Association of Transcription Factor IIA with TATA Binding Protein Is Required for Transcriptional Activation of a Subset of Promoters and Cell Cycle Progression in *Saccharomyces cerevisiae*

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The general transcription factor IIA (TFIIA) interacts with the TATA binding protein (TBP) and promoter DNA to mediate transcription activation in vitro. To determine if this interaction is generally required for activation of all class II genes in vivo, we have constructed substitution mutations in yeast TFIIA which compromise its ability to bind TBP. Substitution mutations in the small subunit of TFIIA (Toa2) at residue Y69 or W76 significantly impaired the ability of TFIIA to stimulate TBP-promoter binding in vitro. Gene replacement of wild-type *TOA2* with a *W76E* or *Y69A/W76A* mutant was lethal in *Saccharomyces cerevisiae*, while the *Y69F/W76F* mutant exhibited extremely low growth at 30°C. Both the *Y69A* and *W76A* mutants were conditionally lethal at higher temperatures. Light microscopy indicated that viable *toa2* mutant strains accumulate as equal-size dumbbells and multibudded clumps. Transcription of the cell cycle-regulatory genes *CLB1*, *CLB2*, *CLN1*, and *CTS1* was significantly reduced in the *toa2* mutant strains, while the noncycling genes *PMA1* and *ENO2* were only modestly affected, suggesting that these *toa2* mutant alleles disrupt cell cycle progression. The differential effect of these *toa2* mutants on gene transcription was examined for a number of other genes. *toa2* mutant strains supported high levels of *CUP1*, *PHO5*, *TRP3*, and *GAL1* gene activation, but the constitutive expression of *DED1* was significantly reduced. Activator-induced start site expression for *HIS3*, *GAL80*, *URA1*, and *URA3* promoters was defective in *toa2* mutant strains, suggesting that the TFIIA-TBP complex is important for promoters which require an activator-dependent start site selection from constitutive to regulated expression. We present evidence to indicate that transcription defects in *toa2* mutants can be both activator and promoter dependent. These results suggest that the association of TFIIA with TBP regulates activator-induced start site selection and cell cycle progression in *S. cerevisiae*.

The RNA polymerase II general transcription factors are an evolutionarily conserved set of proteins required for the regulation and recognition of specific promoter start sites (reviewed in references 56 and 60). In higher eukaryotes, the general transcription factor IID (TFIID) binds to core promoter elements and can nucleate the assembly of an active preinitiation complex in vitro (reviewed in reference 10). TFIID contains the TATA binding protein (TBP) and TBP-associated factors (TAF$_{14}$s), which modify the promoter recognition and transcriptional activities of TBP (reviewed in reference 75). In addition to the TAF$_{14}$s, multiple other factors can associate with TBP and regulate transcription initiation by modulating the binding of TBP to the core promoter (5, 19, 27, 35, 48, 52). The general transcription factor IIA (TFIIA) is a positive modulator of TBP binding to TATA box elements and is essential for regulated transcription in vitro. However, the precise function and general requirement for a TFIIA-TBP association in vivo have not been completely elucidated.

TFIIA stimulates and stabilizes the interaction of TBP with a variety of TATA elements and may make direct contact with promoter DNA upstream of the TATA box (26, 40, 55). TFIIA is required for activator-mediated transcriptional stimulation in reactions reconstituted with human or *Drosophila* TFIIA but appears dispensable for basal-level transcription in reactions reconstituted with TBP (20, 58, 72, 74, 78). Human TFIIA binds directly to at least three viral transcriptional activators (16, 37, 58, 78) and mediates an activator-induced conformational change in TFIID that allows TAF$_{14}$s to interact with promoter sequences downstream of the transcriptional initiation site (42, 58). TFIIA can also induce changes in the interaction of TAF$_{14}$s with promoter sequences in the absence of a transcriptional activator (40, 55). The TFIIA-mediated conformational change in TFIID facilitates the assembly of TFIIB, indicating that TFIIA binding stimulates productive preinitiation complex assembly (13, 14).

TFIIA activity can be reconstituted in vitro by the expression of two evolutionarily conserved genes, referred to as *TOA1* and *TOA2* in yeast or $\alpha\beta$ and $\gamma$ in humans (58, 59, 72, 78). The crystal structure of the yeast TFIIA-TBP-DNA ternary complex revealed that the two subunits of yeast TFIIA fold into a complex heterodimer consisting of a four-helix-bundle domain (FHB) and a $\beta$-sheet domain (22, 73). Contact with TBP is directed through a series of aromatic residues in the $\beta$-sheet domain contributed primarily from the small subunit of TFIIA (Toa2) (22, 73). Mutagenesis of the human TFIIA small subunit ($\gamma$) further corroborated the importance of these aromatic residues in forming a stable TFIIA-TBP-DNA complex in vitro (57). Mutations in these residues of the human TFIIA $\gamma$ subunit were generally defective for transcriptional activation in vitro, indicating that the TFIIA-TBP interaction is absolutely required for transcription function in vitro. Interestingly, conservative mutations in these residues did not disrupt the ter-

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nary TFIIA-TBP-DNA complex in gel electrophoretic mobility shift assays (EMSA), but transcriptional activation for these mutants was still defective in vitro (57). Subsequent biochemical analysis indicated that these mutations in TFIIA increase the dissociation rate or protease sensitivity of the TFIIA-TBP-DNA complex, revealing subtle defects in the stability or conformation of the ternary complex not revealed by EMSA, yet correlating with loss of transcription activation function (58a). TFIIA also functions to derepress transcriptional repression. The stable interaction between TFIIA and TBP precludes the inhibitory association of a variety of transcriptional repressors of TBP-promoter binding, including DR1, NC2, MOT1, DSP1, and HMG1 (5, 21, 27, 35, 53). The derepression function of TFIIA was found to be distinct from its transcriptional coactivating function. Isolation of a smaller form of human TFIIA which lacks the α subunit (the Toa1 amino-terminal homolog) was capable of binding to TBP and derepressing transcriptional inhibitors (47). However, this smaller TFIIA form was incapable of supporting transcription activation in vitro. These results are consistent with mutagenesis studies that implicate the FHB domain as being essential for coactivation function and the β-sheet domain as essential for coactivation, derepression of TBP inhibition, and formation of the TFIIA-TBP-DNA complex (33, 57).

Despite indications that TFIIA is generally important for regulated transcription of all class II promoters in vitro, relatively little is known about how TFIIA functions in vivo. The genes encoding yeast TFIIA, TOA1 and TOA2, are both essential for viability in Saccharomyces cerevisiae (59). Depletion of TFIIA in vivo results in a decrease of several RNA polymerase II-dependent gene transcripts, with no apparent effect on RNA polymerase I- or III-dependent transcripts in yeast (33). Mutations in TBP which disrupt TFIIA binding cause defective transcription activation by acidic activators in yeast and by multiple activators in human cells (8, 68). Mutations in the large subunit of TFIIA which disrupt TBP-DNA binding were found to cause temperature-sensitive phenotypes in yeast (33). However, it is not clear from these previous studies whether a stable TFIIA-TBP interaction was generally required for all class II promoters or activators, or only for a specific subclass of promoters or activators in vivo. To further investigate the general requirements for TFIIA in vivo, we have constructed mutations in TOA2 which compromise the ability of TFIIA to interact with TBP and form a stable TBP-TFIIA-DNA (T-A) complex. These S. cerevisiae mutants were examined for growth phenotypes and specific gene transcription defects in vivo.

**MATERIALS AND METHODS**

**Plasmid constructs and yeast strains.** Wild-type (wt) genomic TOA2 in pSH543 (pRS315 ARS CEN LEU2) and pSH42 (pRS316 ARS CEN URA3) and wt TOA1 in pSH363 (pRS315 ARS CEN LEU2) were kindly provided by S. Hahn (33). toa2 mutants under the control of the wt TOA2 promoter were generated by overlap extension PCR (24, 57). The 2.0-kb PCR fragments containing site-directed mutations in TOA2 were subcloned into the pSH site of pRS15. Escherichia coli pGEM4Z plasmid expression constructs for the wt and toa2 mutants were generated by PCR (Vent polymerase; New England Biotechnology) and subcloned into pRSETA (Invitrogen) with a BamHI restriction site immediately preceding the initiation codon and a HindIII restriction site immediately following the termination codon. pRSETA-Toa1 was constructed by the same cloning strategy. All the wt and toa2 mutant constructs were confirmed by DNA sequencing in both orientations with an ABI automated 373A DNA sequencer. The resulting pRSETA wt or mutant toa2 open reading frames were expressed in E. coli BL21 and purified as previously described (57). S. cerevisiae strain Shy4 (MATA ade1 ura3 his4 leu2 34 TOA2:his3 pSH342 (ARS CEN URA3 TOA2) was kindly provided by S. Hahn (33). The parent strain of Shy94 was BWG1-7a (MATa leu2 his3 ade1 34 ura3 his4 leu2 34 TOA2:his3 pSH342 from Shy94 by streaking the yeast on 5-fluoro-oroic acid (5-FOA) plates. The resulting strains were assayed for a petite phenotype by streaking on several nonfermentable carbon sources (see Table 1). The GAL1(1-147)-VP16 and GAL4(1-147)-HAP4 expression constructs contain the DNA binding domain of GAL4 (amino acids [aa] 1 to 147) fused to the activation domain of herpes simplex virus VP16 (aa 413 to 490) and HAP4 (aa 1 to 413) as described previously (6). Both open reading frames were driven by the yeast ADH1 promoter, which was isolated as a 2-kb BamHI fragment from pBR322 (a gift of S. Berger) and was subcloned into the BamHI site of pRS416 (URA3 CEN) vector (66).

**Protein preparations.** The pRS41 wt or toa2 mutant constructs were purified under denaturing conditions on Ni-nitrotriacetic acid agarose columns (Qiagen). The recombinant wt or Toa2 mutant proteins were isolated by column chromatography with elution denaturant (8 M urea–0.1 M Mops [pH 8.0])–7 M β-mercaptoethanol [β-ME]–1 mM phenylmethylsulfonyl fluoride [PMSF]) of decreasing pH. wt Toa2 was similarly expressed and purified. Purified Toa2 proteins were renatured with equal molar amounts of wt Toa1 by stepwise dialysis into D100 buffer (20 mM HEPES [pH 7.9] [KOH]-20% glycerol-0.2 mM EDTA Na+–100 mM KC1–7 mM β-ME–1 mM PMSF) as described previously (57, 58). Recombinant γ TFIIA was more than 85% pure based on Coomassie blue staining in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (data not shown). The stepwise dialysis protocol yielded approximately 50% higher soluble concentrations of γ TFIIA mutant compared to wt γ TFIIA (data not shown). γ TBP was purified as described elsewhere (44).

**DNA binding reactions.** EMSA of TBP-TFIIA DNA binding reactions with the adenovirus EIB TATA 30-bp oligonucleotide have been described previously (57). TBP (5 ng) was incubated with 50 fmol of 32P-labeled TATA oligonucleotide in a 12.5-μl reaction volume in the absence or presence of 250, 50, or 20 ng of wt or Toa2 mutant TFIIA complexes with TBP, as indicated in Fig. 1B. Complexes were captured by native 6% polyacrylamide–45 mM Tris-base–1.25 mM EDTA gels at 22°C for 2.5 h.

**β-Gal assays.** The β-galactosidase (β-Gal) assays were performed as described elsewhere (61). For the CUP1 expression experiment, the yeast cultures were grown to mid-log phase and were transferred to a 3°C water bath for 10 min prior to being placed on a shaker at 37°C for 4 h. CuSO4 was added to a final concentration of 50 μM, and the cultures were placed for an additional 2 h at 37°C on a shaker prior to taking the extracts for β-galactosidase analysis. β-Gal experiments were performed twice; the averages of these values are shown in Fig. 5, and the error was less than 20% for each sample (wt or mutant). Similar CUP1 expression experiments were performed in duplicate at 30 and 37°C, with shorter preincubation times (20 min and 2.5 h at 37°C) prior to addition (2 add h). The results of all these variations were very similar to those shown in Fig. 5 (data not shown). pLUC (CUP1 LacZ, URA3 CEN) was a gift of S. Berger and D. Thiele. For the PHO5 expression experiments, yeast cultures were grown at 30°C with no apparent effect on TBP binding. The PHO5 expression experiment was performed for 5 h at 30°C in medium without phosphorus prior to extract production. The PHO5 experiments were performed twice; the averages of these values are shown in Fig. 5, and the error was less than 10% for each sample (wt or mutant). β-Gal activity is expressed as units per milligram of protein and was calculated as described elsewhere (12). The PHO5-LacZ construct (pMH13) was a gift of M. Grunstein.

**Yeast phenotype analysis and RNA isolation.** Yeast manipulations and growth protocols are described in detail elsewhere (62). Yeast cultures were grown at 30°C in YPD and YM + 6-azauracil media to OD600 optical density at 600 nm (OD600) range of 0.8 to 1.1 prior to RNA isolation. One hundred-milliliter cultures were pelleted, washed with 0.5 volume of sterile H2O, resuspended in 2.5 ml of sterile filtered TES buffer (10 mM Tris [pH 7.5]–10 mM EDTA–0.5% SDS), frozen on dry ice, and placed at −80°C prior to total RNA purification. The total yeast RNA was isolated as described previously (29). The isolated total RNA was aliquoted into 40-μg quantities, reprecipitated, and stored at −20°C until used for S1 nuclease analysis. For URA1 induction, 10 μg of 6-azauracil/ml (final concentration) was added for 2.5 h prior to harvesting. For galactose induction, samples were grown in synthetic complete (SC) lactate medium overnight and switched to SC galactose medium for 3 h at 30°C. Control samples were grown in SC glucose medium. Doubling times were calculated as described previously (34).

**S1 nuclease analysis.** Oligonucleotides complementary to the genes assayed by S1 nuclease analysis are as follows: CLB1, 5'-TAGAGACAGCCTAATAA-3'; GAL80, 5'-CCAGCTGAAAAAGGAAATATTCCATTTTCTTACGAAAGTGGTGT-3'; CLB2, 5'-CTATGGGTTTGACACTATTAAATATGCAGAGGAGAGGAGGCGG-3'; CLN1, 5'-CAGTGACAAATACCCACCGATTTCCCTATGGTGGTGACGCGGCGGCGG-3'; CTS1, 5'-CGGGAGTCGTAGACGGATCTAGCGTAAACTT-3'; GAL1, 5'-TCATTACTTAAATATGTTCTACTTCTTCCTACAAAAAGGAGGTCGAGGAGGTCGGTGT-3'; GAL10, 5'-GAGAGGATCACGAGGCGGGAAGGAGGCAGAGCAGAGCAGAGGAC-3'; PHO5, 5'-CTTATCCATGGGACACTATTAAATATGCAGAGGAGAGGAGGCGG-3'. The probes were end-labeled with γ32P-ATP (3000 Ci/mmol) using T4 polynucleotide kinase and analyzed by 6% polyacrylamide gels at 22°C for 2.5 h.

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**RESULTS**

**TFIIA-TBP interaction is essential for growth and viability in yeast.** Previous work showed that aromatic residues Y65 and W72 in the small subunit of human TFIIA (γ) were important for forming the TFIIA-TBP-DNA ternary complex (57). Human TFIIA γ is 58% conserved with the yeast TFIIA small subunit (Toa2) (Fig. 1A). Crystal structure revealed that the homologous aromatic residues in Toa2 (Y69 and W76) make the primary stabilizing contact with TBP in the ternary complex with DNA (22, 73). Recombinant yeast TFIIA reconstituted with single-substitution mutants of Toa2 were analyzed for ternary complex formation by EMSA. Substitution of alanine for Y69 and W76 in Toa2 significantly reduced complex formation (~65-fold) (Fig. 1B; compare lane 2 with lanes 5 and 11). Phenylalanine substitution of W76 reduced T-A complex formation 45-fold, and the Y69F mutation reduced it 4.5-fold relative to that in wt TFIIA (Fig. 1B; compare lane 2 with lanes 5 and 11). As mentioned previously, the human homologs of Toa2 Y69F and W76F (Hu Y65F and W76F) stimulate normal relative to that in wt TFIIA (Fig. 1B; compare lane 2 with lanes 5 and 11). As mentioned previously, the human homologs of Toa2 Y69F and W76F (Hu Y65F and W76F) stimulate normal levels of T-A complex but fail to stimulate transcription in vitro with most activators (57). Recent biochemical studies indicate that the Y65F mutant has an increased T-A dissociation rate in EMSA and the W76F mutant forms an altered T-A complex that is highly sensitive to proteolytic digestion (data not shown). These results further indicate that mutations in Toa2 at residues Y69 and W76 affect the stability and/or the conformation of the T-A complex.

To determine the effects of these and related toa2 mutations on cell viability and growth, mutant toa2 alleles were introduced into yeast by the plasmid shuffle technique (62). toa2 mutants with radical substitution of W76 with glutamic acid (W76E) and those with the double alanine substitution mutation (Y69A/W76A) were inviable (Fig. 2A). The mutants with radical substitution of W76 with glutamic acid (W76E) were inviable (Fig. 2A). While the Y69A/W76A mutant fails to complement (Fig. 2A). The Y69A allele is recessive, and the W76A allele is dominant. The Y69A/W76A double mutant fails to complement, indicating that Y69A and W76A are both essential for Toa2 function. The results suggest that Y69A and W76A are both essential for Toa2 function.

**FIG. 1.** TFIIA is a highly conserved heterodimer. (A) The TFIIA subunits from humans (Hu) and yeast (Ya) are aligned. The human TFIIA large subunit (αβ) (aa 1 to 376) is aligned to yeast Toa1 (aa 1 to 286). Hu αβ is proteolyzed in vivo to produce individual α and β subunits, which are indicated. The human α subunit is 58% conserved with the yeast homolog (Toa1; aa 7 to 58), while the β subunit is 72% conserved (Toa1; aa 226 to 286). The γ subunit is 58% conserved throughout its length. The conserved Toa2 residues that are mutated in this study are indicated by stars. (B) Yeast TBP-TFIIA complex formation in EMSA. Recombinant yeast TBP and TFIIA proteins were expressed in E. coli, purified, and used in EMSA. The 32-P-labeled 30-5p adenoviral EB1 TATA box was used as a probe. Decreasing amounts (250, 50, and 20 ng) of TFIIA proteins were added to the EMSA reaction for wt and Toa2 mutant proteins, as indicated above the gel. Arrows point to the TBP-DNA (T) and TBP-TFIIA-DNA (T-A) complexes.
expression could not rescue the transcription defects (data not shown).

The panel of TFIIA substitution mutants was further analyzed for growth defects and conditional lethality (summarized in Table 1). Among the most dramatic growth defects were the failure of Y69F, Y69A, and W76A mutants to grow on galactose-containing media at 30°C, while the F71A, F71E, and W76F mutants grew slowly at 37°C on galactose (Fig. 2B). The Y69 and W76 mutants grew extremely slowly on glycerol at 30°C and were lethal at 37°C on yeast extract-peptone (YP) glycerol plates. In contrast, these mutants grew significantly better on several other nonfermentable carbon sources (Table 1; data not shown). The inability of toa2 alleles to grow on various carbon sources suggests that they are incapable of expressing genes essential for either galactose or glycerol utilization. Furthermore, we examined the ability of toa2 mutants to grow on increasing concentrations of 3-aminotriazole (3-AT), a HIS3 competitor which requires high-level HIS3 gene expression for cell viability. toa2 Y69A and W76A strains showed significantly impaired growth on 15 mM 3-AT, while the Y69F/W76F strain was incapable of growth on 5 mM 3-AT at 30°C (Fig. 2C). We have also generated a mutation in the FHB domain of TFIIA (Toa2 FDK44-46AAA) that causes a temperature-sensitive phenotype in SC media at 37°C (data not shown). In the human system, similar mutants preclude normal interaction of the FHB α and γ helices in glutathione S-transferase assays (57), and TFIIA derivatives lacking the FHB domain stimulate T-A formation in EMSA (47, 58). The TFIIA FHB mutant strain had no effect on 3-AT-dependent growth, indicating that a 3-AT growth defect correlates with mutations in the β-sheet domain of TFIIA which interfere with TBP binding (Fig. 2C). These results suggest that TFIIA must interact efficiently with TBP to support high-level activation of the HIS3 gene.

**TFIIA mutants disrupt cell cycle progression.** Since several yeast TAF11 mutations were found to cause cell cycle arrest phenotypes (3, 77), we further inspected toa2 mutant alleles for effects on cell cycle progression. Light microscopy revealed that toa2 mutants accumulated as fused cell pairs and multipaired clumps with equal-sized buds under permissive condi-
tions, and more so under nonpermissive conditions. We visually counted wt, Y69A, and Y69F/W76F cells (>400 for each experiment) and found a significant decrease in the number of single or small-budded cells in the mutants relative to the wt, with an accumulation of multibudded clumps (Table 2). FACS analysis confirmed that these TFIIA mutants had decreased numbers of cells with a 1N copy of DNA and an increase in multibudded complexes, or clumps (Fig. 3A). The various major FACS peaks of the wt and Y69F/W76F strains were subjected to cell sorting and then analyzed by light microscopy to confirm that the peaks were indeed fused and un budded cell twins (2N) and aggregated multibudded complexes (Fig. 3B). Sonication was capable of disrupting the clumps into unbudded single and fused cell pairs, with a noticeable loss of small-budded cells (Table 2). The clumpy phenotype, accumulation of fused cell pairs, and the loss of small-budded cells after sonication suggest that toa2 mutant strains may be arresting in G2/M or cytokinesis (3, 31, 32, 38, 39, 63).

To further characterize a potential cell cycle defect, we determined if transcription levels of several cell cycle-regulatory genes were reduced in these toa2 mutant strains. We analyzed the CLB1, CLB2, CLN1, SEC72/SIM2, and CTS1 genes by S1 nuclease protection (Fig. 4A). CLB1 and CLB2 are specifically expressed in G1, and their protein products are required for progression into mitosis (2). SEC72/SIM2 is expressed in late G1 and prevents reactivation of the genome prior to mitosis or start (18). CLN1 is expressed in G1 and is required for progression through G1/S (2). CTS1 encodes chitinase and is required for completion of cytokinesis (39). In the toa2 Y69A strain, CLB1, CLB2, CLN1, and CTS1 expression were reduced significantly (to less than 20% of that of the wt), while SEC72/SIM2 expression was reduced to 63% of that of the wt (Fig. 4A). In contrast, expression of ENO2 and PMA1, which are not cell cycle dependent (3), was unaffected by the toa2 Y69A allele (Fig. 4A). In the toa2 Y69F/W76F double mutant, which has a more severe growth arrest phenotype, the cell cycle-specific CLB1, CLB2, and CLN1 transcripts were 0.6, 4, and 6% of wt levels, respectively, while the cell cycle-independent ENO2 and PMA1 transcripts were reduced to only ~35% of wt levels (Fig. 4B). These results indicate that some cell cycle-specific promoters and/or activators are preferentially sensitive to TFIIA mutations and that a stable association of TFIIA with TBP is required for efficient transcription of genes required for cell cycle progression.

A stable TFIIA-TBP interaction is not generally required for transcription of all genes in vivo. S1 analysis of cell cycle-regulatory genes suggested that transcription of some genes is preferentially affected by toa2 mutations (Fig. 4). To better characterize the class of genes affected by toa2 mutations, we assayed the ability of the PHO5 and CUP1 promoters to respond to inducing agents. Using β′-Gal assays, we found that viable mutants with substitutions at residues Y69, F71, and W76 had no significant transcriptional defect with PHO5 induction on medium without phosphorus, or with CUP1 induction in the presence of copper ions (Fig. 5A and B and data not shown). Figures 5A and B show that the most severely growth-defective toa2 mutants, Y69A and Y69F/W76F, were indistinguishable from wt strains with regard to PHO5 and CUP1 induction, even at the nonpermissive temperature (Fig. 5B). This suggests that a compromised TFIIA-TBP interaction is not generally required for all class II transcription. To determine if a subset of genes were affected by these TFIIA mutants, we examined two constitutively active promoters and another inducible promoter by S1 analysis. Steady-state RNA levels of the TRP3 gene were only slightly reduced (83%) in the Y69A mutant relative to the wt (Fig. 5C). In contrast, DED1 RNA levels were dramatically reduced in the Y69A mutant to just 3% of wt RNA levels (Fig. 5C), wt GAL1 mRNA could be induced almost 600-fold by switching to galactose- from lactate-containing medium, while in the Y69A mutant, GAL1 induction was impaired ~2-fold, to 49% of wt levels (Fig. 5D). In the S1 assays, RNA levels were normalized for tRNA expression, which appears to be unaffected by mutations in TFIIA (33). Together, these results indicate that a subset of promot-

### Table 1. Growth phenotypes of toa2 mutants in different media

<table>
<thead>
<tr>
<th>Mutant</th>
<th>OD600 doubling time (min) at 30°C in YPD</th>
<th>SC medium</th>
<th>YPGly</th>
<th>Lactate, EtOH, KAc (30°C)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>30°C</td>
<td>37°C</td>
<td>30°C</td>
</tr>
<tr>
<td>wt</td>
<td>Yes</td>
<td>111</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Y69A</td>
<td>Yes</td>
<td>191</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Y69F</td>
<td>Yes</td>
<td>142</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>F71A</td>
<td>Yes</td>
<td>128</td>
<td>++++</td>
<td>++++</td>
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<tr>
<td>F71E</td>
<td>Yes</td>
<td>141</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
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<td>Yes</td>
<td>170</td>
<td>++</td>
<td>+++</td>
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<td>W76F</td>
<td>Yes</td>
<td>ND</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Y69F/W76F</td>
<td>Yes</td>
<td>244</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>W76E</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y69A/W76A</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* YPD, YP dextrose medium; YPGly, YP medium with glycerol as a nonfermentable carbon source; KAc, potassium acetate.

** - no growth; +, pinpoint colonies; ++, very slow growth; ++++, slow growth; +++, wt growth; ND, not determined.

### Table 2. Cell cycle phenotypes of toa2 mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>% of cells</th>
<th>% of cells after sonication:</th>
<th>% of cells with the following bud size after sonication:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Double</td>
<td>Clumpy</td>
</tr>
<tr>
<td>wt</td>
<td>22</td>
<td>59</td>
<td>19</td>
</tr>
<tr>
<td>Y69A</td>
<td>9</td>
<td>51</td>
<td>40</td>
</tr>
<tr>
<td>Y69F/W76F</td>
<td>5</td>
<td>41</td>
<td>54</td>
</tr>
</tbody>
</table>

* At least 400 cells were counted for each mutant in each experiment.

** Determined by light microscopy from unsonicated cell cultures grown at 37°C in SC medium.

Clumpy cells were disrupted by sonication.
ers are highly sensitive to defects in the TFIIA-TBP interaction (e.g., DED1), while other promoters are seemingly unaffected in vivo (e.g., CUP1).

TFIIA-TBP interaction is required for promoter selection in vivo. For a subset of promoters in yeast, inducible expression is characterized by the utilization of several transcriptional initiation sites (71). Constitutive transcription (TC) initiates from the furthest upstream start site, while inducible transcription initiates from one or multiple downstream initiation sites (TR). Utilization of TR start sites is important for high-level activator-dependent gene expression of the HIS3, GAL80, URA3, and URA1 promoters (46, 64, 70). These promoters were all examined for activator-induced start site selection in mutant versus wt toa2 strains by S1 analysis (Fig. 6). The HIS3 gene has been extensively studied for selection from the constitutive +1 initiation site (TC) to the activator-induced +13 and +22 initiation site (TR) (28, 49, 70). In wt strains, addition of 3-AT induced transcription by ~16-fold at +13 and 9-fold at +22. In contrast, transcription was induced only 1.5-fold at +1 (Fig. 6A). In wt strains, the utilization of TR (+13) after induction with 3-AT was ~11-fold greater than transcription from TC. In contrast, the utilization of TR (+13) was only 2.5-fold greater than that of TC for the toa2 Y69F/W76F strain (Fig. 6A). While utilization of TC was modestly reduced, the ability to activate transcription at TR was most significantly affected by the toa2 Y69F/W76F allele. Similar HIS3 results were seen for the toa2 Y69A single mutant allele (data not shown). Thus, TFIIA mutants in which TBP binding is compromised disrupt high-level transcription initiating from the TR start sites.

Galactose induction of the GAL80 gene results in the stimulation of multiple TR start sites (+37, +47, +56, and +67) which are downstream of the constitutive TC (+1) start site (64). Only GAL80 TC expression is observed under glucose repression (64). Surprisingly, in SC medium with glucose, GAL80 TC levels are severalfold higher for the toa2 Y69A mutant compared to the wt (Fig. 6B and data not shown). In the presence of galactose, GAL80 TR expression was significantly reduced in the toa2 Y69A strain relative to the wt (Fig. 6B). For the GAL80 +56 start site, the ratio of the TR to the TC level was fourfold lower in the mutant toa2 Y69A strain relative to the wt (Fig. 6B).

The URA3 TC start site at −60 (relative to AUG) is weakly expressed in the absence of the PPR1 activator, while the TR start sites at the −56, −38, and −33 positions are induced to high levels by the PPR1 activator (46). We found that yeast strains carrying the toa2 Y69F/W76F mutant allele were able to express the URA3 TC transcript but were severely defective in mediating activator-dependent expression from multiple TR sites for this gene (Fig. 6C).

Similarly, URA1 gene TC expression (~68) was barely affected by the toa2 Y69F/W76F allele, but TR expression at the −54, −43, and −33 start sites was significantly defective in the mutant strain (Fig. 6D). URA1 expression at the −43 start site (TR/TC ratio) was more than eightfold lower in the toa2 Y69F/
Results of representative experiments are shown. tRNA levels were used to detect the mutant shown as a percentage of expression, and 40 μg of total RNA was used for the other S1 reactions. S1 assays were performed in duplicate at least three times, and PhosphorImager quantitation showed less than 20% error. PhosphorImager quantitation (expression in the mutant as a percentage of that in the wt strain) is given below each panel. Results of representative experiments are shown. tRNAW levels were used as a control for intact RNA and are indicated for all samples. (B) Expression of cell cycle-regulatory genes is severely reduced in the mutant. S1 analyses had wt levels of both PMA1 and ENO2 expression (data not shown). These results clearly show that yeast strains carrying a TFIIA mutation, with compromised TBP binding, exhibited defective high-level transcriptional activation of the inducible Tc start sites, with modest Tc defects, for multiple genes in vivo. 

**Activator- and promoter-dependent defects with mutant TFIIA.** Activation of GAL1 and GAL80 genes is largely dependent upon the interaction of GAL4 with the UASG of each promoter (64). The toa2 Y69A mutation affected the steady-state transcription level of GAL1 by 2-fold (Fig. 5) and the start site selection of GAL80 Tc sites up to 4-fold (Fig. 6B). To determine if these defects were partly a result of the transcriptional activator, we compared the ability of two distinct transcriptional-activation domains to activate the same promoter in a wt or a toa2 mutant strain. The activation domains of the herpesvirus VP16 and yeast HAP4 transcriptional activators were fused to the GAL4 DNA binding domain and expressed at high levels by the ADH1 promoter under conditions of glucose repression (Fig. 7A) (6). Both the HAP4 and VP16 activation domains stimulated GAL1 expression to similar levels in the wt strain (Fig. 7A). However, in the toa2 W76A mutant strain, stimulation of GAL1 by the HAP4 activation domain was reduced to 11% of that of the wt, while stimulation by the VP16 activation domain was identical to that observed in the wt strain (Fig. 7A). Western blotting confirmed that the GAL4-fusion proteins were expressed at similar levels in the wt and mutant toa2 strains (Fig. 7C). These results indicate that

**FIG. 4.** Reduced expression of cell cycle-regulatory genes in toa2 mutant strains. (A) wt and toa2 Y69A samples were grown at the nonpermissive temperature (37°C). The cell cycle-regulatory genes CLB1, CLB2, CLN1, and CTS1, and the noncycling genes PMA1 and ENO2, were assayed by S1 nuclease protection. Eighty micrograms of total RNA was used for the other S1 reactions. S1 assays were performed in duplicate at least three times, and PhosphorImager quantitation showed less than 20% error. PhosphorImager quantitation (expression in the mutant as a percentage of that in the wt strain) is given below each panel. Results of representative experiments are shown. tRNAW levels were used as a control for intact RNA and are indicated for all samples. (B) Expression of cell cycle-regulatory genes is severely reduced in the mutant. S1 analyses had wt levels of both PMA1 and ENO2 expression (data not shown). These results clearly show that yeast strains carrying a TFIIA mutation, with compromised TBP binding, exhibited defective high-level transcriptional activation of the inducible Tc start sites, with modest Tc defects, for multiple genes in vivo. 

**FIG. 5.** A stable T-A complex is not generally required for transcription in vivo. (A) PHO5-driven LacZ expression at 30°C in SC medium was assayed for wt, toa2 Y69A, and Y69F/W76F strains by β-Gal assays. In high-PO4 medium, PHO5 expression was repressed. In synthetic medium in the absence of PO4, PHO5 induction is shown. β-Gal activity is expressed as units per milligram of protein. (B) CUP1-driven β-Gal activity was assayed under conditions similar to those for the experiment for which results are shown in panel A, except that after reaching mid-log phase, samples were grown for an additional 4 h at the nonpermissive temperature (37°C) prior to Cu-ion addition. (C) The toa2 Y69A mutant shows defective expression of endogenous DED1, but not of TRP3, in vivo. The wt and toa2 Y69A mutant strains were grown to mid-log phase in SC medium at 30°C and were shifted to the nonpermissive temperature (37°C) for 3 h prior to RNA isolation. Endogenous DED1 and TRP3 expression was assayed by S1 nuclease protection. (D) GAL1 expression is induced in the toa2 Y69A mutant. The wt and toa2 Y69A strains were used to assay GAL1 expression in vivo. Expression levels were measured after conditions of glucose repression (+ GLU) and under galactose induction (+ GAL). PhosphorImager quantitation indicates that GAL1 expression was induced about 600-fold in the wt and 300-fold in the toa2 Y69A strain. For the GAL1 samples, 4 μg of total RNA was used per reaction, while 40 μg of total RNA was used for all other S1 reactions. PhosphorImager quantitation (expression in the mutant as a percentage of that in the wt) is shown in panels C and D.
different activation domains have different requirements for a stable TFIIA-TBP interaction.

Promoter structure is also likely to contribute to the differential requirement for TFIIA in transcription activation. We have already observed that GAL4-mediated activation of GAL80 was more sensitive to a2 mutation than was activation of GAL1 (compare Fig. 5D and 6B). We now show that a2 mutations affect activation of GAL80 but not of GAL1 when the two genes are activated by GAL4-VP16 under identical conditions (Fig. 7B). The GAL4-VP16 fusion protein was expressed by the ADH1 promoter under conditions of glucose repression, and levels of transcription of GAL80 and GAL1 were directly compared. Activation of the GAL1 promoter was similar in the wt and a2 W76A mutant strains (Fig. 7B). In contrast, GAL80 transcription was highly sensitive to a2 mutation (Fig. 7B). RNA levels at all of the GAL80 start sites were significantly reduced in the W76A strain relative to the wt, with the most dramatic defects occurring at the most distal start site, +67, which was reduced to 8% of wt activation levels. These results indicate that different promoters regulated by the same activator can have differential requirements for TFIIA.

**DISCUSSION**

TFIIA-TBP complex formation is essential for a subset of promoters in vivo. The interaction of TFIIA with TBP has
been shown to be important for transcriptional activation in vivo in both human and yeast systems (8, 67). Selection of random mutations in TBP which specifically affect response to acidic activators in yeast predominantly affect the DNA binding surface of TBP or disrupt the association of TBP with TFIIA (4, 68, 69). Facilitated recruitment of TBP to the promoter can bypass the need for an activator in yeast, indicating that some promoters require enhancement of TBP binding in vivo (11, 36). TFIIA has been shown to augment TBP binding to TATA sequences and to function as a coactivator for several human and viral activators in vitro (25, 37, 58). Consistent with this, we found that TFIIA mutants in which binding to TBP was compromised were defective for transcription at a subset of promoters in vivo. TFIIA mutations had dramatic effects on the expression of DED1, GAL80, URA1, and URA3 (Fig. 5 and 6). Cell cycle-regulated CLB1, CLB2, CLN1, and CTS1 expression was significantly reduced in TFIIA mutant strains, while SEC72 levels were modestly reduced (Fig. 4). The interaction of TFIIA with TBP may regulate the activity of these promoters by mediating an association between activators and TBP, or by directly enhancing TBP binding to specific core promoters. For these promoters, the interaction of TFIIA with TBP is likely to be rate limiting in vivo.

TFIIA mutants in which TBP binding was compromised did not generally defective in transcription activation of all class II promoters. Activation of the CUP1 and PHO5 promoters was unaffected by TFIIA mutations, as was expression of the constitutively expressed TRP3, ENO2, and PMA1 genes (Fig. 4 and 5). Previously, we had found that human TFIIA mutants in which TBP binding was compromised exhibited defective transcription from all promoters and most activators tested in vitro (57). The finding that homologous yeast TFIIA mutants have more complex phenotypes in vivo is not unprecedented. TFIIB has been reported to be a rate-limiting target of several eukaryotic activators in vitro, yet mutations in TBP which compromise TFIIB binding had no detectable effect on transcription activation in vivo in yeast (15, 41, 45). Similarly, TAFIIIs are essential for activated transcription in vitro but may be dispensable for regulation of many genes in vivo in yeast (54, 76). More recent examination of the TAFIIIs in yeast indicate that core promoter differences contribute to the requirement for particular TAFIIIs (65, 77). Thus, promoter structure may dictate which general factors and coactivators are rate limiting for transcriptional regulation. Our results indicate that a subset of promoters, but not all, require a stable interaction between TFIIA and TBP for efficient expression in vivo in yeast.

Role of TFIIA in the regulation of cell cycle progression. TFIIA mutants with compromise TBP binding accumulated as aggregated clumps which, when sonicated, were reduced to...
single or twin buds of equal size. S1 analysis of cell cycle-regulated genes revealed a significant reduction in RNA levels of cyclin genes required for cell cycle progression, with little or no effect on several genes not involved in the cell cycle. The chitinase-encoding CTS1 RNA was also significantly reduced in toa2 mutant strains. Reduction in CTS1 expression may account for the clumpy phenotype, since chitinase is required for progression through cytokinesis (39). Similar clumpy phenotypes with reductions in CTS1 transcriptional levels have been observed for yeast strains in which the SIN4 and RGR1 transcriptional regulators were deleted (31, 32, 63). The large accumulation of clumpy cells suggests that cytokinesis is blocked in toa2 mutant strains, and the lack of small- and medium-budded cells after sonication supports this conclusion (Table 2). However, we cannot exclude the possibility that toa2 mutants may be arresting at additional points in the cell cycle. Interestingly, mutations causing temperature-sensitive phenotypes in VtAf90 cause a G2/M arrest, while deletion of TAFI145 causes a G1/S cell cycle arrest, indicating that different TAFIs are required for transcription of distinct subclasses of cell cycle-regulated genes (3, 77). TFIIA, like TAFIIs, appears to also be required for the transcriptional regulation of multiple genes controlling cell cycle progression.

TFIIA and TAFIIs have different effects on start site switching. TFIIA mutants with compromised TBP binding showed defective activation of genes with inducible start sites. Several extensively characterized yeast core promoters have two control elements referred to as TR and TC (28, 64, 70). TR resembles a consensus TATA element and is important for regulated transcriptional initiation in vivo. TC does not have a clear consensus sequence but is important for directing constitutive transcription from the proximal initiation site in vivo. The TC element has been hypothesized to consist of a collection of weak TATA elements, but it is also conceivable that TBP does not directly bind to this sequence (28). Our results indicate that a stable TFIIA-TBP interaction is important for the efficient utilization of the consensus TATA element in TR. TFIIA has also been shown to be important for the selection of the proximal promoter start site found in the Drosophila ADH promoter, which appears to possess a consensus TATA element relative to the nonconsensus TATA element at the distal promoter (23). Our results further suggest that TFIIA is required for the efficient utilization of consensus TATA elements found in many eukaryotic promoters.

Genetic and biochemical evidence clearly indicate that TAFIIs play a regulatory role in transcriptional activation and promoter selection and that this function may be largely dependent upon the presence of TFIIA (23, 54, 76). TAFIIs allow TFIID to utilize the initiator element found in many higher eukaryotic TATA-less promoters (51, 79). The GAL80 core promoter consists of two control elements, a consensus TATA at −20 and an Inr-like element at +1 (64). Mutagenesis of the TATA element results in an abrogation of activator-inducible transcription from the downstream (TR) start sites, while the Inr controls the constitutive expression of the +1 start site (TC) (64). Mutations in TFIIA resulted in reduced TR transcription and a slight but reproducible increase in GAL80 TC expression (Fig. 6 and 7). This is consistent with the findings of Sakurai et al. (64), who describe a competition between the TC and TR initiation sites. For GAL80 expression, TFIIA may function to mediate an isomerization of TFII D from a TCR/Inr recognition complex to a TR/TATA binding complex that allows high-level transcription.

TFIIA interacts biochemically and genetically with other TBP-associated polypeptides. Mot1p/ADI is an ATP-dependent inhibitor of TBP-DNA binding in vitro. The inhibition of TBP binding by Mot1p could be prevented by TFIIA in vitro (5). The MOT1 gene was originally identified as a global negative regulator of a class of genes in yeast (19). Interestingly, Mot1 mutants showed defects in +1 (TR) start site expression of the HIS3 gene (17). In contrast, our data show that TFIIA mutants were defective primarily for +13 (Tp) start site expression of HIS3. Thus, Mot1 and TFIIA appear to affect the two distinct promoter start sites of the HIS3 gene. Similarly, TAFIIs may also compete with TFIIA for directing TBP activation function. Depletion of yeast TAFI145 and TAFI19 in vivo caused phenotypes similar to those of Mot1 mutants, resulting in a decrease in Tc expression, but had no effect on Tp expression of the HIS3 gene (54). Collart has proposed that Mot1p dissociates TBP from consensus TATA elements and that this release may be important for the activation of genes with nonconsensus TATA elements (17). This interpretation is consistent with our findings and suggests that TFIIA promotes TBP interactions at a class of activator-dependent consensus TATA elements (TR) in vivo.

Our results also reveal differences between constitutively expressed promoters in their sensitivity to TFIIA mutants. We found that TRP3 expression was relatively insensitive, while DED1 expression was dramatically reduced by the Y69A allele. TRP3 has been shown to contain a nonconsensus TATA element similar to Tc (50). In contrast, strong constitutive expression of DED1 is synergistically activated by a T-rich element and a UAS which binds to ABF1 (9). Interestingly, DED1 expression is also significantly reduced in a TBP mutant (P109A) which binds poorly to the TATA box in EMSA and may affect TFIIA binding, since the mutated residue is in close proximity to the TFIIA recognition site (4, 22, 73). Thus, even constitutively expressed genes may show differential sensitivity to mutations which compromise TFIIA-TBP complex formation.

Activator and promoter dependence of TFIIA defects. The differential requirements for TFIIA-TBP interaction may depend on either the activator or the promoter structure. Our results suggest that both activator structure and promoter structure contribute to the requirement for a stable TFIIA-TBP interaction. Activation of the GAL4 gene by the VP16 activation domain was unaffected by the toa2 W76A mutation, while the HAP4 activation domain was extremely sensitive to it (Fig. 7A). Thus, different activation domains may require more stable interactions between TFIIA and TBP to execute their function. We found that the GAL80 promoter was significantly more sensitive to the toa2 W76A mutation than was the GAL1 promoter when both were activated by the same activator, GAL4-VP16 (Fig. 7B). This indicates that different promoters can have differential requirement for a stable interaction between TFIIA and TBP. A similar observation has been made for the Zta transcriptional activator in vitro. Zta stimulates TFIIA-TFIID-promoter complex formation and can partially overcome a transcriptional defect resulting from similar human TFIIA mutants in which TBP interaction is compromised in vitro (43, 57). Other activation domains fail to stimulate this interaction and cannot overcome TFIIA mutant transcriptional defects. This suggests that some activators can overcome defects in TFIIA-TBP interaction by introducing compensatory and stabilizing interactions. TFIIA recruitment by Zta was also found to be important for a subset of promoters, further indicating that promoter structure contributes to a requirement for TFIIA recruitment by an activator (43). Together, these results demonstrate that TFIIA can be used variably by different activators and promoters to regulate transcription initiation.

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Conclusion. The interaction of TFIIA with TBP is highly conserved between humans and yeast and is likely to be important for multiple levels of gene regulation. Using site-directed mutagenesis of TFIIA amino acid residues critical for stable interaction with TBP, we were able to characterize the importance of this interaction for the growth phenotypes and RNA expression of several class II genes in S. cerevisiae. In this study, the stable interaction of TFIIA with TBP was found to be particularly important for activator-induced expression of promoters with consensus TATA elements that direct multiple downstream initiation sites (T\_\text{\textsubscript{D}}) and for a subset of cell-specific genes. These results confirm biochemical studies which suggest that TFIIA is a core promoter-dependent coactivator and further suggest that the TFIIA-TBP interaction is rate limiting for the transcriptional regulation of a subset of genes in vivo.

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