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The Involvement of the Histone Fold Motifs in the Mutual Interaction between Human $TAF_{II}80$ and $TAF_{II}22$[1]

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The TATA box-binding factor TFIID mediates transcriptional regulation through interactions with various regulatory factors and putative participation in reconfiguration of nucleosomes, utilizing its components, which include TATA box-binding protein (TBP) and TBP-associated factors (TAFs). Our and other previous studies have elucidated that there exist histone-similar TAFs. Studies on TAFs similar to histone H3 and H4 have revealed their biochemical and structural similarities to the corresponding histones. However, the existence of histone-like interactions involving the other TAFs is still ambiguous. Here we report the analyses with a two-hybrid system of the mutually interacting regions between $hTAF_{II}80$ and $hTAF_{II}22$, which resemble histone H4 and H2B, respectively. The results demonstrate the indispensability of the histone fold motifs of these two TAFs for mutual interaction. Together with earlier biochemical or structural studies, the present results suggest the presence of a histone octamer-like partial TAF complex and its involvement in transcription from chromatin templates.

Key words: histone fold motif, histone octamer, TAF, TFIID, transcription factor.

The TATA box-binding factor TFIID is a multisubunit complex, which is composed of the TATA box-binding protein (TBP) and TBP-associated factors (TAFs) (for reviews, *1*). Previous studies on TFIID using naked DNA templates proved that (i) TFIID facilitates assembly of general transcription factors and RNA polymerase II into a preinitiation complex (*2, 3*) and that (ii) TFIID plays a pivotal role in transcriptional regulation as a major target of various transcription factors (*4-6*).

Most previous studies concerning transcriptional regulation have focused on naked DNA. In fact, eukaryotic genomic DNA is packed into chromatin, which consists of histones and non-histone chromatin proteins (*7*). Studies of the effects of nuclease digestion upon several promoters have clarified that the nucleosome, the smallest unit of chromatin, must be reconfigured on gene activation (for reviews, *8*). Moreover, recent studies have identified various kinds of factors, such as SWI/SNF complex and Nurf, that are involved in reconfiguration of nucleosomes and chromatin (*9, 10*). Therefore the mechanism of transcription from chromatin DNA still remains to be elucidated.

TFIID may play a crucial role in the reconfiguration of nucleosomes in view of the previous findings. For example, (i) TFIID can release transcriptional repression when it binds to DNA before the reconstitution of chromatin *in vitro* (*11*), suggesting that TFIID overcomes the hindrance caused by nucleosomes in transcription. (ii) DNase I digestion of downstream promoter regions bound with TFIID shows hypersensitive sites at approximately 10-bp intervals (*12*). These patterns are quite similar to those observed in digestion of nucleosomal DNA with DNase I, implying that part of TFIID contains similar structure to nucleosomes.

To understand these characteristics of TFIID, we analyzed the primary structures of TBP and TAFs. Interestingly, two TAFs were found to contain regions similar to histones H3 and H4 (*13, 14*). Considering that nucleosomes consist of histone octamer (histone H2A, H2B, H3, and H4) and DNA, these histone-similar regions of the TAFs may have important roles in transcription from chromatin templates. Actually, these histone-similar TAFs can interact with each other *via* their histone-similar regions *in vitro* (*15*). In addition, recent X-ray diffraction analysis has shown that the complex of *Drosophila* $TAF_{II}62$ and $TAF_{II}42$ ($dTAF_{II}62$ and $dTAF_{II}42$, respectively) has almost the same structure as the tetramer of histone H3 and H4 (*16*). These studies imply the importance of the histone-similar regions of TAFs in the formation of the TFIID complex. It is quite clear that the structural relationships between the TAFs resembling histone H3 and H4 are comparable to those between histone H3 and H4. However, little is known about the interactions among other TAFs containing his-

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Abbreviations: 3-AT, 3-amino-1,2,4-triazole; aa, amino acid; β-gal, β-galactosidase; d, *Drosophila*; GAL4-AD, Gal4 activation domain; GAL4-DBD, Gal4 DNA-binding domain; h, human; TAF, TBP-associated factor; TBP, TATA box-binding protein; TFIID, transcription factor IID.

tone H2A and/or H2B similarity regions.

We performed yeast two-hybrid screening to search for interactors with the histone H4-similar region of hTAF$_{II}$80, and isolated cDNA encoding another TAF, hTAF$_{II}$22 (*17*), the homologue of dTAF$_{II}$28/22 (*13, 18*). We pointed out that this TAF has similarities to histone H2B/H3 (*17*). If we hypothesize that four distinct TAFs that possess histone similarity form a complex analogous to the histone octamer, the question arises as to whether these TAFs, especially the TAFs that contain regions similar to histone H2A and/or H2B, interact with each other *via* those regions. To examine this issue, we investigated the regions of hTAF$_{II}$80 and hTAF$_{II}$22 required for mutual interaction. We applied the two-hybrid system for the detection of this interaction, since it seems to be favorable for maintaining the three-dimensional structure of the expressed protein as it is in eukaryotic cells.

MATERIALS AND METHODS

Yeast Two-Hybrid System—The yeast two-hybrid system is a method used to detect physical interaction between two proteins. The protein of interest fused with the Gal4 DNA binding domain (GAL4-DBD) as a bait and another protein fused with the Gal4 activation domain (GAL4-AD) as a prey are expressed in yeast. Physical association between bait and prey leads to activated transcription from reporter genes containing the coding region of *HIS3* and *lacZ*. The *Saccharomyces cerevisiae* strain used for this two-hybrid system is Y190 (*MATa, gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3,-112 URA3::GAL→lacZ, LYS2::GAL→HIS3 cyh*^{r}) (a gift from Dr. S. Elledge; the same strain is now available from CLONTECH).

Construction of the Plasmids Expressing hTAF$_{II}$22 Derivatives—The plasmids carrying the cDNA encoding hTAF$_{II}$22 and its derivatives shown in Fig. 1 were constructed by polymerase chain reaction to create both the *Eco*RI-*Nde*I (GAATTCATATG) restriction sites overlapping the initiation codon and the *Xho*I-*Eco*RI (CTCGAGAATTC) recognition sequence following the created stop codon. The fragment of wild-type hTAF$_{II}$22 was amplified using a p22N primer (5′-CCGGAATTCATATGAACCAGTTTGGCCCCTC-3′) and a p22C primer (5′-GGCGAATTCTCGAGCTATTTCTTGGTTGTTTTCCGG-3′). The oligonucleotide primers used for creating other derivatives are listed in Table I. The fragments generated by the polymerase chain reaction were subcloned into pBluescript followed by confirmation of the sequences from both strands. The *Eco*RI-*Xho*I fragment derived from each construct was subcloned between the *Eco*RI and *Xho*I sites of the plasmid pACTII to express the fusion protein with GAL4-AD.

Construction of the Plasmids Expressing hTAF$_{II}$80 Derivatives—The plasmid expressing hTAF$_{II}$80 and its derivatives fused with Gal4-DBD shown in Fig. 4 were constructed by the oligonucleotide-directed mutagenesis. Each of pBSp80 or pBSp80⊿C225, containing the cDNA encoding the full length (pBSp80) or N-terminal region (aa 1-224; pBSp80⊿C225, constructed from pBSp80 by the following experimental procedure) of hTAF$_{II}$80, respectively, with the already created *Nde*I site at the position corresponding to the first methionine, was transfected into *Escherichia coli* strain BW313 and single-stranded DNA was prepared. Each oligonucleotide DNA (see Table II) was phosphorylated with T4 polynucleotide kinase and annealed to the single-stranded DNA to create the *Nde*I site following the newly created stop codon (TAGCATATG) at the C-terminal end of the hTAF$_{II}$80 derivatives. This

TABLE II. **The sequences of oligonucleotide DNA used for constructing the hTAF$_{II}$80 deletion mutants by site-directed mutagenesis.**

hTAFII80 mutant	oligonucleotide sequence
ΔC225	5' CACGCAGGCCTCGGTGAGCTCCTAGTAGTAGAGCTGCTG 3'
ΔC183	5' GTCGGCTGTGGTGGCCATATGCTATTTGCCCTTCAGGGG 3'
ΔC159	5' CTTCAGGGGTTCTGTCATATGCTACTTCTGTTGCTCTTT 3'
ΔC78	5' GCCATAGAGTGGCTCCATATGCTATAGCTTCAAGGCGTA 3'
ΔN77	
ΔN123	5' GCAGACGTCCAGGGGCATATGCTACAGAGGGGTATTGAT 3'

TABLE I. **The sequences of oligonucleotide DNA used for constructing the hTAF$_{II}$22 deletion mutants by PCR-mediated mutagenesis.**

hTAFII22 mutant	oligonucleotide sequence	
	forward primer	reverse primer
ΔN30	5' CCGGAATTCATATGCCTGAAAACAATCAGGTATTGAC 3'	5' GGCGAATTCTCGAGCTATTTCTTGGTTGTTTTCCGG 3'
ΔN52	5' CCTGGGGCAGGAGGTCGAATTCATATGGAAAACAATCAGGTA 3'	
ΔN74	5' TTAGTAAGAGAAGTGGGAATTCATATGCAGTTGGATGAAGAT 3'	
ΔN115	5' AAGTCTAGCACCCTGGGAATTCATATGGTCCAGCTGCATTTA 3'	
ΔC141	5' CCGGAATTCATATGAACCAGTTTGGCCCCTC 3'	5' CTGTTTGTGAGCTTCTGAATTCTCGAGCTATTTGTAGGGTCGGAT 3'
ΔC116		5' GTTCCACTGGCGCTCTGAATTCTCGAGCTAATCTTTCACCTCCAG 3'
ΔC75		5' CAGCATCTCCTCCACAGAATTCTCGAGCTACTCATTAGGATCCAC 3'
ΔC53		5' TTTCTTCTTGGTCAATGAATTCTCGAGCTAAGGGCTAAGACGACC 3'

template-primer mixture was rendered double-stranded by treatment with Sequenase version 2.0 (Amersham) and T4 DNA ligase. The resultant DNA was transfected into DH5α cells to select for the mutated plasmid. After confirmation of the sequences, the mutated plasmids were used for the construction of the derivatives. The DNA fragments derived from these mutated plasmids by *Nde*I or *Nde*I-*Sal*I digestion, were subcloned between the *Nde*I/*Nde*I or *Nde*I/*Sal*I sites of pAS1CYH2 to express the fusion protein with GAL4-DBD.

Detection of the β-Galactosidase Activity Caused by Association between Derivatives of hTAF$_{II}$80 and hTAF$_{II}$22—The yeast strain Y190 was co-transfected to tryptophan (Trp) and leucine (Leu) prototrophy with the pAS1CYH2-hTAF$_{II}$80 derivative as a bait plasmid (Trp) and the pACTII-hTAF$_{II}$22 derivative as a prey plasmid (Leu) by the Li-acetate method. After incubation on the selection plate (SC-Trp-Leu) for 4 days at 30°C, a single colony was streaked on an SC plate lacking Trp and Leu, and again incubated for 4 days at 30°C. To examine the β-galactosidase (β-gal) activity caused by the interaction between the bait and the prey, we used the β-gal filter lift assay (*19*).

Detection of the HIS3 Activity Caused by Association between Derivatives of hTAF$_{II}$80 and hTAF$_{II}$22—On the other hand, to examine the HIS3 activity, we streaked a single colony derived from the transformant shown above, and incubated it for 10 days at 30°C on SC plates containing 100 mM 3-amino-1,2,4-triazole (3-AT) and lacking Trp and Leu, with or without (−/+) histidine. The expression of *HIS3* gene enables cell growth on the plate lacking histidine in the presence of 3-AT (*20*).

RESULTS AND DISCUSSION

(A) Determination of Region of hTAF$_{II}$22 Engaged in Interaction with hTAF$_{II}$80—In order to identify the regions of hTAF$_{II}$22 essential for interaction with hTAF$_{II}$80, we prepared N- and C-terminal deletion mutants of hTAF$_{II}$22, and examined their interactions by the yeast two-hybrid system. In this system, physical association between bait and prey proteins activates transcription from reporter genes *HIS3* or *lacZ* (*21*). We applied both detection measures to estimate specific interaction in order to avoid the effect of the difference of DNA sequence in the promoter regions.

The N- and C-terminal deletion series of hTAF$_{II}$22 mutants were constructed considering the putative secondary structure predicted from the crystal structure of the histone fold motif (*22*) of corresponding histones (*23*). Figure 1 summarizes of the deletion mutants. Both the plasmid encoding a hTAF$_{II}$22 deletion mutant and the one encoding hTAF$_{II}$80ΔC225 were co-transformed into yeast strain Y190 for the two-hybrid system. hTAF$_{II}$80ΔC225 is sufficient for interaction with hTAF$_{II}$22 as previously reported (*16*). As a control, we utilized pSE1111 encoding the fusion protein of SNF4 with Gal4 activation domain (GAL4-AD), and pSE1112 encoding the fusion protein of SNF1 with Gal4 DNA-binding domain (GAL4-DBD), since both proteins specifically interact with each other (*21*).

Figure 2 shows the results obtained by the filter lift assay for β-galactosidase activity (*19*), which indicates the transcription of *lacZ*, the consequence of the interaction between hTAF$_{II}$22 derivatives and hTAF$_{II}$80ΔC225. The wild-type hTAF$_{II}$22 shows the blue coloration, which signifies interaction between hTAF$_{II}$22 and hTAF$_{II}$80ΔC225. Among the N-terminal deletion series, hTAF$_{II}$22ΔN30 and ΔN52 interact with hTAF$_{II}$80ΔC225, whereas hTAF$_{II}$22ΔN74 and ΔN115 do not. These results show that the region corresponding to the first helix of the histone fold motif is indispensable for interaction. Among the C-terminal deletion series, hTAF$_{II}$22ΔC141 shows interacting activity, while other deletion series do not. These results verify that the three putative helices that compose the histone fold motif are essential for interaction, and that the putative last helix of this subunit is dispensable. This putative helix is not within the histone fold motif. These results clearly indicate that the entire histone fold motif is essential for hTAF$_{II}$22 to interact with hTAF$_{II}$80.

Figure 3 shows the results obtained from the growth of the transformed yeast against 100 mM 3-amino-1,2,4-triazole (3-AT) in the absence of histidine. 3-AT is a chemical

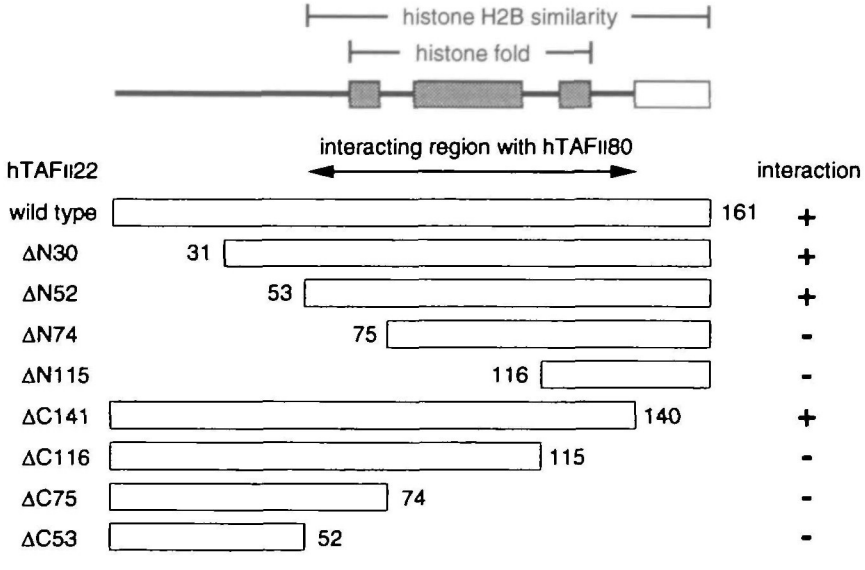

Fig. 1. **Schematic diagram of hTAF$_{II}$22 and its deletion mutants used in this study and summary of the interactions between hTAF$_{II}$80ΔC225 and hTAF$_{II}$22 derivatives.** The coding regions are presented as open boxes with the numbers of the terminal amino acid (aa) residues. The interactions of hTAF$_{II}$80ΔC225 with hTAF$_{II}$22 derivatives (see Fig. 2 and Fig. 3) are indicated by +/− at the right side of each derivative. The arrow above the diagram indicates the minimum region required for the interaction with hTAF$_{II}$80 determined in this study. Predicted α-helices are shown as four boxes, and the three shaded boxes indicate the helices composing the putative histone fold. This prediction was based on the similarity of aa sequences of histone H2B and hTAF$_{II}$22, and the crystal structure of the histone octamer core (*23*).

inhibitor of IGP dehydratase encoded by *HIS3*, one of the enzymes of the histidine synthesis pathway (*20*). The interaction between the two expressed proteins activates the transcription of *HIS3* gene, and leads to better growth on media lacking histidine. The transformed yeasts that show blue coloration in Fig. 2 exhibit significantly better growth than the others. Since all the transformants show growth in the presence of 100 mM 3-AT together with histidine (data not shown), the difference of growth among transformants is confirmed to be caused by *HIS3* expression, not by the toxicity of 3-AT. These results indicate that the hTAF$_{II}$22 region which encompasses amino acid residues 53 to 140 is required for interaction with hTAF$_{II}$80-ΔC225, and are totally consistent with the results obtained by means of the β-gal assay described above.

Therefore we conclude that this histone fold motif region of hTAF$_{II}$22 is involved in the interaction with hTAF$_{II}$80, suggesting that the interaction between two TAFs is quite similar to that of histones in histone octamer. This conclusion would not be affected even if the negative results were caused by poor expression of the mutant protein, because the positive results are sufficient to prove the involvement. We previously pointed out the similarity between hTAF$_{II}$22 or its homologues and histone H2B/H3, based on the primary structures (*16, 24*). However, considering the overall similarity between TAFs and histone octamer, our present results and the studies which proved the similarity of hTAF$_{II}$31 to histone H3 in biochemical and structural respects (*14, 16, 25*) imply that hTAF$_{II}$22 corresponds to histone H2B, not to H3. These results also imply that the tertiary structure of hTAF$_{II}$22 may resemble that of histone H2B.

(B) Determination of Region of hTAF$_{II}$80 Engaged in Interaction with hTAF$_{II}$22—To determine whether the histone-similar region of hTAF$_{II}$80 is essential for the interaction with hTAF$_{II}$22, we prepared deletion mutants of hTAF$_{II}$80, and introduced them into the *S. cerevisiae* expression vectors of the fusion protein in the same way as described in section (A).

The mutant series of hTAF$_{II}$80 were constructed mainly on the basis of conservation between homologues from four different species (*13, 14, 26-29*), except for mutants that contain or lack the N-terminal 77 amino acids, which is in accordance with the histone H4 similarity. Figure 4 shows the deletion mutants used in this study. Interaction studies were performed in the same manner as described above.

Figure 5 shows the results obtained by the filter lift assay of β-galactosidase activity consequent on the interaction between hTAF$_{II}$80 derivatives and hTAF$_{II}$22. The wild-type hTAF$_{II}$80 shows interacting activity with hTAF$_{II}$22. The C-terminal deletion series, including the mutant that contains only the 77 amino acid residues of hTAF$_{II}$80, show blue coloration in comparison with the negative control (SNF1). On the other hand, N-terminal deletion mutants show no coloration. These results apparently indicate that only the N-terminal 77 amino acids of hTAF$_{II}$80 are sufficient for interaction with hTAF$_{II}$22, which demonstrates that the histone H4-similar region of hTAF$_{II}$80 is indispensable for interaction.

Figure 6 shows the growth in the presence of 100 mM 3-AT, but in the absence of histidine. All the co-transformants showed growth in the presence of histidine, suggesting that the toxicity of 3-AT itself is negligible [data not shown, refer to section (A)]. Among the N-terminal deletion mutants, hTAF$_{II}$80ΔC225, ΔC183, and ΔC159 showed apparently better growth than the corresponding negative controls. The wild type and hTAF$_{II}$80ΔC78 exhibited better growth, even though the difference was not as apparent as those of the other three mutants described above. This experiment was performed four times, and the

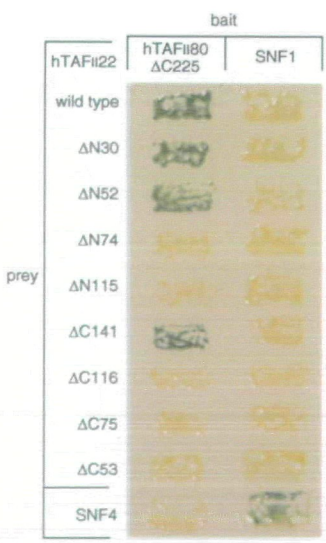

Fig. 2. Interactions between hTAF$_{II}$22 deletion mutants and hTAF$_{II}$80ΔC225 detected by β-gal filter lift assay. Yeasts from a single colony containing both bait and prey plasmids were cultured on SC plates lacking tryptophan and leucine for 4 days at 30°C, and then assayed (see "MATERIALS AND METHODS"). Lines show the proteins expressed from the prey plasmid, columns indicate the proteins from the bait plasmid. The combination of SNF1 and SNF4 was employed as a positive control; they interact with each other. This figure shows the status obtained by incubation for 3 h at 30°C. The difference between the left and the right columns was as clear as that shown in this figure after incubation for 16 h (data not shown).

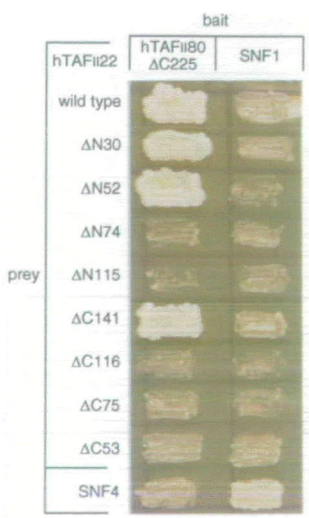

Fig. 3. Interactions between hTAF$_{II}$22 deletion mutants and hTAF$_{II}$80ΔC225 observed by resistance assay to 100 mM 3-AT in the yeast two-hybrid system. Yeasts containing both bait and prey plasmids derived from a single colony were incubated on SC plate containing 100 mM 3-AT and lacking tryptophan, leucine, and histidine for 10 days at 30°C. Presentation of the bait and prey is the same as in Fig. 2.

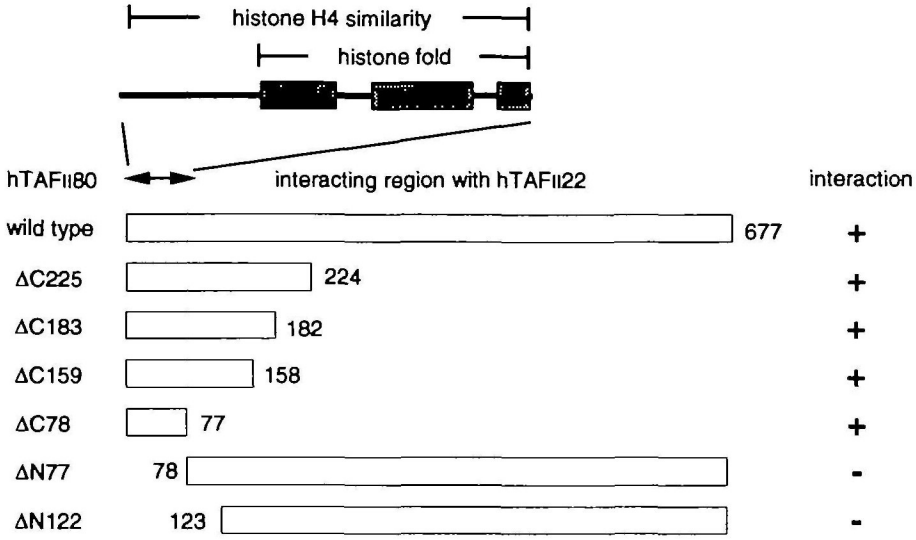

Fig. 4. **Schematic diagram of hTAF$_{II}$80 and its deletion mutants used in this study and summary of the interactions between hTAF$_{II}$22 and hTAF$_{II}$80 derivatives.** The aa number of the terminal residue in each derivative is shown in the figure. The three shaded boxes indicate the predicted α-helices constituting the histone fold motif in the same manner as in Fig. 1. The interactions of hTAF$_{II}$80 derivatives (see Fig. 5 and Fig. 6) with hTAF$_{II}$22 are indicated by +/− at the right side. The arrow above the diagram shows the region interacting with hTAF$_{II}$22 determined in this study.

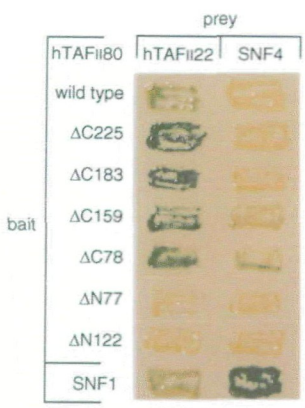

Fig. 5. **Interactions between hTAF$_{II}$80 deletion mutants and hTAF$_{II}$22 detected by β-gal filter lift assay.** Yeasts containing both bait and prey plasmids from a single colony were cultured on SC plates lacking tryptophan and leucine for 4 days at 30°C, and then assayed. Lines indicate the proteins expressed from the bait plasmid, columns show the proteins from the prey plasmid. The colony carrying the plasmids expressing SNF1 and SNF4 was employed as a positive control. The assay was performed in the same way as in Fig. 2. The difference between the left and the right columns was apparent after incubation for 16 h at 30°C (data not shown).

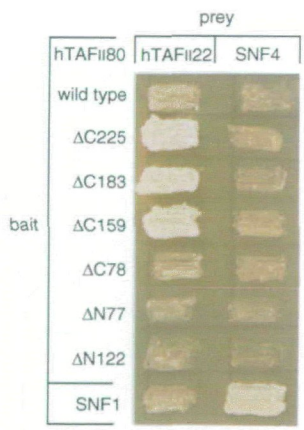

Fig. 6. **Interactions between hTAF$_{II}$80 deletion mutants and hTAF$_{II}$22 observed by resistance assay to 100 mM 3-AT in the yeast two-hybrid system.** Yeasts co-transformed with both bait and prey plasmids were patched on SC plates containing 100 mM 3-AT and lacking tryptophan, leucine, and histidine for 10 days at 30°C. Presentation of the bait and prey is the same as in Fig. 5.

results were highly reproducible. These results are also consistent with those obtained by the β-gal assay. The slight difference between the two results is supposed to be due to the difference between the two detection measures for interaction. Therefore, we conclude that the wild type and ΔC78 can interact with hTAF$_{II}$22.

These results demonstrate that the N-terminal 77 amino acid residues of hTAF$_{II}$80 are sufficient to allow interaction with hTAF$_{II}$22, even if the negative data were due to poor expression of the mutant protein. This is the region similar to histone H4. Conservation of the primary structure of hTAF$_{II}$80 among different species is less significant in the N-terminal region than in the C-terminal region, but the histone H4 similarity is well-conserved among all hTAF$_{II}$80 homologues (*13, 14, 27-29*). Since the histone H4 similarity region of hTAF$_{II}$80 alone possesses the ability to interact with hTAF$_{II}$22, the less conserved N-terminal region may form the association surface with other histone-similar TAFs. Therefore we suppose that the more conserved C-terminal region of this subunit is involved in interactions with other regulatory factors, and these associations might affect the interactions with TAFs and/or other factors through conformational changes. We have already identified a nuclear factor interacting with the C-terminal region of hTAF$_{II}$80 as a potential regulatory factor (Yamamoto, T. and Horikoshi, M., unpublished observations).

Taken collectively, the results described in sections (A) and (B) demonstrate that histone similar regions of these two TAFs are involved in the mutual interaction. Our results significantly extend earlier studies on interactions between histone H3/H4-like TAFs, and strongly support our idea of a histone octamer-like TFIID partial structure, not just a histone H3/H4-like tetrameric structure.

During the preparation of this manuscript, Hoffmann and Roeder reported on the regions of these two TAFs required for mutual interaction by using a different *in vitro* system (*30*). Their results are essentially consistent with ours,

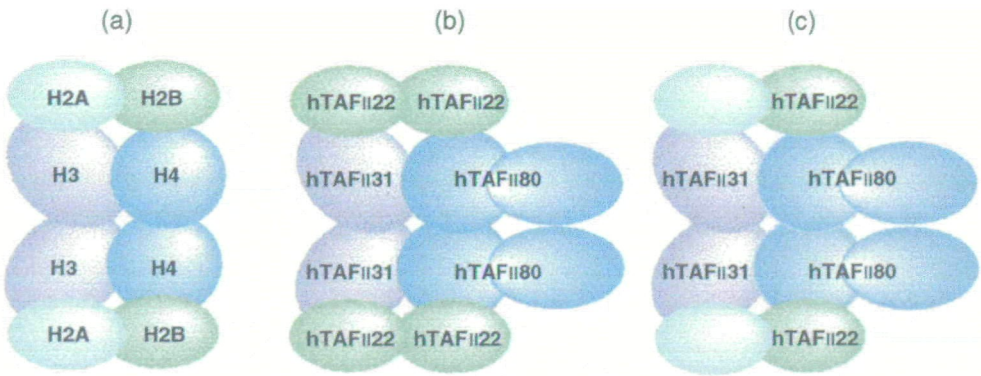

Fig. 7. **Schematic models of the histone-like octamer in TFIID composed of four TAFs containing the regions similar to histones.** (a) Schematic model of histone octamer based on biochemical and structural (*23*) analyses. (b) Histone-like octamer model in TFIID containing hTAF$_{II}$80, hTAF$_{II}$31, and hTAF$_{II}$22 proposed by Hoffmann *et al.* (*25*). hTAF$_{II}$22 in this model is called hTAF$_{II}$20/15 in their studies and is assumed to dimerize. (c) Our model of the histone-like octamer in TFIID. This complex is composed of four different histone-like TAFs, hTAF$_{II}$80, hTAF$_{II}$31, hTAF$_{II}$22, and presumably hTAF$_{II}$30β. The involvement of the fourth TAF, hTAF$_{II}$30β, containing similar regions to histones H2A should be confirmed.

except as regards the requirement of the first helix of the histone fold motif, which our results indicate that to be indispensable for the interaction. The difference may have arisen from the large excess of the TAF mutants employed in their *in vitro* system. We consider that the entire histone fold motif is indispensable for maintaining the physiological tertiary structure of the interaction surfaces. Our system works in eukaryotic cells, and the results obtained are consistent with the concept of the histone fold structural motif (*31*) within histones.

(C) Model of Histone-Similar TAF Complex Analogous to Histone Octamer—Hoffmann and Roeder proposed a model of the histone-octamer-like complex containing hTAF$_{II}$80, hTAF$_{II}$31, and hTAF$_{II}$22, which are all the TAFs already elucidated to possess similarities to histones, based on the studies of the *in vitro* interactions among these molecules and histones, implying that these three TAFs correspond to histone H4, H3, and H2B, respectively (*25*). This model is basically based on the hypothesis that TAFs form histone-like structure within TFIID, and on the crystal structure of dTAF$_{II}$62/dTAF$_{II}$42 complex which resembles that of histone H4/H3 tetrameric complex (*16*). They assume that either hTAF$_{II}$22 or hTAF$_{II}$15 corresponds to histone H2A, based on the interaction of the C-terminal region of hTAF$_{II}$22/15 (*25*) and that the fact that no TAF similar to histone H2A has been reported. However, the evidence supporting their model seems insufficient, particularly as regards the fact that significant similarity has not been presented between histone H2A and hTAF$_{II}$22/15. When we consider the analogy between TAFs and histone octamer, the existence of the TAF which is similar to histone H2A is to be presupposed.

To solve this problem, we surveyed the similarity between histones and TAFs, and found that hTAF$_{II}$30β possesses similarity to histone H2A, as well as H4, which we previously suggested (*32*). In general, due to the similarities among histones themselves, definitive determination of the matching histone to each TAF is difficult only from the primary structures. However, the structural similarity between histone H4 and dTAF$_{II}$62 revealed by crystal structure determination (*16*) strongly suggests that dTAF$_{II}$62 and its human homologue hTAF$_{II}$80 should correspond to histone H4 in the TAF octamer model. This implies that the matching of hTAF$_{II}$30β to histone H2A is more rational than to histone H4. Considering our present results in addition to the previous studies and the overall similarity between TAFs and the histone octamer, we favor a TAF complex containing hTAF$_{II}$30β (or another unidentified TAF) as the histone H2A counterpart (Fig. 7).

To confirm the existence of the histone octamer-like TAF complex, the biochemical characteristics and functional significance of TAFs that is similar to histones should be analyzed. Studies of interactions between histone-similar TAFs and involvement of their histone fold motifs in the interactions are needed. Definitive evidence for our model could be sought by the X-ray crystallography of the whole TAF octamer.

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