Yeast TAF_{II}90 is required for cell-cycle progression through G₂/M but not for general transcription activation

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The RNA polymerase II general transcription factor TFIID is a multisubunit complex comprising TATA-box binding protein and associated factors (TAF_{II}s). In vitro experiments have suggested that TAF_{II}s are essential coactivators required for RNA polymerase II-directed transcription activation. Here, for the first time, we analyze systematically the in vivo function of a specific TAF_{II}, yeast TAF_{II}90 (yTAF_{II}90). We show that functional inactivation of yTAF_{II}90 by temperature-sensitive mutations or depletion leads to arrest at the G₂/M phase of the cell cycle. Unexpectedly, in the absence of functional yTAF_{II}90, a variety of endogenous yeast genes were all transcribed normally, including those driven by well-characterized activators. Taken together, our results indicate that yTAF_{II}90 is not required for transcription activation in general, and reveal linkages between TAF_{II} function and cell-cycle progression.

[Key Words: Transcription; TAFs; yeast; cell cycle]

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Factors involved in the accurate transcription of eukaryotic structural genes by RNA polymerase II can be classified into at least three groups. First, general (or basic) transcription factors (GTFs) are necessary and can be sufficient for accurate transcription initiation (for review, see Zawel and Reinberg 1992; Tjian and Maniatis 1994). These GTFs include RNA polymerase II itself and a variety of auxiliary components such as TFIIA, TFIIB, TFIID, TFIIE, TFIIIF, and TFIIH. The GTFs are capable of supporting a low level of transcription in vitro and function by assembling in a stepwise fashion on the promoter to form a preinitiation complex (PIC) (Zawel and Reinberg 1992). Transcriptional activity is stimulated greatly by the second class of factors, promoter-specific activator proteins (activators). In general, activators are sequence-specific DNA binding proteins whose sites are present within the vicinity of their target promoters (Ptashne 1988; Mitchell and Tjian 1989; Ptashne and Gann 1990). Transcriptional activators are thought to function by interacting, directly or indirectly, with one or more GTFs to increase PIC assembly.

A third group of factors are transcriptional coactivators. Coactivators have been defined operationally as components that are required in vitro for activator-directed (activated) transcription but are dispensable for an activator-independent (basal) transcription reaction. To date, the best-characterized coactivators are components of the TFIID complex.

TFIID is a multisubunit complex comprising the

TATA-box binding protein (TBP) and at least eight associated factors known as TAF_{II}s (for review, see Burley and Roeder 1996). Binding of TFIID to the TATA box constitutes the first step in the assembly of a PIC. While TBP is capable of substituting for TFIID in a basal transcription reaction in vitro, in general it cannot support activated transcription (Burley and Roeder 1996). This observation has led to the proposal that one or more TAF_{II}s are essential coactivators that bridge the interactions between activators and GTFs. In support of this idea, individual TAF_{II}s have been reported to interact with distinct activators (Goodrich et al. 1993; Hoey et al. 1993; Jacq et al. 1994; Thut et al. 1995), and in vitro TFIID reconstitution experiments have demonstrated a requirement for particular TAF_{II}s to support the function of specific activators (Chen et al. 1994).

While higher eukaryotic TFIID and its associated TAF_{II}s have been studied extensively in vitro, very little is known about the function of individual TAF_{II}s in vivo. Our group (Reese et al. 1994) and others (Poon et al. 1995) recently have isolated and cloned TAF_{II}s from the yeast *Saccharomyces cerevisiae*. Most yeast TAF_{II}s (yTAF_{II}s) have recognizable higher eukaryotic homologs and are encoded by genes that are essential for viability.

Yeast TAF_{II}90 (yTAF_{II}90) is a component of the yTAF_{II} complex, required for viability in yeast (Reese et al. 1994; Poon et al. 1995), and has been highly conserved (Burley and Roeder 1996). The most highly conserved portion of yTAF_{II}90 is within its carboxy-terminal region, which contains WD40 (also called β -transducin) repeats that are believed to mediate protein-protein in-

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teractions (van der Voorn and Ploegh 1992). To date, in vitro transcription experiments have failed to provide insights into the function of this highly conserved TAF_{II}, with regard to either its activities, targets, or mechanism of action. In this study we have taken advantage of the genetics available in yeast to analyze the role of yTAF_{II}90 in vivo.

Results

Isolation of taf90 temperature-sensitive alleles

yTAF_{II}90 is encoded by an essential gene (Reese et al. 1994; Poon et al. 1995). To determine the cellular defects associated with the loss of its function, we constructed and isolated two temperature-sensitive taf90 alleles. Then the plasmid shuffle technique (Boeke et al. 1984) was used to create strains of yeast bearing only a mutant taf90 allele. Figure 1A shows that both ytaf_{II}90 temperature-sensitive strains grew at 25°C but not 37°C. Figure 1B shows that in liquid culture these strains displayed a rapid growth arrest upon transfer from the permissive to the nonpermissive temperature. Even at the permissive temperature the ytaf_{II}90 mutant strains grew more slowly than wild-type (data not shown).

Sequencing of the mutants identified double-point substitutions in each of the two alleles. ytaf_{II}90^{ts2-1} (strain LY20) contains an asparagine substituted for a serine at amino acid 703 and an arginine substituted for a glycine at position 793; ytaf_{II}90^{ts3-1} (strain LY21) contains a glutamic acid and a serine substituted for glycines at positions 713 and 714, respectively (Fig. 1C). Significantly, all of the mutations are within the highly conserved WD40 domains.

Strains bearing ytaf_{II}90 temperature-sensitive mutants arrest as large budded cells at the nonpermissive temperature

To gain insight into the nature of the temperature-sensitive growth defect, the cells were analyzed for morphological differences at the nonpermissive temperature. Within 4 hr after shifting to 37°C there was a striking difference in the appearance of the wild-type and mutant strains. Cells from both mutant strains were twice as large as wild-type, and consisted of a majority of large budded cells (Fig. 2A). Approximately 62% of ts2-1 and 84% of ts3-1 cells contained large buds, whereas only 20% of the wild-type strain possessed large buds (Fig. 2B).

Cells arresting with large buds could be blocked in S, G₂, or M phase (Pringle and Hartwell 1981). To distinguish between these possibilities, we performed Hoechst staining and Fluorescence-activated cell sorting (FACS) analysis. Hoechst staining performed on cells incubated for 4 hr at 37°C identified a single nucleus in the mutant cells localized near the neck of the bud (Fig. 2A). The FACS analysis of Figure 2C shows that following incubation for 4 hr at 37°C the majority of wild-type cells contained a 1N DNA content, while the majority of ts2-1 cells and virtually all of the ts3-1 cells contained a 2N DNA content. On the basis of these combined data we conclude that the mutant cells are capable of replicating their DNA and of nuclear migration to the neck of the bud, consistent with a G₂/M block.

Even at the permissive temperature it was evident that a much greater than normal percentage of mutant cells had a 2N DNA content. The inability of these cells to progress efficiently through G₂ helps explain their slow

Figure 1. Temperature-sensitive alleles of taf90. (A) Growth of strains containing wild-type (LY3) and temperature-sensitive alleles of TAF90 (ts2-1 and ts3-1) at 25°C and 37°C. (B) Growth curve of wild-type (□) and temperature-sensitive strains (◇, ts2-1; ○, ts3-1) at 37°C. (C) Sequence of ytaf_{II}90 temperature-sensitive alleles. The segment of the yTAF_{II}90 WD40 domain containing the temperature-sensitive mutations is compared with the corresponding sequence in the *Drosophila* homolog dTAF_{II}80. Mutated residues are marked with a dot. The amino acid changes and the strains containing the mutations are indicated above.

Figure 2. Cell-cycle phenotype of strains bearing temperature-sensitive alleles of *taf90*. (A) Nomarski optics or Hoechst staining was used to visualize the morphology and DNA, respectively, of wild-type and strains harboring temperature-sensitive *yTAF_{II}90* mutants 4 hr after transfer from 25°C to 37°C. (B) Percentage of unbudded, small, medium and large budded cells after 4 hr at 37°C. (Light bars) Wild-type; (medium bars) *ts2-1*; (dark bars) *ts3-1*. (C) FACS analysis of strains containing wild-type and temperature-sensitive alleles of *TAF90* immediately before and 4 hr after transfer from 25°C to 37°C.

growth phenotype and in addition indicates that *yTAF_{II}90* function is impaired even under permissive conditions. Lack of viability at higher temperatures could be explained either by a complete loss of *yTAF_{II}90* function or an inability of unhealthy cells to survive additional stress.

General inhibition of RNA polymerase II-directed transcription does not lead to a cell-cycle phenotype

In *S. cerevisiae* there are several phases of the cell cycle at which transcription is required for proper progression (Koch and Nasmyth 1994). Therefore, it seemed unlikely that a general transcription defect would result in a specific cell-cycle phenotype. To verify this notion we inhibited RNA polymerase II-directed transcription by several strategies and analyzed the effect on cell-cycle progression. As shown in Figure 3A, when transcription was inhibited using RNA polymerase II (Nonet et al. 1987), TBP (Cormack and Struhl 1992), or SRB2 (Koleske et al. 1992) temperature-sensitive strains, or with the RNA polymerase II chemical inhibitor thiolutin (Parker et al. 1991), the cells did not uniformly arrest at a specific stage of the cell cycle. In several instances there was a modest G₁ shift, probably reflecting the elaborate transcriptional program required to traverse G₁ (Rowley et

al. 1993). Thus, a specific cell-cycle phenotype cannot be explained by a general defect in RNA polymerase II-directed transcription.

Strains bearing activation-defective TBP mutants do not have a cell-cycle phenotype

The experiment of Figure 3A involved mutant GTFs, which are completely defective in supporting RNA polymerase II-directed transcription. To ask whether interfering specifically with transcription activation affected cell-cycle progression, we analyzed several previously described TBP mutants that are unable to support activated transcription of some or all genes (Arndt et al. 1995; Lee and Struhl 1995). The FACS analysis of Figure 3B shows that cells harboring three such TBP mutants did not arrest at a specific stage of the cell cycle. Thus, the cell-cycle phenotype of *ytaf_{II}90* mutants is also not readily explained by an activation-specific defect in RNA polymerase II-directed transcription.

Unabated transcription following yTAF_{II}90 inactivation

Numerous experiments have shown that in vitro TAF_{II}s are required for activated, but not basal, transcription

Figure 3. Inhibition of RNA polymerase II-directed transcription does not lead to a cell-cycle phenotype. (A) FACS analysis was performed on strains before and 4 hr after transfer from 25°C to 37°C, or 4 hr after addition of thiolutin to wild-type strain CY245. (B) FACS analysis was performed on strains harboring activation-defective TBP mutants grown to midlog phase at 30°C.

(Burley and Roeder 1996). We therefore sought to determine whether cells bearing the *ytaf_{II}90* temperature-sensitive mutants were responsive to activators. We first chose to analyze the well-characterized acidic activator GAL4 according to the experimental strategy outlined in Figure 4A. As expected, Figure 4B shows that transcription of the *GAL10* gene was induced in all strains under permissive conditions (25°C). Under nonpermissive conditions (37°C), the wild-type (LY3), but not the RNA polymerase II mutant strain (*rpb1-1*), supported *GAL10* transcription. Significantly, in both *ytaf_{II}90* temperature-sensitive strains (*ts2-1* and *ts3-1*) *GAL10* was transcriptionally activated at 37°C. We note that in strain *ts3-1* there was a ~50% decrease of *GAL10* transcription 4 hr following the temperature shift, a time at which the cells had been arrested for at least 3 hr. This modest effect most likely reflects a general deterioration of all activities in dying cells. In particular, RNA polymerase III-directed transcription also undergoes a comparable decrease at this time point (data not shown).

We next asked whether yTAF_{II}90 was required for the function of another, unrelated activator. The acidic activator ACE1 induces transcription of the *CUP1* gene in the presence of copper (Butt et al. 1984; Furst et al. 1988). The experimental design was analogous to the *GAL10* experiment except that the cells were grown in glucose

and the inducer was 100 μM copper sulfate (see Fig. 4A). The results of Figure 4C show that all strains supported copper-inducible transcriptional activation of the *CUP1* gene at 25°C. At 37°C, only the *rpb1-1* strain failed to support normal *CUP1* transcription.

Figure 4D analyzes four other endogenous genes whose transcription is controlled by a diverse set of activators: *PMA1* is regulated by the activators MCM1 and RAP1 (Capieaux et al. 1989; Kuo and Grayhack 1994); *TRP3* is regulated by the activator GCN4 (Aebi et al. 1984; Hope and Struhl 1985; Arndt and Fink 1986); *DED1* is under the control of the activator ABF1 (Buchman and Kornberg 1990); and *ENO2* is glucose-regulatable (Johnston and Carlson 1992). Once again, transcription of these genes was unaffected following inactivation of yTAF_{II}90. We conclude that in vivo yTAF_{II}90 is dispensable for normal transcription of many yeast genes.

Cell-cycle and transcriptional properties of strains conditionally depleted of yTAF_{II}90

Immunoblot analysis of strains bearing the *ytaf_{II}90* temperature-sensitive mutants revealed that at the nonpermissive temperature they contained significant levels of ytaf_{II}90, as well as several other yTAF_{II}s (data not shown). Therefore, a possible explanation for the lack of a transcriptional defect in the above experiments was that yTAF_{II}90 contained multiple functional domains and the mutations were within a domain not required for transcription of those genes tested. To rule out this possibility, we constructed a strain in which yTAF_{II}90 could be depleted conditionally. In this strain (LYC-1) *TAF90* was under the control of the galactose-inducible *GAL1* promoter. The immunoblot of Figure 5A shows that upon transfer from galactose- to glucose-containing medium, yTAF_{II}90 was rapidly depleted, and by 12 hr was undetectable. The quantitative immunoblot analysis of Figure 5B shows that by 12 hr yTAF_{II}90 was below our level of detection, which was 2% of the wild-type level. As expected, LYC-1 cells grew in the presence of galactose but not glucose (Fig. 5C), and upon transfer to glucose-containing medium, the LYC-1 cells displayed a rapid growth arrest (Fig. 5D).

We next analyzed the effect of yTAF_{II}90 depletion on cell-cycle progression and transcription. Analogous to the results with the temperature-sensitive mutants, cells depleted of yTAF_{II}90 arrested with large buds (Fig. 6A). After incubation in glucose for 12 hr, 80% of strain LYC-1 consisted of budded cells, 74% of which contained large buds. By comparison, the wild-type strain possessed only 47% budded cells, only 18% of which contained large buds (Fig. 6B).

Transcription was determined by S1 nuclease analysis of RNA isolated following transfer from galactose- to glucose-containing medium. The half-life of each of these mRNAs is <30 min (Cormack and Struhl 1992; Thompson and Young 1995; see also Fig. 4D). As mentioned above, transcription of *PMA1* is regulated by the activators MCM1 and RAP1 (Capieaux et al. 1989; Kuo

Figure 4. Transcription in strains bearing *ytaf₁₉₀* temperature-sensitive mutants. (A) Experimental design. (B) Transcriptional activation of *GAL10* was measured by primer-extension analysis in cells 60 min after induction by 3% galactose. (C) Transcriptional activation of *CUP1* was measured by S1 nuclease analysis in cells 30 min after induction by 100 μ M copper sulfate. (D) Transcription of the indicated endogenous genes was measured by S1 nuclease analysis before and at the indicated times after transfer from 25°C to 37°C.

and Grayhack 1994); transcription of *ENO2* is glucose-regulatable and involves the activator ABF1 (Johnston and Carlson 1992); whereas transcriptional regulation of *RAD23* is not well understood. Figure 6B shows that following *yTAF₁₉₀* depletion, each gene tested was transcribed at wild-type levels (lanes 6–10) even at 12 hr, a time at which there was no detectable *yTAF₁₉₀* (Fig. 5A,B). In some cases, a small transcriptional decrease was observed 14 hr after transfer, a time at which the cells had been arrested for ~6 hr. At these late times, RNA polymerase I- and III-directed transcription was also impaired modestly. Thus, this small decrease in transcription reflects a general deterioration of function in dying cells, and not a specific defect in RNA polymerase II-directed transcription.

Transcription of genes required for *G₂/M* progression following *yTAF₁₉₀* inactivation

The results described above raised the possibility that *yTAF₁₉₀* might be required not for transcription in general but rather for a subset of genes involved in progression through the *G₂/M* phase of the cell cycle. As a first test of this possibility, we analyzed the transcription of two key genes required for progression through *G₂/M*.

CLB2 encodes a cyclin involved in *G₂/M* progression and is transcribed only during *G₂/M* (Surana et al. 1991). *CSE4* encodes a protein required for proper chromosome segregation, and, significantly, the phenotype of cells bearing *cse4* mutants is identical to that of the *yTAF₁₉₀* mutant strains described here (Stoler et al. 1995). The Northern blot of Figure 7 shows that transcription of *CLB2* and *CSE4* and as a control, *CLN3*, which encodes a constitutively expressed *G₁* cyclin (Nash et al. 1988), was unaffected following temperature-sensitive inactivation of *yTAF₁₉₀*. Thus, failure to transcribe the *CLB2* and *CSE4* genes cannot explain the cell-cycle arrest phenotype of strains harboring *ytaf₁₉₀* mutants.

yTAF₁₉₀ is a component of the PIC and can activate transcription as a LexA fusion protein

A possible explanation for the lack of a transcriptional effect in the above experiments was that *yTAF₁₉₀* was not a component of an RNA polymerase II PIC. To address this possibility we performed both biochemical and in vivo transcription experiments. First, we asked whether *yTAF₁₉₀* was associated with TBP and other TAF_{II}s. The immunoprecipitation experiment of Figure

Figure 5. Characterization of strains conditionally expressing $yTAF_{II}90$. (A) Immunoblot analysis of whole-cell extracts prepared from wild-type (LY3) and LYC-1 (GAL1-HA- $yTAF_{II}90$) at the indicated times following transfer from galactose- to glucose-containing medium. (B) Quantitative immunoblot analysis. Extracts prepared from the wild-type strain (right) were diluted as indicated and $yTAF_{II}90$ detected by immunoblotting. Extracts prepared from LYC-1 (left) were analyzed undiluted (8 and 12 hr) or diluted 1:20 (0 hr) following transfer from galactose- to glucose-containing medium. HA-tagged $yTAF_{II}90$, expressed from the GAL1 promoter, was detected by immunoblotting. The proteins are indicated by arrows. (C) Growth of wild-type and LYC-1 on plates containing galactose or glucose at 30°C. (D) Growth curve of wild-type and LYC-1 before and after transfer from galactose- to glucose-containing medium.

8A shows that an α - $yTAF_{II}90$ antiserum immunoprecipitated TBP and $yTAF_{II}145$, the $yTAF_{II}$ that directly contacts TBP (Reese et al. 1994). Likewise, an α - $yTAF_{II}145$ antiserum immunoprecipitated both TBP and $yTAF_{II}90$. Thus, in a yeast whole-cell extract $yTAF_{II}90$ is associated with TBP and other $yTAF_{II}$ s, as expected for a component of the TFIID complex.

Second, and more important, we obtained direct evidence that $yTAF_{II}90$ was a component of an RNA polymerase II PIC formed on a class II promoter. Previously, we have described methods for quantitating transcription factors that are stable components of PICs using immobilized DNA templates (Choy and Green 1993). Figure 8B analyzes by immunoblotting the presence of several GTFs, RNA polymerase II, and $yTAF_{II}90$ in PICs formed under standard transcription conditions in a yeast whole-cell extract. As expected, and consistent with previous results (Choy and Green 1993; Kim et al. 1994), TBP, TFIIB, and RNA polymerase II were associated stably with a DNA fragment containing a class-II promoter (G5E4T) but not an irrelevant DNA (pGEM3).

Significantly, $yTAF_{II}90$ was also stably and specifically associated with the promoter, indicating that $yTAF_{II}90$ is a component of the PIC.

Recent experiments have shown that PIC components, such as TBP and GAL11, can activate transcription when tethered to the promoter via a heterologous DNA-binding domain (Barberis et al. 1995; Chatterjee and Struhl 1995; Klages and Strubin 1995; Xiao et al. 1995). We used a similar strategy to determine whether in vivo $yTAF_{II}90$ entered into a transcriptionally competent PIC. Fusion proteins containing the DNA-binding domain of LexA attached to $yTAF_{II}90$ were assayed for their ability to activate transcription from an integrated reporter bearing LexA binding sites upstream of the *GAL1* TATA box and the *lacZ* gene. Figure 8C shows that a LexA- $yTAF_{II}90$ fusion protein activated transcription, whereas the LexA DNA-binding domain alone (vector) did not.

It was important to rule out the possibility that $yTAF_{II}90$ contained a cryptic activation domain, which in yeast are short, acidic sequences (Ma and Ptashne

Figure 6. Cell-cycle and transcriptional properties of strains conditionally expressing yTAF_{II}90. (A) Wild-type and LYC-1 cells were visualized by light microscopy 12 hr after transfer from galactose- to glucose-containing medium. (B) Percentage of unbudded, small, medium, and large budded cells was determined for wild-type (light bars) and LYC-1 (dark bars) immediately before and 12 hr after transfer from galactose- to glucose-containing medium. (C) Transcription was measured by S1 nuclease analysis of RNA isolated from wild-type and LYC-1 cells after transfer from galactose- to glucose-containing medium.

1987; Ptashne 1988). We therefore constructed LexA fusion proteins containing an amino-terminal (1–491) or carboxy-terminal (492–798) fragment of yTAF_{II}90. Significantly, neither of these yTAF_{II}90 fragments had yTAF_{II}90 function (data not shown). Figure 8C shows that whereas the LexA fusion of full-length yTAF_{II}90 activated transcription, the amino- and carboxy-terminal derivatives did not. These results suggest strongly that activated transcription by LexA–yTAF_{II}90 was not caused by a cryptic activation domain in yTAF_{II}90.

The results of Figure 8D provide additional support for this conclusion. LexA–yTAF_{II}90^{ts2-1} and LexA–yTAF_{II}90^{ts3-1} fusion proteins were constructed and tested for their ability to activate transcription from a promoter bearing LexA binding sites upstream of the *GAL1* TATA box and *LEU2* gene. The ability to support growth on selective media provided a sensitive assay for transcriptional activation. The results of this experiment demonstrate that when fused to the DNA-binding domain of LexA, the temperature-sensitive alleles of *taf90* supported growth on leucine-deficient media at 25°C but not 37°C. Therefore, these *taf90* mutants enter into a transcriptionally competent PIC at the permissive but not at the nonpermissive temperature. The

immunoblot analysis of Figure 8E indicates that all of the LexA–yTAF_{II}90 fusion proteins described above were expressed at comparable levels. Taken together, these results indicate that in vivo yTAF_{II}90 is a component of a PIC and further suggest that the molecular defect of the *taf90* mutants is failure to engage in a protein–protein interaction(s) at the nonpermissive temperature.

Discussion

Although extensive in vitro data have demonstrated a requirement for TAF_{II}s in transcription activation, their functions in vivo have not been evaluated critically. Here we report that cells in which a highly conserved and essential TAF_{II}, yTAF_{II}90, was inactivated by either temperature-sensitive mutations or conditional depletion had no general defect in RNA polymerase II-directed transcription. We estimate from immunoblotting that yeast cells normally contain 3000–6000 copies of yTAF_{II}90, which was decreased >98% by glucose depletion (Fig. 5B and data not shown). The maximal 120 copies of yTAF_{II}90 remaining is far below the ~4000 actively transcribed genes in a yeast cell (see Lewin 1990). Whereas our experiments do not rule out that yTAF_{II}90

Figure 7. Transcription of genes required for progression through G₂/M following yTAF_{II}90 inactivation. Transcription of the indicated genes was measured by Northern blot analysis.

is required for transcription of a particular subset of genes, they do indicate a lack of a general requirement for transcription activation. Consistent with our in vivo results are in vitro experiments demonstrating that partial TFIID complexes lacking TAF_{II}90 can support transcription directed by several types of activators (Chen et al. 1994; Sauer et al. 1995a,b).

Our results are also not inconsistent with the limited in vivo analysis of other TAF_{II}s in higher eukaryotes. For example, mammalian cell lines harboring a temperature-sensitive TAF_{II}250 allele do not have a global defect in RNA polymerase II-directed transcription under nonpermissive conditions (see, e.g., Liu et al. 1985). Furthermore, inactivation of TAF_{II}250 did not prevent transcriptional activation of the *c-fos* gene, which contains a highly inducible and well-characterized promoter (Wang and Tjian 1994).

yTAF_{II}90 function and cell-cycle progression

The results described in this study point to a role for yTAF_{II}90 at a specific stage of the cell cycle. Significantly, an identical cell-cycle phenotype was observed when yTAF_{II}90 was inactivated by two independent strategies: temperature-sensitive inactivation or conditional depletion. We emphasize that the cell-cycle phenotype resulting from yTAF_{II}90 inactivation is not typical of a transcription defect. Blockage of transcription by exposure to a chemical inhibitor or through the use of temperature-sensitive alleles of RNA polymerase II, TBP, or SRB2 did not lead to a uniform cell-cycle arrest. Most significant, strains harboring activation-defective TBP mutants also failed to display a cell-cycle phenotype. Interestingly, mammalian cell lines expressing a temperature-sensitive allele of TAF_{II}250 also displayed a cell-cycle phenotype at the nonpermissive temperature, although in this instance the arrest was in G₁ (Sekiguchi et al. 1988, 1991; Hisatake et al. 1993; Ruppert et al. 1993). Taken together, these results raise the intriguing possibility that in both yeast and mammalian cells TAF_{II}s may have distinct and essential functions in cell-cycle progression, and that different TAF_{II}s may act during different stages of the cell cycle.

It will be important to determine how TAF_{II}s facilitate cell-cycle progression. In yeast, cell-cycle progression requires the temporally regulated transcription of particular genes (reviewed in Koch and Nasmyth 1994). Perhaps yTAF_{II}s are required not for transcription in general, but rather for transcription of specific genes involved in cell-cycle progression. Although we have analyzed the ability of yTAF_{II}90 mutant cells to transcribe two important genes required for G₂/M progression, our analysis is far from exhaustive. Thus, it still remains possible that yTAF_{II}90 is required for transcription of one or more genes required for progression through G₂/M, and experiments to test this idea are in progress.

However, it is also possible that yTAF_{II}90 does not act directly in transcription activation. For example, a *Drosophila* TAF_{II}, dTAF_{II}250, recently has been reported to have a protein kinase activity (Dikstein et al. 1996). This or other biochemical activities could serve to coordinate transcription with cell division or other cellular processes. Thus, yTAF_{II}90 and other TAF_{II}s may function in the cell cycle by a mechanism that does not operate directly through a transcription pathway.

An additional suggestion that yTAF_{II}90 may be performing a function other than as a coactivator is that the phenotype of yeast strains unable to support activated transcription differ substantially from that of the *taf90* mutant strains described here. Activation-defective TBP mutant strains are viable, do not support activated transcription of several genes tested (Kim et al. 1994; Arndt et al. 1995; Lee and Struhl 1995; Stargell and Struhl 1995), and do not have a cell-cycle phenotype (Fig. 3B); conversely, the *taf90* mutant strains are inviable, support activated transcription of all genes tested, and specifically arrest in G₂/M. Collectively, these observations raise the possibility that yTAF_{II}90 may function other than as a general coactivator.

Transcription activation by tethered PIC components

yTAF_{II}90 activates transcription when tethered to the promoter through a LexA DNA-binding domain. The portions of yTAF_{II}90 required to support activated transcription in this assay are identical to those required for yTAF_{II}90 function. In particular, substitutions in the WD40 repeats, presumptive protein-protein interaction sites, affected yTAF_{II}90 activity and LexA-yTAF_{II}90 activity comparably. We interpret these results to mean that to activate transcription as a LexA fusion protein, yTAF_{II}90 engages in the same interactions with other PIC components as it does when it functions as yTAF_{II}90.

Previous studies have shown that both TBP and GAL11, a component of the holoenzyme, can activate transcription in analogous tethering experiments (Barberis et al. 1995; Chatterjee and Struhl 1995; Klages and Strubin 1995; Xiao et al. 1995). Thus, TBP, a GTF; yTAF_{II}90, a component of the TAF_{II} complex; GAL11, a component of the holoenzyme; and TFIIB (data not shown) can all activate transcription when fused to a DNA binding domain. Thus, appropriate tethering of di-

Figure 8. $yTAF_{II}90$ is a component of the PIC and can activate transcription as a LexA fusion protein. (A) Immunoprecipitation analysis. Immunoprecipitates formed with α - $yTAF_{II}90$ or α - $yTAF_{II}145$ antisera were analyzed by immunoblotting for the presence of TBP, $yTAF_{II}90$, and $yTAF_{II}145$. (B) Immobilized DNA template assay. Promoter (G5E4T) or control (pGEM3) DNA was coupled to Dynal streptavidin beads, and following incubation in a yeast whole-cell extract under standard transcription conditions. Factors associated stably were detected by immunoblot analysis. (C) Transcription activation by LexA- $yTAF_{II}90$ fusion proteins. Wild-type strains containing an integrated *lacZ* reporter with two LexA binding sites upstream of the GAL1 TATA box and the DNA binding domain of LexA alone fused to full-length $yTAF_{II}90$ or amino-terminal (1–491) or carboxy-terminal (492–798) portions of $yTAF_{II}90$ were analyzed for β -galactosidase activity. (D) LexA- $yTAF_{II}90$ fusion proteins containing wild-type or temperature-sensitive alleles of $yTAF_{II}90$ attached to the DNA binding domain of LexA were transformed into strain EGY48. Strain EGY48 contains a single chromosomal *LEU2* gene whose expression is controlled by three LexA-binding sites. Transformants were streaked on selective medium lacking leucine and tested for growth at 25°C and 37°C. (E) Immunoblot analysis using an α -LexA antibody was performed on extracts prepared from strains harboring the various LexA- $yTAF_{II}90$ fusion proteins. The proteins are indicated by arrows.

verse PIC components can activate transcription, presumably by nucleating formation of a PIC on the promoter. Based solely on these types of experiments, it is difficult to draw strong conclusions about the target of an activator or the steps an activator affects.

Materials and methods

Yeast strains

Strains CY245, LY3, LY20, LY21, and LYC-1 are isogenic, derivatives of S288C, and listed in Table 1. Strains *rbp1-1* (Nonet et al. 1987), *srb2Δ1* (Koleske et al. 1992), EGY48 (Gyuris et al. 1993), and strains harboring the TBP mutants P109A (Arndt et al. 1995), N159L, and V161A (Lee and Struhl 1995) have been described previously. Mutant *taf90* strains were made by transformation of strain LY3 with various mutant plasmids (Ito et al. 1983), followed by screening for cells that had lost the wild-type plasmid on 5-fluoro-orotic acid (5-FOA) (Boeke et al. 1984). Strain LGY7 was constructed by digestion of the plasmid *Jp167* (J. Pearlberg, unpubl.) with *ApaI* followed by transformation into yeast strain JYY45 (J. Pearlberg, unpubl.). Cultures were grown in YEPD unless selection was necessary, in which

case all cultures were grown in the appropriate selective medium.

Plasmid constructions

The *TAF90* mutagenized plasmid Lp7 was constructed by cloning the *HindIII* fragment of plasmid Lp6 (Reese et al. 1994), which contains the entire coding region of *TAF90*, into the *HindIII* site of pGAD (Chien et al. 1991). Mutants generated in plasmid Lp7, *ytaf_{II}90^{ts2-1}* and *ytaf_{II}90^{ts3-1}*, were liberated from the plasmid by *HindIII* digestion and cloned into the *HindIII* site of plasmid Lp5, to generate plasmids Lp12 and Lp11, respectively. Lp5 is a derivative of the *LEU2* marked CEN plasmid RS415 (Sikorski and Hieter 1989), which contains the ADH1 promoter and terminator. Plasmid Lp16, in which *TAF90* is under the control of the GAL1 promoter, was constructed by ligating the *HindIII* fragment from plasmid Lp7 into the *HindIII* site of plasmid Lp15. Lp15 is also a derivative of RS415 with the addition of the GAL1–GAL10 UAS. LexA-*TAF90* fusions containing the wild-type and temperature-sensitive alleles were constructed by cloning the *XbaI*–*XhoI* fragment of plasmid Lp1, which contains the entire coding region of *TAF90* in-frame with LexA(1–87). LexA-*TAF90*, amino-terminal (Δ) and carboxy-

Table 1. Yeast strains

Strain	Genotype	Source
CY245	MATa, lys2-801, ade2-101, leu2- Δ 1, his3 Δ 200, ura3- Δ 99	C. Peterson
LY3	MATa, lys2-801, ade2-101, leu2- Δ 1, his3 Δ 200, ura3- Δ 99, <i>ytaf_{II}90::HIS3</i> , Lp6(2u URA3,ADH1-TAF90)	Reese et al. 1994
LY20	MATa, lys2-801, ade2-101, leu2- Δ 1, his3 Δ 200, ura3- Δ 99, <i>ytaf_{II}90::HIS3</i> , Lp12(CEN, ARS1, LEU2, ts2-1)	This study
LY21	MATa, lys2-801, ade2-101, leu2- Δ 1, his3 Δ 200, ura3- Δ 99, <i>ytaf_{II}90::HIS3</i> , Lp11(CEN, ARS1, LEU2, ts3-1)	This study
LYC-1	MATa, lys2-801, ade2-101, leu2- Δ 1, his3 Δ 200, ura3- Δ 99, <i>ytaf_{II}90::HIS3</i> , Lp16(CEN, ARS1, LEU2, GAL1-TAF90)	This study
LGY7	Mata, lys2D385, leu2- Δ 1, his3 Δ 200, ura3-52, trp1 Δ 63, URA3::JP167	L.G. Burns

terminal (Δ) deletion mutants were constructed by cloning the *Xba*I-*Bgl*II fragment and the *Bgl*II-*Xho*I fragment of the wild-type *TAF90* coding region respectively, in frame with LexA(1–87). Expression of all LexA-TAF90 constructs are under control of the *ADH1* promoter cloned into plasmid pRS423.

Isolation of temperature-sensitive TAF90 alleles

A plasmid containing the coding region of TAF90 under the control of the *ADH1* promoter (Lp7) was treated with 0.5 M hydroxylamine for 1 hr at 70°C. The mutagenized DNA was then transformed into the yeast strain LY3 and grown at room temperature on selective plates. Three thousand colonies from the transformation were then patched to selective plates, grown at room temperature, and replica plated to two plates containing 5-FOA to screen for cells that have lost the wild-type copy of *yTAF_{II}90*. One plate was grown at room temperature and the other at 37°C. Colonies growing at room temperature but not 37°C were restreaked and rescreened for growth at the two temperatures. Two colonies were identified that grew at room temperature but not at 37°C. The mutagenized plasmids from each of these strains were isolated and the *TAF90* genes subcloned. The plasmid shuffle technique was repeated to create strains containing only the mutagenized copy of *TAF90*. Liquid cultures of the mutant cells that had been incubated at 37°C for 4 hr displayed 100% recovery if transferred back to 25°C. Survival was reduced to 2% if the cultures were allowed to grow for 24 hr at 37°C before transfer to 25°C.

Temperature-shift experiments

Unless otherwise stated, cells were grown at room temperature to midlog phase (0.3–0.6) followed by transfer to 37°C either directly or after the addition of an equal volume of the appropriate media, which had been warmed to 37°C. Similar results were obtained by both methods. For galactose induction, cells were grown at room temperature in 3% raffinose to midlog phase. Each culture was then divided into four flasks, two of which were incubated at room temperature and the other two at 37°C. After 3 hr one of the two flasks at each temperature received galactose to a final concentration of 3% and the other remained as the uninduced control. The cells were incubated for an additional hr then harvested at 4°C, frozen on dry ice, and stored at –80°C. Copper induction was performed identically to the galactose induction with the following exceptions. The cells were grown in the presence of 3% glucose, the inducer was 100 μ M copper sulfate, and the cells were harvested 30 min after induction.

Conditional depletion of γ TAF_{II}90

Cultures were grown at 30°C in the presence of 3% galactose and 0.25% sucrose to midlog phase. The cells were then harvested, washed 2 $\times$ with sterile water, resuspended in selective media containing 3% glucose, and incubated for an additional 16 hr. Aliquots of cells were taken throughout this time period, frozen on dry ice, and stored at –80°C to be used for protein and RNA analysis.

RNA analysis

Total RNA was isolated as described previously (Peterson et al. 1991) and quantitated by absorbance at 260 nm and visualization on an agarose gel. S1 nuclease analysis, primer extension analysis, and Northern blotting were performed as described previously (McKnight and Kingsbury 1982; Cormack and Struhl 1992). In each case, 10–20 μ g of total RNA was used. Probes for Northern blot analysis were generated by using the primer pairs 5'-CGGGATCCGGATCAAGCACTGAGGTAG-3' and 5'-CCCTCTTCTCATTTCATGCAAG-3', 5'-GCGGATCCGGAGATCACTCAGTAACGTC-3' and 5'-GAAGATCTGCGGAGAGCACTTGTGCGAACC-3', 5'-GCGTCTAGATCAGCGAGTTTCTTGAGGTT-3' and 5'-CGGGATCCCAATGGCCATATTGAAGGATACC-3', and 5'-ATCGTCTAGACGATTTT-TTCTAAACCGTGGAATATTTCG-3' and 5'-CTCGGATCCGGACGGATTACAACAGGTATTGTCC-3' to generate PCR fragments of *CLB2*, *CSE4*, *CLN3*, and *ADH1* respectively. The rRNA, tRNA^f, *DED1*, *RAD23*, *TRP3*, and *GAL10* probes have been reported previously (Cormack and Struhl 1992; Peterson and Herskowitz 1992; Thompson and Young 1995). The sequences of the remaining probes are as follows: CUP1: 5'-GGCATTGGCACTCATGACCTTCATTTTGGAA-GTTAATTAATTGCACTTGG-3'; ENO2: 5'-CGGGAGTCGTAGACGGATCTAGCGTAACTTTAGAGACAGCCTAATAA-3'; and PMA1: 5'-GCAGGCTTTTCTTGAGTTGGCTGATGAGCTGAAACAGAAGATGCACTTCT-3'.

Whole-cell extracts and immunoblot analysis

Cultures were grown to midlog phase and aliquots harvested by centrifugation for 5 min at 3400 rpm. 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 50 μ l of extraction buffer (0.1 M Tris at pH 8.0, 0.5 M NaCl, 5 mM EDTA, 0.01% NP40, and 15% glycerol) with freshly added protease inhibitors (10 mM DTT, 10 μ g/ml leupeptin, 10 μ g/ml aprotinin, 2 mM benzamidine-HCl, 1.0 mM PMSF and 2 μ g/ml pepstatin), followed by vortexing six times

for 10 sec with 1 min of cooling on ice in the presence of 50–75 μ l of glass beads. The extracts were then clarified 2 $\times$ for 10 min at 4°C and protein concentration determined by the Bradford Assay (Bradford 1976). Proteins were visualized by the ECL detection system from Amersham Life Science (Arlington Heights, IL).

Immunoprecipitation analysis

Yeast whole-cell extracts (Reese et al. 1994) were diluted to 10 mg/ml protein and adjusted to 0.25 M potassium acetate buffer T plus 0.01% NP40. The extracts were clarified and 100 μ l was incubated with 2 μ l of rabbit polyclonal antisera for 4 hr on ice. Immune complexes were recovered by the addition of 3 μ l protein-A-agarose beads followed by a 4-hr incubation at 4°C with rotation. The beads were recovered and washed 4 $\times$ in ice-cold 0.75 M potassium acetate buffer T plus 0.01% NP40. Proteins were solubilized with SDS-PAGE loading dye.

Immobilized DNA template assays

Yeast whole-cell extracts (600 μ g protein in 100 μ l) were incubated under transcription conditions (Reese et al. 1994) with a fragment of biotinylated pGEM3 or G5E4T (0.15 pmole) complexed to Dynal streptavidin beads prepared as described (Choy and Green 1993); 200 ng of poly[d(I-C)] was added. The reactions with G5E4T contained 200 ng of GAL4-VP16 to stimulate the formation of the PIC. After a 40-min incubation at 23°C, the beads were collected and washed 4 $\times$ with wash buffer (20 mM HEPES-KOH at pH 7.5, 10 mM magnesium acetate, 2.5 mM EGTA, 100 mM potassium acetate, 1 mM DTT, 0.003% NP40, and 10% glycerol). The proteins were eluted from the beads by digestion with 5 units of micrococcal nuclease for 15 min at 23°C, and analyzed by immunoblotting.

Analysis of LexA-yTAF_{II}90 fusion proteins

LexA-yTAF_{II}90 fusion proteins containing full-length, amino-terminal and carboxy-terminal deletion were transformed into LGY7, which contains an integrated *lacZ* reporter with two LexA operators upstream of the TATA box. β -Galactosidase activities were assayed as described (Kaiser et al. 1994). Wild-type and LexA-yTAF_{II}90 temperature-sensitive hybrids were transformed into strain EGY48 (Gyuris et al. 1993). Transformants were restreaked on minimal medium without leucine and tested for the ability to grow at 25°C and 30°C.

Flow cytometry

Samples were prepared for FACS analysis as described previously (Lew et al. 1992), and FACS analysis was performed by the Flow Cytometry Facility at the University of Massachusetts Medical Center.

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Yeast TAF(II)90 is required for cell-cycle progression through G2/M but not for general transcription activation.

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