Molecular Cell, Vol. 2, 663–673, November, 1998, Copyright ©1998 by Cell Press

Histone-like TAFs Are Essential for Transcription In Vivo

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Summary

In yeast, the TBP-associated factors (TAFs) Taf17, Taf60, and Taf61(68) resemble histones H3, H4, and H2B, respectively. To analyze their roles in vivo, conditional alleles were isolated by mutagenizing their histone homology domains. Conditional alleles of *TAF17* or *TAF60* can be specifically suppressed by overexpression of any of the other histone-like TAFs. This and other genetic evidence supports the model of a histone octamer-like structure within TFIID. Shifting strains carrying the conditional TAF alleles to nonpermissive conditions results in degradation of TFIID components and the rapid loss of mRNA production. Therefore, in contrast to previous studies in yeast that found only limited roles for TAFs in transcription, we find that the histone-like TAFs are generally required for in vivo transcription.

Introduction

Gene transcription by RNA polymerase II (pol II) requires a set of accessory factors that position the polymerase at the promoter. A key component is TFIID, the factor that recognizes and binds to basal promoter elements (Hernandez, 1993). TFIID is composed of the TATA-binding protein (TBP), which binds the consensus promoter sequence TATA, and a set of associated proteins known as TBP-associated factors (TAFs) (Burley and Roeder, 1996). Homologous TAFs have been identified in *Drosophila*, mammals, and yeast, indicating that the architecture of TFIID is conserved over evolution (Burley and Roeder, 1996). Although the requirement for TBP in pol II transcription has been demonstrated both in vivo and in vitro, the role of the TAFs is less clear.

TAFs apparently recognize basal promoter elements other than the TATA element. *Drosophila* $TAF_{II}150$ binds to certain initiator elements (Verrijzer et al., 1994), and $TAF_{II}40$ and $TAF_{II}60$ proteins may recognize a promoter element located downstream of the transcription start site (Burke and Kadonaga, 1997). Surprisingly, a subset of TAFs are also components of histone acetyltransferase complexes, and it has been suggested that the TAF subunits may target acetylation to promoter regions (Grant et al., 1998a; Ogryzko et al., 1998).

In vitro experiments with mammalian or *Drosophila* factors have shown that TAFs are dispensable for basal transcription on a TATA-containing promoter but are required for response to transcriptional activators. This has led to the proposal that TAFs function as "coactivators": intermediates between transcriptional activators and the basal transcriptional machinery (Chen et al., 1994).

As in higher eukaryotes, TAFs can mediate activation in a yeast in vitro transcription system (Reese et al., 1994; Poon et al., 1995). However, activation in the absence of TAFs can also be supported by a "mediator" activity consisting of a set of pol II–associating proteins (Kim et al., 1994; Koleske and Young, 1994). In vivo studies in yeast show that transcription of most genes occurs in the apparent absence of some TAFs, leading to the conclusion that TAFs are not generally required for transcriptional activation (Moqtaderi et al., 1996a; Walker et al., 1996, 1997; Shen and Green, 1997). Rather, some TAFs may function as gene-specific regulators. For example, yeast TAF145 is required for expression of G1 and B-type cyclins, as well as some ribosomal protein genes (Walker et al., 1997). Interestingly, dependence on TAF145 is conferred by sequences in the basal promoter rather than the particular activator used (Shen and Green, 1997).

Sequence analysis has revealed some similarity between three TAFs and the histones H3, H4, and H2B (Table 1; see Burley and Roeder, 1996, for review). Supporting the sequence alignment, a crystal structure of *Drosophila* $TAF_{II}40$ and $TAF_{II}60$ fragments is strikingly similar to the H3/H4 heterotetramer within the nucleosome (Xie et al., 1996). Both contain a "histone fold," which consists of three α helices (Arents and Moudrianakis, 1995; Xie et al., 1996; Luger et al., 1997). In addition, biochemical experiments suggest a histone-like pattern of association between human $TAF_{II}31$, $TAF_{II}80$, and $TAF_{II}20$ (Hoffmann et al., 1996). Interestingly, complexes can also be formed between some of these TAFs and histones (Hoffmann et al., 1996), raising the question of whether the histone fold is a generic dimerization domain. Recently, a second histone fold pair between human $TAF_{II}28$ and $TAF_{II}18$ has been reported (Birck et al., 1998).

To investigate whether a histone-like TAF subcomplex exists in vivo and whether it has a role in transcription, temperature-sensitive (TS) alleles of *TAF17*, *TAF60*, and *TAF61* were analyzed. We present genetic evidence for specific in vivo interactions between the three histone-like Tafs. We also find that, in marked contrast to previous reports of TAF inactivation, the histone-like TAFs are generally required for pol II transcription in vivo.

Results

Isolation and Characterization of Histone-like TAF Conditional Alleles

To explore their in vivo role, we isolated conditional alleles of *TAF17*, *TAF60*, and *TAF61* using a PCR-based misincorporation method and plasmid shuffling. The entire open reading frame of *TAF17* was subjected to mutagenesis, whereas only the histone homology domains

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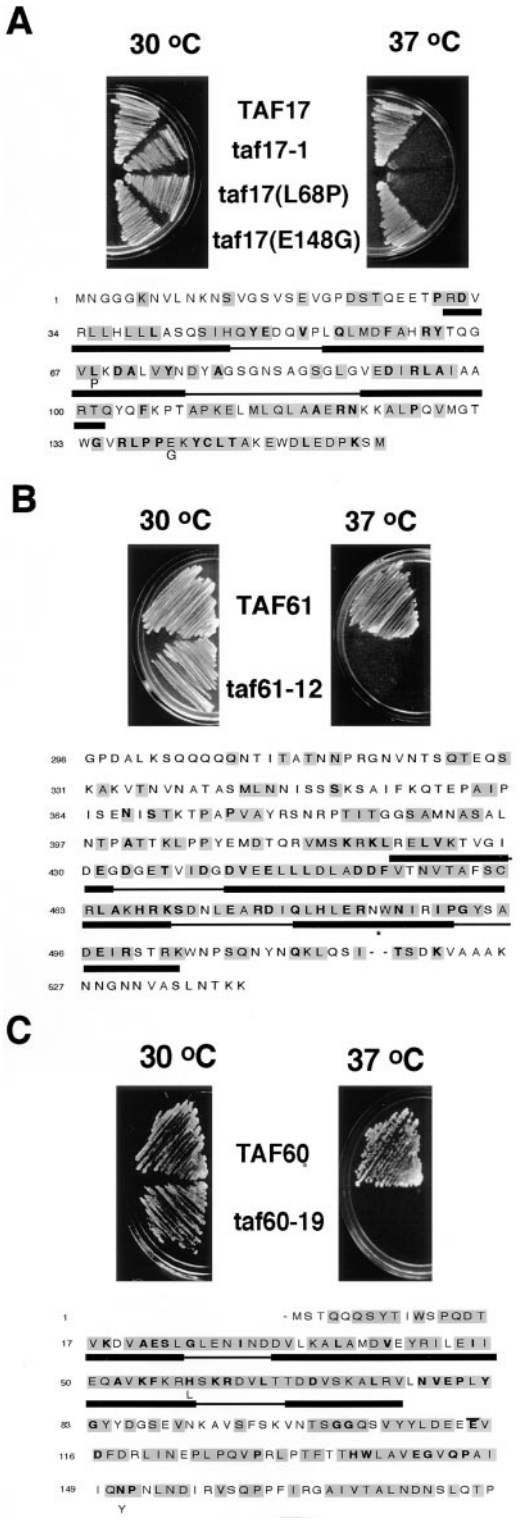

Figure 1. Conditional Alleles of Histone-like TAFs

Strains were grown at either 30°C or 37°C for 3 days. The sequence of each TAF is shown below the plates. Residues that are identical between yeast, human, and *Drosophila* TAFs are shown in bold; similar residues are shaded. Thick bars underneath the sequence represent predicted α-helical regions based on Xie et al. (1996) and Luger et al. (1997). The changed sequence of the mutants is shown underneath the corresponding wild-type residue.

Table 1. Correspondence between TAFs and Histones

Histone	Human	*Drosophila*	Yeast
H2B	$TAF_{II}20/15$	$TAF_{II}30\alpha(28/22)$	TAF61/68
H3	$TAF_{II}31(32)$	$TAF_{II}40(42)$	TAF17
H4	$TAF_{II}80(70)$	$TAF_{II}60(62)$	TAF60

of *TAF60* and *TAF61* were targeted. The phenotypes of the wild-type and conditional alleles of *TAF17*, *TAF61*, and *TAF60* at permissive and nonpermissive temperatures are shown in Figures 1A, 1B, and 1C, respectively. We note that the genetic screens produced conditional alleles with indistinguishable plate phenotypes but different responses upon shift to nonpermissive temperature in liquid media. Some mutants immediately ceased growing upon transfer to 37°C, some divided once and then stopped growing, and others continued growth with very long doubling times. All three strains shown in Figure 1 displayed a rapid growth arrest (0.5–2.0 hr) at 37°C (data not shown). All of the conditional alleles used in this report were recessive (data not shown).

DNA sequencing of the *taf17-1* allele found two point mutations: leucine 68 to proline (L68P) and glutamine 148 to glycine (E148G) (Figure 1A). When the two mutations were tested separately, only the L68P strain was TS. Interestingly, in liquid culture, this single point mutant is very sick but still grows very slowly at 37°C (see below). The E148G strain behaved like wild type at nonpermissive conditions. Leucine 68 is conserved in both the *Drosophila* and human homologs of Taf17. L68P is located within, and would be predicted to "kink," the central α helix of the histone motif (based on the $dTAF_{II}40$ and histone H3 structures). E148G is a semiconserved residue located outside the histone-like region (Figure 1A).

Sequencing of the *taf61-12* mutant allele (Figure 1B) revealed a deletion of two nucleotides that changes the reading frame and results in truncation of the protein by 51 amino acids. Another tight TS allele (*taf61-23*, not shown) of *TAF61* was also found to be a C-terminal deletion of approximately 50 residues. Taf61 is similar to histone H2B, which has an extra helical extension C-terminal to the main histone fold (Luger et al., 1997). If Taf61 is structurally similar to histone H2B, the Taf61 deletions are predicted to remove a corresponding extension as well as the last few amino acids of the third α helix.

Sequencing of the *taf60-19* mutant allele (Figure 1C) revealed two mutations that change histidine 59 to leucine (H59L) and asparagine 151 to tyrosine (N151Y).

(A) The *taf17-1* allele carries two substitutions, which were then separated (L68P and E148G).

(B) The *taf61-12* allele is a truncation that results in a stop codon at position W486 (designated with an asterisk). Note that only amino acids 298–539 of the protein are shown. A speculative fourth α helix is shown based on the existence of such an extension in the histone H2B structure (Luger et al., 1997).

(C) The *taf60-19* allele has two amino acid substitutions (H59L and N151Y). Note that only the first 181 amino acids of the protein are shown.

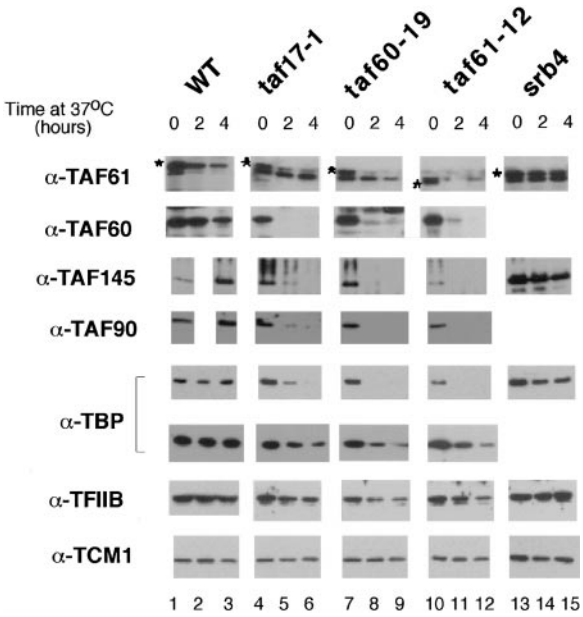

Figure 2. TAF and TBP Protein Levels Are Dramatically Reduced in Histone-like TAF Mutant Strains at the Nonpermissive Temperature

Aliquots of each TAF mutant strain were taken after the indicated amount of time at 37°C. Total protein was blotted and probed with antibodies to TAF60, TAF61, TAF90, TAF145, TBP, TFIIB, and TCM1. Extracts from a TS *srb4-138* mutant strain were included as a control. The asterisk in the α-Taf61 blots indicates the position of the Taf61 band; note that the wild-type protein runs just above while the truncated Taf61-12 runs just below a background band. For TBP, two amounts of extract were immunoblotted. The top row is 25 μg of extract and corresponds to the same level shown for all the other proteins. The bottom row is 50 μg of extract and shows that TBP levels do not decrease to 0.

Histidine 59 is conserved in the *Drosophila* and human homologs, and it is also present in histone H4, where it forms a hydrogen bond between H4 and H2B (Luger et al., 1997). Based on sequence alignments, H59 is predicted to be at the C terminus of the central α helix in the histone fold (Xie et al., 1996; Luger et al., 1997). N151 is located outside the histone fold motif and is also conserved in Taf60 *Drosophila* and human homologs. The relative contribution of the two mutations to the phenotype has not yet been determined. Some experiments were also performed using the *taf60-12* allele. This allele has six amino acid changes throughout the mutagenized region. Although we did not determine the contribution of individual mutations, this strain was used for some experiments because of its very tight TS phenotype.

Histone-like TAFs Are Required for Stability of the TFIID Complex

Immunoblotting was used to monitor TAF protein levels after shifting strains carrying either *taf17-1*, *taf60-19*, or *taf61-12* mutant alleles to nonpermissive conditions. The Taf60-19 and Taf61-12 proteins were rapidly degraded at 37°C (Figure 2, lanes 7–9 and 10–12). After 2 hr at 37°C, the steady-state levels of the proteins were dramatically reduced, and by 4 hr they were almost undetectable. Interestingly, Taf61 and Taf60 protein levels were also dramatically reduced in strains carrying *taf60-19* (lanes 7–9), *taf61-12* (lanes 10–12), or *taf17-1* (lanes 4–6). This was not due to a block in cell division or transcription, because TAF levels were not affected in an *srb4-138* conditional strain shifted to nonpermissive temperature. Therefore, the histone-like TAFs are dependent upon each other for stability.

Levels of two other TAFs were assayed (TAF90 and TAF145). They also degraded with kinetics paralleling those of the histone-like TAFs. In the *srb4* mutant, TAF145 was stable. Thus, loss of any of the histone-like TAFs results in degradation of the entire TFIID complex.

TBP levels were also significantly reduced in the TAF mutant strains after temperature shift (Figure 2). In contrast to Taf60 and Taf61, which decreased to undetectable levels at the nonpermissive temperature, at least 15%–30% of TBP remained stable (as quantitated by densitometry). This may represent TBP within the pol I and pol III transcription complexes. TFIIB levels were also moderately reduced in these strains. Levels of a control protein, the ribosomal protein Tcm1, were unaffected. TFIID loss was not seen in an *srb4* TS strain (lanes 13–15), arguing that the cause is TAF inactivation and not a nonspecific effect of cessation of transcription or cell division.

Unfortunately, anti-TAF17 antibodies were unavailable. Since Taf60, Taf61, and TBP were degraded under nonpermissive conditions in a *taf17-1* strain, it is likely that Taf17-1 protein is either degraded or dissociated from TFIID at 37°C. Taken together, the immunoblotting results indicate that all three histone-like TAFs are required for the in vivo stability of the TFIID complex.

Genetic Interactions between Histone-like TAFs

The model of a histone octamer-like subcomplex within TFIID predicts that Taf17 (the H3-like protein) dimerizes with Taf60 (the H4 analog) and that Taf61 (which resembles H2B but may be analogous to both H2A and H2B) interacts with both Taf17 and Taf60. To test for genetic interactions between these TAFs, a strain carrying the *taf17(L68P)* allele was transformed with high-copy plasmids carrying different TFIID subunit genes. Overexpression of either *TAF61* or *TAF60* suppresses the conditional lethality of *taf17(L68P)* allele (Figure 3A). Upon longer incubation, very weak growth was also observed with high-copy *TAF90*. No suppression was observed upon overexpressing any of the other TAFs, histone genes, or TBP (Figure 3A and data not shown).

High-copy suppression of *taf17(L68P)* by *TAF61* was stronger than suppression by *TAF60*. Taf61 contains an N-terminal extension that is not present in its human or *Drosophila* homologs. The conserved carboxyl terminus of the yeast protein includes the histone H2B homology region, and the conserved region is necessary and sufficient for cell viability (Moqtaderi et al., 1996b). Overexpression of this domain of Taf61 suppressed *taf17(L68P)* lethality at 37°C, although several extra days of incubation were required relative to wild-type Taf61 (data not shown). Therefore, *TAF17* interacts genetically with both *TAF60* and the histone-like region of *TAF61*.

Based on the sequence of the *taf17(L68P)* TS allele,

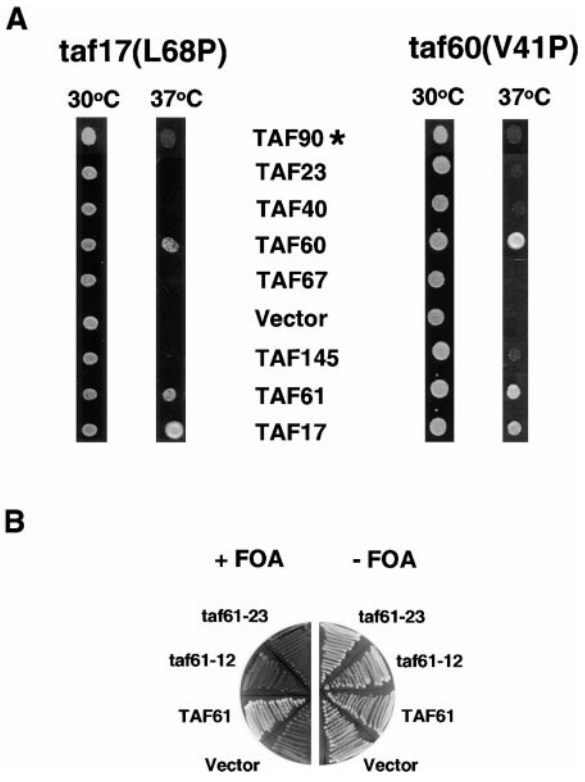

Figure 3. Genetic Interactions between the Histone-like TAF Genes

(A) High-copy suppression of *taf17(L68P)* and *taf60(V41P)* mutant strains. Cells were transformed with high-copy plasmids carrying the indicated TAF genes and then spotted at 30°C and 37°C. Photos were taken after 2 days of growth. The asterisk near TAF90 denotes a partial suppression upon prolonged incubation at 37°C. Also tested and found to be negative (not shown) were TAF19, TBP (SPT15), and various combinations of histone genes.

(B) Synthetic lethality between *taf61* TS alleles and *taf17-1*. YSB586 contains *taf17-1* as well as a deletion of the chromosomal copy of *TAF61* and wild-type *TAF61* carried on a *URA3* plasmid. This strain was transformed with *HIS3*-marked plasmids carrying either a *TAF61* TS allele (*taf61-19* and *taf61-23*) or wild-type *TAF61*. The resulting strains were tested on FOA plates for the ability to lose the URA3 plasmid carrying the wild-type copy of *TAF61*.

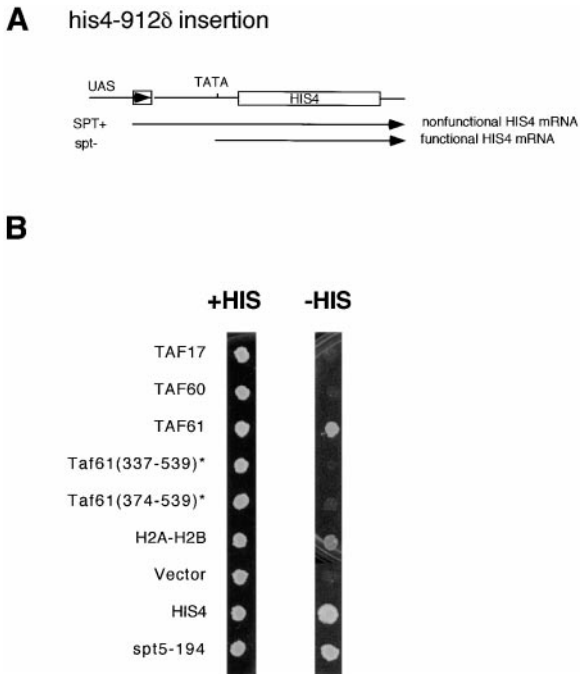

Figure 4. Suppression of the *his4-912*δ Insertion by Overexpression of TAF61

(A) Schematic diagram of the *his4-912*δ locus. The delta insertion is pictured as the arrow within a box. Transcripts arising from the wild-type (SPT+) and suppressed (spt−) conditions are shown as arrows. See Winston (1992) for more details.

(B) The yeast strain FY119 was transformed with high-copy plasmid carrying the indicated TAF genes, two amino-terminal truncations of Taf61 (the remaining amino acids in the protein are indicated), the genes encoding histones H2A and H2B, or the vector alone. Cells were spotted on media containing (+HIS) or lacking (−HIS) histidine to select for suppression of the delta insertion. As positive controls, cells from a strain encoding a wild-type allele of the gene *HIS4* and from a strain carrying a *spt5-194* allele are shown (Swanson and Winston, 1992).

we predicted that a proline inserted into the central α helix of *TAF60* should also generate a temperature-sensitive allele. Valine 41 was chosen for mutagenesis because it is conserved in both the *Drosophila* and human homologs. As predicted, a strain carrying the *taf60(V41P)* allele is temperature sensitive. This strain was transformed with various high-copy TAF plasmids (Figure 3A). The *taf60(V41P)* conditional phenotype is suppressed by overexpression of *TAF61* or *TAF17* specifically, confirming a specific genetic interaction between the histone-like TAFs. Other TAFs, histones, or TBP did not suppress the TS phenotype when overexpressed (data not shown). As seen with *taf17(L68P)*, *TAF90* overexpression gave a very slight improvement in growth of the *taf60(V41P)* mutant at the nonpermissive temperature (data not shown).

Interestingly, the TAF mutants that showed rapid arrest of growth when shifted to nonpermissive conditions (*taf17-1*, *taf60-19*, or *taf61-12*) were not suppressed by overexpressing other histone-like TAFs (data not shown). This is presumably because the mutant proteins are too defective at the nonpermissive temperature to be rescued by increased concentrations of their binding partners.

A second type of genetic interaction between TAF17 and TAF61 was discovered when the *taf17-1* allele was combined with either of two different *taf61* TS alleles (Figure 3B). Using plasmid shuffling to exchange the *taf61* mutants for the wild-type gene, it was seen that the *taf17-1, taf61* double mutants were inviable when grown under conditions permissive for either single mutant. We also observed synthetic lethality between *taf17-1* and several *taf60* conditional alleles (data not shown). This synthetic lethality indicates that the mutant TAF alleles are partially impaired even at the permissive temperature and that their combined effects result in a nonfunctional TFIID complex.

Taf61 Overexpression Suppresses a *his4–912*δ Insertion Mutation

Retrotransposon Ty integration into a promoter renders a gene nonfunctional. Mutations in SPT genes suppress

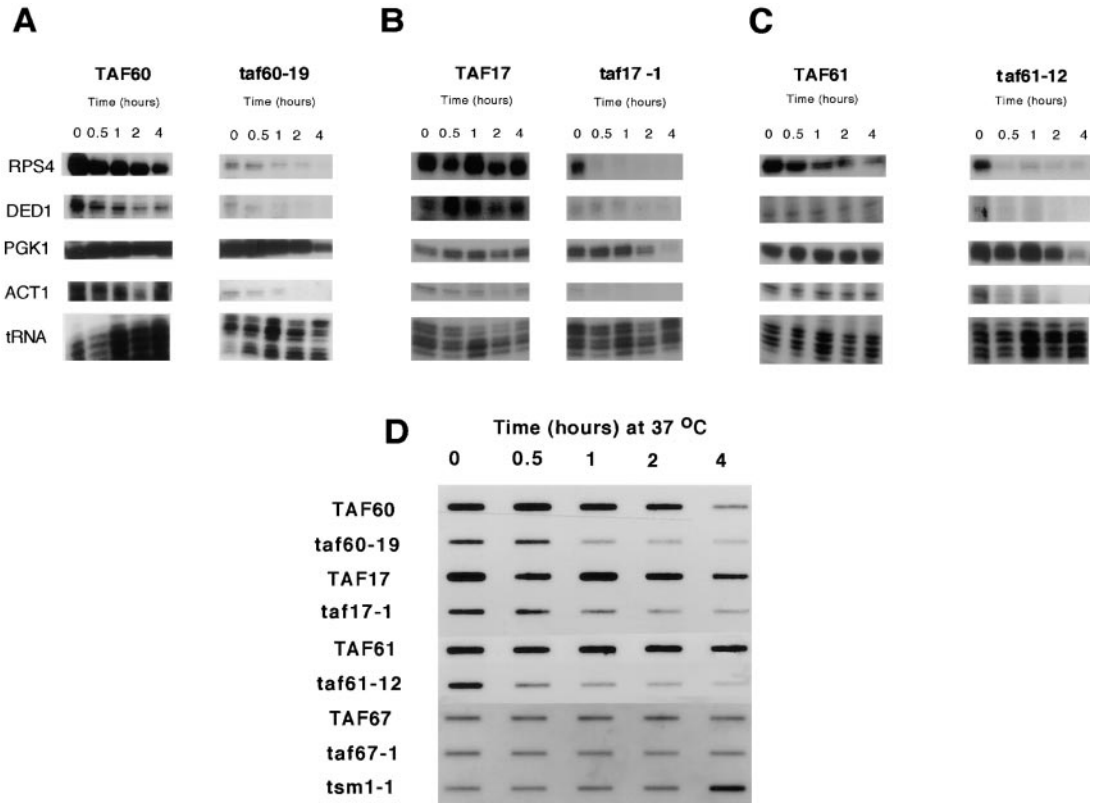

Figure 5. Histone-like TAFs Are Generally Required for In Vivo Transcription by Pol II

(A) S1 nuclease protection assay of mRNAs in the *taf60-19* mutant strain. RNA was isolated from wild-type or mutant strains incubated at 37°C for the indicated amount of time. Probes for the *RPS4*, *DED1*, *PGK1*, and *ACT1* genes were used to monitor levels of those transcripts. A probe for pol III–transcribed $tRNA^W$ was used as an internal control for RNA normalization.

(B) S1 nuclease protection assay of mRNAs in the *taf17-1* mutant strain.

(C) S1 nuclease protection assay of mRNAs in the *taf61-12* mutant strain.

(D) Total poly(A)$^+$ levels in strains carrying either the indicated wild-type or the mutant TAF allele. Total RNA from wild-type or mutant TAF strains were isolated as described in (A) and slot-blotted to a membrane. Poly(A)$^+$ levels were assayed by hybridization with a radioactive oligo-dT probe (Thompson and Young, 1995; Kuldell and Buratowski, 1997). This figure shows a composite of three different experiments, but each result was reproduced at least three times.

Ty and δ (a remnant of Ty) insertions by altering sites of transcription initiation (Winston, 1992). The SPT screen has revealed that either mutations in the TATA binding protein (*SPT15*), certain components of the SAGA complex, or altered dosage of histones can change patterns of transcription in vivo.

We tested whether high-copy expression of histone-like TAFs could suppress the *his4–912*δ or *lys2–128*δ insertion mutations. Figure 4 shows that overexpression of *TAF61* suppressed a *his4–912*δ insertion at least as well as overexpressing histones H2A and H2B (i.e., growth on histidine lacking plates after 3 days). Taf61 truncations that remove the nonconserved N-terminal domain were also able to suppress this insertion mutation, although growth on plates lacking histidine required 4–5 days incubation. In contrast to H2A/H2B, overexpression of *TAF61* did not suppress a *lys2–128*δ insertion (data not shown). Neither *TAF17* nor *TAF60*, overexpressed alone or together, was able to suppress a *his4–912*δ or a *lys2–128*δ insertion.

Suppression of *his4–912*δ provides evidence for *TAF61* playing a role in transcription in vivo. However, at this time it is not clear whether this effect is mediated via the TFIID complex or Taf61's function in the SAGA histone acetyltransferase complex (Grant et al., 1998a; Ogryzko et al., 1998). It may be that overexpression of Taf61 perturbs its balance between the two complexes and that this leads to the Spt− phenotype.

Histone-like TAFs Are Generally Required for Pol II Transcription In Vivo

Previous analyses of conditional TAF alleles revealed only limited effects on in vivo transcription (Moqtaderi et al., 1996a; Walker et al., 1996). However, only a subset of TAFs was analyzed. To test whether conditional growth of histone-like TAF mutants was due to a defect in pol II transcription, RNA was analyzed from wild-type and mutant TAF strains grown at 37°C. Specific mRNAs were analyzed using an S1 nuclease protection assay, and total pol II transcription was monitored by measuring levels of poly(A)$^+$ RNA.

Figure 5 shows that shifting cells to 37°C substantially reduced transcription in strains carrying the TS *taf60-19*, *taf17-1*, and *taf61-12* alleles compared to wild-type cells. By 2 hr after temperature shift, transcript levels of *RPS4*, *DED1*, and *ACT1* were barely detectable while

tRNA levels were unaffected (Figures 5A–5C). Similar results were obtained using probes for the genes *ENO2*, *TCM1*, and *HTA2* (data not shown). Transcript levels of *PGK1*, a highly expressed gene (Velculescu et al., 1997), were also affected, although the effect was most pronounced only after 4 hr at 37°C. It is noteworthy that levels of several transcripts are already significantly reduced in the mutant strain relative to wild-type even under permissive conditions (time 0).

To determine whether histone-like TAFs were generally required for pol II transcription, the mutant TAF strains were shifted to nonpermissive conditions, and total poly(A)$^+$ mRNA levels were monitored (Figure 5D). Consistent with the nuclease protection assays, the mutants had slightly reduced levels of mRNA at the permissive temperature (time 0). By 1–2 hr after shift, there was a substantial reduction in mRNA levels. By 4 hr, the poly(A)$^+$ signal was reduced to the level of background. The rate of the poly(A)$^+$ reduction parallels the inhibition of cell growth (see below). In our hands, the loss of poly(A)$^+$ RNA in the histone-like TAF mutants occurred just as rapidly as with a TS *srb4* strain, an essential component of the pol II holoenzyme (data not shown, see Thompson and Young, 1995). Based on these results, we conclude that the histone-like TAFs are required for transcription at most, if not all, promoters.

Allele Specificity of the Transcription Effect

Since our findings were different from results obtained by other labs analyzing other TAF subunits, we tested TS alleles in two other TFIID components: *TAF67* and *TSM1*. Poly(A)$^+$ mRNA levels from a *taf67* TS allele and from *tsm1-1* were not affected when the cells were shifted to the nonpermissive temperature (Figure 5D).

Furthermore, not every TS allele of the histone-like TAF genes exhibited complete loss of transcription. For example, the *taf17-1* mutation (which contains both L68P and a second mutation that makes the conditional phenotype slightly "tighter"; Figures 1 and 6A) caused a dramatic loss of transcription (Figure 5). Levels of *RPS4* (Figure 6B) and *DED1* (Figure 6C) transcripts were severely reduced. In contrast, a strain carrying the single point mutation *taf17(L68P)* continued very slow growth at 37°C in liquid media (Figure 6A), despite the fact that its conditional phenotype was "tight" on plates (see Figure 1). In this mutant, there was essentially no effect on *DED1* or *RPS4* transcription at the nonpermissive temperature (Figures 6B and 6C). Total poly(A)$^+$ RNA blots confirmed that the transcription defect was only manifested in the double mutant (data not shown). Similarly, no dramatic transcriptional defect was observed in the *taf60(V41P)* strain, while there was a severe loss of transcription for strains carrying either allele *taf60-12* or *taf60-19* (data not shown and Figure 5). Interestingly, these weaker alleles did not result in dramatic loss of TFIID proteins (data not shown).

Therefore, careful characterization and choice of conditional alleles or expression systems must be exercised before making conclusions based on the lack of an observed transcription effect. In our hands, the rapid loss of transcription was observed only with conditional TAF mutants that caused a rapid and complete cessation of growth.

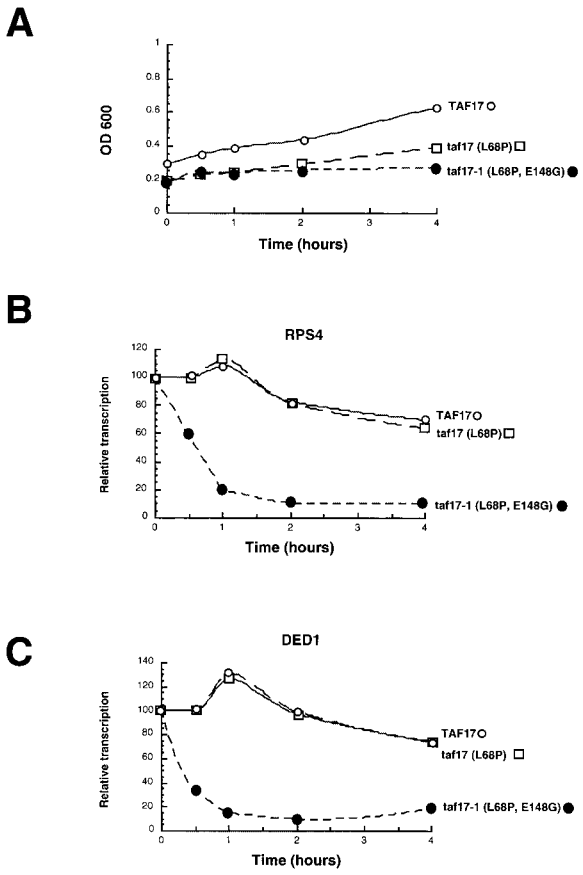

Figure 6. Allele Specificity of the Transcription Defect

(A) Growth curves of *TAF17* wild-type, *taf17(L68P)*, and *taf17 (L68P, E148G)* strains at 37°C.

(B) Quantitation of *RPS4* mRNA levels.

(C) Quantitation of *DED1* mRNA levels. Transcription was monitored by S1 analysis as shown in Figure 5 and quantitated using a phosphorimager. Levels were plotted relative to the time 0 amount (set to 100%). tRNA transcription was used to normalize for RNA recovery and gel loading.

Effect of Histone-like TAF Mutants on the SAGA Complex

Interpretation of results involving the histone-like TAFs has been complicated by the recent discovery that they are also components of the SAGA histone acetyltransferase complex (Grant et al., 1998a). Indeed, one *TAF61 (68)* allele leads to a partially defective SAGA complex that can acetylate free histones but not nucleosomes (Grant et al., 1998a). To test the effects of our histone-like TAF mutants on SAGA, we assayed levels of two SAGA components, Ada1 and Ada2, at both permissive and nonpermissive temperatures (Figure 7A). At 30°C, the TAF mutants have slightly reduced Ada1 and Ada2 levels relative to wild-type (lanes 1, 3, and 7). After 2 hr at 37°C, the SAGA components are essentially undetectable (lanes 4 and 6). Therefore, the histone-like TAFs are essential for integrity of the SAGA complex. In contrast,

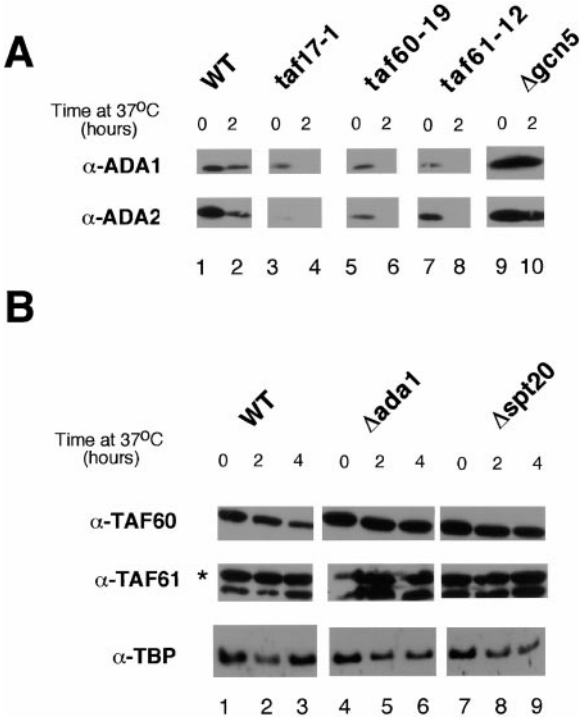

Figure 7. Effect of Histone-like TAF Mutations on the SAGA Complex

(A) Extracts were prepared from the indicated strains grown at 30°C or shifted to 30°C for 2 hr. Proteins were electrophoresed and immunoblotted to detect levels of the Ada1 and Ada2 subunits of SAGA. (B) Extacts were prepared from a wild-type strain (FY839), as well as an ADA1 and SPT20 deletion strain. Cells were grown at 30°C and shifted to 37°C for 2 or 4 hr. The levels of TAF60, TAF61, and TBP were assayed by immunoblotting.

these SAGA components remain stable in a Gcn5 deletion strain (lanes 9 and 10, see also Grant et al., 1998b).

Although all the Spt and Ada components of SAGA are encoded by nonessential genes, some of the deletion strains are TS. We shifted two of these (ADA1Δ and SPT20Δ, see Figure 7B) to the nonpermissive temperature and assayed levels of Taf60, Taf61, and TBP. Even after 4 hr, levels of these proteins remained relatively constant. Therefore, an intact SAGA complex is not required for TAF stability and suggests that the majority of the histone-like TAFs in vivo are not part of SAGA.

Could some or all of the transcription defects in the histone-like TAF mutants be due to the loss of SAGA? While we cannot rule out some contribution, we think it is unlikely that SAGA is the major factor for the following reasons. First, except for the histone-like TAFs, none of the SAGA component genes (*SPTs*, *ADAs*, or *GCN5*) are essential for viability. Therefore, they cannot individually be generally required for transcription. Second, deletion of the *SPT20* gene causes total loss of the SAGA complex and temperature sensitivity (see Grant et al., 1998b). However, when we assayed either the *ADA1* or *SPT20* deletion strains, we did not observe the overall reductions in mRNA levels seen with histone-like TAFs (data not shown). Therefore, to maintain that the transcription defect in the histone-like TAF mutants is due to effects on SAGA, one must postulate a TAF function that does not require the GCN5 acetyltransferase or any of the other SAGA components.

Discussion

In Vivo Evidence for an Octamer-like Structure within TFIID

In this report, we provide in vivo evidence for a subcomplex within TFIID made up of the three histone-like TAFs. Mutant alleles of *TAF17* and *TAF60* were isolated, which, by homology to the nucleosome and the *Drosophila* $TAF_{II}40/TAF_{II}60$ structure, are predicted to disrupt the intermolecular interactions of an octamer-like structure. In the case of Taf17 and Taf60, a proline residue inserted within the central helix of the histone fold domain causes a TS phenotype. This phenotype can be suppressed by overexpression of its dimerization partner or by the third histone-like TAF, Taf61. The suppression is specific for the histone-like TAFs, since none of the other TAFs could suppress the *TAF17* or *TAF60* mutants when overexpressed. Furthermore, overexpression of the histone-like TAFs did not suppress TS alleles in *TAF40*, *TAF67*, or *TSM1* (S. B., unpublished data). Synthetic lethal interactions between *taf61* and *taf17* mutant alleles provides further evidence for interactions between these proteins.

Structural and biochemical evidence also supports a model in which Taf17 and Taf60 form heterotetramers. The histone-like domains of the *Drosophila* homologs have been cocrystallized and form a histone H3/H4–like structure (Xie et al., 1996). In vitro binding studies show that the *Drosophila* and human histone-like TAFs can interact (Hoffmann et al., 1996; Nakatani et al., 1996), although the histone-like TAFs could also interact with histones themselves, raising questions about physiological relevance (Hoffmann et al., 1996). Another pair of TAFs without obvious sequence similarity to histones has also been shown to interact via a histone fold dimer (Birck et al., 1998). Thus, the histone fold may represent a fairly generic and nonspecific dimerization motif. Our genetic results provide evidence for the physiological relevance of the in vitro interactions between the three histone-like TAFs.

Although the case for a histone octamer-like TAF structure appears strong, it is not clear whether the similarity extends to the entire nucleosome. The DNAse I protection pattern of TFIID suggests that DNA is wrapped around some component of TFIID (Nakajima et al., 1988). DNA cross-linking of a downstream promoter element (DPE) to the *Drosophila* TAF60 and TAF40 (the histone H4 and H3-like TAFs) further supports a nucleosome-like model for TFIID interaction with DNA (Burke and Kadonaga, 1997). However, the recently published nucleosome structure shows that histones bind DNA predominantly through arginine side chains inserted into the minor groove of DNA (Luger et al., 1997). These arginines are not conserved in histone-like TAFs, and therefore, any TAF–DNA interactions cannot be mediated by similar contacts.

Histone-like TAFs Are Required for TFIID Integrity

At the nonpermissive temperature, the histone-like TAF mutants cause rapid degradation of both the mutant protein, its interacting partners, and other TAF and SAGA proteins (Figures 2 and 7). TBP levels are also significantly reduced, although 15%–30% remains (Figure 2). This probably represents TBP within the pol I and pol III transcription complexes, since pol III transcription is not affected by the histone-like TAF mutants (Figure 5). This drop in levels of TFIID components is not an indirect effect due to a block in pol II transcription, since a TS *srb4* strain did not exhibit similar effects.

We propose that the conditional phenotypes associated with the histone-like TAF mutants result from their weakened association with the TFIID complex. At the nonpermissive temperature, the mutant proteins cause dissociation of the complex, resulting in degradation. Suppression by overexpression of either the presumed dimerization partner or the third histone-like TAF occurs by driving the equilibrium toward the formation of the histone-like TAF complex. In support of this model, we find that overexpression of the Taf17-1 mutant protein from a strong galactose-inducible promoter abrogates the TS phenotype. Our results also suggest that TBP does not exist free in the cell but, rather, is complexed with other proteins that regulate its stability and function (Lee and Young, 1998).

Histone-like TAFs Are Essential for Pol II Transcription In Vivo

Our results show that the histone-like TAFs are generally required for pol II transcription in vivo. The effect of shifting the TAF mutants to the nonpermissive temperature is rapid and dramatic; 30 min after the shift, there is already a significant loss of transcription. Furthermore, the kinetics of TAF and TBP depletion closely correlate with the loss of mRNA transcription. Therefore, pol II cannot transcribe in the absence of a functional histone-like TAF complex, and presumably a functional TFIID complex. Our results strengthen the view that TFIID (not TBP alone) is the component of the transcriptional machinery acting at promoters in vivo.

The recent discovery of the histone-like TAFs as well as Taf90 and Taf23/25 within the SAGA histone acetyltransferase complex (Grant et al., 1998a) raises the question of whether the transcription effects we observe in our TAF mutants could be mediated by the SAGA complex. We think it unlikely that the loss of transcription observed in the histone-like TAF mutants is primarily mediated by the SAGA complex because none of the other components of the SAGA complex are essential for viability, and their deletion does not cause such a general loss of transcription (Grant et al., 1998b; and data not shown).

Are Histone-like TAFs Different from Other TAFs?

The requirement of histone-like TAFs for pol II transcription distinguishes them from a number of other TAFs analyzed either by inducible shut-off systems or by use of conditional alleles (Apone et al., 1996; Moqtaderi et al., 1996a; Walker et al., 1996, 1997; Shen and Green, 1997). In those studies, transcription effects were only seen at a very limited set of promoters. There are two possible explanations for this apparent discrepancy.

One possibility is that the histone-like TAFs are fundamentally different from other TAFs in being generally required for transcription. The earlier studies looked at only a subset of the TAFs, and it was premature to generalize that none of the TAFs were required based on these few. Moqtaderi et al. (1996a) previously observed little effect of Taf130 or Taf90 depletion using an inducible transcription repression/protein degradation system. However, when similar experiments were performed depleting Taf17, a general loss of transcription was observed (Moqtaderi et al., 1998 [this issue of *Molecular Cell*]). Although this supports the assertion that the Taf17 may be unique, shutoff of Taf60 using the same system did not produce a general loss of transcription (Moqtaderi, 1996a). Using a glucose-repressible system, Walker et al. (1996) depleted Taf61 (68 in that paper) and found that the *CUP1* and *HSP70* genes could still be induced. Since Taf17, Taf60, and Taf61 are bound to each other in an octamer-like structure and thereby function interdependently (see Figure 2), it is difficult to explain how only Taf17 would be required for transcription in vivo.

A second possible explanation for differences between our studies and others is in the method of TAF inactivation. In vivo experiments have been performed using either TS mutants or inducible systems that allow depletion over time. We have noted that different TS alleles in the same TAF gene can give dramatically different transcription results at the nonpermissive temperature (Figure 6). As a rule, we observe transcription loss only with TS alleles that cause a rapid and absolute cessation of growth. Invariably, these alleles also result in reduction of TBP levels.

It must be kept in mind that a conditional allele under nonpermissive conditions is not necessarily the equivalent of a null allele. The studies postulating a limited role for TAFs in transcription assayed the loss of TAF proteins by immunoblotting and concluded that no more than 5% of wild-type TAF levels remained (Moqtaderi et al., 1996a; Walker et al., 1996). If these TAFs are actually required for transcription at most promoters, one must conclude that TFIID exists in vivo in excess of the concentration required for most promoters or that some of the TAF breakdown species were stable and functional. In the case of TAF130/145, a stable degradation product was observed (Moqtaderi et al., 1996a). Since the affinity of particular promoters for TFIID will vary greatly in vivo, a conditional allele or expression system that does not completely abolish TAF function might cause loss of transcription only from some genes. These would probably be those genes with the lowest in vivo affinity for TFIID; therefore, TAF dependence would map to a particular TATA box or other basal promoter element. If even one of these affected genes were essential for cell division or viability, conditional growth would be observed. However, many other promoters might still be transcribed despite the much lower levels of TFIID present in the cell.

We conclude that at least the histone-like TAFs form a complex essential for pol II transcription in vivo. At this point, we cannot distinguish whether the essential role is in basal promoter recognition, response to activators, or simply to keep TBP available for binding to pol

Table 2. Yeast Strains Used in This Study

YSB286	MATa/MATα, ura3-52/ura3-52, leu2Δ1/leu2-3,112, TRP1/trp1Δ63, his3Δ200/his3Δ200
YSB377	MATa, ura3-52, leu2::PET56, trp1, his3Δ200, ade2, taf17Δ::ADE2, {pJA73-TAF17}
YF85	MATa, ura3-52, leu2-3,112, his3Δ200, srb4Δ2::HIS3, {pCT181 = srb4-138, LEU2, CEN/ARS}
YSB380	MATa, ura3-52, leu2::PET56, trp1, his3Δ200, ade2, taf17Δ::ADE2, {pRS314-TAF17}
YSB463	MATa, ura3-52, leu2::PET56, trp1, his3Δ200, ade2, taf17Δ::ADE2, {pRS314-taf17(L68P, E148G)}
YSB492	MATa, ura3-52, leu::PET56, trp1, his3Δ200, ade2, taf17Δ::ADE2, {pRS314-taf17(L68P)}
YSB460	MATa, ura3-52, leu2Δ1 or leu2-3,112, his3Δ200, taf60Δ::LEU2 {pRS316-TAF60b}
YSB522	MATa, ura3-52, leu2Δ1 or leu 2-3,112, his3Δ200, taf60Δ::LEU2, {pJA70-taf60(V41P)}
YSB452	MATa, ura3-52, leu2::PET56, trp1Δ1, his3Δ200, ade2, taf61Δ259::LEU2 {pJA73-TAF61a}
YSB547	MATa, ura3-52, leu2::PET56, trp1Δ1, his3Δ200, ade2, taf61Δ259::LEU2, {pRS313-taf61-23}
YSB 590	MATα, ura3-52, leu2::PET56, trp1, his3Δ200, ade2, taf61Δ259::LEU2, {pJA73-TAF61}
YSB586	MATa, ura3-52, leu2::PET56, trp1, his3Δ200, ade2, taf17Δ::ADE2, taf61Δ259::LEU2, {pRS314-taf17(L68P, E148G), pJA73-TAF61}
YSB270	MATa, ura3-52, leu2Δ1, lys2, tsm1-1
FY119	MATα, ura3-52, leu2Δ1, trp1Δ63, his4-912δ, lys2-128δ
FY300	MATa, ura3-52, leu2Δ1, his4-912δ, lys2-128δ, spt5-194
FY839	MATa, ura3-52, leu2Δ1, his3Δ200
FY 1558	MATa, ura3-52, leu2Δ1, his3Δ200, lys2-128δ, ada1Δ::HIS3
FY1076	MATa, ura3-52, leu2Δ1, his3Δ200, spt20Δ::URA3
FY1354	MATα, ura3-52, leu2Δ1, his3Δ200, lys2-173r2, gcn5Δ::HIS3

II–transcribed promoters. However, it is clear that results obtained for one TAF cannot be immediately applied to all TAFs, and it will be necessary to identify the functions of individual TAFs to completely understand TFIID function.

Experimental Procedures

Yeast Strains, Genetic Manipulations, and Media

Yeast strains used in this study are listed in Table 2. Parentheses indicate episomal plasmids. Yeast strains YSB377 and YSB452 (Moqtaderi, 1996b) were used for plasmid shuffling of *TAF17* and *TAF61*, respectively. One copy of the *TAF60* gene was deleted from the diploid strain YSB286 by one-step gene disruption using a *LEU2* marker. The resulting strain was transformed with a *URA3*-marked plasmid carrying a wild-type copy of *TAF60*, followed by sporulation and tetrad dissection. A resulting Leu+Ura+ haploid strain (YSB460) was used for plasmid shuffling of *TAF60* alleles.

To test for synthetic lethality between *taf17-1* and *taf61-ts* alleles, a strain deleted for genomic copies of both *TAF17* and *TAF61* was constructed by crossing YSB463 to YSB590. Diploids were sporulated, and a segregant containing the correct set of markers (YSB586) and displaying both temperature sensitivity (due to *taf17-1*) and FOA sensitivity (due to the *TAF61* shuffling system) was used for plasmid shuffling.

Yeast strains were transformed by lithium acetate procedure (Gietz et al., 1992). Standard methods for media preparation, sporulation, and tetrad analyses were used (Ausubel et al., 1991; Guthrie and Fink, 1991).

Isolation of Conditional Alleles in TAF17, TAF61, and TAF60

TAF genes were randomly mutated by a polymerase chain reaction (PCR)-based misincorporation method (Mulhard et al., 1992). Reactions contained 1 U Taq polymerase, 0.3 μM primers, 0.25 mM $MnCl_2$, and biased dNTP concentrations (0.4 mM dG,dC,dTTP and 0.1 mM dATP). The reaction was cycled 30 times for 1′ at 94°C, 1′ at 57°C, and 2′ at 72°C. To introduce the PCR products into yeast, a single-step method based on gap repair was used (Mulhard et al., 1992; Hirschhorn et al., 1995).

For *TAF17*, a PCR product spanning 459 bp upstream of the ATG to 530 bp downstream of the termination codon was amplified using primers yTAF17-B (5′-GCGGATCCGTTATCGTTGGCGGAGGCAGT AGCAGTT-3′) and yTAF17-A (5′-GCGGATCCAAACGCATTATCAGA AAGGTTCACAAT-3′). The mutagenized PCR product was transformed into YSB377 together with a 5239 bp BglII/HpaI fragment of pRS314-TAF17, which contains a CEN/ARS origin, a *TRP1* marker, and approximately 300 bp of overlap with each end of the PCR product. Because this fragment lacked *TAF17* coding sequence, plasmids carrying *TAF17* sequences could only arise through recombination between the PCR fragment and the gapped plasmid.

For *TAF61*, mutagenic PCR was performed using primers yTAF61-C (5′-GCGGATCCAAGCTTGAAGATCAACTCAGAAG-3′) and yTAF61-D (5′-GGATCCATGGTGAATAATATAAGTTCGAGC-3′). These primers amplified a 936 bp fragment containing the C-terminal 631 bp of the *TAF61* open reading frame as well as 258 bp downstream the termination codon. The mutagenized PCR products were transformed into yeast strain YSB452 together with a 6696 bp AgeI/PacI fragment of pRS313-TAF61, which contains a CEN/ARS origin, a *HIS3* marker, and approximately 200 bp of overlap with each end of the PCR product. This linearized plasmid lacks 549 bp encoding the carboxy-terminal region that contains the histone homology region.

For *TAF60*, mutagenic PCR was performed using primers yTAF60-A (5′-CCAGATCTCCTGAATTCCTTGCTAAGATG-3′) and yTAF60-E (5′-GGTTGAAATGACCTTATTGAAATAAATCTG-3′). These primers generated a 1121 bp fragment beginning 442 bp upstream the ATG and including the first 699 coding nucleotides of TAF60 containing the N-terminal histone homology region. The mutagenized PCR products were transformed into yeast strain YSB460 together with a 6133bp BamHI/EneI fragment of pRS313-TAF60b that contains a CEN/ARS origin, a *HIS3* marker, and approximately 150 bp of overlap with each end of the PCR product. This linearized plasmid lacks 857 bp encoding the amino-terminal histone homology region of Taf60.

After transformation, the wild-type TAF/URA3 plasmid was shuffled out on media containing 5-fluororotic acid (5-FOA). Approximately 5% of cells were 5-FOA sensitive, indicating they did not regenerate a functional copy of the TAF gene. 5-FOA-resistant cells were replica-plated at 37°C and 30°C, and TS colonies were isolated. Plasmid DNA was isolated and retransformed to confirm plasmid linkage. Conditional strains were further characterized for growth in liquid media at permissive and nonpermissive temperatures. Several tight TS alleles were chosen for further analysis and sequenced (Figure 1).

Site-Directed Mutagenesis

One conditional allele of *TAF60* was generated using PCR-mediated site-directed mutagenesis (Ho et al., 1989). Degenerate oligonucleotide Taf60(V41) (5′-TAGAATTCGGTATTCABNGTCCATAG-3′) and oligonucleotide yTAF60-A were used to amplify the 5′ end of the *TAF60* gene. A second PCR reaction was performed using the 581 bp amplified fragment containing the V41 substitutions and oligonucleotide yTAF60-C (5′-CCAGATCTGAATTCGCAGTTCGGGGGCCT TTGA-3′) as primers. The resulting 2100 bp amplified fragments were cloned into PCR-Script (Stratagene). Mutant clones were identified by the presence of an EcoRI restriction site, and specific valine substitutions were identified by sequencing. An SpeI/HpaI fragment

containing the V41 substitutions was used to replace the SpeI/HpaI fragment in plasmid pJA70-TAF60. The resulting plasmids were shuffled into yeast strain YSB460 to test for complementation and temperature sensitivity.

Protein Analysis

Whole-cell extracts were prepared by glass bead disruption of cells in lysis buffer (10 mM Tris–HCl [pH 7.4], 1 mM EDTA, 0.5% SDS) supplemented with 1 mM PMSF. Equivalent amounts of protein from each sample were then subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting. Detection was by enhanced chemiluminescence using standard methods.

RNA Analysis

Cells were grown to early log phase at 30°C, briefly heated to nullify heat shock artifacts (Cormack and Struhl, 1992), and after 30 additional min at 30°C, cells were shifted to 37°C. Cell density was determined, and equal numbers of cells were harvested at the indicated times. Cells were washed once with cold water, and RNA was isolated using hot acid phenol extraction (Ausubel et al., 1991). The concentration of each RNA sample was determined by measuring the A260. The integrity of the RNA was confirmed by ethidium bromide staining of RNA in agarose gels.

To monitor specific genes, S1 nuclease protection assays were carried out with 50 μg of RNA and 0.1 pmol oligonucleotide probes as described (Cormack and Struhl, 1992). Sequences of the oligonucleotides DED1, RPS4, PGK1, and $tRNA^W$ are described in Cormack and Struhl (1992). Sequences for the oligonucleotides ACT1 and TCM1 are described in Thompson and Young (1995).

To monitor overall pol II transcription, slot blot analysis of total poly(A)$^+$ RNA was performed according to Thompson and Young (1995) using 2 μg of RNA for each time point. Poly d(T) labeling and hybridization were performed as described (Kuldell and Buratowski, 1997).

Acknowledgments

We thank Joe Reese for anti-TAF antibodies, Lenny Guarente for anti-Ada antibodies, Elaine Elion for anti-TCM1, Nathan Swilling for the TAF67 conditional strain, and Fred Winston for yeast strains and helpful discussions. We thank Song Tan for help in sequence/structure comparisons. We are particularly grateful to Zarmik Moqtaderi, Kevin Struhl, and other members of the Struhl lab for sharing information and materials. B. M. is supported by a fellowship from CONACYT (Mexico). S. B. is a Pew Scholar in the Biomedical Sciences and an American Cancer Society Junior Faculty Research Award recipient. This work was supported by a grant from the NIH (GM46498).

Received July 24, 1998; revised September 21, 1998.

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