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Functional Analysis of the TFIID-specific Yeast TAF4 (yTAF_{II}48) Reveals an Unexpected Organization of Its Histone-fold Domain*

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Yeast TFIID comprises the TATA binding protein and 14 TBP-associated factors (TAF_{II}s), nine of which contain histone-fold domains (HFDs). The C-terminal region of the TFIID-specific yTAF4 (yTAF_{II}48) containing the HFD shares strong sequence similarity with *Drosophila* (d)TAF4 (dTAF_{II}110) and human TAF4 (hTAF_{II}135). A structure/function analysis of yTAF4 demonstrates that the HFD, a short conserved C-terminal domain (CCTD), and the region separating them are all required for yTAF4 function. Temperature-sensitive mutations in the yTAF4 HFD α 2 helix or the CCTD can be suppressed upon overexpression of yTAF12 (yTAF_{II}68). Moreover, coexpression in *Escherichia coli* indicates direct yTAF4-yTAF12 heterodimerization optimally requires both the yTAF4 HFD and CCTD. The x-ray crystal structure of the orthologous hTAF4-hTAF12 histone-like heterodimer indicates that the α 3 region within the predicted TAF4 HFD is unstructured and does not correspond to the *bona fide* α 3 helix. Our functional and biochemical analysis of yTAF4, rather provides strong evidence that the HFD α 3 helix of the TAF4 family lies within the CCTD. These results reveal an unexpected and novel HFD organization in which the α 3 helix is separated from the α 2 helix by an extended loop containing a conserved functional domain.

Accurate transcription initiation at protein-coding genes by RNA polymerase II requires the assembly of a multiprotein complex around the mRNA start site (1). Transcription factor TFIID is one of the general factors involved in this process. TFIID comprises the TATA binding protein (TBP),¹ responsible for specific binding to the TATA element found in many RNA

polymerase II promoters, and a set of TBP-associated factors (TAF_{II}s) (2, 3). A subset of TAF_{II}s are present not only in TFIID but also in the SAGA, PCAF, STAGA, and TFTC complexes that lack TBP but are involved in RNA polymerase II transcription (4–8). A subset of TAF_{II}s are also found in a macromolecular complex containing *Drosophila* polycomb group proteins (9).

The function of TAF_{II}s has been studied in several organisms. In yeast, the genes encoding all of the TFIID components, with the exception of yTAF14, are essential for viability. Genetic studies have shown that TAF_{II}s play an important role in transcriptional regulation of many genes (10). Temperature-sensitive (TS) mutations in yTAF1 and yTAF5 provoke cell cycle arrest where the expression of only a small number of genes is affected (11, 12). In contrast, TS mutations in yTAF6, yTAF9, yTAF10, and yTAF12 or the TFIID-specific yTAF11 lead to a dramatic decrease in overall transcription levels (13–18). Studies in *Drosophila* have shown that TAF_{II}s are involved in the transcriptional regulation of genes during development (19–22). In mammalian cells, TAF_{II}s again seem to be involved in cell cycle control and are essential for the viability of proliferating cells (23–26).

The histone-fold domain (HFD) plays an important role in the structural organization of TFIID. Sequence comparisons and structural studies indicated that TAF6 and TAF9 contain HFDs similar to those of core histones H4 and H3, which interact to form an H3-H4-like heterotetramer (27, 28). hTAF4 and hTAF12 heterodimerize via HFDs similar to those of H2A and H2B, respectively (29). It has been suggested that the TAF6-TAF9 heterotetramer may associate with the TAF4-TAF12 heterodimer to form an octameric substructure within TFIID (28, 29). The equivalent yTAF_{II}s have been shown to assemble *in vitro* to form a macromolecular complex with stoichiometry (yTAF6-yTAF9)₂-(yTAF4-yTAF12) consistent with that of a histone-like octamer (30). The possible existence of such a structure *in vivo* is supported by high copy suppressor genetic interactions in yeast (15, 30) and the finding that these four yTAF_{II}s colocalize within the same subdomain of native yeast TFIID (31).

X-ray crystallography has shown that hTAF11 and hTAF13 heterodimerize via HFDs, and this interaction is in agreement with a corresponding *in vivo* genetic interaction between their yeast orthologues (14, 32). Biochemical studies have also indicated that yTAF3 and yTAF8 contain HFDs that heterodimerize with a HFD in yTAF10 (33). A similar result was obtained in studies of their metazoan orthologues (21, 34). The existence of these heterodimers in native yeast TFIID is supported by immunoelectron microscopy, which shows colocalization of these proteins (31). Hence, nine yTAF_{II}s contain HFDs that specifically heterodimerize to form five histone-like pairs (for review, see Ref. 3).

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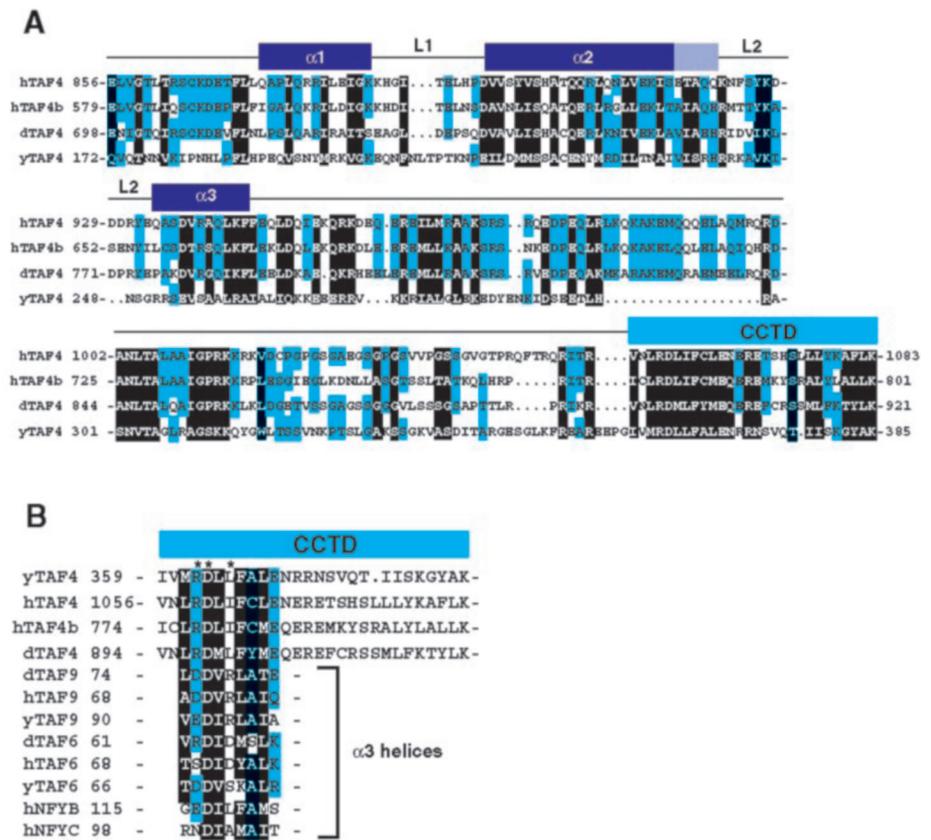
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¹ The abbreviations used are: TBP, TATA-binding protein; TAF_{II}, TBP-associated factor; HFD, histone-fold domain; TS, temperature sensitive; CCTD, conserved C-terminal domain; y, yeast; h, human.

FIG. 1. Sequence comparison of yTAF4 with its metazoan orthologues. The sequence of yTAF4 is compared with that of hTAF4, hTAF4b, and dTAF4. Highly conserved positions are shown in *white* against a *black* background and additional residues conserved among at least three of the family members (mostly the metazoan homologues) are boxed in *light blue*. Amino acids were classified as follows: small residues, P, A, G, S, T; hydrophobic, L, I, V, A, F, M, C, Y, W; polar/acidic, D, E, Q, N; basic, R, K, H. Threonine residues are occasionally present in otherwise hydrophobic positions. The locations of α -helices 1 and 2 are based on the known crystal structure where the α 2 helix seen in the crystal is shown by the *dark blue box* and its expected length is indicated by the *light blue box*. The previously proposed α 3 helix and the L1 and L2 loops within the HFD are indicated along with the CCTD. **B**, the sequence homology between the CCTD and other experimentally determined α 3 helices of dTAF6 and dTAF9 (along with their human and yeast orthologues) and NFY B and C is indicated. The conserved D(L/V/M/I) pair is shown in *white* against a *black* background along with other highly conserved hydrophobic positions. Positions where there are predominantly conserved charged or polar residues are shown with *blue* shading. The *asterisks* indicate amino acids mutated in yTAF4m5 described here or in Selleck *et al.* (30), whose TS phenotype can be suppressed by overexpression of yTAF12.



TSG2/yTAF4 shows significant sequence similarity to the metazoan TAF4 and TAF4b proteins (35, 36). Overexpression of yTAF4 suppresses the TS phenotype of a mutation in yTAF12, and these TAF_{II}s interact physically with one another (30, 35), although the precise domains required for their interaction have not been described.

Although the yTAF4 HFD shows sequence similarity to those of its metazoan counterparts, additional shared regions of similarity exist in the C terminus. To better understand the function of each region and their contribution to heterodimerization with yTAF12, we have made a detailed structure-function analysis of yTAF4. We show that the HFD is essential, but not sufficient, for yTAF4 function, which additionally requires the short highly conserved C-terminal domain (CCTD) and the intervening linker region. We demonstrate a strong genetic interaction between the yTAF4 CCTD and yTAF12 *in vivo*, and coexpression in *Escherichia coli* shows that the CCTD also contributes to direct yTAF4-yTAF12 heterodimerization *in vitro*. Together with the structure of the orthologous hTAF4-hTAF12 heterodimer (48), our results provide evidence that the α 3 helix of the TAF4 HFD is located within the CCTD and reveal a novel HFD organization in which the α 2 helix is separated from the α 3 helix by an extended L2 loop containing a functional domain.

EXPERIMENTAL PROCEDURES

Yeast Strains—The yeast strains used in this study are: YLS46 (*MATa leu2 Δ 0 ura3 Δ 0 his3 Δ 1 met15 Δ 0 KAN Δ taf4* [pRS416ADH-TAF4]), used for plasmid shuffling of TAF4, was derived from YLS40 (36) by sporulation and tetrad dissection; YLS46/4 (*MATa leu2 Δ 0 ura3 Δ 0 his3 Δ 1 met15 Δ 0 KAN Δ taf4* [pAS3-TAF4]); YLS46/4m4 (*MATa leu2 Δ 0 ura3 Δ 0 his3 Δ 1 met15 Δ 0 KAN Δ taf4* [pAS3-TAF4(M219P)]); YLS46/4 (186–388) (*MATa leu2 Δ 0 ura3 Δ 0 his3 Δ 1 met15 Δ 0 KAN Δ taf4* [pAS3-TAF4 (186–388)]); and YLS46/4m5 (*MATa leu2 Δ 0 ura3 Δ 0 his3 Δ 1 met15 Δ 0 KAN Δ taf4* [pAS3-TAF4 (186–388)](R362A, D363A)).

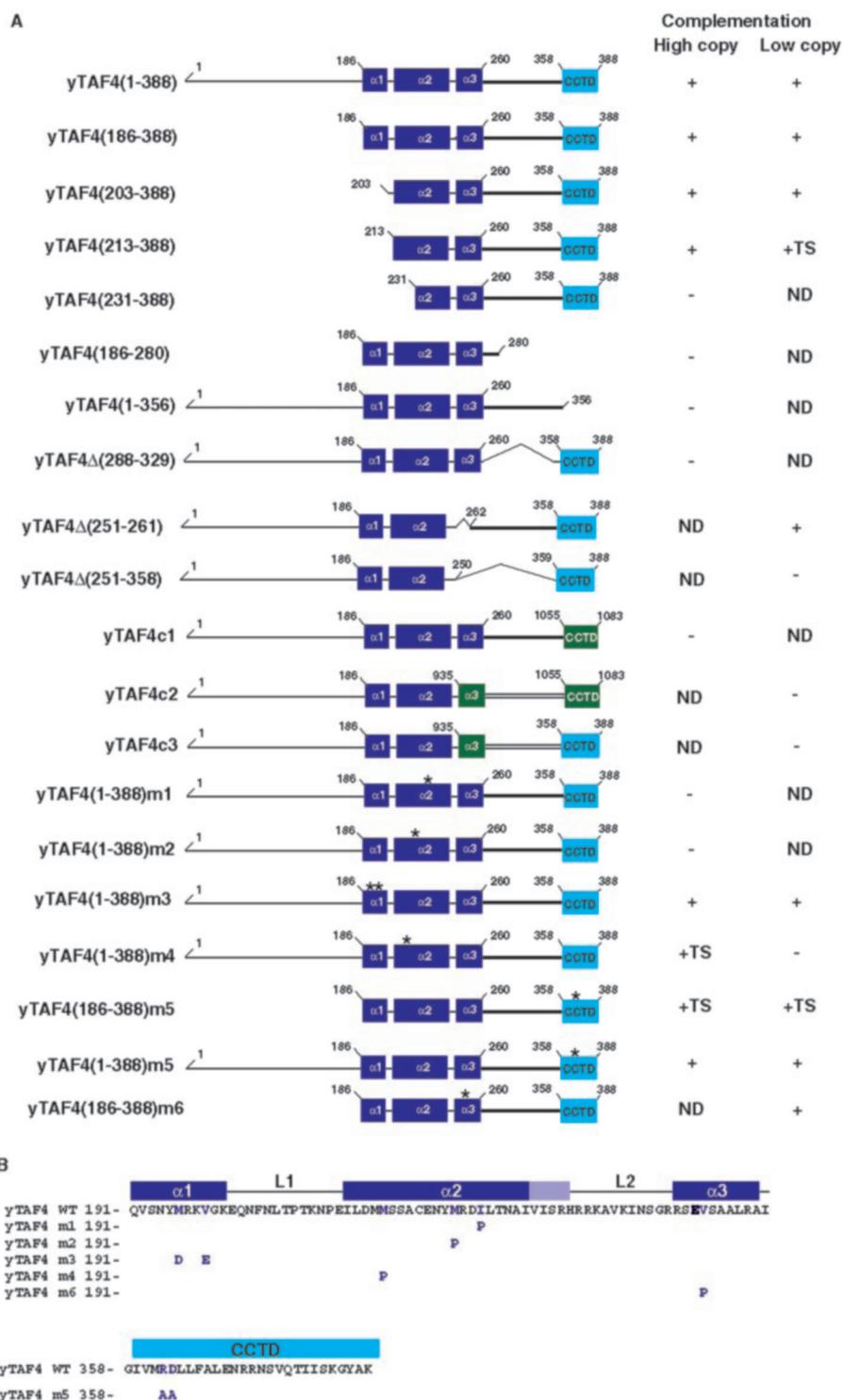
Construction of Recombinant Plasmids—All yeast and bacterial expression vectors were constructed by PCR using oligonucleotide primers with the appropriate restriction sites (details available on request). All constructs were verified by restriction enzyme analysis and automated sequencing. For complementation experiments, wild-type or mutated TAF_{II}s were cloned in pAS3 with a Leu marker as previously described (33). A low copy expression plasmid containing the native yTAF4 promoter was constructed by introducing a cassette comprising 500 bp of sequence upstream of the yTAF4 ATG into the pRS415 vector (37) (pRS415taf4p). Downstream of the ATG, a sequence encoding the FLAG epitope was inserted followed by restriction sites allowing the in-frame cloning of the wild-type or mutated yTAF4 derivatives.

Yeast Complementation Assays and High-copy-number Temperature-sensitive Suppression Assays—All strains were transformed by the LiAc technique. For complementation assays, the indicated plasmids were transformed and the wild-type yTAF4 URA3 plasmid was shuffled out by two passes on media containing 5-fluorouracil as previously described (33). For suppression of the yTAF4 mutant strains, cells were transformed with high-copy-number vectors with URA3 or HIS3 markers expressing the indicated yTAF_{II}. Transformed yeast were then grown at the indicated temperatures. In all experiments, cultures were grown in yeast extract-peptone dextrose medium. Auxotrophic selections were performed in the appropriate synthetic dextrose medium.

RNA Isolation and Quantitation of Poly(A)⁺ RNA—Total RNA was isolated from strains grown at the indicated temperatures for the indicated times as described above. In each case the cultures were immediately cooled, pelleted for 5 min at 4000 rpm, washed twice in cold H₂O, repelleted, and frozen at -80°C . Total RNA was isolated by the hot phenol/chloroform method and subsequently quantified (38). Analysis of poly(A)⁺ RNA was performed on slot blots by hybridization with an oligo(dT) probe and quantified by Phosphorimager analysis (Amersham Biosciences) as previously described (18). All samples were analyzed in triplicate, and the average values \pm standard error are shown.

Coexpression in *E. coli*—Coexpression in *E. coli* was performed as previously described (29). The derivative of yTAF12 was expressed as a histidine-tagged fusion protein in pET-15b. Native untagged derivatives of yTAF4 were expressed from a modified version of the vector pACYC184 (PerkinElmer Life Sciences) (39).

FIG. 2. Schematic description of *yTAF4* deletion mutants. *A*, yeast *TAF4* is schematically depicted. Non-conserved regions are shown as a *thin black line*, the linker region between the HFD and the CCTD as a *thick black line*, the α -helices of the HFD are indicated as *dark blue boxes*, and the CCTD by a *light blue box*. In *yTAF4c1–3*, the hTAF4 CCTD and/or $\alpha3$ helices are differentiated from their *yTAF4* counterparts by *coloring* and in *yTAF4c2* and *c3* the hTAF4 linker region is shown by an *open box*. The presence of substituted amino acids is indicated with an *asterisk*. The amino acid coordinates of the deletion end points, the HFD, and the CCTD are indicated. The ability of each construct to complement the growth of the *taf4* Δ strain when expressed from a high copy vector or a low copy vector under the control of the *TAF4* promoter is indicated to the right. *TS* indicates temperature sensitive growth. *B*, the location of amino acid substitutions within the HFD and CCTD is indicated. The wild-type amino acid sequence is indicated on the first line and the mutated residues are shown below.



RESULTS

Evolutionarily Conserved Regions of yTAF4 Are Required for Function in Vivo—Comparison of the amino acid sequence of *yTAF4* to those of its metazoan orthologues hTAF4, dTAF4, and hTAF4b indicates strong similarity in the C-terminal domain of the proteins. This region can be divided into three sub-domains, the HFD, a short strongly conserved domain at the extreme C terminus (Conserved C-Terminal Domain, CCTD), and an intervening linker region (Fig. 1). To address the requirement of these domains for function *in vivo*, a set of deletion mutants were constructed (Fig. 2A) and tested for their ability to rescue the growth of the yeast *taf4* Δ null strain.

In plasmid shuffle experiments, full-length wild-type *yTAF4* rescued the growth of the *taf4* Δ null strain (Fig. 3A, sector 1, summarized in Fig. 2A), although no rescue was seen with the expression vector alone (Fig. 3A, sector 2). Growth was also seen when the non-conserved N-terminal region upstream of the HFD was deleted (Fig. 3A, sector 4 and Fig. 3B, sector 1; *yTAF4* (186–388)). Similarly, deletion of the $\alpha1$ helix and the $\alpha1$ helix and loop L1 of the HFD did not abolish *yTAF4* function (Fig. 3B, sectors 2 and 3; *yTAF4* (203–388) and *yTAF4* (213–388)), which was lost upon additional deletion of the N-terminal half of the $\alpha2$ helix (Fig. 3B, sector 4; *yTAF4* (231–388)), summarized in Fig. 2A). In agreement with the observation

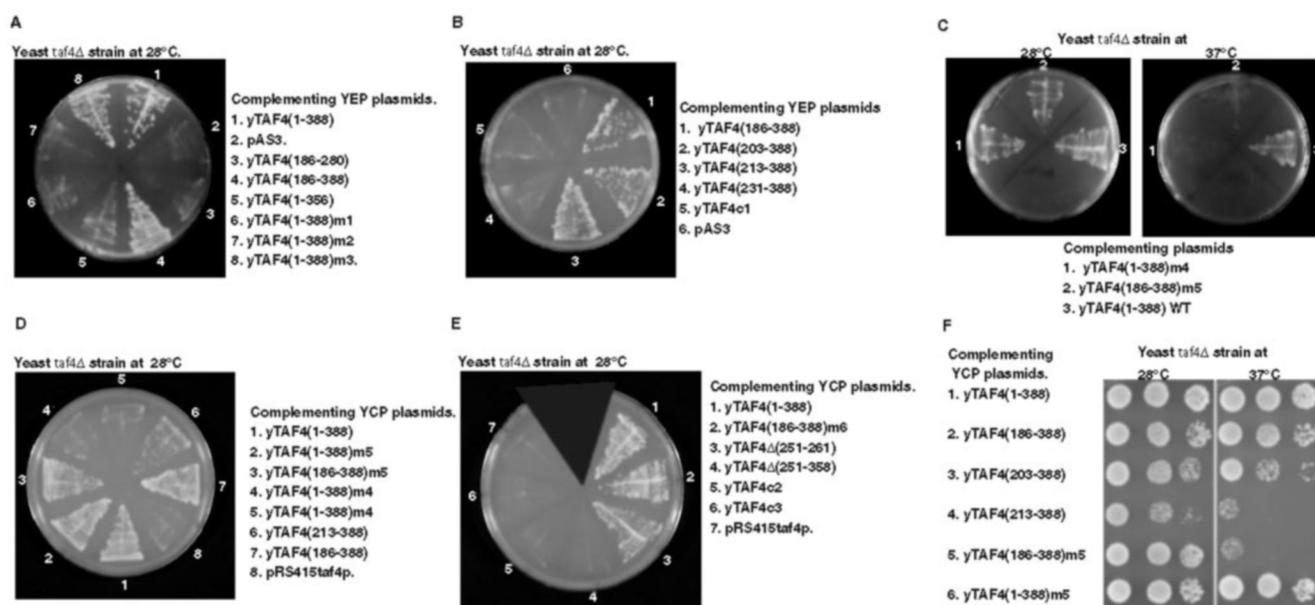


FIG. 3. **Complementation of the *taf4Δ* strain.** The growth of yeast plated at the indicated temperatures is shown. The complementing plasmids used are indicated alongside the plates. A–C show the results obtained with the high copy vector, and D–F shows the low copy vector. In panel F, serial 10-fold dilutions are shown.

that the $\alpha 1$ helix could be deleted without abolishing function, a mutation within this helix also did not affect growth (Fig. 2B, *yTAF4m3* and Fig. 3A, sector 8). In contrast, mutations in the $\alpha 2$ helix either completely abolished function (Fig. 2B, *yTAF4m1*, *m2* and Fig. 3A, sectors 6 and 7) or led to a TS phenotype (*yTAF4m4*, Fig. 3C, sector 1). Hence, the central $\alpha 2$ helix of the HFD is critical for *yTAF4* function. Surprisingly however, mutation (*yTAF4* (186–388)m6, Fig. 2, A and B) or deletion (*yTAF4Δ* (251–261), Fig. 2A) of the $\alpha 3$ helix did not affect the ability to efficiently complement (Fig. 3E, sectors 2 and 3, summarized in Fig. 2A).

Although the above results show that *yTAF4* HFD is required for function, no complementation is seen with the construct *yTAF4* (186–280) containing the HFD alone (Fig. 3A, sector 3), indicating that the linker region and/or the CCTD are also required. Indeed, deletion of the CCTD completely abolished function (Fig. 3A, sector 5; *yTAF4* (1–356)). Mutation of two highly conserved residues within the *yTAF4* CCTD led to a TS phenotype when introduced into the (186–388) deletion mutant (Fig. 3C, sector 2; mutation *yTAF4* (186–388)m5). In addition, deletion of the linker region between the presumed $\alpha 3$ helix of the HFD and the CCTD led to a loss of function (*yTAF4Δ* (288–329), summarized in Fig. 2A). Similarly, deletion of the entire linker region and the presumed $\alpha 3$ helix (*yTAF4Δ* (251–358), Fig. 2A), which brings the CCTD into close proximity to the $\alpha 2$ helix (see also below), also led to a loss of function (Fig. 3E, sector 4). Interestingly, neither the hTAF4 CCTD alone, nor the combination of the hTAF4 CCTD and linker regions can substitute for the equivalent *yTAF4* regions and support yeast growth (*yTAF4c1* and *yTAF4c2*, Fig. 3B, sector 5 and Fig. 3E, sector 5). Finally, exchanging the *yTAF4* linker region by that of hTAF4 did not permit complementation (*yTAF4c3*, Fig. 3E, sector 6).

Some of the above complementation results were performed with a high copy expression vector containing the strong alcohol dehydrogenase promoter. To exclude the possibility that some of the derivatives may complement due to increased expression, those bearing deletion and point mutations for which complementation was observed were also expressed under the control of the natural *yTAF4* promoter sequence in a low copy vector (as described under “Experimental Procedures”).

As observed above, wild-type *yTAF4* and the (186–388) derivative containing only the conserved regions both supported growth (Fig. 3D, sector 1 and 7). When expressed from this vector, deletion or mutation of the $\alpha 1$ helix did not affect complementation (summarized in Fig. 2A and 3F, sector 3). However, deletion of the $\alpha 1$ helix and L1 loop of the HFD led to impaired growth at 28 °C and a TS phenotype (Fig. 3D, sector 6 and Fig. 3F, sector 4). Similar to the high copy vector, complementation and a TS phenotype were observed with *yTAF4* (186–388)m5 (Fig. 3D, sector 3 and Fig. 3F, sector 5). No TS phenotype was, however, observed when the mutation was introduced in the context of the full-length protein (Fig. 3F, *yTAF4* (1–388)m5). The only significant difference with the low copy vector was observed with *yTAF4* (1–388)m4 containing a mutation in the $\alpha 2$ helix. When expressed from a high copy expression vector, this derivative complemented at 28 °C and led to a TS phenotype (summarized in Fig. 2A), whereas when expressed from the low copy vector, no complementation was seen (Fig. 3D, sectors 4 and 5).

Taken together, the above results show that the $\alpha 2$ helix of the HFD is critical for function, but that the $\alpha 1$ helix is not absolutely required. The CCTD and the intervening region are also essential domains, because their deletion results in a loss of function even when expressed from a high copy vector. Therefore, the evolutionarily conserved regions of *yTAF4* all contribute to function *in vivo*.

Selective Genetic Interactions between the HFD and CCTD of *yTAF4* and *yTAF12*—As shown above, we have isolated mutations in the CCTD or in the HFD with a TS phenotype. We overexpressed the other histone-like *yTAF_{II}s* in strains harboring each mutation in order to test their ability to suppress this TS phenotype and hence to interact genetically with *yTAF4*. With the strain harboring the *yTAF4* (1–388)m4 mutation in the HFD, overexpression of wild-type *yTAF4* efficiently rescued growth as expected (summarized in Fig. 4A). However, of the other *yTAF_{II}s* tested, only the expression of *yTAF12* was able to rescue the growth at the non-permissive temperature, whereas all strains grew at the permissive temperature (summarized in Fig. 4A). A similar result was obtained using mutation *yTAF4* (186–388)m5 in the CCTD (Fig. 4, A and B). These results show strong and

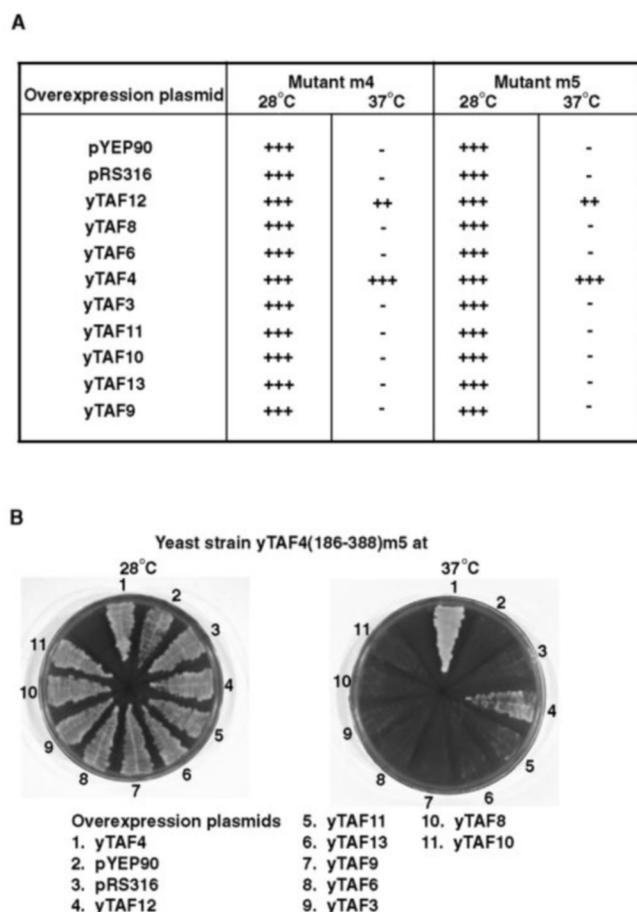


FIG. 4. High copy suppression of the yTAF4 m4 and m5 strains. A, the ability of the overexpressed yTAF_{II}s shown on the left column of the table to suppress the TS phenotype of the yTAF4 m4 or m5 (expressed from the high copy vector) strains is summarized. B, the experimental result for the high copy suppression of the yTAF4 m5 strain is shown. Yeast were plated at the indicated temperatures, and the overexpression plasmids used are indicated below the plates.

selective genetic interactions between yTAF12 and the HFD and CCTD of yTAF4.

The CCTD Is Required for Heterodimerization of yTAF4 and yTAF12—Direct physical interactions between yTAF4 and yTAF12 have previously been observed (30, 35), however, the domains of yTAF4 required for this have not been determined. To address this question, yTAF4 deletion mutants were tested for their ability to heterodimerize with a histidine-tagged derivative of the yTAF12 HFD when coexpressed in *E. coli*, a powerful method for investigating heterodimerization of TAF_{II}s (33, 39). After coexpression, the bacterial extracts were separated on cobalt agarose beads, and the retained proteins were analyzed by electrophoresis and staining with Coomassie Brilliant Blue.

When coexpressed with the histidine-tagged yTAF12 (409–491), efficient heterodimerization was observed with full-length wild-type yTAF4, which was retained on the beads only in the presence of yTAF12 (Fig. 5B, lanes 2 and 3). As previously observed with other HFDs, coexpression and heterodimerization also led to an increase in solubility of the yTAF12 HFD (lanes 1 and 3). Heterodimerization was also observed with yTAF4 (144–388) in which the N-terminal region has been deleted (lane 5). In contrast, only small amounts of heterodimer were seen with the construct yTAF4 (144–280) in which the conserved C-terminal sequence was deleted (lane 7). We also tested the ability of several other constructs equivalent to those tested in the complementation experiments for

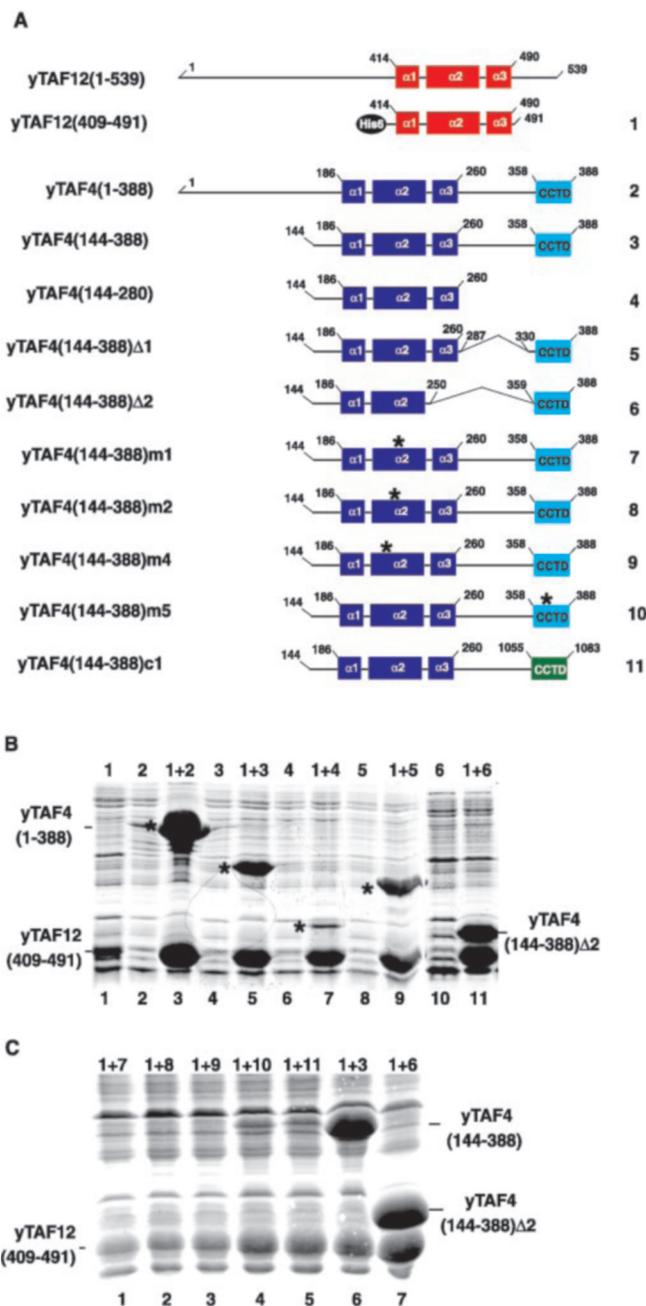


FIG. 5. Direct heterodimerization of yTAF4 with yTAF12. A, The expression plasmids used are depicted schematically. The presence of a 6-histidine tag at the N terminus of the yTAF12 derivatives is indicated. B and C, after coexpression of the proteins indicated above each lane, the bacterial extracts were separated over Co²⁺ agarose, and the retained proteins were analyzed by SDS-PAGE and staining with Coomassie Brilliant Blue. The positions of migration of the yTAF4 and yTAF12 derivatives are indicated to the right and left of the figure or with asterisks.

their ability to heterodimerize. Deletion of the linker region between the presumed α 3 helix and the CCTD resulted in efficient production of the heterodimer (yTAF4 (144–388) Δ 1, lane 9), showing that this domain is not required for heterodimerization. Moreover, a deletion of the presumed α 3 helix and linker region, leaving only a short loop region and bringing the CCTD in close proximity to the α 2 helix also resulted in highly efficient heterodimer production (yTAF4 (144–388) Δ 2, lane 11). These results show that deletion of the CCTD, but not the linker region strongly impairs heterodimer production while bringing the CCTD close to the α 2 helix results in highly efficient heterodimer production.

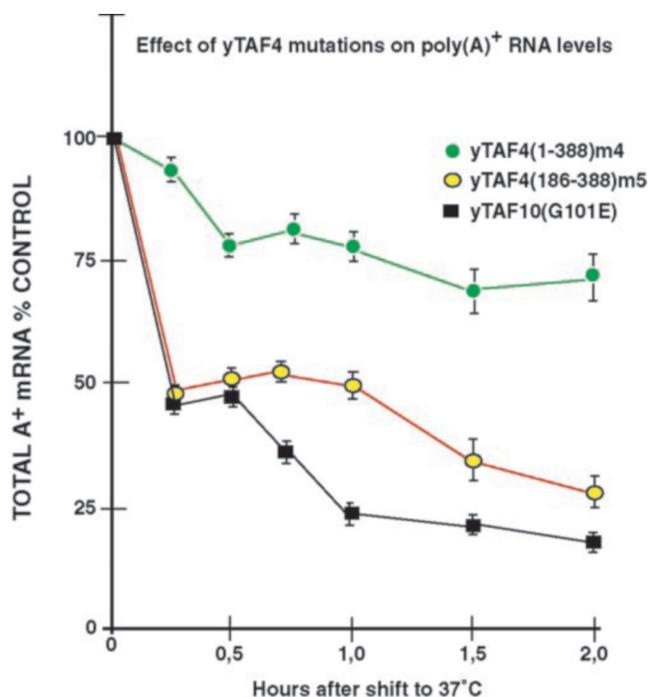


FIG. 6. Bulk mRNA levels in the mutant strains. A quantitative graphical representation of bulk poly(A)⁺ levels in mutant strains is shown. The level of poly(A)⁺ RNA in each strain measured at the time of shift to 37 °C is taken as 100%. The relative levels of Poly(A)⁺ RNA (vertical axis) measured at the times after shift to 37 °C on the horizontal axis are shown for each mutant.

Heterodimerization with yTAF12 was strongly impaired by mutations m1, m2, and m4 within the α 2 helix of the yTAF4 HFD (Fig. 5C, lanes 1–3). Similarly, mutation m5 in the CCTD and replacement of the yTAF4 CCTD by that of hTAF4 (yTAF4 c1) also impaired heterodimerization compared with that seen with the equivalent wild-type construction (compare lanes 4, 5, and 6). This observation shows that the CCTD plays a direct role in heterodimerization with yTAF12.

Mutation of the yTAF4 HFD and CCTD Affects Gene Expression—Here we describe two novel TS mutants in yTAF4. To determine the effect of each mutation on overall RNA polymerase II transcription, the levels of poly(A)⁺ RNA were examined in each strain following the shift to 37 °C. As a control in these experiments, we included the previously characterized yTAF10 mutant strain (G210E) bearing a mutation in the HFD. In this strain, bulk poly(A)⁺ levels are strongly reduced (18). Total RNA was prepared from each yeast strain grown at 28 °C, and after growth at 37 °C for between 15 min and 2 h, poly(A)⁺ levels were quantified by hybridization with an oligo(dT) probe and PhosphorImaging.

As previously described, the level of poly(A)⁺ mRNA fell off rapidly to less than 25% of the control value after shift of the yTAF10(G210E) mutant strain to 37 °C (Fig. 6). Mutation of the CCTD also led to a strong reduction in poly(A)⁺ mRNA levels, which decreased to around 30% (Fig. 6). In contrast, the mutation in the α 2 helix of the HFD had a less dramatic effect (Fig. 6). These results show that the integrity of the CCTD is rather broadly required for transcription.

DISCUSSION

In this report, we show that the conserved region of yTAF4 is required for yeast viability. We demonstrate that the CCTD of yTAF4 interacts genetically with yTAF12, is broadly required for transcription *in vivo*, and is required for optimal heterodimerization with yTAF12 *in vitro*. Together with the structure of the human TAF4-TAF12 heterodimer in the accompa-

nying paper (48), our results provide evidence that the HFD of the TAF4 family has an unexpected organization where the α 3 helix is located within the CCTD.

Functional Domains of yTAF4—We have previously proposed that TAF4 contains a HFD that mediates heterodimerization with that of TAF12 (29). Analysis of yTAF4 shows that the HFD is essential for function *in vivo*. Mutations in the α 2 helix of the HFD abolish function or lead to a TS phenotype. These mutations also impair heterodimerization in *in vitro* coexpression experiments. In parallel with our study, the x-ray crystal structure of the hTAF4-hTAF12 histone-like pair was determined (48), indicating that hTAF12 adopts a canonical histone fold and showing the presence of an α 1-L1- α 2 in hTAF4. From the structure, it can be seen that the above mutations are indeed located within the heterodimerization interface. The correlation with complementation indicates that yTAF4-yTAF12 heterodimerization is essential for function *in vivo*. It is, however, surprising that deletion or mutation of the α 1 helix that contributes significantly to the heterodimerization interface seen in the structure does not have a more radical effect.

In contrast to yTAF12, yTAF10, or yTAF3 (16–18, 33, 40), the previously ascribed yTAF4 HFD is not by itself sufficient for growth, which requires the entire conserved C-terminal region. Deletion of the CCTD results in a loss of function. Impaired heterodimerization with yTAF12 is also seen when the CCTD is deleted and with mutant m5 in the CCTD which shows a TS phenotype *in vivo*. The yTAF4c1 in which the yTAF4 CCTD is replaced by that of hTAF4 does not complement *in vivo* and displays impaired heterodimerization. The hTAF4 and yTAF4 CCTDs are closely related but not identical. There are several amino acid substitutions and an additional amino acid in the human sequence. Any one or several of these evolutionary changes may hinder heterodimerization and any other function(s) of the CCTD and lead to a loss of the ability to complement.

In contrast to the above, deletion of the linker region does not affect heterodimerization but abolishes function *in vivo*. Hence, this loss of function cannot be ascribed to a loss of heterodimerization with yTAF12 suggesting that the conserved linker region plays a distinct role *in vivo* perhaps interacting with other TFIID subunits or mediating interactions of TFIID with other cellular proteins. As for the CCTD, species specificity is also observed, as the hTAF4 linker region cannot substitute for the equivalent region of yTAF4.

Mutation of the yTAF4 CCTD has a dramatic effect on bulk mRNA levels upon shift to 37 °C, indicating that this domain is rather broadly required for transcription. This mutation is more detrimental than the m4 mutation in the yTAF4 HFD. However, it is important to note that the m5 mutation is present in the context of a deletion of the N-terminal region. In fact, the m5 mutation has an effect only in this context, but not in the context of the full-length protein. The fact that deletion of the N-terminal region alone has no effect suggests that there is a sequence within the N-terminal region, perhaps an additional α N helix as found in histone H3 or hTAF11 (32), which plays a partially redundant role with the CCTD and can suppress the effect of mutation, but not deletion of the CCTD. Nevertheless, our results suggest that distinct mutations within yTAF4 can have differential effects on gene expression, a conclusion confirmed by gene-specific effects of these yTAF4 mutations.² The conclusion that the full spectrum of activity of a given TAF cannot be derived from the study of a single mutated allele has been underscored by analysis of distinct

² S. T. and I. D., manuscript in preparation.

mutations in yTAF10 or yTAF5, which have dramatically different effects both on gene transcription and cell-cycle progression (40–42).

An Unexpected Organization of the yTAF4 HFD—A mutation in the yTAF4 α 2 helix (M219P) leads to a TS phenotype. The introduction of proline residues in the α 2 helix of several other yTAF_{II}s has also previously been shown to generate TS phenotypes and has proven useful for examining genetic interactions in yeast (15, 33). Using the TS mutation in the yTAF4 α 2 helix, we find a strong and selective genetic interaction with yTAF12, whose overexpression rescues the growth at restrictive temperature. These results are complementary to those of Reese *et al.* (35), who have shown that overexpression of yTAF4 could rescue the TS phenotype of a yTAF12 TS mutant containing a partial deletion of the α 3 helix. Hence, there is a reciprocal genetic interaction *in vivo* between the HFDs of yTAF4 and yTAF12, suggesting that this pair exists within the native TFIID. The existence of this pair *in vivo* is also supported by the colocalization of yTAF4 and yTAF12 observed in immunoelectron microscopy within native yeast TFIID (31).

Our results extend those cited above by demonstrating a further selective genetic interaction between yTAF12 and the yTAF4 CCTD. While this work was in progress, Selleck *et al.* (30) reported that a TS mutation in the HFD of yTAF4 could be suppressed by overexpression of yTAF12. A reevaluation of this observation (43) indicates that the mutation conferring temperature sensitivity to this yTAF4 derivative is L365P, which does not lie within the presumed HFD region, but within the CCTD (see Fig. 1B). This observation provides independent corroboration of a genetic interaction between yTAF12 and the yTAF4 CCTD.

Given this strong genetic interaction, it was not surprising to find that yTAF4 directly heterodimerizes with yTAF12 when coexpressed in *E. coli*. These results extend those of Reese *et al.* (35), who showed an interaction between yTAF4 and yTAF12 in glutathione *S*-transferase-pulldown experiments. Selleck *et al.* (30) have reported heterodimerization of full-length yTAF4 and an N-terminally deleted derivative of yTAF12 upon coexpression in *E. coli*. Here we show that heterodimerization requires only the yTAF12 HFD, but that for yTAF4 the CCTD is additionally required. Full-length yTAF4 or only the conserved region heterodimerize efficiently with the HFD of yTAF12. In contrast, with only the yTAF4 HFD greatly reduced production of the heterodimer was obtained. Mutations in the CCTD impaired heterodimerization, whereas deletion of the presumed α 3 helix as well as the linker region did not impair heterodimer production, but rather led to an optimal yield. Hence, when the CCTD is deleted or mutated, but not when the intervening linker region including the putative α 3 helix is deleted, a reduction in heterodimer production is observed. This observation is in agreement with genetic suppressor results *in vivo* and shows that the CCTD directly contributes to efficient heterodimerization with yTAF12.

With the determination of the hTAF4-hTAF12 structure, it was noted that the amino acids encoding the presumed hTAF4 α 3 helix were present within the crystal, but were disorganized, suggesting that this region probably does not correspond to the α 3 helix. Therefore, either hTAF4 belongs to a novel class of HFD which does not contain an α 3 helix or an α 3 helix is located elsewhere within the C-terminal domain.

Several observations made during the analysis of yTAF4 suggest that an α 3 helix is located within the CCTD. The CCTD is highly conserved in the TAF4 family. Within the CCTD, a region of strong similarity to other experimentally determined HFD α 3 helices can be observed with a conserved D(L/V/M/I) pair (Fig. 1B). This Asp residue, which is mutated in our m5

derivative plays an important role in several HFDs by forming an intramolecular bond with an arginine residue in the L2 loop, whereas the L365P mutation described by Selleck *et al.* (30) is located immediately following the DL pair (see *asterisks* in Fig. 1B). Hence, the mutations conferring a TS phenotype and impairing heterodimerization lie within a region showing high sequence similarity to known α 3 helices (Fig. 1B). The suppression of these mutations upon overexpression of yTAF12 and the impaired heterodimerization seen *in vitro* provides strong evidence that this is indeed the α 3 helix of the TAF4 HFD. In contrast, deletion or mutation of the previously ascribed α 3 helix has no effect on yTAF4 function *in vivo* and does not affect heterodimerization *in vitro*. Together, these observations point to the possible presence of an α 3 helix within the CCTD, showing that the TAF4 HFD has an unexpected organization with an extended linker between the α 2 and α 3 helices.

The presence of an extended L2 loop within the HFD of yTAF10 and yTAF11 has previously been noted (30, 33, 40). However, in contrast to yTAF4, these loops are not present in the metazoan orthologues and can be deleted in the yeast proteins without loss of function. In the TAF4 family, the extended loop region is conserved and cannot be deleted without loss of function. Hence, the yTAF4 HFD has a novel organization in which a functional domain is located within the L2 loop.

In addition to mediating heterodimerization with TAF12, the conserved region of TAF4 also mediates interaction with the Q-rich region of the cAMP-response element-binding protein transcriptional activator (44). It has been suggested that proteins harboring poly(Q) expansions interact with the conserved region of TAF4 and provoke the development of neurodegenerative diseases by sequestration of TAF4 and interference with cAMP-response element-binding protein function (45). Moreover, TAF4 interacts with the adenovirus E1A protein, and this interaction absolutely requires the CCTD (46). Finally, the TAF4 linker and CCTD regions interact with the general transcription factor TFIIA (47). Given this plethora of interactions, it is not surprising that the conserved region of TAF4 has a complex organization and contains several functional elements.

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