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 non-permissive 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 TAF4,50 binds to certain initiator elements (Verrijzer et al., 1994), and TAF4,50 and TAF60 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-associated 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 (Mqtaderi 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 TAF40 and TAF60 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 TAF31, TAF80, and TAF20 (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 TAF28 and TAF18 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...
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 DtaFII40 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).

Table 1. Correspondence between TAFs and Histones

<table>
<thead>
<tr>
<th>Histone</th>
<th>Human</th>
<th>Drosophila</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2B</td>
<td>TAF20/15</td>
<td>TAF30/28/22</td>
<td>TAF61/68</td>
</tr>
<tr>
<td>H3</td>
<td>TAF31/32</td>
<td>TAF40/42</td>
<td>TAF17</td>
</tr>
<tr>
<td>H4</td>
<td>TAF80/70</td>
<td>TAF60/62</td>
<td>TAF60</td>
</tr>
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</table>

(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.
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 mutated 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 TFII D 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 TFII D 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. TFII B levels were also moderately reduced in these strains. Levels of a control protein, the ribosomal protein Tcm1, were unaffected. TFII D 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 TFII D at 37°C. Taken together, the immunoblotting results indicate that all three histone-like TAFs are required for the in vivo stability of the TFII D complex.

Genetic Interactions between Histone-like TAFs

The model of a histone octamer-like subcomplex within TFII D 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 TFII D 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 homolog. 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,
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 hu-
man 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 spe-
cifically, confirming a specific genetic interaction be-
tween the histone-like TAFs. Other TAFs, histones, or
TBP did not suppress the TS phenotype when overex-
pressed (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 ar-
rest 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, we 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 TFII D complex.

Taf61 Overexpression Suppresses
a his4-912Δ Insertion Mutation
Retrotransposon Ty integration into a promoter renders
a gene nonfunctional. Mutations in SPT genes suppress
Histone-like TAFs Are Generally Required for In Vivo Transcription by Pol II

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 his4-912δ or lys2-128δ insertion mutations. Figure 4 shows that overexpression of TAF61 suppressed a his4-912δ 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 lys2-128δ insertion (data not shown). Neither TAF17 nor TAF60, overexpressed alone or together, was able to suppress a his4-912δ or a lys2-128δ insertion.

Suppression of his4-912δ 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; Ogrzyko 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.

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,
Histone-like TAFs Required for Transcription

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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 TAFII40/TAFII60 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 provide further evidence for interactions between these proteins.

Structural and biochemical evidence also supports a model in which Taf17 and Taf60 form heterotetramers. 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.

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Histone-like TAFs Are Required for TFII D 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 TFII D 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 TFII D 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 TFII D complex. Our results strengthen the view that TFII D (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, shut-off of Taf60 using the same system did not produce a general loss of transcription (Moqtaderi et al., 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 TFII D 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 TFII D 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 TFII D; 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 TFII D 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
TAF17 (Moqtaderi, 1996b) were used for plasmid shuffling of HIS3.

**Experimental Procedures**

The diploid strain YSB286 by one-step gene disruption using a URA3-marked plasmid carrying a wild-type copy of TAF60, followed by sporulation and tetrad dissection. A resulting Ura- haploid strain (YSB460) was used for plasmid shuffling of TAF60 alleles.

To test for synthetic lethality between tafl7-1 and tafl6-ts alleles, a strain deleted for genomic copies of both TAF17 and TAF61 was generated 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 tafl7-1) and FOA sensitivity (due to the TAF61 shuffling system) was used for plasmid shuffling.

Yeast strains were transformed by lithium acetate procedure. After transformation, the wild-type TAF/URA3 plasmid was shuffled into the genome and the carboxy-terminal region that contains the histone homology region of TAF60 was amplified using oligonucleotide yTAF60-A were used to amplify the 5'-6133bp BamHI/EcoRI fragment of pRS313-TAF60b that contains a 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 tafl7-1) 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**

For TAF60, mutagenic PCR was performed using primers yTAF60-A (5' -GCGGATCAGCTCTGAGTATGTAAGTTCCAG-3') and yTAF60-B (5'-GGATCCATGGTGAATATTATGTCGC-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 of the termination codon. The mutagenized PCR products were transformed into yeast strain YSB452 together with a 696 bp Agel/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' -CATACAGCTTCGAGTTACCTGTAAGTTCCAG-3') and yTAF60-B (5'-GGATCCATGGTGAATATTATGTCGC-3'). These primers generated a 1121 bp fragment beginning 442 bp upstream of 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 containing a 613bp BamHI/EcoRI 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 TAF17/URA3 plasmid was shuffled out on media containing 5-fluoroorotic 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.

**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'-TAGAATTCGTGATGATGTAAGTTCCAG-3') and oligonucleotide Taf60-A were used to amplify the C' end of the TAF60 gene. A second PCR reaction was performed using the 581 bp amplified fragment containing the V41 substitutions and oligonucleotide Taf60-C (5'-CCAGATCAGCTGTGTAAGTTCCAGGAGG-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 Spel/HpaI fragment

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**Table 2. Yeast Strains Used in This Study**

<table>
<thead>
<tr>
<th>Yeast Strains Used in This Study</th>
<th>Description</th>
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<tr>
<td>YSB286</td>
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containing the V41 substitutions was used to replace the Spel/HpaI fragment in plasmid pJ A70-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* 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 (dT) labeling and hybridization were performed as described (Kuldell and Buratowski, 1997).

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**References**


