

Broad, but Not Universal, Transcriptional Requirement for yTAF_{II}17, a Histone H3-like TAF_{II} Present in TFIID and SAGA

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Summary

The RNA polymerase II general transcription factor TFIID is a multisubunit complex comprising TATA box-binding protein (TBP) and associated factors (TAF_{II}s). Experiments in yeast have shown that although most TAF_{II}s are required for viability, many genes are transcribed normally upon inactivation of individual and even multiple yTAF_{II}s. Here we analyze yTAF_{II}17, recently found to be present in both the SAGA HAT complex as well as TFIID. Functional inactivation of yTAF_{II}17 by temperature-sensitive mutation or depletion results in loss of transcription of many, but not all, genes. The upstream activating sequence (UAS), which contains the activator binding sites, is the region that renders a gene yTAF_{II}17 dependent. In conjunction with previous studies, our results reveal that different TAF_{II}s have remarkably distinct properties.

Introduction

Transcription initiation by RNA polymerase II involves the assembly of general transcription factors (GTFs) on the core promoter to form a preinitiation complex (PIC). A variety of studies indicate that promoter-specific activator proteins (activators) work, at least in part, by increasing PIC formation (reviewed in Tjian and Maniatis, 1994; Zawel and Reinberg, 1995). The first step of PIC assembly is binding of the GTF TFIID to the TATA box. TFIID is a multisubunit complex comprising the TATA box-binding protein (TBP) and 8–12 tightly associated TAF_{II}s (reviewed in Burley and Roeder, 1996). Like other components of the general transcription machinery, both TBP and TAF_{II}s have been well conserved from yeast to man.

Early *in vitro* transcription experiments suggested that TAF_{II}s were universal and obligatory coactivators (Burley and Roeder, 1996). Also suggestive of a critical role, almost all yeast TAF_{II}s are essential for viability, indicating that they each perform at least one essential, nonredundant function. However, subsequent studies in yeast demonstrated that in contrast to the results of

in vitro transcription experiments, a variety of genes were transcribed normally *in vivo* in the absence of multiple yTAF_{II}s (Apone et al., 1996; Moqtaderi et al., 1996a; Walker et al., 1996, 1997). To date, the analysis for yTAF_{II}145 has been most extensive. Although yTAF_{II}145 is dispensable for transcription of most yeast genes, a subset of genes require yTAF_{II}145, and the determinant of yTAF_{II}145 dependence maps to the core promoter, not the UAS (Shen and Green, 1997). Whether the properties of other TAF_{II}s are similar to or different from those of yTAF_{II}145 remains to be determined.

Sequence homologies, biochemical studies, and structural analysis have revealed that a subset of TAF_{II}s have properties reminiscent of nonlinker histones (Burley and Roeder, 1996). Specifically, dTAF_{II}40/hTAF_{II}31/yTAF_{II}17 resembles histone H3, dTAF_{II}62/hTAF_{II}80/yTAF_{II}60 resembles histone H4, and dTAF_{II}30 α /hTAF_{II}20/yTAF_{II}68 resembles histone H2B. To date, a histone H2A homolog has not been identified. It has been proposed that these histone-like TAF_{II}s assume an octamer-like structure comprising two dimers of the histone H2B-like TAF_{II} complexed to a tetramer of the histone H3/H4-like TAF_{II}s (Burley and Roeder, 1996).

Recently, in both yeast and human cells, the histone-like TAF_{II}s have been found in large nuclear complexes in addition to TFIID. In yeast, the three histone-like TAF_{II}s (yTAF_{II}17, yTAF_{II}60, and yTAF_{II}68) and two other non-histone-like TAF_{II}s (yTAF_{II}25 and yTAF_{II}90) are integral components of the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex (Grant et al., 1998a). Likewise, human cells contain a PCAF complex that contains several histone-like TAF_{II}s and other subunits bearing TAF_{II} homologies (Ogryzko et al., 1998). Significantly, both SAGA and PCAF contain a single subunit with a histone acetyltransferase (HAT) activity, GCN5 and PCAF, respectively.

This study focuses on yTAF_{II}17, which for several reasons is of particular interest. First, the higher eukaryotic homologs of yTAF_{II}17, dTAF_{II}40, and hTAF_{II}31 have been reported to interact with acidic activation domains (Goodrich et al., 1993; Lu and Levine, 1995; Thut et al., 1995; Uesugi et al., 1997). Acidic activators are the major, and perhaps only, class of activator in yeast (reviewed in Ptashne, 1988; Struhl, 1995), raising the possibility that yTAF_{II}17 may have an important general role in yeast transcription. Second, as discussed above, yTAF_{II}17 is one of the histone-like TAF_{II}s that is an integral component of both TFIID and the SAGA HAT complex. Here we analyze the transcriptional properties of yTAF_{II}17 in living cells and find substantial differences from those of other yTAF_{II}s.

Results

Isolation of yTAF_{II}17 Temperature-Sensitive Mutants
yTAF_{II}17 is encoded by an essential gene (Moqtaderi et al., 1996b; our unpublished data and see below). To determine the cellular defects associated with loss of yTAF_{II}17 function, we isolated three temperature-sensitive (ts) TAF_{II}17 alleles. Strains containing these alleles

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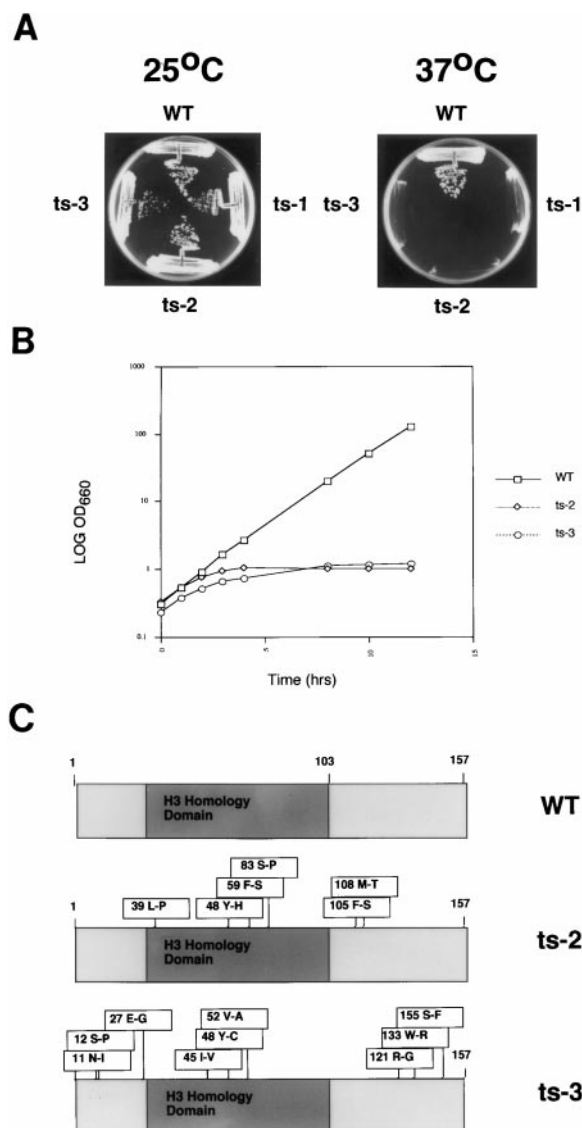


Figure 1. Temperature-Sensitive yTAF_{II}17 Mutants
(A) Strains containing WT (LY740) and temperature-sensitive alleles of yTAF_{II}17, ts-1 (LY722), ts-2 (LY761), and ts-3 (LY766) were streaked onto plates containing rich medium and incubated at 25°C and 37°C.
(B) Growth curve of wild-type and temperature-sensitive strains of yTAF_{II}17 following transfer from 25°C to 37°C.
(C) yTAF_{II}17 temperature-sensitive alleles were sequenced to determine the nucleotide changes generated by mutagenesis. The relevant amino acid substitutions for each allele are indicated.

grew at 25°C but not 37°C (Figure 1A). However, one allele, *ts-1*, displayed only a weak temperature-sensitive phenotype as measured by growth characteristics and initial transcription experiments (data not shown) and was therefore not analyzed further in the experiments presented below. Figure 1B shows that the two remaining temperature-sensitive strains, *ytaf_{II}17^{ts-2}* (strain LY761) and *ytaf_{II}17^{ts-3}* (strain LY766), displayed a rapid growth arrest upon transfer from the permissive to the nonpermissive temperature.

Sequences of the two temperature-sensitive mutants

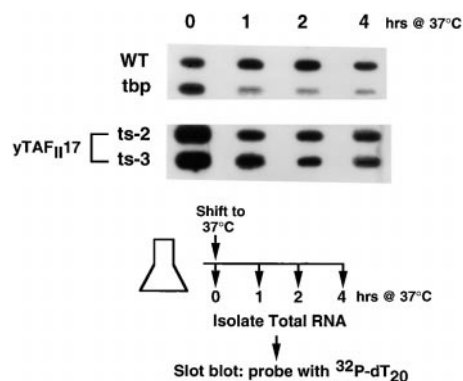


Figure 2. Role of yTAF_{II}17 in Global RNA Polymerase II-Directed Transcription

(Upper panel) Total RNA was isolated from wild-type, temperature-sensitive yTAF_{II}17 strains and as a control, a *tbp* temperature-sensitive strain (*tbp^{ts-1}*), transferred to a filter, and hybridized with a ³²P-oligo-dT₂₀ probe to measure total poly(A)⁺ mRNA.
(Lower panel) Schematic representation of the experimental design.

are shown in Figure 1C. *ytaf_{II}17^{ts-2}* contains six amino acid substitutions, four within and two outside of the histone H3 homology domain; *ytaf_{II}17^{ts-3}* contains nine amino acid substitutions, three within and six outside of the histone H3 homology domain. Thus, diverse substitutions are associated with the temperature-sensitive phenotype of the yTAF_{II}17 mutants.

Role of yTAF_{II}17 in Global RNA Polymerase II-Directed Transcription

As an initial step to determine the role of yTAF_{II}17 in transcription of mRNA-encoding genes, we analyzed the levels of poly(A)⁺ mRNA in the mutant strains by hybridization of total RNA with a ³²P-labeled oligo(dT) probe. Because the half-lives of most yeast mRNAs are relatively short (<15 min; Chia and McLaughlin, 1979; Herrick et al., 1990), this assay is a measure of transcription initiation. We have previously used this assay to show that there was no significant difference in the synthesis of total poly(A)⁺ mRNA following temperature-sensitive inactivation of yTAF_{II}145, TSM1, or yTAF_{II}90 (Walker et al., 1997).

Figure 2 shows the analysis of global RNA polymerase II-directed transcription in yeast strains harboring the two temperature-sensitive yTAF_{II}17 mutants, or as a control, a temperature-sensitive TBP mutant. As expected, inactivation of TBP resulted in a rapid and dramatic loss of transcription. Significantly, there was also a substantial decline (~60%–78%) in the synthesis of total poly(A)⁺ RNA in the temperature-sensitive yTAF_{II}17 mutant strains. However, a fraction of total poly(A)⁺ mRNA synthesis was unaffected by yTAF_{II}17 inactivation.

Differential Requirement of yTAF_{II}17 for Transcription of Actively Expressed Yeast Genes

The results of Figure 2 indicate that upon yTAF_{II}17 inactivation, poly(A)⁺ mRNA synthesis substantially declined but was not abolished. One explanation for these results is that transcription of all genes decreased uniformly following yTAF_{II}17 inactivation. Alternatively, the results

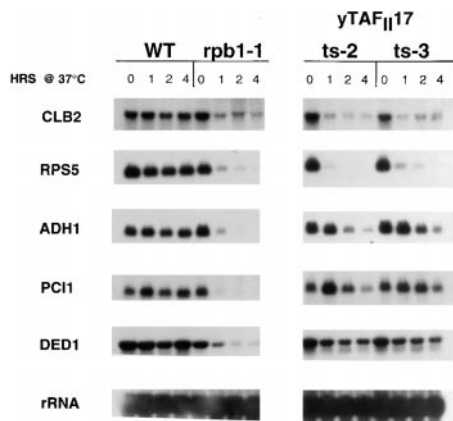


Figure 3. Transcriptional Analysis of Specific Genes Following yTAF_{II}17 Inactivation

Northern blotting was used to measure the transcription levels of five genes (indicated on the left) in the yTAF_{II}17 temperature-sensitive strains following transfer from 25°C to 37°C. A temperature-sensitive *rpb1* strain was used as a control.

could be explained by the existence of two classes of genes, one that required yTAF_{II}17 for transcription and a second that did not. To distinguish between these two possibilities, we analyzed the expression of specific, actively transcribed genes. Yeast strains harboring a yTAF_{II}17 mutant or, as a control, a temperature-sensitive RNA polymerase II mutant (*rpb1-1*), were shifted to 37°C, and RNA was isolated at various times. Transcription was measured by Northern blotting using probes specific to five representative, well-studied yeast genes whose expression is directed by diverse activators: *ADH1*, *CLB2*, *PCL1*, *DED1*, and *RPS5*.

The results of Figure 3 show, as expected, that in the *rpb1^s* strain, transcription of all five genes was reduced to virtually undetectable levels within 1 hr after temperature shift. In the yTAF_{II}17 temperature-sensitive mutant strains, however, the results were highly gene-specific: *CLB2* and *RPS5* were significantly affected; *ADH1* was moderately affected; and *DED1* and *PCL1* were relatively unaffected, by yTAF_{II}17 inactivation. We note that there was a modest decrease in *DED1* and *PCL1* transcription 4 hr following the temperature shift, a time at which the cells had been arrested for at least 3 hr (see Figure 1B). This modest effect most likely reflects a general deterioration of all activities and, in particular, loss of TBP (see below). The results of Figure 3 also show, as expected, that expression of an rRNA gene, which is transcribed by RNA polymerase I, was unaffected by yTAF_{II}17 inactivation.

Immunoblot Analysis of Other yTAF_{II}s and TBP Following Temperature-Sensitive Inactivation of yTAF_{II}17

We have previously shown that temperature-sensitive inactivation or depletion of an individual yTAF_{II} can disrupt TFIID leading to degradation of other yTAF_{II}s and ultimately TBP (Walker et al., 1996). Thus, one explanation for the broad transcriptional defect observed upon yTAF_{II}17 inactivation is the loss of TBP, and perhaps other yTAF_{II}s.

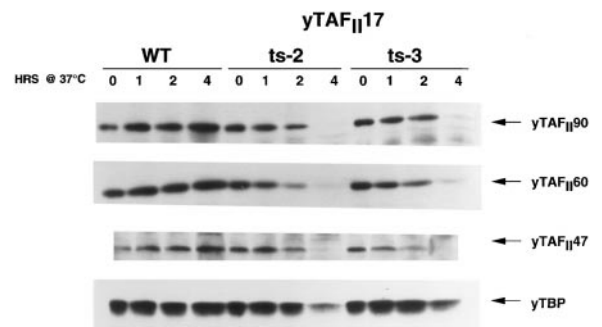


Figure 4. Immunoblot Analysis Following Temperature-Sensitive Inactivation of yTAF_{II}17

The levels of TBP and the indicated yTAF_{II}s (right) in wild-type and yTAF_{II}17 temperature-sensitive strains were quantitated by immunoblot analysis of whole-cell extracts prepared following transfer from 25°C to 37°C.

To address this possibility, we performed immunoblot analysis in the yTAF_{II}17 temperature-sensitive strains using antibodies to TBP, the core subunit of TFIID; yTAF_{II}47, which is present in TFIID but not SAGA; and yTAF_{II}60 and yTAF_{II}90, which are present in both TFIID and SAGA. The results of Figure 4 show that following 1 hr incubation at the nonpermissive temperature, the levels of TBP and the other yTAF_{II}s were unaffected. However, at later times there was a progressive and substantial reduction in the levels of all other yTAF_{II}s, and by 4 hr there was also a significant loss of TBP.

These results indicate that the transcriptional decrease of genes such as *CLB2* and *RPS5* occurring at 1 hr cannot be explained by loss of TBP or other TAF_{II}s. Based upon these data, our previous studies with other yTAF_{II}s (Apone et al., 1996; Walker et al., 1996, 1997; Shen and Green, 1997), and experiments presented below, we believe that the transcriptional decline of these genes is a primary effect of yTAF_{II}17 inactivation. In addition, these results indicate that the modest transcriptional decrease of other genes, such as *DED1* and *PCL1*, at 4 hr is most likely due to loss of TBP and perhaps other TAF_{II}s. Finally, there was almost complete loss of yTAF_{II}60 and yTAF_{II}90 (as well as yTAF_{II}47) upon yTAF_{II}17 inactivation. This result is explained by their presence, along with yTAF_{II}17, in TFIID and SAGA (Grant et al., 1998a), which are both disrupted upon inactivation of yTAF_{II}17.

Gene-Specific Transcriptional Defects Following yTAF_{II}17 Depletion

To confirm and extend the results obtained with the yTAF_{II}17 temperature-sensitive mutants, we inactivated yTAF_{II}17 using an independent, conditional depletion strategy. We constructed a strain in which yTAF_{II}17 was under the control of the glucose-repressible GAL1 promoter (*LY101*). Following transfer to glucose-containing medium, these cells displayed a rapid growth arrest (Figure 5A). The immunoblot data of Figure 5B shows that yTAF_{II}17 was rapidly depleted and by 12 hr was undetectable. These data also show that although yTAF_{II}17 was depleted at 12 hr, TBP levels were normal.

We next analyzed the effect of yTAF_{II}17 depletion on

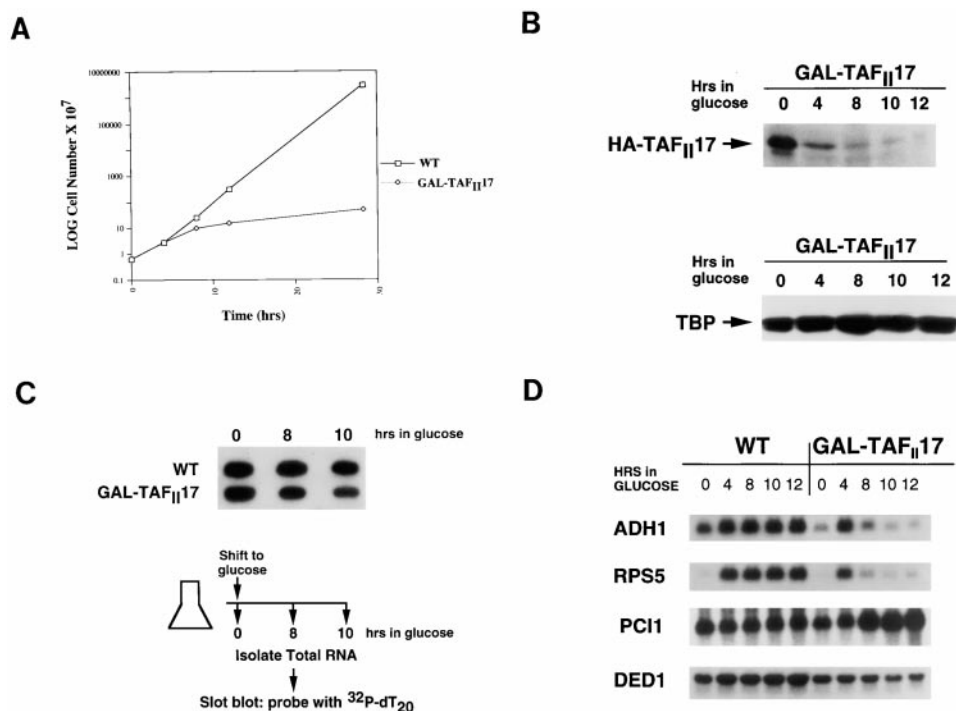


Figure 5. Characterization of Strains Following Conditional Depletion of *yTAF_{II}17*

(A) Growth curve of strains LY90 (WT) and LY101 (GAL1-HA-*yTAF_{II}17*), following transfer from galactose to glucose-containing media.

(B) Whole-cell extracts were prepared from strain LY101 (GAL1-HA-*yTAF_{II}17*) at the indicated times following transfer from galactose to glucose-containing media. *yTAF_{II}17* was detected by immunoblotting with an antibody (12CA5) that recognizes the HA epitope, and TBP was detected with a polyclonal α -TBP antibody.

(C) (Upper panel) Total RNA was isolated from strains LY90 (WT) and LY101 (GAL1-HA-*yTAF_{II}17*) grown in glucose-containing media for the indicated times, transferred to a filter, and hybridized with a 32 P-oligo-dT₂₀ probe to measure total poly(A)⁺ mRNA.

(Lower panel) Schematic representation of the experimental design.

(D) Northern blot analysis was used to analyze the transcription levels of four genes (indicated on the left) in strains LY90 (WT) and LY101 (GAL1-HA-*yTAF_{II}17*) at the indicated times following transfer from galactose to glucose-containing media.

transcription. Similar to the results obtained with the temperature-sensitive *yTAF_{II}17* mutants, there was a significant loss (~70%) of total poly(A)⁺ mRNA synthesis following *yTAF_{II}17* depletion, although once again, a fraction of poly(A)⁺ RNA appeared unaffected (Figure 5C). Likewise, the analysis of specific genes was completely consistent with the results obtained using the *yTAF_{II}17* temperature-sensitive mutants: transcription of *RPS5* and *ADH1* was compromised by *yTAF_{II}17* depletion, whereas transcription of *PCL1* and *DED1* was unaffected (Figure 5D). We note that transcription of *RPS5* and *ADH1* increased in both wild-type and GAL-*TAF_{II}17*-containing strains at 4 hr following shift to glucose-containing media. This increase most likely reflects the change in carbon source and resultant increase in growth rate.

Identification of *yTAF_{II}17*-Dependent Genes by High-Density Oligonucleotide Array Analysis

The results presented above define two classes of genes distinguished by their transcriptional requirement for *yTAF_{II}17*. To confirm this conclusion and identify all genes that require *yTAF_{II}17* for transcription, we employed high-density oligonucleotide arrays to measure

transcription from all yeast genes in a single experiment (Wodicka et al., 1997).

The genome-wide expression profile of *ytaf_{II}17^{ts-2}* cells was compared to isogenic wild-type cells 45 min after both populations were shifted to 37°C. The transcription levels of 5349 genes were analyzed in *ytaf_{II}17^{ts-2}* cells and isogenic wild-type cells in two independent experiments, and these data are summarized in Table 1. Of the 5349 genes, the levels of 3877 mRNA species decreased by more than 2-fold in the mutant relative to wild-type cells; the levels of the remaining 1472 genes were not significantly affected. Thus, 72% of the genes analyzed displayed a transcriptional defect following loss of *yTAF_{II}17*, whereas the remaining 28% did not.

Because the time required to produce most secondary effects involves a substantial reduction in both a transcript and its translation product, the reduction in mRNA levels observed in temperature-sensitive mutants soon after a temperature shift (e.g., 45 min) is likely due to a primary effect. Nonetheless, the results obtained in this experiment could be due to both primary and secondary effects following *yTAF_{II}17* inactivation. An approach to identify the set of genes whose change in expression is almost certainly a direct consequence of the loss of function of the temperature-sensitive factor has been developed (Holstege et al., 1998). The rate of

Table 1. Dependence of Genome-Wide Transcription on yTAF_{II}17

Gene Set	Results
Total yeast genes	6173
Total genes analyzed	5349
Genes whose mRNA decreased >2-fold	3877 (72%)
Genes appropriate for comparison with <i>rpb1-1</i>	4736
Genes whose mRNA decay rates fit those of <i>rpb1-1</i>	3180 (67%)

The results of genome-wide transcription analysis following temperature-sensitive inactivation of yTAF_{II}17 in the yTAF_{II}17^{ts-2} mutant strain are summarized. Approximately 6% of yeast genes produce transcripts with half-lives greater than 45 min, and in these cases the requirement for yTAF_{II}17 has not been assessed.

loss of all transcripts is determined for the RNA polymerase II temperature-sensitive mutant *rpb1-1* under identical conditions. These data are then used to identify the set of transcripts with equivalent decay kinetics in, for example, the yTAF_{II}17^{ts-2} mutant. Thus, this method identifies the set of genes whose expression is equivalently dependent on the factor of interest and RNA polymerase II itself.

Of the 5349 transcript levels scored in the yTAF_{II}17^{ts-2} experiment, 4736 fit our criteria for an accurate comparison with the levels of mRNAs after inactivation of RNA polymerase II (Table 1). Of these 4736, 3180 transcripts (67%) were reduced with similar kinetics in both the yTAF_{II}17^{ts-2} mutant and the RNA polymerase II mutant *rpb1-1*. We conclude that the expression of approximately 67% of actively expressed yeast genes is as dependent on yTAF_{II}17 function as it is on core RNA polymerase II itself.

Differential Requirement of yTAF_{II}17 for Transcription of Inducible Genes

The genes analyzed in the above experiments were actively transcribed at the time of the temperature shift or following depletion of yTAF_{II}17 by switching to glucose-containing media. To examine the requirements of yTAF_{II}17 for transcription of inducible genes, we performed the experiments shown in Figure 6. The two yTAF_{II}17 temperature-sensitive mutants, and the conditional depletion strategy, were used to analyze three well-characterized inducible genes: *CUP1*, which is inducible by copper; *SSA4*, which is inducible by heat shock; and *HIS4*, which is inducible by 3-aminotriazole (3AT). In all instances, yTAF_{II}17 was either inactivated or depleted prior to adding the inducer.

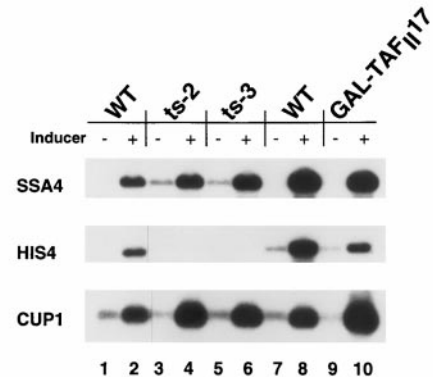
The results indicate that neither temperature-sensitive inactivation nor depletion of yTAF_{II}17 affected transcriptional activation of either copper-inducible *CUP1* or heat-inducible *SSA4*. However, the two yTAF_{II}17 temperature-sensitive strains were completely unable to support *HIS4* induction. Following depletion of yTAF_{II}17, *HIS4* induction was also significantly reduced (compare lanes 8 and 10). We conclude that, as with constitutively expressed genes, transcription of some but not all inducible genes is dependent upon yTAF_{II}17.

Mapping the Promoter Region that Confers yTAF_{II}17 Dependence

The results presented above define two classes of genes distinguished by their transcriptional requirement for

yTAF_{II}17. We next sought to delineate the promoter region that conferred these distinct transcriptional properties. We focused on two genes whose promoters and UAS elements have been well characterized: *HIS4*, which requires yTAF_{II}17, and *CUP1*, which does not. We constructed chimeric promoters in which the *HIS4* UAS was fused to the *CUP1* core promoter, or conversely, the *CUP1* UAS was fused to the *HIS4* core promoter.

A



B

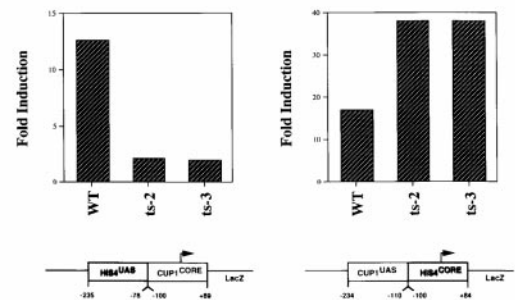


Figure 6. Transcription Induction Following yTAF_{II}17 Inactivation

(A) Northern blotting was used to measure transcription levels of *SSA4*, *HIS4*, and *CUP1*, in wild-type and conditional yTAF_{II}17 strains. The inducer was added either following incubation at 37°C for 2 hr (ts-2 and ts-3), or following transfer to glucose-containing media for 10 hr (GAL-TAF_{II}17).

(B) Chimeric promoter analysis. Transcriptional induction was determined by measurement of β -galactosidase activity. Schematic diagrams of the *HIS4*^{UAS}-*CUP1*^{CORE} promoter-lacZ fusion gene and *CUP1*^{UAS}-*HIS4*^{CORE} promoter-lacZ fusion gene are shown below the bar graph.

The results of Figure 6B show, as expected, that in wild-type cells transcription from the *HIS4^{UAS}-CUP1^{core}* chimeric promoter was activated following addition of the inducer, 3AT. However, following inactivation of yTAF_{II}17, transcriptional inducibility of the *HIS4^{UAS}-CUP1^{core}* chimeric promoter, like intact *HIS4*, was severely compromised. In the reciprocal experiment, the *CUP1^{UAS}-HIS4^{core}* promoter was, as expected, activated in wild-type cells following addition of copper. In this case, following inactivation of yTAF_{II}17, transcriptional induction by copper still occurred and was, in fact, modestly increased (~2-fold). A similar increased inducibility following yTAF_{II}17 inactivation was also evident for the endogenous *CUP1* gene (see Figure 6A). Based upon these combined results, we conclude that the UAS is the critical determinant of yTAF_{II}17 dependence.

Discussion

We have presented a series of experiments demonstrating that yTAF_{II}17 is required for transcription of many, but not all, yeast genes. Whole-genome analysis using DNA chip microarrays indicates that approximately 67% of yeast genes directly require yTAF_{II}17 for transcription. In this respect, although yTAF_{II}17 is clearly not a universally required GTF, it provides a more general transcriptional function than all other yTAF_{II}s analyzed to date. These results contrast sharply with those obtained for previously analyzed yTAF_{II}s: for example, depletion of yTAF_{II}145 (Walker et al., 1996) and yTAF_{II}90 (Apone et al., 1996) to below 300 copies per cell, far less than the ~4500 actively transcribed yeast genes, did not result in a general loss of transcription. yTAF_{II}17 also differs from the previously analyzed yTAF_{II}145, by the promoter element required for yTAF_{II}17 dependence: whereas yTAF_{II}145 dependence maps to the core promoter (Shen and Green, 1997), it is the UAS that confers the yTAF_{II}17 requirement. Several lines of evidence strongly suggest that the loss of transcription upon yTAF_{II}17 inactivation is a primary effect and not an indirect consequence of, for example, disrupting yTAF_{II}17-containing complexes or failing to synthesize another factor, which in turn, is required for transcription of yTAF_{II}17-dependent genes. First, comparable results were observed using two independent strategies to functionally inactivate yTAF_{II}17: temperature-sensitive mutations and conditional depletion. Second, the loss of transcription following temperature-sensitive inactivation of yTAF_{II}17 first occurs when the levels of other yTAF_{II}s and TBP are normal. Third, and relatedly, the requirement for yTAF_{II}17 is much broader than any other yTAF_{II} or SAGA component analyzed to date. Therefore, the transcriptional requirement for yTAF_{II}17 cannot be attributed to action through these other components. Finally, and most importantly, upon inactivation of yTAF_{II}17, yTAF_{II}17-dependent genes cease transcription with kinetics identical to those observed upon loss of RNA polymerase II activity. A transcriptional defect resulting from a secondary effect would exhibit delayed kinetics.

Perhaps histone-like TAF_{II}s will, in general, provide a broader transcriptional function than their non-histone-like counterparts. It has recently been reported that a

mammalian extract immunodepleted of TAF_{II}s can support transcription activation (Oelgeschläger et al., 1998). Interestingly, the extracts used in this study still contained histone-like TAF_{II}s, leaving open the possibility that they were required for the activated transcription.

Several yTAF_{II}s, including yTAF_{II}145, TSM1, and yTAF_{II}90, are required for progression through specific phases of the cell cycle, and not for general RNA polymerase II-directed transcription (Apone et al., 1996; Walker et al., 1996). For yTAF_{II}145, the cell cycle phenotype results from a failure to transcribe a specific subset of genes required for G1/S phase progression (Walker et al., 1997). In contrast, blockage of general RNA polymerase II transcription either by chemical inhibitors or GTF mutants leads to a random arrest throughout the cell cycle (Apone et al., 1996). Likewise, yTAF_{II}17, which is required for transcription of many yeast genes, does not have a cell cycle phenotype (data not shown).

Possible Mechanisms of yTAF_{II}17 Action

Although it is clear that the requirement for yTAF_{II}17 is relatively broad, its mechanism(s) of action remains to be determined. One possibility is that yTAF_{II}17 functions through direct interactions with the activator. As mentioned above, the higher eukaryotic homologs of yTAF_{II}17 have been reported to interact with acidic activators (Goodrich et al., 1993; Lu and Levine, 1995; Thut et al., 1995; Uesugi et al., 1997), the predominant and perhaps exclusive class of activator in yeast (Ptashne, 1988; Struhl, 1995). Consistent with this possibility, we have found that the promoter element required for yTAF_{II}17 dependence maps to the UAS. However, it has not been shown that yTAF_{II}17 interacts with acidic activation domains; yTAF_{II}17 is substantially smaller than its higher eukaryotic homologs, and whether the interaction site has been conserved is unknown. Furthermore, yTAF_{II}17 is not required for the function of two well-characterized acidic activators: heat shock transcription factor and Ace 1.

Alternatively, yTAF_{II}17 may function by interacting with promoter DNA. The histone homology present in yTAF_{II}17 is suggestive of a DNA binding function, and several, independent lines of evidence have revealed contacts between TAF_{II}s and the promoter (Burley and Roeder, 1996; Oelgeschläger et al., 1996). Most relevant to this study, the *Drosophila* homolog of yTAF_{II}17, dTAF_{II}40, and its heterodimeric partner, dTAF_{II}60, both contact the promoter in a sequence-specific fashion (Burke and Kadonaga, 1997).

An understanding of the mechanism of yTAF_{II}17 action is further complicated by its presence in at least two complexes, TFIID and SAGA (Grant et al., 1998a), raising the question as to which complex mediates the yTAF_{II}17 transcriptional effects. Previous studies have shown that inactivation of several yTAF_{II}s leads to disruption of the TFIID complex without compromising transcription (Walker et al., 1996). Furthermore, in TFIID the function of yTAF_{II}17 and other TAF_{II}s is expected to depend on yTAF_{II}145: yTAF_{II}145 is the only TAF_{II} known to contact TBP directly (Reese et al., 1994), and its higher eukaryotic homolog, TAF_{II}250, is always required to reconstitute TFIID activity in vitro (Chen et al., 1994). However, the

transcriptional effect of yTAF_{II}145 inactivation is significantly more limited than that of yTAF_{II}17. Collectively, these considerations raise the possibility that TAF_{II}17 may function in some other complex.

SAGA is the most likely alternative complex in which TAF_{II}17 would function. Models of SAGA typically invoke a targeted recruitment of the complex that positions the GCN5 HAT activity at the promoter (reviewed in Grant et al., 1998b). However, GCN5 is not essential and is required for transcription of relatively few genes (see, for example, Georgakopoulos and Thireos, 1992). Similarly, other SAGA components such as *ada1*, *ada2*, *ada3*, and *spt20* are also not essential and are not required for transcription of most genes (reviewed in Grant et al., 1998b; Hampsey, 1998). It is therefore difficult to attribute the requirement of TAF_{II}17 for viability and its relatively broad transcriptional requirements to action through these known SAGA components. Similarly, yTAF_{II}90, which is dispensable for transcription of most yeast genes (Apone et al., 1996; Walker et al., 1996, 1997), is also a component of the SAGA complex.

In summary, additional experiments will be required to determine whether yTAF_{II}17 exerts its function in TFIID or SAGA. Perhaps disruption of both TFIID and SAGA is required to obtain the broad transcriptional defects observed following yTAF_{II}17 inactivation. It is also possible that yTAF_{II}17 could exist in additional complexes or even function alone, as inferred by the report that unlike yTAF_{II}17, its heterodimeric partner, yTAF_{II}60, is dispensable for transcription of most yeast genes (Moqtaderi et al., 1996a).

Implications for the Function of Multicomponent Transcription Complexes

Multisubunit transcription complexes are generally believed to mediate a single activity: for example, PIC assembly for TFIID and chromatin modification for SAGA. The results with yTAF_{II}17 underscore the unexpected diversity of the individual subunits of these complexes. As an example of this diversity, comparison of our previous results for yTAF_{II}145 (Shen and Green, 1997) with the current results for yTAF_{II}17 reveals that both yTAF_{II}s are required for transcription of *RPS5*, neither is required for transcription of *DED1*, yTAF_{II}145 but not yTAF_{II}17 is required for transcription of *PCL1*, and yTAF_{II}17 but not yTAF_{II}145 is required for transcription of *ADH1*.

Although the above discussion has focused upon TFIID and SAGA, the conclusions are also applicable to other multisubunit transcription complexes. For example, there are striking differences in the properties of individual subunits of the SRB/mediator complex, with regard to both requirements for viability and transcriptional effects (Hengartner et al., 1995; Myers et al., 1998).

These observations raise the possibility that it may be the intrinsic activity of the subunits, rather than their presence in a single complex, that is critical. The intrinsic activity of a subunit could enable it to function when present in multiple complexes, in a partially disrupted complex, or on its own. We suggest that in some instances components may be present in a single complex not to provide a single activity, but rather for other reasons, such as coregulation or efficient corecruitment to the promoter.

Experimental Procedures

Yeast Strains

All strains are isogenic derivatives of W303, with the exception of *rpb1-1* (Nonet et al., 1987) and TBpts-1 (Cormack and Struhl, 1992), which have been previously described. The *TAF17* disruption strain, LY80, was constructed by transformation of strain W303 with an EcoRI fragment isolated from plasmid Lp21, which contains a disrupted copy of *TAF17*, followed by selection on leucine-deficient medium. PCR and Southern blotting were used to confirm proper integration of the disruption construct. Strain LY80 was then transformed with plasmid Lp19, which contains a wild-type copy of *TAF17* on a URA3-marked plasmid, to generate strain LY87. LY87 was then sporulated and the resulting tetrads were dissected. Spores that grew on media lacking uracil and leucine were selected. One such strain, called LY90, was used to generate additional wild-type and conditional yTAF_{II}17 strains using the plasmid shuffle technique (Ito et al., 1983). LY740, LY722, LY761, LY766, and LY101 were derived by transformation of LY90 with plasmids Lp20, Lp34, Lp35, Lp36, and Lp37, respectively, followed by screening on 5-fluoroorotic acid (5-FOA) containing media to select for cells that had lost the wild-type plasmid. Cultures were grown in YEPD, unless selection was necessary, in which case all cultures were grown in the appropriate selective (SD) medium.

Plasmid Constructions

A PCR fragment containing wild-type *TAF17* was generated from genomic DNA using the primers 5'-GGCCTGAATTCACCTTTTACCG-3' and 5'-CGGAATTCGCGGAAAGTGCTCTCAAGAAGATG-3'. The primers were designed to generate an EcoRI restriction site on both ends of the PCR fragment. The EcoRI-digested PCR fragment was cloned into the EcoRI site of the *HIS3*-marked, single copy number plasmid pRS413 (Sikorski and Hieter, 1989) to generate plasmid Lp20; into the URA3-marked single copy number plasmid pRS416 (Sikorski and Hieter, 1989), to generate Lp19; and into the plasmid Bluescript (Stratagene) to generate plasmid Lp17. Lp17 was then digested with BsmI, and the ends were made blunt with T4 DNA polymerase. A DNA fragment containing the *LEU2* gene was isolated from plasmid pJJ282 (Jones and Prakash, 1990) by digestion with SspI and cloned into the blunted Lp17 plasmid to generate the *TAF17* disruption construct Lp21. To construct Lp37, in which *TAF17* expression is under the control of the GAL1 promoter, a PCR fragment containing the coding region of *TAF17* was generated from genomic DNA using the primers 5'-GAGGACTTCACAGGAATTCATGAACGGC-3' and 5'-CGGAATTCGCGGAAAGTGCTCTCAAGAAGATG-3'. The fragment was digested with EcoRI and cloned into the EcoRI site of plasmid pRD54 (Apone et al., 1996).

LacZ fusion constructs were generated by cloning PCR fragments containing the *CUP1* (-100 to +89) and *HIS4* (-100 to +84) promoter regions into the XhoI and BamHI sites of the vector LRD101 (Yocum et al., 1984) to replace the *GAL1* promoter region. PCR was also used to generate fragments containing the *HIS4* (-235 to -76) and *CUP1* (-234 to -110) UAS regions, which were cloned into the NheI and BamHI sites of the *CUP1* and *HIS4* promoter-containing constructs, respectively.

Isolation of Temperature-Sensitive Alleles

The *taf17 ts-1* allele (Lp34) was generated by treatment of plasmid Lp20 with 0.5 M hydroxylamine for 3 hr at 75°C. The *taf17 ts-2* (Lp35) and *ts-3* (Lp36) alleles were generated using PCR mutagenesis protocol 2 described previously (Leung et al., 1989). The ends of the PCR-generated fragment were made blunt with T4 DNA polymerase and then cloned into the EcoRV site of the *HIS3*-marked vector, pRS313 (Sikorski and Hieter, 1989). In each case, the mutagenized DNA was then transformed into strain LY90, and the cells were grown at room temperature on media lacking uracil. Transformants were then screened for growth at room temperature and at 37°C on media containing 5-FOA. Plasmids from colonies that maintained the temperature-sensitive phenotype upon rescreening were isolated and sequenced.

Temperature-Shift Experiments

Cells were grown at room temperature to log phase; an equal volume of media, prewarmed to 37°C, was added; and the cultures were

transferred to 37°C. Aliquots of cells were taken at various times following shift to 37°C. For transcription induction experiments, cells were incubated at 37°C for 2 hr prior to the addition of inducer. For *CUP1* induction, copper sulfate was added to a final concentration of 1 mM, and the cultures were incubated at 37°C for an additional 30 min. For *HIS4* induction, cells were washed once in media lacking histidine and resuspended in the same media with the addition of 3-amino-1-2-4-triazole (3AT) to a final concentration of 10 mM if cultures were to be induced and complete media for cultures not induced. For *SSA4* induction, cells were transferred to 39°C for 15 min prior to harvesting. In each case, cells were harvested at 4°C and stored at -80°C. Induction of *LacZ* fusion constructs was carried out as described above for the endogenous genes with the following exceptions. Cells were grown in SD-URA, and the *CUP1^{UAS}* was induced for 1 hr after transfer to the nonpermissive temperature, followed by a 3 hr incubation at the nonpermissive temperature. *HIS4^{UAS}* induction was carried out as described above, and also with a 30 min and a 1 hr incubation at the nonpermissive temperature prior to induction. Similar results were obtained using the three different induction protocols. β -galactosidase activity was assayed as described previously (Kaiser et al., 1994).

Conditional Depletion of γ TAF_{II}s

Cultures were grown at 30°C to log phase in the presence of 3% galactose. Cells were then harvested, washed two times with sterile water, and resuspended in media containing 3% glucose. For transcription induction experiments, cells were maintained in glucose for 10 hr, after which time inductions were carried out as described above for the temperature-sensitive strains.

RNA Analysis

Total RNA was isolated and Northern blotting performed as previously described (Apone et al., 1996). Analysis of poly(A)⁺ mRNA was performed as follows. Total RNA (5 μ g) was transferred to GeneScreen (NEN Life Science Products, Boston, MA) as recommended by the manufacturer (Hoefer Scientific Instruments, San Francisco, CA). RNA was fixed to the filter by UV cross-linking using a Stratalink (Stratagene, La Jolla, CA), and the filter hybridized overnight in prehybridization buffer consisting of 6 \times SSC, 5 \times Denhardt's, 0.5% SDS, and 0.2 mg/ml salmon sperm DNA. The filter was then hybridized for at least 18 hr in fresh prehybridization buffer containing 5 \times 10⁵ cpm/ml of end-labeled oligo dT20 probe. The filter was then washed once for 15 min at 37°C in prehybridization buffer, followed by three washes at room temperature for 20 min each in 1 \times SSC, 0.1% SDS.

Whole-Cell Extracts and Immunoblot Analysis

Cultures were grown to mid-log phase and aliquots harvested by centrifugation for 5 min at 3.4 K. The cells were then washed with cold ddH₂O, transferred to a microfuge tube, and frozen at -80°C until all aliquots were taken. The cells were resuspended in 200 μ l of extraction buffer (0.1 M Tris [pH 8.0], 1 mM DTT, and 20% glycerol) with freshly added protease inhibitors (Boehringer Mannheim, Germany), transferred to a tube containing 200 μ l of acid-washed glass beads, and vortexed six times for 20 s with 1 min of cooling, on ice, between vortexing. Extraction buffer (50 μ l) plus protease inhibitors was then added, and the extracts were vortexed briefly to mix. Extracts were then clarified twice for 10 min at 4°C, and the protein concentration was determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories Hercules, CA). Proteins were visualized by the ECL detection system from Amersham Life Science.

Whole-Genome Transcription Analysis Using High-Density Oligonucleotide Arrays

Wild-type and mutant yeast strains were grown to an OD 600 of 0.5 in YPD at 30°C. The cultures were diluted with an equal volume of YPD at 44°C and allowed to grow for 45 min at 37°C. Total RNA was isolated, and the genomic expression profile of each strain was determined using four Affymetrix GeneChip arrays. Poly(A)⁺ mRNA was isolated, converted to biotin-cRNA, hybridized, washed, stained, and scanned as described previously (Wodicka et al., 1997). Five poly(A)-tagged control RNAs were added to equal amounts of total RNA from each preparation. The levels of these controls were then

used to normalize each wild-type and mutant expression profile to total RNA. Two independent experiments were performed for each wild-type versus mutant comparison. Individual mRNA levels were scored if the computer algorithm analyzing the scanned results (Wodicka et al., 1997) returned a "present" call in both the two wild-type and both the two mutant expression profiles for that gene, or if the expression levels of that gene changed in the same direction and greater than background levels in both wild-type and mutant comparisons. A decrease was called if an mRNA dropped more than 2-fold in both comparisons. An identical experiment performed with *rpb1-1* that harbors a temperature-sensitive mutation within the largest subunit of RNA polymerase II resulted in an operational half-life for the decay rate of all detectable mRNAs. For those mRNAs whose levels were called in the *rpb1-1* experiment and where the average decrease was greater than 2-fold, a comparison was made between the operational half-life and the decrease observed in the *ytaf_{II}17^{ts-2}* experiment. In the experiments described here, this comparison was successfully performed for 89% of the mRNAs whose levels had been called. The decrease in the *ytaf_{II}17^{ts-2}* experiment was determined to fit the *rpb1-1* decay rate if the decrease observed was within one operational half-life of the decrease observed with *rpb1-1*. A less stringent fit of one and a half half-lives was used for signals that approached the lower limit of detection to accommodate the greater variation observed in such instances. Complete experimental details will be described elsewhere (Holstege et al., 1998).

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References

- Apone, L.M., Virbasius, C.A., Reese, J.C., and Green, M.R. (1996). Yeast TAF_{II}90 is required for cell-cycle progression through G₂/M but not for general transcription activation. *Genes Dev.* 10, 2368-2380.
- Burley, S.K., and Roeder, R.G. (1996). Biochemistry and structural biology of transcription factor IID (TFIID). *Annu. Rev. Biochem.* 65, 769-799.
- Burke, T.W., and Kadonaga, J.T. (1997). The downstream core promoter element DPE is conserved from *Drosophila* to humans and is recognized by TAF_{II}60 of *Drosophila*. *Genes Dev.* 11, 3020-3031.
- Chen, J.-L., Attardi, L.D., Verrijzer, C.P., Yokomori, K., and Tjian, R. (1994). Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* 79, 93-105.
- Chia, L.L., and McLaughlin, C. (1979). The half-life of mRNA in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 170, 137-144.
- Cormack, B.P., and Struhl, K. (1992). The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. *Cell* 69, 685-696.
- Georgakopoulos, T., and Thireos, G. (1992). Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.* 11, 4145-4152.
- Goodrich, J., Hoey, T., Thut, C.J., Admon, A., and Tjian, R. (1993). *Drosophila* TAF_{II}40 interacts with both a VP16 activation domain and the basal transcription factor TFIIB. *Cell* 75, 519-530.
- Grant, P.A., Schieltz, D., Pray-Grant, M.G., Steger, D.J., Reese, J.C., Yates, J.R., III, and Workman, J.L. (1998a). A subset of TAF_{II}s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell* 94, 45-53.

- Grant, P.A., Sterner, D.E., Duggan, L.J., Workman, J.L., and Berger, S.L. (1998b). The SAGA unfolds: convergence of transcription regulators in chromatin-modifying complexes. *Trends Cell Biol.* **8**, 193–197.
- Hampsey, M. (1998). Molecular genetics of the RNA polymerase II general transcriptional machinery. *Microbiol. Mol. Biol. Rev.* **62**, 465–503.
- Hengartner, C.J., Thompson, C.M., Zhang, J., Chao, D.M., Liao, S.M., Koleske, A.J., Okamura, S., and Young, R.A. (1995). Association of an activator with an RNA polymerase II holoenzyme. *Genes Dev.* **9**, 897–910.
- Herrick, D., Parker, R., and Jacobson, A. (1990). Identification and comparison of stable and unstable mRNAs in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10**, 2269–2284.
- Holstege, F.C.P., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.S., Lander, E.R., and Young, R.A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**, in press.
- Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**, 163–168.
- Jones, J.S., and Prakash L. (1990). Yeast *Saccharomyces cerevisiae* selectable markers in pUC18 polylinkers. *Yeast* **6**, 363–366.
- Kaiser, C., Michaelis, S., and Mitchell, A. (1994). *Methods in Yeast Genetics* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).
- Leung, D.W., Chen, E., and Goeddel, D.V. (1989). A method for random mutagenesis of a defined DNA segment using a modified polymerase chain reaction. *Technique* **1**, 11–15.
- Lu, H., and Levine, A. (1995). Human TAF_{II}31 protein is a transcriptional coactivator of the p53 protein. *Proc. Natl. Acad. Sci. USA* **92**, 5154–5158.
- Moqtaderi, Z., Bai, Y., Poon, D., Weil, P.A., and Struhl, K. (1996a). TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature* **383**, 188–191.
- Moqtaderi, Z., Yale, J.D., Struhl, K., and Buratowski, S. (1996b). Yeast homologues of higher eukaryotic TFIID subunits. *Proc. Natl. Acad. Sci. USA* **93**, 14654–14658.
- Myers, L.C., Gustafsson, C.M., Bushnell, D.A., Lui, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R.D. (1998). The Med proteins of yeast and their function through the RNA polymerase II carboxy-terminal domain. *Genes Dev.* **12**, 45–54.
- Nonet, M., Scafe, C., Sexton, J., and Young, R. (1987). Eukaryotic RNA polymerase conditional mutants that rapidly cease mRNA synthesis. *Mol. Cell. Biol.* **7**, 1602–1611.
- Ogryzko, V.V., Kotani, T., Zhang, X., Schiltz, R.L., Howard, T., Yang, X.-J., Howard, B.H., Qin, J., and Nakatani, Y. (1998). Histone-like TAFs within the PCAF histone acetylase complex. *Cell* **94**, 35–44.
- Oelgeschläger, T., Chiang, C.-M., and Roeder, R.G. (1996). Topology and reorganization of a human TFIID-promoter complex. *Nature* **382**, 735–738.
- Oelgeschläger, T., Tao, Y., Kang, Y.K., and Roeder, R.G. (1998). Transcription activation via enhanced preinitiation complex assembly in a human cell-free system lacking TAF_{II}s. *Mol. Cell* **1**, 925–931.
- Ptashne, M. (1988). How eukaryotic transcription activators work. *Nature* **335**, 683–689.
- Reese, J.C., Apone, L., Walker, S.S., Griffin, L.A., and Green, M.R. (1994). Yeast TAF_{II}s in a multisubunit complex required for activated transcription. *Nature* **371**, 523–527.
- Shen, W.-C., and Green, M.R. (1997). Yeast TAF_{II}145 functions as a core promoter selectivity factor, not a general coactivator. *Cell* **90**, 615–624.
- Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**, 19–27.
- Struhl, K. (1995). Yeast transcriptional regulatory mechanisms. *Annu. Rev. Genetics* **29**, 651–674.
- Thut, C., Chen, J., Klemm, R., and Tjian, R. (1995). p53 transcriptional activation mediated by coactivators TAF_{II}40 and TAF_{II}60. *Science* **267**, 100–104.
- Tjian, R., and Maniatis, T. (1994). Transcriptional activation: a complex puzzle with few easy pieces. *Cell* **77**, 5–8.
- Uesugi, M., Nyanguile, O., Lu, H., Levine, A.J., and Verdine, G.L. (1997). Induced alpha helix in the VP16 activation domain upon binding to a human TAF. *Science* **277**, 1310–1313.
- Walker, S.S., Reese, J.C., Apone, L.M., and Green, M.R. (1996). Transcription activation in cells lacking TAF_{II}s. *Nature* **383**, 185–188.
- Walker, S.S., Shen, W.-C., Reese, J.C., Apone, L.M., and Green, M.R. (1997). Yeast TAF_{II}145 required for transcription of G1/S cyclin genes and regulated by the cellular growth state. *Cell* **90**, 607–614.
- Wodicka, L., Dong, H., Mittmann, M., Ho, M.H., and Lockhart, D.J. (1997). Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nat. Biotechnol.* **15**, 1359–1367.
- Yocum, R.R., Hanley, S., West, R., and Ptashne, M. (1984). Use of *lacZ* fusions to delimit regulatory elements of the inducible divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **4**, 1985–1988.
- Zawel, L., and Reinberg, D. (1995). Common themes in assembly and function of eukaryotic transcription complexes. *Annu. Rev. Biochem.* **64**, 533–561.