Transcriptional activation is weakened when Taf1p N-terminal domain 1 is substituted with its *Drosophila* counterpart in yeast TFIID

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Transcription factor II D (TFIID), a multiprotein complex consisting of TATA-binding protein (TBP) and 13–14 TBP-associated factors (Tafs), plays a central role in transcription and regulates nearly all class II genes. The N-terminal domain of Taf1p (TAND) can be divided into two subdomains, TAND1 and TAND2, which bind to the concave and convex surfaces of TBP, respectively. The interaction between TAND and TBP is thought to be regulated by TFIIA, activators and/or DNA during transcriptional activation, as the TAND1-bound form of TBP cannot bind to the TATA box. We previously demonstrated that *Drosophila* TAND1 binds to TBP with a much stronger affinity than yeast TAND1 and that the expression levels of full-length chimeric Taf1p, whose TAND1 is replaced with the *Drosophila* counterpart, can be varied *in vivo* by substituting several methionine residues downstream of TAND2 with alanine residues in various combinations. In this study, we examined the transcriptional activation of the *GAL1-lacZ* reporter or endogenous genes such as *RNR3* or *GAL1* in yeast cells expressing various levels of full-length chimeric Taf1p. The results showed that the substitution of TAND1 with the *Drosophila* counterpart in yeast TFIID weakened the transcriptional activation of *GAL1-lacZ* and *RNR3* but not that of *GAL1*. These findings strongly support a model in which TBP must be released efficiently from TAND1 within TFIID upon transcriptional activation.

Key words: *Saccharomyces cerevisiae*, TAND, TFIID, transcriptional activation

INTRODUCTION

Transcription factor II D (TFIID) is a general transcription factor (GTF) that plays a central role in transcription and regulates nearly all class II genes (Baptista et al., 2017; Warfield et al., 2017). This factor can directly recognize a variety of core promoter structures such as the TATA box, initiator (Inr), motif ten element (MTE), downstream promoter element (DPE) or downstream core element (DCE) to initiate accurate transcription from transcriptional start sites (TSSs) (Thomas and Chiang, 2006; Kadonaga, 2012; Lenhard et al., 2012; Nogales et al., 2017). Furthermore, when TFIID interacts with

transcriptional activators on the promoter DNA, its conformation may be altered to recruit other GTFs (i.e., TFIIA, B, E, F and H) as well as RNA polymerase II to the core promoter region, culminating in assembly of the preinitiation complex (PIC) (Johnson and Carey, 2003; Liu et al., 2009; Coleman et al., 2017).

TFIID is a large multiprotein complex consisting of the TATA-binding protein (TBP) and 13–14 TBP-associated factors (Tafs) (Tora, 2002; Thomas and Chiang, 2006; Bieniossek et al., 2013; Sainsbury et al., 2015). Recent structural analyses of TFIID through cryo-electron microscopy revealed that TFIID consists of three lobes (lobes A, B and C) and alters its conformation dynamically by changing the orientation of lobe A to lobe B/C upon recognizing the downstream region of the core promoter, making it possible to form a functional PIC and initiate accurate transcription from TSSs (Nogales et al.,

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2017; Kolesnikova et al., 2018; Patel et al., 2018). We previously found that TAND (Taf1p N-terminal domain), which is comprised of TAND1 and TAND2, can prevent TBP from binding to the TATA box (Kokubo et al., 1998; Kotani et al., 1998; Liu et al., 1998; Mal et al., 2007). Importantly, TAND1 and TAND2 bind to the concave and convex surfaces of TBP in competition with DNA and TFIIA, respectively (Nishikawa et al., 1997; Kokubo et al., 1998; Kotani et al., 1998; Kotani et al., 2000; Mal et al., 2004; Anandapadamanaban et al., 2013). In a recent model proposed by Patel et al. (2018), TFIID adopts five distinct conformations during the process of binding to the core promoter, named the canonical, extended, scanning, rearranged and engaged states. In the canonical and extended states, TAND1 and TAND2 remain associated with TBP to inhibit TBP–DNA interactions. Interestingly, these subdomains are successively displaced with DNA or TFIIA in (or just prior to the formation of) the scanning and rearranged states, respectively, to allow TBP binding to the TATA box. Furthermore, this model also suggested that transcriptional activators have a dual role: TFIID recruitment to the core promoter and stabilization of the rearranged state on the core promoter, such as via the interaction with the N-terminal Q-rich or TAFH domains of Taf4p (Wang et al., 2007; Hibino et al., 2017; Patel et al., 2018).

In a previous study, we found that yeast TAND1 (y1) activated transcription in yeast cells when it was artificially recruited to the promoter by fusion with an appropriate DNA-binding domain (Kotani et al., 2000). Furthermore, the activation domains (ADs) derived from several transcriptional activators functioned as y1 in TBP-binding and yeast growth assays, indicating that y1 and AD are functionally equivalent (Kotani et al., 2000). Based on these observations, we proposed a two-step hand-off model, in which TBP is first handed from TAND1 to AD and then from AD to DNA during transcriptional activation (Kotani et al., 2000). Consistent with this model, *Drosophila* TAND1 (d1) was found to function as a weak AD in yeast cells (Kotani et al., 2000), with the efficiency of the second hand-off step likely lower because of its strong affinity to TBP (Kokubo et al., 1998; Kotani et al., 1998). Similarly, yeast TAND2 (y2) fused to the C-terminus of y1 also decreased the AD activity of y1 (Kotani et al., 2000). To confirm this model, we examined whether the function of AD becomes weaker in yeast cells expressing Taf1p containing d1 rather than y1 (designated here as d1y2-Taf1p), which may lower the efficiency of the first hand-off step. However, these efforts were unsuccessful because yeast cells could not express enough full-length d1y2-Taf1p. This was later found to be because of a downstream shift of the TSSs and an accompanying downstream shift of translational initiation sites (Kasahara et al., 2004), although the precise mechanism underlying these effects remains unclear.

In this study, we further evaluated the possibility that d1 in yeast TFIID could lower the AD activity by examining the efficiencies of transcriptional activation in yeast cells expressing various levels of full-length d1y2-Taf1p. In these cells, several methionine residues downstream of y2 were substituted with alanine residues in various combinations, such that they could not function as alternative translational initiation sites and thereby restore the expression levels of full-length d1y2-Taf1p. We found a strong inverse correlation between the expression levels of full-length d1y2-Taf1p and the activation efficiencies of a reporter gene (*GAL1-lacZ*) and an endogenous gene (*RNR3*), suggesting that d1 in yeast TFIID inhibits transcriptional activation. These observations strongly support the two-step hand-off model, although it remains unknown whether the hand-off reactions occur in the same scanning state as proposed for TFIID in the absence of transcriptional activators.

MATERIALS AND METHODS

Yeast strains and cultures Standard techniques were used for yeast growth and transformation. Yeast strains used in this study were generated from Y22.1 (*taf1Δ* strain) using a plasmid shuffle technique as described previously (Kasahara et al., 2004).

Preparation of plasmids All plasmids encoding wild-type (y1y2) or mutant (d1y2 and its derivatives) Taf1p were prepared as described previously (Kasahara et al., 2004). Plasmids encoding the activation domain (VP16 or y1) fused to the Gal4 DNA-binding domain were described previously (Kobayashi et al., 2001). pB20, a multi-copy *URA3* plasmid with a *GAL1* promoter upstream of *lacZ*, was kindly provided by Dr. A. G. Hinnebusch (NIH, USA).

Induction of *RNR3* and *GAL1* *RNR3* induction was conducted as follows. Yeast strains were grown in YPD medium (containing 2% glucose as a carbon source) at 25 °C. When the OD₆₀₀ reached ~0.7, hydroxyurea (HU) was added at a final concentration of 100 mM and cultivation was continued. Some of the yeast cells were harvested at 0, 1, 2 and 3 h after adding HU and used for Northern blot analysis. *GAL1* induction was conducted as follows. Yeast strains were grown in YPR medium (containing 2% raffinose as a carbon source) at 25 °C. When the OD₆₀₀ reached ~0.7, an equal volume of YPG medium (containing 4% galactose as a carbon source) was added and cultivation was continued. Some yeast cells were harvested at 0, 20, 40 and 80 min after adding galactose and used for Northern blot analysis.

Northern blot analysis Northern blot analyses were conducted as described previously (Kasahara et al., 2007). To prepare probes to detect *RNR3*, *GAL1*,

ADH1 and *SCR1* RNAs, DNA fragments were amplified by PCR from yeast genomic DNA using the primer pairs TK6663-TK6664, TK253-TK254, TK1224-TK1225, TK1186-TK1187 and TK9507-TK9508, respectively, and then 32 P-labeled by a random priming method. The raw data were obtained and quantified using a BAS-2500 image analyzer and Multi Gauge version 3.0 software (Fujifilm). The PCR primers are listed in Supplementary Table S1.

Immunoblot analysis Immunoblot analyses were conducted as described previously (Kotani et al., 1998). Briefly, whole cell extracts were prepared from yeast cells cultured in YPD medium at 30 °C to mid log-phase, electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel, and transferred to a nitrocellulose membrane. Taf1p proteins tagged at the C-terminus with the HA epitope were detected with anti-HA monoclonal antibodies (F7, Santa Cruz Biotechnology).

β -Galactosidase assay *TAF1* or *taf1* mutant strains were transformed with the activator expression plasmid (Gal4-VP16 or Gal4-y1) along with the reporter plasmid pB20. The resulting yeast cells were grown in SD medium at 25 °C to mid log-phase, shifted to YPD medium, and cultured for 3 h. The cells were harvested

and subjected to β -galactosidase assays as described previously (Kobayashi et al., 2001).

RESULTS

Expression of Taf1p containing *Drosophila* TAND1 affects yeast growth at higher temperatures Our previous studies demonstrated that replacement of TAND1 or TAND2 of yeast Taf1p (y1 and y2) with their *Drosophila* counterparts d1 and d2 induced truncation of TAND, resulting in the accumulation of several specific TAND-less forms of Taf1p in yeast cells (Kotani et al., 1998; Kasahara et al., 2004). Taf1p contains ten methionine residues within a small region downstream of TAND, i.e., M82, 83, 85, 107, 137, 139, 176, 184, 188 and 249, as described in Fig. 1A. Our previous mutation analyses, in which these methionine residues were substituted with alanine residues in various combinations (Fig. 1A), demonstrated that they can be used as alternative translational initiation sites to produce several specific TAND-less forms of Taf1p (Fig. 1B) (Kasahara et al., 2004).

The expression profiles of these substitution mutants were determined in our previous study (Kasahara et al., 2004). Consistent with this, Taf1p containing d1 rather than y1 (d1y2-WT) was produced primarily as a truncated protein (Fig. 1B, open triangle), likely because of

Fig. 1. *Drosophila* TAND1 does not function as yeast TAND1 in yeast cells. (A) Schematic illustration of the yeast (y1y2) or chimeric (d1y2) Taf1p proteins whose properties were examined in this study. For d1y2 mutant proteins (mut #1–11), methionine residues at the X-marked positions were substituted with alanine residues as indicated on the right. Note that mut #2 harbors an additional mutation, G86M (glycine-to-methionine substitution at position 86), marked with a red square in the diagram, to restore the expression level of TAND-lacking Taf1p as described in B. (B) Expression profiles of y1y2 or d1y2 Taf1p examined by immunoblot analysis. These Taf1p proteins were visualized with antibodies specific for the HA epitope tagged at their C-termini. A filled triangle and open triangle/square bracket at the left indicate the positions of full-length and truncated Taf1p, respectively. (C) Growth phenotypes of the yeast strains described in B. Cells were preincubated in YPD medium, spotted on YPD plates at three different dilutions, and incubated for 3 days at the indicated temperatures.

the downstream shift of the transcriptional initiation sites (Kasahara et al., 2004). Importantly, the expression levels of full-length d1y2-Taf1p (filled triangle) were increased significantly in d1y2-mut#1, #3, #4, #5, #6 and #7 compared to the level in d1y2-WT (Fig. 1B). Notably, a G86M substitution, which was introduced into d1y2-mut#1 to generate d1y2-mut#2, restored the expression profile of d1y2-WT (Fig. 1B). Specifically, this substitution increased the expression level of a truncated form of Taf1p (open triangle) but decreased that of full-length d1y2-Taf1p (filled triangle). Similar to d1y2-mut#1, the altered expression profiles of the truncated forms of Taf1p appeared to increase the expression levels of full-length d1y2-Taf1p in d1y2-mut#3, #4, #5, #6 and #7, although how the expression ratio of full-length to truncated forms of Taf1p was altered remains unknown.

In our previous study, we examined the growth phenotypes of yeast cells harboring *taf1* mutant alleles at 30 or 37 °C and found that they were temperature-

sensitive and did not differ significantly (Kasahara et al., 2004). We examined their growth phenotypes in more detail and found that yeast cells harboring d1y2-mut#6 or #7 showed more growth defects at 36.5 °C than those harboring d1y2-WT (Fig. 1C). Interestingly, the G86M substitution in d1y2-mut#2 restored the subtle growth defects observed at 36.5 °C in d1y2-mut#1 to a similar level as d1y2-WT (Fig. 1C). These observations suggest that higher expression of full-length d1y2-Taf1p has an inhibitory effect on yeast growth, particularly at higher temperatures.

Taf1p containing *Drosophila* TAND1 shows lower activity in transcriptional activation in a reporter assay system To examine the transcriptional activities of full-length d1y2-Taf1p, we conducted a reporter assay as described previously (Kobayashi et al., 2001). Specifically, yeast cells expressing various types of Taf1p were transformed with a plasmid expressing either of

Fig. 2. Taf1p containing *Drosophila* TAND1 inhibits transcriptional activation of a reporter gene. (A) Schematic illustration of the reporter assay system used to examine the effect of Taf1p containing d1 on transcriptional activation *in vivo*. Yeast cells described in Fig. 1B and 1C were transformed with a plasmid expressing one of the two activators (Gal4-VP16 or Gal4-y1), along with a reporter plasmid containing the *GAL1* promoter fused to *lacZ* (pB20). This system is useful for testing how strongly these two activators can activate transcription via TFIID containing y1y2, d1y2 or TAND-lacking Taf1p. DBD indicates DNA-binding domain. (B) Effect of the TAND deletion of Taf1p on transcriptional activation examined by this system. Yeast cells expressing wild-type (y1y2-WT) or TAND-lacking Taf1p (ΔTAND) were subjected to the β-galactosidase assay. For each activator, β-galactosidase activities were measured and are represented as values relative to those obtained for y1y2-WT. The experiment was performed in technical triplicate, and the mean was calculated. Standard deviation is indicated as error bars. (C) Effect of dTAND1 in Taf1p/TFIID on transcriptional activation examined by this system. β-Galactosidase assays were conducted for the strains used in Fig. 1B and 1C (y1y2, d1y2 and d1y2-mut #1-#11) as described in B.

the two activators (Gal4-VP16 or Gal4-y1), along with a reporter plasmid containing the *GAL1* promoter fused to *lacZ* (pB20) as schematically illustrated in Fig. 2A. The transcriptional activities at the *GAL1* promoter were quantified as β -galactosidase activity in these cells. As a control, we examined the transcriptional activities in yeast cells expressing the TAND-less form of Taf1p (Δ TAND) and observed comparable levels of activities as in wild-type cells (y1y2-WT) (Fig. 2B), indicating that TAND is not required for transcriptional activation by these two activators.

Next, we examined transcriptional activities in yeast cells expressing various levels of full-length d1y2-Taf1p and found an inverse correlation (Fig. 2C). Yeast cells expressing lower amounts of full-length d1y2-Taf1p (e.g., d1y2-WT, mut#2, #8, #9, #10 and #11 in Fig. 1B)

showed stronger transcriptional activities than those expressing larger amounts of full-length d1y2-Taf1p (e.g., d1y2-mut#1, #3, #4, #5, #6 and #7 in Fig. 1B) (Fig. 2C). Furthermore, even cells belonging to the former group showed weaker transcriptional activities than wild-type y1y2-WT cells (Fig. 2C). Given that the expression level of truncated Taf1p in d1y2-WT was similar to that in Δ TAND (Kasahara et al., 2004), the lowered activities observed in cells belonging to the latter group were not because of the lowered expression of truncated forms of Taf1p, but rather because of dominant inhibitory effects mediated by full-length d1y2-Taf1p. Collectively, these results indicate that dTAND1 in yeast TFIID could not mediate transcriptional activation efficiently, at least in this reporter assay system.

Fig. 3. Taf1p containing *Drosophila* TAND1 inhibits the induction of endogenous gene(s). (A) Effect of d1 in Taf1p/TFIID on *RNR3* induction by adding hydroxyurea (HU). Experimental procedures are schematically illustrated in the panel above the blots. Yeast cells of the four strains (y1y2, d1y2, d1y2-#6 and Δ TAND) were harvested at the indicated times after adding HU. Total RNA (10 μ g) was isolated, electrophoresed, transferred to a nylon membrane, and detected using 32 P-labeled gene-specific probes for *RNR3*, *SCR1* and *ADH1*. (B) Quantification of *RNR3* and *SCR1* transcripts. The values for *RNR3* transcripts were normalized to those of *SCR1* transcripts and are plotted as a relative ratio to the highest value of y1y2. (C) Effect of d1 in Taf1p/TFIID on *GAL1* induction by the carbon source shift from raffinose to galactose. Experimental procedures are schematically illustrated in the panel above the blots. Yeast cells of the same set of strains as described in A were harvested at the indicated times after the shift. Northern blot analyses were conducted as described in A using 32 P-labeled gene-specific probes for *GAL1*, *SCR1* and *ADH1*. (D) Quantification of *GAL1* and *SCR1* transcripts. The values for *GAL1* transcripts were normalized to those of *SCR1* transcripts and are plotted as a relative ratio to the highest value.

Taf1p containing *Drosophila* TAND1 shows promoter-specific defects in transcriptional activation of endogenous genes It is less likely that expression of full-length d1y2-Taf1p has global inhibitory effects on transcriptional activation of class II genes, as its effect on growth appears to be very limited (Fig. 1C). Thus, we examined its inhibitory effects on the transcriptional activation of endogenous genes involved in nonessential functions for growth on glucose-containing medium.

According to this criterion, we first examined the transcriptional activation of *RNR3*, which encodes a minor isoform of the large subunit of the ribonucleotide reductase complex and is known to be transcriptionally induced by HU in a TFIID-dependent manner (Fig. 3A) (Li and Reese, 2000; Zhang et al., 2008). Interestingly, we found that yeast cells expressing larger amounts of full-length d1y2-Taf1p had slower activation kinetics in response to HU (compare d1y2-mut#6/#7 with d1y2-WT or y1y2-WT in Fig. 3A, 3B).

We also examined transcriptional activation of *GAL1*,

which encodes galactokinase; this enzyme is essential for galactose catabolism, and therefore can be induced by a carbon source shift from raffinose to galactose (Fig. 3C) (Lohr et al., 1995). In contrast to *RNR3*, we observed no significant effects of the expression of full-length d1y2-Taf1p on *GAL1* activation (Fig. 3C, 3D). These observations indicate that dTAND1 in yeast TFIID inhibits transcriptional activation not only of the reporter gene but also of other specific endogenous genes.

DISCUSSION

In this study, we showed that d1 in yeast TFIID weakened the transcriptional activation of specific genes, such as *GAL1-lacZ* on a reporter plasmid (Fig. 2) or endogenous *RNR3* on the chromosome (Fig. 3). Although it is currently unclear why the inhibitory effects of d1 appear to be gene-specific, they may not be related to specific transcriptional activators or core promoter structures, as these are common or very similar (Gal4, Gal4-VP16 and

Fig. 4. Model of transcriptional activation in which TBP is released from TAND1 and transferred to the TATA box via the action of transcriptional activators. The postulated molecular functions of y1, d1 and the activation domain (AD) of VP16 as a component of TFIID (A–C) or AD (D–F) are summarized. Levels of transcriptional activation are indicated as “+++” (strong) or “+” (weak). In wild-type cells, y1 binds to the concave surface of TBP. Upon transcriptional activation, TBP is handed from TAND1 to AD, and then to the TATA box (two-step hand-off model) (Kotani et al., 2000) (A). When y1 in TFIID is replaced by VP16, which binds to TBP with similar affinity as y1, TFIID can mediate strong transcriptional activation (B). In contrast, when y1 in TFIID is replaced with d1, which binds to TBP with much stronger affinity than y1, TFIID can mediate only weak transcriptional activation (C). When y1 or VP16 are used as the AD, they induce strong transcriptional activation (D and E). In contrast, when d1 is used as the AD, it can induce only weak transcriptional activation, as the second hand-off step from AD to the TATA box is inefficient because of its strong affinity to TBP (F). Note that only the cartoon depicted in C summarizes the results obtained in this study, and is thus marked with a bold rectangle.

Gal4-y1 are all acidic-type activators) between the two genes, d1-sensitive *GAL1-lacZ* (Fig. 2) and d1-insensitive endogenous *GAL1* (Fig. 3). Earlier studies showed that only a subset of genes is transcriptionally affected in various *taf* mutants (Shen and Green, 1997; Holstege et al., 1998; Lee et al., 2000; Shen et al., 2003; Huisinga and Pugh, 2004). However, more recent studies have demonstrated that TFIID is involved in the transcription of nearly all class II genes (Grünberg et al., 2016; Baptista et al., 2017; Warfield et al., 2017). This discrepancy can be explained by the “transcript buffering” phenomenon, in which the steady-state levels of mRNAs are generally buffered by their stabilization in the cytoplasm when mRNA synthesis is impaired in the nucleus (Timmers and Tora, 2018). The transcription of genes less buffered by this mechanism tends to be more affected in a specific *taf* mutant. Therefore, the gene-specific inhibitory effect of d1 may be explained by the same mechanism, as the mRNA sequences derived from *GAL1-lacZ* and endogenous *GAL1* differ and, thus, may have different stabilizing effects in the cytoplasm. To test this hypothesis, nascent mRNA levels should be examined in cDTA (comparative dynamic transcriptome analysis) experiments (Sun et al., 2012).

Our current model for transcriptional activation in yeast is summarized in Fig. 4. In wild-type cells (Fig. 4A), AD first displaces y1 transiently based on their functional interchangeability (they may bind to the concave surface of TBP via a similar fuzzy interaction mode (Warfield et al., 2014)). Subsequently, TBP is released from AD and then binds to the TATA box to induce strong activation; this was previously proposed as a two-step hand-off model (Kotani et al., 2000). As demonstrated previously (Kotani et al., 2000) and described in the main text, AD (e.g., VP16) functions not only as a classical activator (Fig. 4E) but also as y1 in yeast TFIID (Fig. 4B). In contrast, y1 functioned as a strong AD (Fig. 4D), while d1 functioned only as a weak AD because of its strong affinity for TBP (Fig. 4F). According to this scenario, d1 in yeast TFIID may decrease activation efficiencies, as d1 is difficult to displace by AD for the same reason (Fig. 4C). Although expressing large amounts of d1y2-Taf1p in yeast cells was difficult, we overcame this problem by utilizing yeast cells expressing various mutant d1y2-Taf1p polypeptides (Fig. 2). Our results (Fig. 2, Fig. 3) agree with the prediction that d1 in yeast TFIID decreases activation efficiencies (Fig. 4C), thus further supporting the two-step hand-off model (Kotani et al., 2000).

Previous studies have demonstrated that transcriptional activation of *RNR3* by Crt1p is highly dependent on Tafs in TFIID, which are required for the recruitment of the SWI/SNF complex and for nucleosome remodeling (Zhang and Reese, 2005, 2007). It has also been shown that SAGA, rather than TFIID, is responsible for TBP

binding to the TATA box of the *RNR3* promoter (Zhang et al., 2008). However, the recruitment of TBP and Taf1p to the *RNR3* promoter was affected to similar degrees by the *Aspt3* mutation (Zhang et al., 2008), suggesting that SAGA is required to recruit the entire TFIID complex and that TAND of Taf1p mediates the final delivery step of TBP to this promoter. This model is consistent with our results showing that d1 in yeast TFIID weakened the transcriptional activation of endogenous *RNR3* (Fig. 3).

Another previous study showed that non-classical activators, which are generated by connecting some components of the Mediator to an appropriate DNA-binding domain, activate transcription very strongly in yeast cells expressing TAND-less forms of Taf1p (Cheng et al., 2002). Similar effects were not observed (Fig. 2B) or were very weak (Cheng et al., 2002) for classical activators. Although the molecular reason for the difference between non-classical and classical activators remains unclear, it is likely that classical activators must perform dual tasks during activation: they remove TAND from the concave surface of TBP and recruit the Mediator to the promoter. If this is the case, we expect much stronger activation of non-classical activators specifically in the *taf1-ΔTAND* strain, as these tasks can be bypassed by the *taf1-ΔTAND* mutation and artificial recruitment of the Mediator.

Although the NMR or X-ray structures of the d1-TBP or y1y2-TBP complexes have been determined (Liu et al., 1998; Anandapadamanaban et al., 2013), a high-resolution structure of the entire complex containing TFIID, TFIIA, DNA and activators has not yet been elucidated (Papai et al., 2010). To confirm our proposed model, further detailed structural analyses of TFIID are necessary.

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