Mapping key functional sites within yeast TFIID

Claire Leurent¹, Steven L Sanders²,
Máté A Demény¹, Krassimira A Garbett²,
Christine Ruhlmann^{3,4}, P Anthony Weil²,
László Tora¹ and Patrick Schultz^{3,4,*}

¹Department of transcription, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Illkirch, France,

²Department of Molecular Physiology and Biophysics, Vanderbilt University, School of Medicine, Nashville, TN, USA, ³Department of Structural Biology and Genomics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Illkirch, France and

⁴Ecole Supérieure de Biotechnologie de Strasbourg, Pôle API, Illkirch, France

The transcription factor TFIID, composed of the TATA box-binding protein (TBP) and 14 TBP-associated factors (TAFs), plays a key role in the regulation of gene expression by RNA polymerase II. The structure of yeast TFIID, as determined by electron microscopy and digital image analysis, is formed by three lobes, labelled A–C, connected by thin linking domains. Immunomapping revealed that TFIID contains two copies of the WD-40 repeat-containing TAF5 and that TAF5 contributes to the linkers since its C- and N-termini were found in different lobes. This property was confirmed by the finding that a recombinant complex containing TAF5 complexed with six histone fold containing TAFs was able to form a trilobed structure. Moreover, the N-terminal domain of TAF1 was mapped in lobe C, whereas the histone acetyltransferase domain resides in lobe A along with TAF7. TBP was found in the linker domain between lobes A and C in a way that the N-terminal 100 residues of TAF1 are spanned over it. The implications of these data with regard to TFIID function are discussed.

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Introduction

In eukaryotes, transcription of protein-coding genes requires the formation of a preinitiation complex (PIC) containing the TATA box-binding protein (TBP) and the five basal transcription factors TFII-A, -B, -E, -F and -H (Buratowski *et al*, 1989; Hampsey and Reinberg, 1999). *In vitro*, the PIC positions precisely RNA polymerase II, which can then accurately

transcribe DNA from a TATA box-containing promoter (Orphanides *et al*, 1996). However, such a transcription system poorly reflects the *in vivo* situation since it often fails to respond to activators and only inefficiently transcribes TATA-less promoters and/or chromatinized DNA templates. In this respect, cellular TBP is not found in a free form, but instead is stably incorporated into multiple complexes that carry some of the above-mentioned missing functions. In *Saccharomyces cerevisiae*, TBP and 14 TBP-associated factors (TAFs) form the general transcription factor TFIID, which is 20–50 times more active than TBP for activated transcription *in vitro* (Sanders *et al*, 2002). The *in vivo* importance of TFIID is demonstrated by the observations that all, but one, yeast TAF-encoding genes are essential for viability and that the subunit composition of TFIID is highly conserved throughout evolution (Tora, 2002).

TFIID is instrumental in core promoter recognition through the binding of TBP to the TATA box and also because several TAFs interact with promoter sequences such as the initiator (INR) or the downstream promoter element (Verrijzer *et al*, 1994; Oelgeschlager *et al*, 1996; Burke and Kadonaga, 1997). In this respect, TAF1 and TAF2, the largest subunits of TFIID, were found to contribute to promoter recognition *in vivo* by targeting preferentially the INR sequences (Purnell *et al*, 1994; Sypes and Gilmour, 1994; Chalkley and Verrijzer, 1999). TAF1 was also reported to interact with TBP and to regulate negatively its binding to the TATA box through a TAND domain placed in the N-terminal region of TAF1 (Bai *et al*, 1997; Kotani *et al*, 1998; Liu *et al*, 1998; Banik *et al*, 2001). Furthermore, TAF1 post-transcriptionally modifies histones and general transcription factors through several enzymatic functions (reviewed in Wassarman and Sauer, 2001). Metazoan TAF1 contains a Ser/Thr kinase activity that phosphorylates TFIIF α and TFIIA *in vitro* (Dikstein *et al*, 1996; Solow *et al*, 2001). In addition, an acetyltransferase (AT) domain mapping to the C-terminal portion of TAF1 is able to acetylate free and nucleosomal histones *in vitro* as well as the transcription factors TFIIE β and TFIIF (Mizzen *et al*, 1996; Imhof *et al*, 1997).

Based on interaction studies between recombinant TFIID subunits, it was suggested that TAF1 could serve as a scaffold for TFIID assembly since a *Drosophila* TFIID subcomplex comprising TAF2, 4, 5, 6, 9, 11 and 12 could be assembled on TAF1 (Chen *et al*, 1994). However, TAF5 is also a good candidate for organizing the TFIID complex since it forms a stable subcomplex with TAF6 and TAF9 (Tao *et al*, 1997). *In vitro* experiments further showed that TAF5 binds strongly to TAF1, TAF7 and TAF11, and with lower affinity to TAF12, TAF13 and TBP (Dubrovskaya *et al*, 1996). The C-terminal end of TAF5 contains multiple WD-40 repeats, an evolutionary conserved structural motif whose exact function is not understood in TAF5 but which has been shown in other systems to form a propeller-like platform dedicated to protein interactions (Dynlacht *et al*, 1993; Dubrovskaya *et al*, 1996; Smith *et al*, 1999).

A three-dimensional (3-D) model of yTFIID was recently solved at 3.5 nm resolution from electron microscopy images

*Corresponding author. Department of Structural Biology and Genomics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, 1, rue Laurent Fries, BP10142, F-67404 Illkirch, France. Tel.: +33 3 90 24 4800; Fax: +33 3 88 65 3201; E-mail: patrick.schultz@igbmc.u-strasbg.fr

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of isolated particles (Leurent *et al*, 2002). In this model, the TFIID subunits are organized as a molecular clamp composed of three lobes linked by thin connections. An immunolabelling approach, used to position nine TAFs containing histone fold domains (HFD), showed that these HFD-TAFs were distributed in each of the three lobes of yTFIID and raised the question as to which subunits form the structural linkers between the three domains and are responsible for the clamp shape. In this report, we mapped TBP, TAF1, TAF5 and TAF7 within the yTFIID complex. The results show that in contrast to previous suggestions, not only TAF1 but also TAF5 play a key structural role by bridging the three TFIID lobes and thus forming a molecular scaffold on which other TAFs can assemble. Furthermore, we demonstrate that the N-terminal domain of TAF1 is spanned over TBP that is itself located between the two lobes A and C. Moreover, we have colocalized the AT domain of TAF1 with TAF7 in the native TFIID, in good agreement with previous interaction experiments.

Results

Immunolocalization of TBP

In order to locate the still unmapped yTFIID subunits in the native complex, we employed an immunolabelling approach using subunit-specific antibodies (Leurent *et al*, 2002). The endogenous TFIID was incubated with a five-fold molar excess of a TBP-specific polyclonal antibody (pAb), and 1464 electron microscopy images of labelled complexes were recorded and analysed (n1 in Table I). A subset of 607 images representing a unique orientation of TFIID with a characteristic triangular shape (Figure 1A and n2 in Table I) was extracted and further analysed. To demonstrate binding specificity, the antibody interaction sites were plotted onto a map of the TFIID view composed of nine sectors obtained by dividing each lobe (A, B or C) into three sectors of similar size (Figure 1B and Table I). Sector 1 bound antibodies with highest frequency (33.7%), and this value was found to be significant since it was greater than the average background binding frequency of the eight other sectors (8.3%) by 3.2 sigmas (Figure 1B and Table I). To determine more accurately the site where antibodies contacted TFIID, the images were partitioned according to the peripheral density variation, which reflects the presence of the antibody. Finally, a density difference map was calculated between the unlabelled and

the labelled class averages in order to locate the tip of the antibody when superimposed on TFIID. Using this approach, anti-TBP IgGs were found to bind to two sites, separated by 4–5 nm, one in lobe A and the other in lobe C, suggesting that TBP is located within the linker domain between the two lobes (Figure 1C and D). The detection of two antibody-binding sites could reflect (i) two different, predominant epitopes recognized by the pAb population, (ii) two TBP molecules present per TFIID complex or (iii) two distinct positions of TBP in the complex. In order to discriminate between these possibilities, a yeast strain expressing an N-terminally HA-tagged TBP was generated and used for the purification of TFIID. Upon incubation with monoclonal anti-HA antibodies, a single specific labelling site was detected in lobe C₁ (Figure 1E), a result that argues in favour of the presence of a single copy of TBP in the TFIID complex located in the connecting region between domains A and C, consistent with previous genetic and immunological analyses of the subunit composition of the complex (Sanders *et al*, 2002).

Mapping of yTAF1

Four different antibody probes were used to map the functional domains of yTAF1 within the yTFIID complex (Figure 2A). The first probe was a purified pAb raised against the entire yTAF1 subunit (Ab1-TAF1). These antibodies specifically bound to an extended region in lobe A and part of lobe C (> than 75% of the total labelling mapped to less than 40% of the contour). Sector 2 showed the highest labelling frequency (29%), and sectors 9, 1 and 3 also showed high antibody-binding scores (13, 15 and 19%, respectively), whereas the average background antibody-binding frequency of the five remaining sectors was 4.6%. The analysis of the peripheral density variation revealed that Ab1-TAF1 bound to several sites encompassing the whole of lobe A and a region of lobe C close to the connection between A and C (Figure 2B). These multiple binding sites of the pAb probably delineate the spread of yTAF1 in the yTFIID complex and suggest that yTAF1 is an extended protein, localized mainly in lobe A, but which could contribute to the linking domain between lobes A and C. This idea is supported by the observation that yTAF1 is extremely sensitive to proteolysis (data not shown). To map the N-terminus of yTAF1, which has been shown to interact *in vitro* with TBP, a pAb directed

Table I Labelling statistics

yTAFs labelled	No. of images		Lobe A			Lobe B			Lobe C		
	n1	n2	Sector 1	Sector 2	Sector 3	Sector 4	Sector 5	Sector 6	Sector 7	Sector 8	Sector 9
TBP	1464	607	3.2	0.9	0.8	1.3	0.8	1.2	0.8	0.8	0.9
HA-TBP	908	599	1.1	5.9	0.3	1	0.5	1.5	0.6	6.7	8.1
Ab1-TAF1	1144	420	6.0	15.1	8.8	0.5	0	0.9	1.4	0.9	4.9
Ab2-TAF1	1180	590	0.4	0.2	0.7	1.3	2	0.7	0.3	3.4	0.2
Ab3-TAF1	286	126	0.4	0.4	0.7	0.6	2	0.9	0.5	5.1	0.9
Ab4-TAF1	729	422	0.5	1.8	5.2	0.2	0.7	-0.6	-0.3	-0.8	-1.3
N-term TAF5	888	248	-0.8	-1	0.8	-0.8	1	0.3	5.6	-0.8	1.3
C-term TAF5	639	202	0.2	3.6	3.9	1.7	3.4	0.9	0.2	0.7	0.7
TAF7	750	164	0.1	1.0	5.7	0.4	1.3	1.3	0.4	1.0	1.0

For each antibody used to detect the position of TBP or yTAFs, the number of recorded images (n1) and the number of trilobed views (n2) are indicated. Each lobe was divided into three sectors as shown in Figure 1B. The values reported for each sector represent deviations from the average binding frequency (in sigma folds). Bold characters represent values higher than 3 sigmas.

Figure 1 Immunolabelling of TBP. (A) Characteristic trilobed view of TFIID that is used throughout this report to identify the antibody-labelled site, represented with isodensity contour levels. (B) Mapping of the TBP-specific pAb-binding site at the outer contour of the trilobed TFIID view. Each lobe A, B and C is divided into three sectors labelled as in Table I (A 1,2,3; B 4,5,6; C 7,8,9). The values indicated for each sector represent deviations from the average binding frequency (in sigma folds). (C, D) Average images of TFIID molecules specifically labelled with the anti-TBP pAb (left panel) and density difference map with an unlabelled TFIID (right panel). (E) Average image of TFIID molecules in which the N-terminally HA-tagged TBP is labelled with an anti-HA antibody (left panel) and difference map with an unlabelled TFIID molecule (right panel). The bar represents 15 nm.

against the first 100 amino acids of γ TAF1 was used (Ab2-TAF1). In this case, only sector 8 was labelled (Table I), and the analysis of the peripheral density variation revealed antibodies bound to lobes C_I and C_{II} (Figure 2C). The position of the N-terminus of TAF1 was confirmed using an anti-HA antibody (Ab3-TAF1) to label the N-terminally HA-tagged TAF1 (Sanders and Weil, 2000). In this experiment, only lobe C_{II} was specifically labelled (Table I and Figure 2D). In order to map the position of the C-terminal part of TAF1, a yeast strain was constructed that carried an HA tag at the C-terminus of TAF1. Immunolabelling experiments with monoclonal anti-HA antibodies showed a significant labelling of sector 3, demonstrating that the C-terminal end of TAF1 was

Figure 2 Immunolabelling of TAF1 domains. (A) Schematic representation of the structural and functional features of γ TAF1. In (B–E), the stars below the inset indicate the portion of TAF1 recognized by the antibodies used. (B) Average images of TFIID molecules specifically labelled with a pAb raised against full-length TAF1 (Ab1-TAF1). Several classes of images differing by the position of the antibody-binding site were found and likely correspond to distinct epitopes (left panel). The density difference maps between the various labelled classes and the unlabelled TFIID were aggregated into a single diagram for clarity (upper right panel). The lower right panel summarized the results obtained with Ab1-TAF1 by mapping the labelled sites on the previously obtained 3-D model of TFIID (Leurent *et al*, 2002). (C) Average images of TFIID molecules specifically labelled with a pAb raised against the first 100 amino acids of TAF1 (Ab2-TAF1) are depicted in the left panel. The aggregated density difference maps are shown in the right panel and indicate that lobe C is labelled in different regions. (D) Average image of TFIID molecules HA-tagged on the N-terminus of TAF1 and labelled with an anti-HA antibody (Ab3-TAF1). The difference map (right panel) indicates that a single site is labelled in lobe C_{II}. (E) Average image of TFIID molecules HA-tagged on the C-terminus of TAF1 and labelled with an anti-HA antibody (Ab4-TAF1). The difference map (right panel) indicates that a single site is labelled in lobe A.

located in lobe A thus orienting TAF1 in the complex (Figure 2E). Altogether, these experiments show that TAF1 has an extended conformation and connects the two lobes A and C. The C-terminal part lies in lobe A and at least the first 100 residues are located in lobe C. In this situation, the N-terminal region of TAF1 is spanned over TBP consistent with the fact that the TAF1 TAND binds TBP.

Immunolocalization of TAF7

Biochemical experiments indicate that TAF7 interacts with the C-terminal part of TAF1. In order to test this hypothesis, we mapped the position of TAF7 within TFIID by using subunit-specific pAbs. The TFIID molecules were incubated with the TAF7-specific antibodies and the immune-complexes were analysed as described above. The labelling statistics clearly indicate that the antibodies bind preferentially to sector 3 within lobe A (Table I) and are substantiated by the noise-free views of the labelled TFIID complexes and the

Figure 3 Immunolabelling of TAF7 with a subunit-specific pAb. (A) Average images of yTFIID molecules specifically labelled with a pAb raised against yTAF7. (B) Statistically significant difference map between the labelled and the unlabelled yTFIID molecules. (C) Position of the labelled sites on the 3-D model of TFIID highlighted by an asterisk.

density difference map with the unlabelled molecules (Figure 3). This experiment shows that TAF7 is located close to the C-terminus of TAF1, which is in good agreement with recent genetic and biochemical data (Yatherajam *et al*, 2003; Singh *et al*, submitted).

Domain mapping of yTAF5

As TAF1 was only found to bridge lobes A and C, further experiments were performed to investigate how the three TFIID lobes are connected. Biochemical and genetic evidence indicates that the large 90 kDa, WD-40-containing TAF5 is present as two copies in TFIID and could therefore also be implicated in linking the three TFIID lobes (Sanders *et al*, 2002). In order to map the C-terminal region of TAF5, which contains the WD-40 repeats, a yeast strain was generated in which the C-terminus of TAF5 was fused to a calmodulin-binding protein–protein A tag (TAP tag) in an HA-TAF1 background (Figure 4A). The TFIID complex was purified via standard methods using HA-specific antibodies so as to leave intact the 30 kDa TAP tag on TAF5. The purified TAP-tagged TFIID appeared less homogeneous than the wild-type TFIID, and the complexes had a slight tendency to dissociate, suggesting that the insertion of a large protein moiety at the C-terminus of TAF5 decreases the stability of TFIID. There is however no reason to believe that the molecular organization of the complex is modified, since the yeast strain grows normally and the purified complex has a characteristic subunit composition. Despite this lower stability, characteristic trilobed particles were identified and a data set of 903 molecular images was collected and analysed. The average trilobed view (Figure 4B) closely resembled the shape of the wild-type TFIID molecule. In an attempt to locate the additional mass generated by the TAP tag, a density difference map was calculated between the tagged and the native TFIID. This calculation revealed two additional densities of similar size, one in lobe A and the other in lobe B (Figure 4B, right panel), whose size is consistent with that of the tag, confirming that TAF5 is present in two copies in TFIID. To further demonstrate that these densities correspond to the added TAP tag and do not arise from a local reorganization of TFIID, the complex was incubated with a nonspecific anti-rabbit antibody to target the protein A moiety of the tag. Large amounts of labelled complexes were observed, most of which labelled either lobe A or B (Figure 4C) and in some cases both lobes (data not shown). This experiment confirms that (i) the additional densities detected in the tagged TFIID correspond

Figure 4 Labelling of the N- and C-terminal ends of TAF5. (A) Schematic representation of the structural features of TAF5 showing the WD-40 repeats and the 30 kDa tag for tandem affinity purification (TAP) that was introduced at the C-terminus of TAF5. (B) Average image of TFIID molecules in which TAF5 was fused to the TAP tag (left panel) and statistically significant density difference map with wild-type TFIID molecules (right panel). The difference map shows two additional densities located in lobes A and B. (C) Average images of TFIID containing TAP-tagged TAF5 subunits. The TAP tag was localized with an antibody to reveal the protein A moiety (left panels) and difference maps with nonlabelled TFIID molecules (right panels). Two distinct labelled particles were obtained that were labelled either in lobe B (upper panel) or in lobe A (lower panel), thus confirming the position of the TAP tag placed at the C-terminus of TAF5. (D) Average images of yTFIID molecules specifically labelled with a pAb raised against the first 18 residues of TAF5 (left panel) and corresponding difference map (right panel).

to the TAP tag, (ii) TAF5 is present as two copies in TFIID as shown previously (Sanders *et al*, 2002) and (iii) the C-termini of these two TAF5 molecules are located in lobes A and B, respectively.

In order to locate the N-terminal part of TAF5, we raised antibodies against residues 1–18 of yTAF5; these antibodies were incubated with TFIID. The antibodies specifically interacted with lobe C (Table I), and the difference map between the labelled and the unlabelled TFIID complex showed that the N-terminal region of TAF5 is located in lobe C_{II} (Figure 4D). The labelling did not reveal two distinct sites, one for each TAF5 molecule, suggesting either that the N-termini of the molecules are closer to each other than the method can resolve or that one of the two N-termini of TAF5 is hidden in the complex. Altogether, these results show that TAF5 is present as two copies in the complex, that the C-terminal part of the subunit is located in lobes A and B and suggest that the N-terminal ends of both copies are

located in lobe C. Thus, TAF5 appears to be organized in a symmetric way and could form the linking regions between lobes A and C and between lobes B and C.

Analysis of a recombinant TAF-containing complex

To further investigate the putative architectural role of TAF5, different combinations of recombinant mammalian TAFs were coexpressed in Sf9 cells using a baculovirus expression system. The coexpression of six HFD-containing TAFs, which form pairwise interactions (TAF4/12, TAF6/9 and TAF8/10), both together with TAF5 gave rise to a stable complex that could be immunoprecipitated with an anti-TAF10 antibody and was found to migrate as a homogeneous complex with an apparent molecular weight of about 800 kDa on a size exclusion column (Figure 5A). Western blot analysis confirmed that the major bands observed in silver-stained SDS-PAGE correspond to the expected TAF molecules (Figure 5B). When TAF5 is left out of the coexpression experiment, the only subunit co-immunoprecipitated with TAF10 is its heterodimerization partner TAF8 (Gangloff *et al*, 2001), demonstrating that in the absence of TAF5 no large TAF10-containing complex is formed (Figure 5C). When fraction 14 from the size exclusion purification was analysed by electron microscopy, a homogeneous population of particles was observed in which three major domains were visible (Figure 5D). The image analysis of 1250 molecular images revealed a characteristic trilobed organization reminiscent of the architecture of TFIID (Figure 5E). The two external lobes A and C were smaller in the seven-TAF recombinant complex than in TFIID, likely reflecting the smaller subunit composition of the seven-TAF complex. Although it cannot be concluded from these observations that the TAF distribution is identical in the recombinant and in the WT complexes, the present data show that the seven-TAF complex, in which TAF5 is the only subunit without an HFD, is organized as three lobes connected by thin linkers.

Discussion

The present immunoelectron microscopy experiments localize TBP, TAF7 as well as multiple distinct domains of TAF1 and TAF5 within the 3-D model of yTFIID. The location of these subunits, in addition to the previously mapped HFD-containing subunits (Leurent *et al*, 2002), illuminates the complex molecular architecture of TFIID (Figure 6).

TAF5 contributes a scaffold functionality to TFIID

Our results characterize the structural scaffold underlying the trilobed structure found in human and yeast TFIID (Andel *et al*, 1999; Brand *et al*, 1999; Leurent *et al*, 2002) and identify the subunits involved in the interlobe connections. TAF1 and TAF5 are good candidates to form the linker regions since for both subunits, parts of the same polypeptide were found in two adjacent lobes of TFIID. However, TAF1 can only contribute to the A/C linker since it is absent from lobe B whereas TAF5 maps in all three lobes.

The experiments show that TFIID contains two copies of TAF5 since two distinct C-termini were found, one in lobe A and the other in lobe B. This stoichiometry of TAF5 is consistent with the subunit quantification of immunoprecipitated TFIID, with results showing the simultaneous incorporation of TAF5 and Myc-tagged TAF5 molecules into

Figure 5 Analysis of a recombinant complex containing seven TAFs. (A) Seven TAFs (hTAF4, 5, 6, 9, 10, 12 and mTAF8) were coexpressed in Sf9 cells and the TAF10-containing complexes were purified using a TAF10-specific antibody. The bound complexes were eluted and separated by gel filtration on a Superdex 200 column. (A) SDS-PAGE analysis and silver staining of the collected fractions showing the formation of a homogeneous complex in fraction 14 with an estimated molecular weight of 800 kDa. The elution of molecular weight standards is indicated (top). (B) Western blot analysis showing that the seven TAFs coelute in the peak fractions of the Superdex 200 gel filtration profile. (C) Western blot analysis of anti-TAF10 immunoprecipitation (IP) experiments. Lane 1, coexpression of the seven TAFs; lane 2, TAF5 is missing in the coexpression; lane 3, TAF10 is missing in the coexpression. (D) Electron microscopic observation of the peak fraction 14 reveals a homogeneous population of complexes in negative stain. (E) Image analysis of the seven-TAF complex reveals a TFIID-like trilobed structure. The bar represents 50 nm in (D) and 13.5 nm in (E).

yTFIID (Sanders *et al*, 2002) and with the finding that *Schizosaccharomyces pombe* incorporates two TAF5 homologues, spTAF5 (TAF72) and spTAF5b (TAF73), into the same TFIID complex (Mitsuzawa *et al*, 2001). The detection of a single site for the N-termini in lobe C_{II} is most likely due to the proximity of the two ends, which were not resolved.

Figure 6 Summary of the immunolabelling experiments. Proposed location of TAFs within the 3-D model of yTFIID. The location of histone fold-containing TAF pairs is schematically represented by green circles as determined previously (Leurent *et al*, 2002). The extended TAF1 and TAF5 subunits are schematically represented to highlight their presence in two lobes. Note that the size and shape of the coloured areas does not directly correspond to polypeptide mass.

However, we cannot rule out the possibility that one of the TAF5 N-termini is hidden in the complex and thus is not accessible to the antibody. The location of the C- and N-termini of the two TAF5 molecules argues in favour of a symmetric arrangement of the molecules, each of which contributes to the formation of an interlobe linker. The importance of TAF5 in bridging the TFIID lobes is supported by the structure of the recombinant complex formed upon coexpression of TAF5 together with six HFD-TAFs. The trilobed architecture of this seven-TAF assembly indicates that the TAFs missing in this recombinant complexes, and particularly TAF1 and TAF2, are not essential to bridge the lobes. Although TAF5 is the only subunit shown to connect all lobes, we cannot formally exclude that some of the HFD-TAFs also contribute to the linker domain or that additional TFIID subunits stabilize or reinforce the links between the lobes.

The WD-40 motif present in the C-terminus of TAF5 was described as being involved in protein-protein interactions (Smith *et al*, 1999) and could therefore play a role in the assembly of the TAFs in lobes A and B. Temperature-sensitive mutations in the WD-40 repeats of yTAF5 were found to cause broad transcription defects, and their analysis established the importance of these motifs in maintaining the integrity of both the TFIID and SAGA complexes (Durso *et al*, 2001). When shifted to nonpermissive temperature, TFIID complexes purified from these mutants often showed reduced levels of TAF1 that we show here to reside partly in lobe A. This study also revealed lower amounts of several HFD-TAFs (TAF3, TAF9 and TAF6), TFIID subunits that have been mapped to lobes A and B (Leurent *et al*, 2002). These biochemical and genetic experiments are thus fully consistent with the proposed location of the subunits in the complex.

The proposal that the WD-40 repeats play a role in TFIID assembly apparently contradicts a previous report that stated that the C-terminal part of hTAF5 is not required for incorporation into the TFIID complex (Dubrovskaya *et al*, 1996). This study showed that the N-terminal half of TAF5 can be incorporated into TBP-containing complexes whereas the C-terminal part fails to do so and was therefore believed to contact no other TFIID components. The interpretation of these results was flawed since at that time the trilobed architecture of TFIID and the presence of two TAF5 copies was not suspected. With our present knowledge, the results of Dubrovskaya *et al* (1996) are totally consistent with our model, which predicts that the C-terminal part of TAF5 cannot be incorporated into TFIID since its N-terminal 'dimerization' domain is missing. On the other hand, the N-terminal part of TAF5 is predicted to dimerize with a full-length endogenous TAF5 molecule and thus be incorporated into a partial TFIID complex from which one lobe would be truncated.

Architecture of TAF-containing complexes: a tale of symmetry

The importance of TAF5 in forming the trilobed structure of TFIID is reinforced by the fact that TAF5, along with TAF6, 9, 10 and 12, is shared with the SAGA complex whose homologue in humans, the TFTC complex, was shown to have a similar multilobed structure (Grant *et al*, 1998; Wiczyk *et al*, 1998; Brand *et al*, 1999). The proposed symmetric arrangement of TAF5 within TFIID raises two intriguing questions: (i) how is asymmetry introduced into the native TFIID and SAGA/TFTC complexes and (ii) which signals decide whether TFIID or SAGA is assembled.

The TAF6-9 pair adopts the same symmetric position as the C-termini of TAF5 and could thus interact with the WD-40 repeats as predicted by analysis of mutants (Durso *et al*, 2001). A specific event has to introduce asymmetry and direct the assembly towards the recruitment of TFIID- or SAGA-specific subunits since the distribution of the remaining common subunits does not obey the two-fold symmetry. To trigger the specialization of lobe A versus lobe B, we propose that a subset of TAFs interacts with the TAF5-containing subcomplex in a way to prevent its symmetric growth. TAF1 has an extended, directional arrangement within the TFIID where it spans both lobes A and C thus providing the potential for directing a specific and nonsymmetric organization of TFIID by creating a unique environment in each lobe. An interaction between TAF1 and TAF5, consistent with their spatial overlap, was described by several groups (Dubrovskaya *et al*, 1996; Tao *et al*, 1997; Yatherajam *et al*, 2003). Accordingly, deletion analysis of yTAF1 showed that the removal of residues 208-303 impairs cell growth, and co-immunoprecipitation studies revealed that this variant, although capable of 'normal' interaction with TAF7 and TBP, fails to assemble a holo-TFIID complex indicating that this region is important for TFIID assembly/stability (Bai *et al*, 1997; Mencia and Struhl, 2001).

Furthermore, TAF1 interacts with a large number of TFIID components that reinforce the asymmetry of the complex. TAF1 contacts TAF10 and TAF12, which are present as two copies in TFIID but do not obey two-fold symmetry (Jacq *et al*, 1994; Kirschner *et al*, 2002; Kobayashi *et al*, 2003). These

biochemical and genetic data are consistent with our mapping results showing that TAF1 and TAF10 colocalize in lobes A and C, thus providing two possible interaction sites, and that the N-terminal ends of TAF1 and TAF12 are both located in lobe C_{II}. TAF1 was also shown to contact several non-HFD TFIID subunits that are present as single copies in the complex: TBP has several binding sites within the N-terminus of TAF1; hTAF2 was reported to interact with hTAF1 in Sf9 cells (Chalkley and Verrijzer, 1999) and transfection experiments in COS cells showed that TAF7 interacts with TAF1 (Lavigne *et al*, 1996). The latter interaction is supported by co-immunoprecipitation studies with a family of TAF1 deletion showing that C-terminal deletions all decrease the association of TAF7 with TAF1 (Singh *et al*, submitted). All these interactions are consistent with the proposed location of these subunits mostly in lobe A of the native TFIID (this report and Leurent *et al*, 2002).

Positions of TBP and TAF1 define the primary DNA-binding interface

The major DNA-binding subunit, TBP, was found to reside in the thin linker region between lobes A and C_I. Our results are consistent with a previous study locating human TBP in domain C_I at the top of the cavity and facing the groove where the promoter DNA was suggested to bind (Andel *et al*, 1999). Residing in the linking domain, TBP would almost be surrounded by solvent, which would provide a high accessibility for interaction with additional transcription factors consistent with the description of manifold TBP interaction partners.

Several lines of evidence indicate that TAF1 could also directly contact specific sequence elements of the core promoter DNA and may thereby act as a promoter selectivity factor (Shen and Green, 1997). Yeast deletion mutants showed that the C-terminus of TAF1 is instrumental in promoter recognition either by directly contacting the DNA through its basic residues or through an indirect mechanism involving TFIIA (Ranish *et al*, 1999; Mencia and Struhl, 2001). Our observations indicate that the C-terminus of TAF1 is located in lobe A where TFIIA was found to bind (Andel *et al*, 1999) and support the hypothesis that lobe A is likely to contact DNA. The function of lobe A in binding directly or indirectly to DNA is reinforced by the observation that several subunits located in lobe A were shown to interact with transcriptional activators (Lavigne *et al*, 1999; Munz *et al*, 2003).

Our labelling experiments show that lobe A contains the C-terminal end of TAF1, including the conserved AT domain, and that TAF7 resides in close proximity to that part of TAF1. This is consistent with a report showing that hTAF7 interacts and inhibits the acetyltransferase activity of TAF1 (Gegonne *et al*, 2001). The positioning of lobe A close to DNA may assist the AT domain to exert its function to acetylate either histones as was shown *in vitro* for human TFIID (Brand *et al*, 1999) or the transcription factors TFIIE β and TFIIF (Imhof *et al*, 1997).

YTAF1 and the autoinhibitory model

The position of the N-terminal part of yTAF1 is in good agreement with the location of TBP in the A/C linker domain. Several lines of evidences have shown that the N-terminal region of TAF1 interacts with TBP and may modulate its

binding to the TATA box, resulting in a repressed basal transcriptional activity (Kokubo *et al*, 1998; Kotani *et al*, 1998; Liu *et al*, 1998). A region binding TBP with nanomolar affinity was mapped between residues 2 and 115 in yTAF1 and was shown to be of physiological importance for cell growth (Bai *et al*, 1997; Banik *et al*, 2001). In *Drosophila*, the N-terminal TAND region was dissected into subdomain I (residues 11–77) and subdomain II (residues 82–156), and were predicted to bind to the concave and the convex surfaces of TBP, respectively (Kokubo *et al*, 1994; Nishikawa *et al*, 1997). An NMR-generated structure of *Drosophila* subdomain I was solved in the presence of yeast TBP, and showed that the N-terminal TAND I domain of dTAF1 mimics the minor groove surface of the TATA box, a result that could explain its inhibitory effect on promoter recognition (Liu *et al*, 1998). Our results clearly show that the N-terminus of TAF1 is stretched over TBP, thus suggesting that the autoinhibitory model is plausible in the context of the native TFIID. However, while the isolated peptide adopts a compact structure, our results indicate that the N-terminal region of HA-tagged yTAF1 has to be very extended. The N-terminus, visualized with anti-HA antibody, is placed in domain C_{II} at the tip of lobe C, whereas pAbs produced against the 100-residue-long N-terminal portion yTAF1 decorate the outer surface of domain C_I and the C_I/C_{II} junction over a distance of 9 nm. The large distance covered by the first 100 residues suggests that, in the native TFIID, the TBP-binding subdomain I is not folded, as observed by NMR, but may wrap around domain C. This observation opens the possibility that yTAF1 TAND I domain may adopt different conformations possibly regulated by a dedicated mechanism (Kotani *et al*, 2000). Furthermore, the location of TBP does not overlap with the labelling pattern of the first 100 residues of TAF1 (Ab3-TAF1), indicating that TBP resides at some distance from the extreme N-terminus of yTAF1 and interacts more probably with subdomain II of yTAF1. However, we cannot exclude that a particular form of TFIID was selected during the immunolabelling, either for accessibility reasons or because the binding of the antibody was more readily detectable.

Future experiments will address more directly the position of the N-terminus of TAF1 in controlled conditions by making use, for example, of a large tag such as the one employed to map the C-terminus of TAF5. Such experiments, possibly performed in cryo-electron microscopy to preserve the hydration of the specimen, will help to better understand whether the TAND domain can adopt various positions and whether these are affected by the binding of TFIIA or other cofactors.

Materials and methods

Yeast strains and yeast TFIID purification

The yeast strains used to purify TFIID containing an HA tag on the N- or C-terminus of TAF1 or on TBP have been described, and the purification protocol was identical for all strains (Sanders and Weil, 2000). The majority of contaminating proteins in the HA peptide eluate and the eluting HA peptides were eliminated during the Uno-S ion exchange chromatography. The subunit composition and purity of the three TFIID preps were practically indistinguishable when analysed by SDS-PAGE/silver or Sypro stain. Yeast TFIID containing TAF5-TAP and HA-TAF1 was purified using the same protocol except that the 12CA5 anti-HA mAb-sepharose beads were preincubated with 5 mg of protein A (Sigma) per 1 ml beads for 1 h prior to immunoaffinity chromatography. This modification was

necessary to prevent nonspecific binding between the IgG-binding protein A domains from the TAP-tagged TAF5 and the anti-HA antibody crosslinked to the beads.

Purification of the recombinant TAF complex

Sf9 cells were simultaneously infected with seven different baculoviruses that express His-hTAF4, His-hTAF5, Flag-hTAF6, His-mTAF8, hTAF9, hTAF10 and His-hTAF12, respectively. All constructs used to generate the baculoviruses have been described previously (Dubrovskaya *et al*, 1996) except for TAF8. To generate this vector, the coding sequence of the mouse cDNA was amplified by PCR and inserted in frame into the *Xho*I and *Bgl*II restriction sites of the pAcSG His NT-A baculovirus transfer vector (Pharmingen).

Cells were lysed 48 h postinfection in a buffer containing protease inhibitors and 400 mM KCl, 20 mM Tris-HCl (pH 7.9), 20% glycerol and 2 mM DTT by three cycles of freeze-thawing. From the extracted soluble material, TAF10-containing complexes were isolated by immunoprecipitation with the 23TA-1H8 monoclonal antibody attached to protein G-sepharose beads (Wieczorek *et al*, 1998). Bound complexes were eluted from the antibody by incubation with 2 mg/ml of the corresponding epitope peptide. The eluted material was concentrated on a Nanosep OMEGA30K ultrafiltration centrifuge column (Pall Filtron). The proteins were separated on a SUPERDEX 200 size exclusion chromatography column in the SMART FPLC system (Pharmacia). Fractions of 50 μ l were collected and analysed by Western blotting and silver staining. A pAb was raised against TAF8 by immunizing rabbits with the peptide FPDPTYIKTPTYREPVSQ conjugated to ovalbumin (Georgieva *et al*, 2000).

Electron microscopy, immunoelectron microscopy and image processing

The purified yTFIID fractions were diluted to a concentration of 20 μ g/ml in buffer A (20 mM Tris-HCl (pH 7.4), 150 mM NaCl and 20% glycerol). A volume of 10 μ l of this preparation was placed on a 10 nm thick carbon film previously treated by a glow discharge in air. After 2 min of adsorption, the grid was negatively stained with a 2% (w/v) uranyl acetate solution. The images were formed on a Philips CM120 Transmission Electron Microscope operating at 100 kV with a LaB6 filament. Areas covered with individual molecules were recorded under low-dose condition (less than 20 electrons/ $\text{\AA}^2$) at a nominal magnification of $\times 35,000$ on a Pelletier cooled slow scan CCD camera (Model 794, Gatan, Pleasanton). The image processing was performed using the IMAGIC software package (van Heel *et al*, 1996) (Image Science Software, Berlin, Germany).

Unless otherwise stated, all immunolabelling experiments were performed using purified TFIID HA-tagged at the N-terminus of TAF1. Specific rabbit pAbs recognizing TAF1, TAF7 and TBP were

generated, purified and characterized as described previously (Sanders *et al*, 1999; Sanders and Weil, 2000). To generate the anti-TAF5 rabbit pAbs, a peptide corresponding to residues 1–18 was synthesized and used for immunization. For immunoelectron microscopy a 3- to 5-fold molar excess of antibodies was incubated for 30 min at 20°C with purified TFIID at a final protein concentration of 20 μ g/ml. The relative amounts of TFIID and of antibodies were adjusted by electron microscopy inspection of the incubation mixture. The statistical analysis of the immune complexes was performed as described previously (Leurent *et al*, 2002). Briefly, labelled trilobed views were collected and sorted according to their orientation. Distinct features introduce asymmetry in TFIID and allow an unambiguous discrimination of the six possible pseudosymmetric positions. The elongated shape and the separation into two subdomains makes lobe C clearly different from lobes A and B and prevents any in-plane confusion. The asymmetry is reinforced by the relative distances between the lobes, lobe B is closer to lobe C than is lobe A and by the position of lobe A collinearly with the long axis of lobe C whereas lobe B is positioned perpendicular to the long axis of lobe C. The opening of the clamp between lobes A and B is not a discriminating feature because slight variations in staining or in orientation may cause lobes A and B to overlap. As a result of this asymmetry, the two upside-down orientations were always separated into two different classes during the classification step showing that they cannot be mixed up. The antibody-binding specificity was evaluated by dividing the contour of the molecular view into nine equivalent sectors (three for each lobe) and plotting the antibody-binding site of each labelled complex into one of these sectors. The binding was judged as specific when one or more sectors bound antibodies significantly more frequently (average +3 sigma) than the average binding frequency of background sectors.

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