{II}$42 form a heteromeric complex *in vitro*.** *A*, schematic overall structures of dTAF$_{II}$62 and dTAF$_{II}$42. The regions having sequence similarities with nucleosomal core histones H4 and H3 (4) are *shaded*. The regions expressed in *E. coli* for interaction study are depicted by *thick bars*. *B*, interaction between histone-like regions *in vitro*. GST-dTAF$_{II}$62 (aa 1–91) and His-dTAF$_{II}$42 (aa 1–100) were expressed independently as inclusion bodies and co-renatured (*lanes 1-5*). Control extracts without His-dTAF$_{II}$42 (aa 1–100) (*lanes 6–8*) or GST-dTAF$_{II}$62 (aa 1–91) (*lanes 9–11*) were also prepared. Complexes were purified by Ni^{2+}-NTA-agarose chromatography followed by glutathione-Sepharose chromatography (*lanes 2* and *3*; *lanes 7* and *8* for control). Similarly, complexes were also purified by glutathione-Sepharose chromatography followed by Ni^{2+}-NTA-agarose chromatography (*lanes 4* and *5*; *lanes 10* and *11* for control).

rpm for 30 min, the supernatants were analyzed by SDS-polyacrylamide gel electrophoresis to estimate the concentrations of the recombinant proteins. Extracts were mixed in an equimolar ratio and dialyzed against buffer B containing 0.5 M KCl and 1 M guanidine HCl for 12 h. Dialysis was continued for another 12 h in buffer B containing 0.1 M KCl and 0.1 M guanidine HCl. After centrifugation at 12,000 rpm for 10 min, the supernatant was used for interaction experiments. The supernatant was incubated with [1,20] bed volume of either Ni^{2+}-NTA-agarose (Qiagen) or glutathione-Sepharose (Pharmacia) for 30 min with rotation and washed with the same buffer 5 times. Protein was eluted from Ni^{2+}-NTA and glutathione matrices with the same buffer containing 100 mM imidazole or 20 mM glutathione (pH 7.5), respectively.

CD Measurements—For CD measurements, both His- and GST-tags were removed from the dTAF$_{II}$62-dTAF$_{II}$42 complex in the following manner. The complex was immobilized on glutathione-Sepharose as described above and was equilibrated with digestion buffer containing 20 mM Tris-HCl (pH 8.3), 0.15 M KCl, 2.5 mM $CaCl_2$, and 10% glycerol. Cleavage was carried out in 1 bed volume of the same buffer containing thrombin (10 units/ml). The supernatant was dialyzed against buffer B containing 0.5 M KCl. For further purification, 50 μl of the sample was applied to Superose 12 (25-ml bed volume) and chromatographed with the same buffer. The peak fractions (>98% purity) were used for CD measurements.

CD measurements were performed at 25 °C using a Jasco J700 spectropolarimeter. The spectra are presented as the average of eight scans in units of mean residue ellipticity (θ). The concentration of the protein was 15.7 μM in 20 mM sodium phosphate (pH 7.0). Protein concentration was determined by measuring absorbance at 280 nm and using a calculated extinction coefficient. The protein was denatured by the addition of guanidine hydrochloride to a concentration of 6 M. Protein secondary structure was estimated using the software supplied with the Jasco J700 spectropolarimeter.

Yeast Two-hybrid System—Plasmids for the yeast two-hybrid system were transformed into *Saccharomyces cerevisiae* strain Y190 in various combinations as shown in Table I. β-Galactosidase activity was measured as described (18).

RESULTS AND DISCUSSION

TAF Histone-like Regions form a Heteromeric Complex in Vitro—Based on the similarities between dTAF$_{II}$62 and dTAF$_{II}$42, and histones H4 and H3, intermolecular interactions between the histone-like regions in dTAF$_{II}$62 and dTAF$_{II}$42 were examined. dTAF$_{II}$62 (aa 1–91) and dTAF$_{II}$42 (aa 1–100) were expressed independently in *E. coli* as His- and GST-tagged proteins, respectively (Fig. 1*A*). Since both recombinant polypeptides are highly insoluble (data not shown), they were solubilized by disrupting *E. coli* in buffer containing 6 M guanidine. The two extracts were mixed at an equimolar ratio of recombinant polypeptides and co-renatured by dialyzing against buffer containing 0.1 M guanidine (Fig. 1*B*, *lane 1*). Complexes were purified by Ni^{2+}-NTA agarose chromatography followed by glutathione-Sepharose chromatography (*lanes 2* and *3*). GST-dTAF$_{II}$62 (aa 1–91) and His-dTAF$_{II}$42 (aa 1–100) proteins co-purified through these chromatographic steps. Similar results were obtained by glutathione-Sepharose chromatography followed by Ni^{2+}-NTA-agarose chromatography (*lanes 4* and *5*). In contrast, no specific protein was observed after the second chromatography step in controls including single extract (*lanes 6-11*).

The far-UV (250–200 nm) CD spectrum of the dTAF$_{II}$62 (aa 1–91)-dTAF$_{II}$42 (aa 1–100) heterocomplex is shown in Fig. 2. The overall shape of the spectrum, with minima in the mean residue ellipticity at 208 and 222 nm, is characteristic of polypeptides dominated by α-helical secondary structure. In support of this, we used manufacturer-supplied software to obtain the following estimates for the secondary structure: 41.4% α-helix, 8.4% β-strand, 15.8% turn, and 34.4% random.

Histone-like Regions Can Heterodimerize, but They Do Not Homodimerize—The interaction between the histone-like regions was further analyzed in a yeast two-hybrid system. dTAF$_{II}$62 (aa 1–91) and dTAF$_{II}$42 (aa 1–100) were fused to either the GAL4 DNA-binding domain or activation domain. Yeast bearing an integrated *GAL1-lacZ* fusion were transformed with different combinations of plasmids (Table I). The *lacZ* reporter gene is expressed in a manner dependent on the interaction of dTAF$_{II}$62 and dTAF$_{II}$42. Neither dTAF$_{II}$62 nor dTAF$_{II}$42 displayed significant activity when tested for self-interaction. We therefore conclude that dTAF$_{II}$62 and dTAF$_{II}$42 form only heteromeric complexes. It is noteworthy that dTAF$_{II}$62 and dTAF$_{II}$42 inhibited yeast growth (doubling

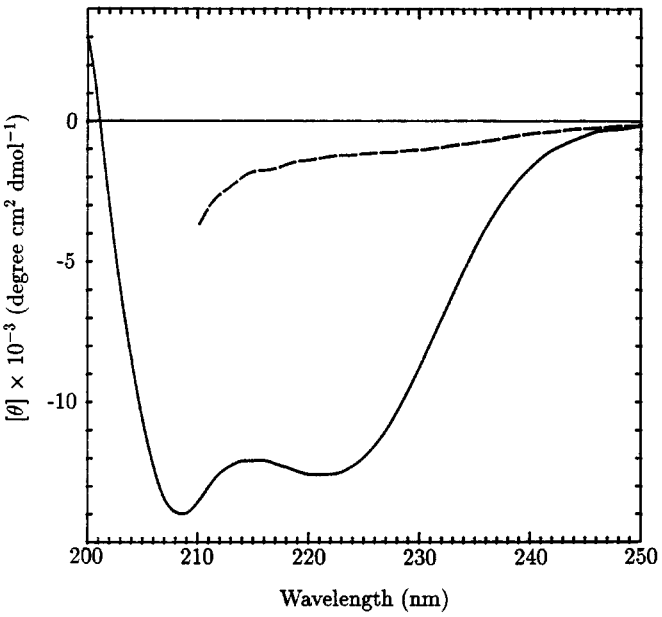

FIG. 2. **Circular dichroism spectra of $dTAF_{II}62$ (aa 1–91)-$dTAF_{II}42$ (aa 1–100) in native (*solid line*) and denatured (*dashed line*) conditions.**

time, >10 h) only when they were transformed together. Overexpression of acidic activators also inhibits yeast growth (19). The growth inhibition may thus be caused by exceptionally strong interactions between $dTAF_{II}62$ and $dTAF_{II}42$, resulting in overproduction of the GAL4 DNA-binding domain-activation domain complex. Alternatively, the heteromeric $dTAF_{II}62$-$dTAF_{II}42$ complex may titrate yeast factors important for growth.

In summary, we have demonstrated that the N-terminal regions of $dTAF_{II}62$ and $dTAF_{II}42$, similar in sequence to nucleosome core histones H4 and H3, form a heteromeric complex both *in vitro* and in a yeast two-hybrid system. Like histones H3 and H4, neither $dTAF_{II}62$ nor $dTAF_{II}42$ forms a homomeric complex, and CD measurements suggest that the heteromeric $dTAF_{II}62$-$dTAF_{II}42$ complex is dominated by α-helical secondary structure.

In some promoters, including the adenovirus major late and human *gfa* promoters (20–22), the TFIID footprint extends from the TATA box through to 35 bp downstream from the initiation site, whereas the TBP footprint extends only over the TATA box region. These results indicate that one or more TAFs contribute to the downstream interaction. Recent studies with recombinant TAFs demonstrated that $dTAF_{II}150$ alone can bind to the initiator element (23). Moreover, the $dTAF_{II}150$-TBP complex gives a footprint from the TATA box through to 35 bp downstream from the initiation site (23, 24), like native TFIID. However, the $dTAF_{II}150$-TBP complex does not produce DNase I-hypersensitive sites as observed for native TFIID (24). Thus, it is likely that other TAFs are also involved in the downstream interaction. We predict that the histone-like region in $dTAF_{II}62$-$dTAF_{II}42$ is bound to the downstream sequence because, similar to histone core, TFIID produces DNase I-hypersensitive sites about every 10 bp. The important question is whether the $dTAF_{II}62$-$dTAF_{II}42$ complex is a heterodimer or heterotetramer in the TFIID complex; the N-terminal histone-like regions of $dTAF_{II}62$-$dTAF_{II}42$ do form heterotetramers under physiological conditions,[4] suggesting that these TAFs could be present as a tetramer in the TFIID complex.

[4] X. Xie and S. K. Burley, personal communication.

TABLE I
Histone-like domains in dTAFII62 and -42 interact in the yeast two-hybrid system

Interaction between dTAFII62 (1–91) and dTAFII42 (1–100) was determined in the yeast two-hybrid system. dTAFII62 (1–91) and dTAFII42 (1–100) were fused to the GAL4 DNA binding domain and the GAL4 activation domain. Yeast bearing an integrated *GAL1-lacZ* fusion (strain SFY 526, Clontech) were transformed with different combinations of two plasmids. GAL4 activation domain with no insert was used as negative control. The resulting β-galactosidase activity was measured and indicated by units.

DNA binding	Activation	β-Galactosidase
		units
$dTAF_{II}42$ (1–100)	None	<5
$dTAF_{II}42$ (1–100)	$dTAF_{II}42$ (1–100)	<5
$dTAF_{II}42$ (1–100)	$dTAF_{II}62$ (1–91)	785
$dTAF_{II}62$ (1–91)	None	<5
$dTAF_{II}62$ (1–91)	$dTAF_{II}42$ (1–100)	6,000
$dTAF_{II}62$ (1–91)	$dTAF_{II}62$ (1–91)	<5

The downstream interaction plays an important role in both basal and activated transcription. TFIID binds to the downstream region in a DNA sequence-dependent fashion, at least in the human *gfa* promoter, contributing to both stable TFIID binding and effective transcription *in vitro* (22, 25). In contrast, in the adenovirus E4 promoter, TFIID does not bind to the downstream region and produces very low basal transcription activity. Importantly, activators induce the downstream interaction and facilitate preinitiation complex formation (26, 27). Furthermore, the histone-like domains may have a more critical role in a natural chromatin environment, *e.g.* stimulation of the displacement of nucleosomal histones near the transcription start site and/or maintenance of histone exclusion from the transcription start site.

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