TFIIA Interacts with TFIIID via Association with TATA-Binding Protein and TAF40

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Received 30 August 2000/Returned for modification 29 October 2000/Accepted 22 November 2000

TFIIA and TATA-binding protein (TBP) associate directly at the TATA element of genes transcribed by RNA polymerase II. In vivo, TBP is complexed with approximately 14 TBP-associated factors (TAFs) to form the general transcription factor TFIIID. How TFIIA and TFIIID communicate is not well understood. We show that in addition to making direct contacts with TBP, yeast TAF40 interacts directly and specifically with TFIIA. Mutational analyses of the Toa2 subunit of TFIIA indicate that loss of functional interaction between TFIIA and TAF40 results in conditional growth defects and effects in transcription. These results demonstrate that the TFIIA-TAF40 interaction is important in vivo and indicate a functional role for TAF40 as a bridging factor between TFIIA and TFIIID.

Transcription by eukaryotic RNA polymerase II (Pol II) involves the assembly of a preinitiation complex consisting of Pol II and the general transcription factors TFIIA, TFIB, TFIID, TFIIE, TFIIF, and TFIIH (for review, see reference 66). An important step of transcription initiation is the binding of TFIIID to the core promoter. TFIIID, a multisubunit protein complex that is highly conserved among eukaryotes, is composed of the TATA-binding protein (TBP) and over a dozen TBP-associated factors (TAFs) (reviewed in references 26 and 27). TBP mediates promoter recognition through the sequence-specific binding of the TATA element found at many promoters. The importance of the TBP-TATA interaction is illustrated by many studies which demonstrate that recruitment of TBP is a rate-limiting step at a majority of promoters (12, 17, 37, 39, 46, 50).

In yeast, 13 TAFs are required for viability, indicating essential roles for individual TAFs (75, 77; reviewed in reference 26). However, the precise functional requirements for the TAFs remain unresolved. In vitro biochemical experiments suggest that TAFs function in higher eukaryotic systems as obligatory coactivators essential for activator response (reviewed in references 9 and 84). In contrast, functional inactivation and depletion studies with certain TAFs in yeast cells demonstrate that the expression of many genes is unaffected by TAF loss, although TAF inactivation results in distinct cell cycle phenotypes (2, 55, 59, 60, 62, 86). In addition, disruption of the TFIIID complex with a temperature-sensitive mutation in TBP results in gene-specific transcriptional defects (71), and promoter occupancy studies indicate that TAFs are not present on certain transcriptionally active promoters in vivo (45, 49). Promoter-specific requirements for particular TAFs are further illustrated by whole-genome transcriptional profiles. For example, inactivation of TAF145/130 has no effect on the expression of a majority of genes, while transcription of a subset of genes is affected (31). This TAF dependence was mapped to the core promoter (78), indicating important TAF functions in promoter activity in vivo. In contrast to these gene-specific effects, inactivation of several other TAFs, namely TAF17 (2, 59, 61), TAF40 (44), TAF60 and TAF61/68 (59, 62), and TAF23/25 (76), results in dramatic effects on a large fraction of genes transcribed by Pol II. The requirement for these particular TAFs is not yet understood, but it is clear that individual TAFs may be generally required for transcription while others function at a subset of promoters. To complicate the issue further, it is apparent that certain TAFs in both human and yeast systems can be found in large protein complexes distinct from TFIIID, such as the SAGA complex (25) and the SWI/SNF complex (10). Taken together, these studies indicate that different TAFs may have distinct functional roles in transcription, yet the nature of the specific functions for a majority of the TAFs remains to be elucidated.

In addition to TAFs, other transcription factors associate with TBP at the core promoter to mediate transcription. One such factor, TFIIA, has been shown to stabilize the interaction between TBP and DNA at the TATA element (reviewed in reference 27). Mutational studies of both TBP and TFIIA demonstrate the importance of the TFIIA-TBP interaction for transcription in vivo (35, 53, 68, 80). TFIIA has been described as a coactivator, since in vitro functions of certain activators as TFIIA dependent (51, 67), and as an antirepressor, because TFIIA can mediate displacement of certain transcriptional inhibitors that act on TBP (4, 5, 22, 32, 42, 56, 58, 69).

A growing body of evidence suggests that the functions of TFIIA and TAFs are connected. DNase I footprinting experiments show that the addition of TFIIA alters the DNA protection pattern of TFIIID (14, 15, 51). Consistent with these findings, UV-cross-linking experiments indicate that TFIIA induces a conformational change in TFIIID that alters specific TAF interactions with the core promoter (64). Moreover, a set of TFIIA mutations that can form a TBP-TFIIA-DNA complex are defective for forming a complex with TFIIID (69).
Finally, the three-dimensional structure of the human TFIID-TFIIA-TFIIIB complex clearly suggests TFIIA-TAF interactions, since TFIID maps to a large noncentral lobe of TFIIH, with TBP being located more centrally in the structure (1). It is not yet understood how this TFIID-TAF communication is established or which particular TAFs are involved.

In this report, we investigate the importance of the interactions between TFIID and yeast TAFs. We demonstrate a direct interaction between TFIID and TAF40, as well as a direct interaction between TAF40 and TBP. We also find that mutations in TFIID that impair the TFIID-TAF40 interaction result in conditional growth phenotypes and defects in transcription in vivo. These results suggest that TAF40 serves as a link between TFIID and TFIIH functions, and they reveal a new role for TAF40 in RNA Pol II transcriptional regulation.

Materials and Methods

DNA constructs. Activation domain (AD) hybrids were cloned into the 2μ LEU2 marked vector, pACT2.2 (19), which contains the ADH1 promoter, a nuclear localization sequence, the hemagglutinin (HA) epitope, and the Gal4 AD sequences. Two DNA-binding domain (DB) hybrids were constructed by subcloning from the corresponding AD constructs into the pPC97-TRP vector (85) (CEN, TRP3), which contains the ADH1 promoter, a nuclear localization sequence, and the Gal4 DB (residues 1 to 147). The TAF40 Escherichia coli expression plasmid was created by cloning the TAF40 open reading frame into the pET15b vector using PCR and designed oligonucleotides. The E. coli expression plasmids for Toa2 glutathione S-transferase (GST)-TFIIB have been described elsewhere (80). GST-Toa1 was constructed by subcloning an EcoRI fragment containing the open reading frame of Toa1 into the pGEX1T vector (Pharmacia); GST-Toa2 was constructed by PCR of TBP followed by cloning into the GST-2T vector (Pharmacia). TOA2-YCP2 contains TOA2 driven by its native promoter and terminator, which were generated by PCR from genomic DNA. A NcoI site was engineered at the ATG start codon and utilized for inserting six myc epitopes (GEOKLISEEDLN), creating myc-Toa2-YCP2. Site-directed Toa2 mutants were created using oligonucleotide primers containing the desired mutation and PCR. Mutant derivatives were subsequently subcloned into the Gal4-DNA binding domain vector (pPC97-TRP1) and into pET15b (Novagen) lacking the histidine tag. All PCR products were completely sequenced.

Yeast strains. All strains used in the yeast two-hybrid assay were transformants of MaV103 (85). MaV103 contains the GAL1 promoter (with four Gal4 binding sites) fused to the HIS3 promoter and structural gene; GAL4 and GAL80 are both deleted in the strain. Viability tests of TOA2 mutant derivatives were conducted in ROY100, a derivative of KY114 (relevant genotype MATa ade2-101 leu2-3:PET56 trp1 Δura3-52), which was created using a two-step gene knock-out of the complete open reading frame of the TOA2 gene and contains TOA2 on a 2 μm, URA3-marked plasmid. The plasmid shuffle technique, which involves transforming ROY100 with TRP1-marked TOA2 derivatives, followed by selection for loss of the URA3-marked plasmid by growth on 5-fluoro-orotic acid (5-FOA), was used to test the mutant derivatives for viability and to create Toa2 mutant strains for further characterization.

Yeast two-hybrid assays and phenotypic studies. Both Gal4 DB and Gal4 AD plasmids were transformed into the yeast strain MaV103 using a standard lithium acetate transformation. The resulting strains were grown in the appropriate selection media, and 10-fold serial dilutions were performed. Cells were spotted on the appropriate plates, which either contained or lacked 3-aminotriazole (AT), and grown at 30°C for 4 to 7 days. For phenotypic studies, 10-fold serial dilutions of strains were spotted on plates with rich media containing either glucose (yeast-peptone-dextrose [YPD]) or galactose (yeast-peptone-galactose [YPG]), and plates were incubated at either 30 or 38°C.

Protein purification. TFIIA was purified as described previously (74) by expressing the GST-Toa1 and -Toa2 subunits separately in E. coli, denaturing both in 8 M urea, combining the subunits, and dialyzing out the urea. GST-TBP, GST-TFIIB, and GST were expressed and purified from bacteria as described elsewhere (80). TAF40 was purified with a denaturing and refolding protocol similar to that used to make recombinant yeast TFIIA. BL21 (DE3) cells containing TAF40 cloned into the bacterial expression plasmid pET15b (His-TAF40) were grown to an optical density at 600 nm (OD600) of 0.6 and induced for 2 h with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). One liter of cells was harvested, washed with 200 ml of buffer A (20 mM Tris-HCl [pH 7.5], 200 mM NaCl), and resuspended in 25 ml of buffer B (1 mM EDTA, 5 mM dithiothreitol [DTT], 0.05% NP-40), and frozen at −70°C. Cells were thawed and sonicated. The insoluble fraction was collected by spinning at 10,000 × g for 15 min at 4°C. Polysomes were resuspended in 30 ml of buffer C (20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 8 M urea, 5 mM DTT). A 7.5-ml volume of buffer C (20 mM Tris-HCl [pH 7.5], 500 mM NaCl, 1 mM DTT, 10% glycerol) was added slowly, and the solution was cleared by spinning at 10,000 × g for 15 min at 4°C. The supernatant was diazylated against buffer C containing 1 mM imidazole. The diazylated proteins were cleared by spinning at 10,000 × g for 15 min at 4°C. The soluble material was bound to Ni-nitrotriacetic acid resin (Qugen), washed with wash buffer (20 mM Tris [pH 7.5], 100 mM KCl, 10% glycerol, 40 mM imidazole, 1 mM DTT), and eluted with elution buffer (20 mM Tris-HCl [pH 7.5], 100 mM KCl, 10% glycerol, 1 mM DTT, 200 mM imidazole). Elution fractions were diazylated against buffer C containing 20 mM HEPES (pH 7.9), 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 10% glycerol, and 1 mM DTT.

In vitro interaction studies. Approximately 12.5 pmol of GST-fusion protein or GST alone was incubated with 10 to 20 pmol of His-TAF40 protein in 200 μl of binding buffer (20 mM HEPES [pH 7.9], 20 mM Tris [pH 7.5], 200 mM NaCl, 50 mM KCl, 10 mM MgCl2, 0.025% NP-40, 10% glycerol, 0.5 mM DTT) for 3 h at 4°C. Complexes were recovered by incubation with glutathione Sepharose for 1 h at 4°C in binding buffer with 3% bovine serum albumin. Complexes were washed twice in 400 μl of binding buffer, incubated with sodium dodecyl sulfate (SDS) loading buffer, and boiled, and 10 μl of sample was separated by SDS-polyacrylamide gel electrophoresis (PAGE). Gels were analyzed by immunoblotting with antibodies specific to His-TAF40 (Santa Cruz Biotechnology) or GST (Sigma) and visualized by chemiluminescence detection (Pierce).

Electrophoretic mobility shift assays were performed using a 32P-labeled 45-bp fragment containing the adenovirus early 1B TATA box, as described previously (80). Purified recombinant yeast TBP (5 nM), TFIIA or TFIIIB (3 nM), TAF40 (19 to 142 nM), and 100 ng of poly(dG-dC) were incubated at 25°C for 30 min in 20 μl of Tris (pH 7.5), 40 mM HEPES (pH 7.9), 100 mM KCl, 1 mM DTT, 0.5 mM phenylmethylsulfonfyl fluoride, and 10% glycerol. Complexes were separated from unbound DNA by 6% nondenaturing acrylamide gel electrophoresis in 0.5x Tris-borate-EDTA and quantified by phosphorimaging.

Transcriptional analysis. Quantitative S1 nuclease analysis was done as described elsewhere (34), with approximately 30 to 50 μg of RNA. For the temperature shift and AT (Sigma) inductions, cells were grown in synthetic complete medium to an OD600 of 0.5 to 1.0. Cells were pre-heat shocked at 38°C for 15 min and incubated at 30°C for 1 h, followed by 38°C for 1 h. AT was added to a concentration of 20 nm, and cells were incubated for an additional hour at 38°C.

Total RNA was prepared by hot-phenol extraction and was quantitated at OD260. RNA amounts in each reaction mixture were normalized to the levels obtained from a probe to the intron of the tryptophan tRNA gene (rRNA).

Results

Yeast TAF40 associates with TFIID in vivo. TFIIA interacts with TBP and DNA at the promoter and stabilizes the TBP-DNA interaction. Yet, within a cell, TBP associates with TAFs to form the TFIID complex. We used a yeast two-hybrid assay to investigate the potential interplay between TFIIA and TAFs. Yeast TFIIA is composed of two subunits encoded by the genes TOA1 and TOA2, both of which are required for viability (73). The DB of Ga14 was fused in frame with Toa2 (DB-Toa2), creating the bait for the two-hybrid assay. TAF proteins were fused in frame to the Ga14 AD. Fusion proteins were expressed in a yeast strain with the HIS3 gene under the control of the GAL1 promoter (which contains four Gal4 binding sites). Interactions between Toa2 and the AD-fusion proteins were determined by examining activation of the HIS3 gene. HIS3 gene activation was assayed by growth in the presence of AT, a competitive inhibitor of the HIS3 gene product (28). Strains in which the HIS3 gene is highly expressed, due to interactions between the DB-fusion protein and the AD-fusion protein, will grow on AT.

In contrast to a DB fusion to Toa1, which activates transcription (79), expression of DB-Toa2 showed no HIS3 gene
activation (Fig. 1A). The difference between the activity of the two subunits is not understood, but it does provide the opportunity to use DB-Toa2 in the two-hybrid assay. Since DB-Toa2 exhibited a strong interaction with AD-Toa1, DB-Toa2 is not defective for subunit interactions with Toa1, and this also suggests that DB-Toa2 interacts with native Toa1 within the cell. We then tested the ability of DB-Toa2 to interact with all 13 of the known essential yeast TAFs found in the TFIID complex (for review, see references 26 and 27). A strong interaction was observed between Toa2 and TAF40. Because two-hybrid interactions can be indirect and TAF40 is a component of the TFIID complex, it was surprising that DB-Toa2 showed no interaction with the other TAF proteins shown in Fig. 1 (TAF17, TAF19, TAF47, and TAF61) or TAF25, TAF48, TAF60, TAF65, TAF67, TAF90, TAF130, and TAF150/TSM1 (data not shown). The lack of interaction was not due to lack of expression, since each of the TAF fusion proteins was easily detectable by immunoblotting of whole-cell extracts with antibodies specific to the HA tag present in the AD vector (data not shown). These results suggest that TAF40 associates with Toa2 within the cell, perhaps directly.

A reciprocal interaction between TAF40 and TFIIA is a component of the TFIID complex, it was surprising that DB-Toa2 showed no interaction with the other TAF proteins shown in Fig. 1 (TAF17, TAF19, TAF47, and TAF61) or TAF25, TAF48, TAF60, TAF65, TAF67, TAF90, TAF130, and TAF150/TSM1 (data not shown). The lack of interaction was not due to lack of expression, since each of the TAF fusion proteins was easily detectable by immunoblotting of whole-cell extracts with antibodies specific to the HA tag present in the AD vector (data not shown). These results suggest that TAF40 associates with Toa2 within the cell, perhaps directly.

FIG. 1. TFIIA associates with TAF40 in vivo. (A) Two-hybrid assays were used to demonstrate that TFIIA interacts specifically with Toa1 and TAF40. The indicated Gal4 AD fusion proteins were tested for the ability to interact with a DB fusion of Toa2 (DB-Toa2). Approximately 10^4 cells were spotted onto synthetic complete plates containing either 0 or 40 mM AT. Growth on AT is indicative of an interaction between the two hybrid proteins. The bottom panel shows that strains containing AD-Toa1 or AD-TAF40 do not grow on AT with the DB vector alone. (B) TAF40 stimulates transcription in an artificial recruitment assay. DB-TBP, DB-40, and DB-Fos each stimulate high levels of transcription when tethered to a promoter via a heterologous DB.

result is in accord with similar recruitment studies that used other TAFs and TBP (3, 7, 12, 21, 24, 36, 37, 89). TFIIA has been shown to interact with certain ADs (16, 40, 70). A trivial explanation for the interaction detected is that DB-Toa2 interacts with the AD of AD-TAF40, and the TAF40 domain stimulates transcription. To examine this possibility, the Fos C-terminal AD, which strongly stimulates transcription when bound to the promoter via the Ga14 DB (Fig. 1B), was cloned into the Ga14 AD vector (AD-Fos). Expression of the AD-Fos hybrid protein, which has two tandem functional ADs, did not yield a positive two-hybrid interaction with DB-Toa2 (Fig. 1A). The inability of DB-Toa2 to interact with AD-Fos eliminates the possibility that Toa2 is simply interacting with the Ga14 AD of AD-TAF40 and, in effect, mimicking the activation by DB-TAF40 seen in the artificial recruitment assay.

TAF40 interacts with TFIIA and TBP. To demonstrate a direct interaction between TAF40 and TFIIA or TBP, recombinant proteins were produced in bacteria and their ability to physically interact was examined using GST pull-down assays. Immunoblot analysis of the isolated complexes revealed that significant amounts of TAF40 interact with the GST-TFIIA (Fig. 2A). In addition, GST-TBP interacts with TAF40 directly. TAF40 does not interact with either GST-TFIIB or GST.

Electrophoretic mobility gel shift assays were used to test whether the presence of TAF40 affects the TBP-TFIIA-DNA ternary complex. In the absence of magnesium, TBP does not form a stable complex with DNA, and the addition of TFIIA stabilizes the TBP-DNA interaction and shifts the TATA-containing probe (Fig. 2B). Under conditions of subsaturating amounts of TFIIA, the addition of TAF40 resulted in a 20-fold enhancement of the TFIIA-TBP-DNA complex (Fig. 2B). Enhancement of the TBP-TFIIA-DNA complex was not observed when equivalent amounts of bovine serum albumin or buffer were added (data not shown). In addition, TAF40 had no effect on the amount of ternary complex formed with TFIIA (Fig. 2C). Excess TAF40 also resulted in no changes in the DNA-binding properties of TBP, TFIIA, or TFIIB alone, either in the presence or absence of magnesium ions (Fig. 2B and C and data not shown). Thus, enhancement of complex formation by TAF40 is specific to the TBP-TFIIA-DNA ternary complex.

Although TAF40 addition results in a significant enhancement of the TBP-TFIIA-DNA complex, it does not alter the mobility of the complex. This result suggests that either the addition of TAF40 to the complex results in a conformational change that masks the added mass of TAF40, or that TAF40 is not stable to the gel running conditions. We feel that the latter hypothesis is supported by the observation that inclusion of antibodies specific to TAF40 does not result in a shift in the complex size but instead abolishes the ability of TAF40 to enhance TBP-TFIIA-DNA complex formation (data not shown).

Mutations in a hydrophobic patch of TFIIA are defective for interacting with TAF40 in vivo. Analysis of the crystal structures of the yeast TFIIA-TBP-DNA ternary complex (23, 81) reveals several striking features. TFIIA consists of two domains, a β domain and a four-helix bundle (4HB) domain (Fig. 3). The β domain makes all of the contacts with TBP and also binds DNA upstream of the TATA element. The 4HB domain of TFIIA projects away from the TBP-TFIIA-DNA complex
into solution. In addition, there are two large solvent-exposed patches of hydrophobic residues on TFIIA; one patch is within the β domain and the other is within the 4HB domain. Hydrophobic interactions are important for many protein-protein interactions. In fact, the hydrophobic region on the β domain contacts TBP. We hypothesized that the hydrophobic patch on the 4HB domain may contribute to other TFIIA functions, possibly for interactions with TAF40. Three residues within this hydrophobic patch were targeted for mutational studies: isoleucine at position 27 of Toa2 was changed to alanine (I27A) or lysine (I27K); methionine at position 38 was changed to alanine (M38A) or lysine (M38K); and leucine at position 41 was substituted with either alanine (L41A) or aspartic acid (L41D).

Each of the mutant derivatives was cloned into the DB vector. To determine whether any of the mutations causes a drastic change in the folding ability of Toa2, the derivatives were tested for the ability to interact with AD-Toa1 (Fig. 4). DB-L41D was defective for interacting with Toa1, indicating that this Toa2 derivative is compromised for TFIIA formation. The five remaining derivatives were indistinguishable from wild-type Toa2 with regard to their ability to interact with Toa1.

We next tested the Toa2 derivatives for defects in interactions with TAF40. I27A, I27K, and L41D showed significantly weakened interactions with TAF40 in the two-hybrid assay (Fig. 4). Since L41D was also defective for interaction with Toa1, the loss of the TAF40 interaction may be the result of global defects in the structure of this protein. In contrast, substitutions at I27 are specifically defective for TAF40 interactions, in that both I27A and I27K are indistinguishable from wild-type Toa2 for Toa1 interactions. The remaining derivatives exhibited interactions with both TAF40 and Toa1 that were comparable to that with wild-type Toa2. These results suggest that the hydrophobic region on the 4HB domain of TFIIA, in particular residue I27 of Toa2, plays an important role in the interaction between TFIIA and TAF40.

Toa2 derivatives impart mutant growth phenotypes. To examine the physiological relevance of the TFIIA-TAF40 interaction, the I27A and I27K Toa2 mutants (under the control of the TOA2 promoter and terminator) were expressed in a TOA2 deletion strain. Both alleles supported cell viability, but each caused a slow-growth phenotype at 30°C and a temperature-sensitive phenotype at 38°C (Fig. 5A). The doubling time for wild-type cells was 2.5 h, whereas I27A- and I27K-containing cells have a doubling time of 3.5 to 4 h at 30°C (data not shown). The slow-growth phenotype at 30°C is consistent with the observation that the TFIIA-TAF40 interaction is disrupted in the two-hybrid assay, which is performed at this same temperature (30°C). Furthermore, the I27K mutant was unable to support growth on galactose-containing medium, suggesting an inability to respond to the Gal4 activator protein. Mutant phenotypes were not the result of a destabilization of Toa2 protein, since the I27A and I27K strains produced amounts of Toa2 protein comparable to that in wild-type Toa2 at 30 and 38°C, as assayed by immunoblot analyses of whole-cell yeast extracts (Fig. 5B). None of the mutant phenotypes could be suppressed by overexpression of TAF40 (data not shown). This suggests that interactions with TAF40 and the I27A and I27K forms of TFIIA are severely compromised, since simple overexpression is not sufficient to counteract the loss of interaction.

In vitro analysis of the TFIIA mutants I27A and I27K. In order to determine the extent to which the mutations at the I27 residue were defective for the interaction with TAF40 in vitro,
GST pull-down assays were performed. Immunoblot analysis of the recovered complexes showed that TFIIA recombinant proteins containing the I27A and I27K substitutions were significantly decreased in their ability to interact with TAF40 when compared to wild-type TFIIA (Fig. 6A). Moreover, TAF40 binding by I27K was indistinguishable from the binding observed with GST alone. These results coincide with those of the two-hybrid studies, since in both the two-hybrid assay and the pull-down assay, I27K displayed a more dramatic defect in interacting with TAF40. In contrast, TFIIA substituted with I27A or I27K is fully functional in forming the TBP-TFIIA-DNA complex (Fig. 6B). In addition, both I27A- and I27K-substituted TFIIA were competent for complex enhancement by TAF40 (Fig. 6B). This result is not unexpected, since the direct interaction between TBP and TAF40 (Fig. 2) may compensate for the loss of interaction between TAF40 and TFIIA.

TFIIA mutants are defective for transcription in vivo. To determine the ramifications of a defect in the TFIIA-TAF40 interaction, we compared wild-type, I27A, and I27K strains for their transcriptional competency. Constitutive transcription of a collection of Pol II-transcribed genes was examined at 30 and 38°C (Fig. 7). When cultured at 30°C, the I27A and I27K strains exhibited a significant reduction in HIS3 gene expression compared to expression in wild-type cells. Levels of transcription of both the +1 and +13 transcripts of HIS3 were decreased. The +1 transcript is generated from a noncanonical promoter element, while the +13 transcribed is derived from a conventional TATA element (13, 33). This suggests that the TAF40-TFIIA interaction is important for transcription from both canonical and noncanonical promoters in vivo. RPS4, DED1, HTA2, and PGK1 mRNA levels also decreased at both 30 and 38°C. The decreases in transcription at the permissive temperature (30°C) are consistent with the fact that the TAF40-TFIIA interaction defect is observed at 30°C in the two-hybrid assay. The transcriptional effects on expression of...
RPS4, HTA2, and DEDI in strains with defective TFIIA-TAF40 interactions are consistent with similar observations on the expression of these genes in a TAF40 mutant strain (44). In contrast to the genes described above, transcription from the ENO2 gene was unaffected. Thus, requirement for the TFIIA-TAF40 interaction is promoter specific. Promoter-specific dependency on TAF40 is also supported by chromatin immunoprecipitation studies, which showed TAF40 occupancy can vary on transcriptionally active promoters (45).

We also tested the ability of the I27A and I27K strains to respond to acidic activators. Gcn4-dependent activation of HIS3 transcription was assayed by growing the cells in AT, a competitive inhibitor of the HIS3 gene product (Fig. 8A). To examine the response at the restrictive temperature, cells were incubated at 38°C for 1 h, AT was added, and the cells were incubated for an additional hour before harvesting. For both the I27A and I27K mutants, activation of HIS3 transcription was decreased compared to that in wild-type cells. Activation by the acidic activator Gal4 was determined by growing cells in galactose-containing medium and assaying for GAL1 transcript levels (Fig. 8B). Both the I27A and I27K mutants displayed a significant decrease in GAL1 transcription. Taken together, these defects in both constitutive and induced transcription for the I27A and I27K derivatives of Toa2 indicate that the TAF40-TFIIA interaction plays an important role in transcription in vivo.

TFIIA mutants defective for interactions with both TAF40 and TBP are not viable. Combining previous work and the results presented above, we conclude that TFIIA interacts with TFIID via TAF40 and TBP. To determine how critical the contacts with TAF40 and TBP are for TFIIA functions in vivo, mutations defective for the TFIIA-TAF40 interaction and the
TFIIA-TBP interaction were engineered into the same Toa2 allele. TFIIA substituted with a Y69A mutation in Toa2 is not competent for forming the TBP-TFIIA complex in vitro and results in a temperature-sensitive phenotype and transcriptional defects in vivo (68). We tested the hypothesis that TFIIA interactions with TFIID are essential by combining mutations in Toa2 at either I27A or I27K (which are TAF40 defective) with Y69A (which is TBP defective). While the I27A, I27K, and Y69A single mutants were able to support cell viability, both the I27A/Y69A and I27K/Y69A double mutants were unable to support cell viability in a strain deleted for TOA2 (Fig. 9). Thus, TFIIA interactions with TFIID via TBP or TAF40 are essential for cell survival.

**DISCUSSION**

**TFIIA and TAF40 interact directly.** The ability of TFIIA to interact with TBP and stabilize it on a promoter is well characterized, but how TFIIA communicates with TBP in the context of TFIID remains unclear. TFIIA is generally required for transcription in the presence of TAFs, yet it is uncertain how the functions of TFIIA and TAFs are connected. We report here that an important link between TFIIA and TFIID resides in TAF40. The interaction between TFIIA and TAF40 was observed both in vivo and in vitro. Thus, the TFIIA-TAF40 interaction may be directly involved in processes that are TFIIA and TAF dependent.

We also identified a direct interaction between TAF40 and TBP. TAF-TBP interactions have been identified in other organisms (29, 30, 41, 57, 63, 82, 87, 88). In addition, a 100-amino-acid fragment of yeast TAF130/145 has been shown to interact with TBP (6, 42), and the conserved C-terminal domain of yeast TAF68 interacts with TBP (75). However, TAF40 is the first full-length TAF that has been shown to interact directly with yeast TBP in solution. A TBP-TAF40 interaction is consistent with studies that show the human homologue of TAF40, human TAF28, interacts with human TBP (47, 48, 57) and indicates that this interaction is conserved from yeast to humans. Human TAF28 has also been shown to interact with human TAF18, TAF55, TAF100, TAF135, and the viral activator Tax (8, 11, 18, 47, 48, 57). Taken together with our studies, these results suggest that TAF40 may have critical functions in TAF-TAF, TAF-TBP, and TAF-TFIIA interactions.

**Mutations in TFIIA affect the interaction with TAF40.** The functional importance for the TFIIA-TAF40 interaction is supported by mutational studies of the Toa2 subunit of TFIIA in the 4HB domain of TFIIA. Mutations at the I27 residue of Toa2 (I27A and I27K) caused a defect in the interaction
Toa2 is conserved in yeast, an important target that facilitates formation of an active transcriptional activator in vitro (54). Taken together with our results, this suggests that the 4HB domain of TFIIA is a functionally important target that facilitates formation of an active transcription initiation complex. Since the isolectric point at position 27 of Toa2 is conserved in yeast, Drosophila, and human TFIIA (23), this surface may play a critical role in the interaction of the higher eukaryotic homologues of TFIIA and TAF40. It is interesting to speculate that an interaction between TFIIA and TAF40 would orient the TAF40-TBP interaction on the N-terminal repeat of TBP. The surface of TBP situated on the same side of the structure as I27 is the substrate for various TAF DNAs. It has also been shown that an interaction between TAF40 and TFIIA is a first step in determining the TFIID complex downstream of the transcriptional start site and alters the cross-linking pattern of several TAFs to promoter sequences (14, 15, 20, 64). It has also been shown that an interaction between TFIIA and TFIID results in the generation of a productive form of TFIID that is capable of stably interacting with the promoter (72). Moreover, under certain conditions, TAFs have been shown to inhibit the ability of TBP to bind DNA (42, 43, 63, 83). Yet the addition of TFIIA reverses this TAF inhibition (42, 69).

The identification and characterization of an interaction between TAF40 and TFIIA is a first step in determining the mechanistic requirement for TAF40 in transcription. It is clear that TAF40 performs an essential and nonredundant function in yeast cells, since it is required for cell viability (38) and TAF40 inactivation appears to affect transcription from RNA Pol II promoters in yeast (44, 61). To date, TAF40 appears to be a component specific to the TFIID complex (25, 44, 62, 65), and thus the TFIID-TAF40 interaction has the potential to serve as a critical link between TFIIA and TFIID functions in vivo.

ACKNOWLEDGMENTS

This work was supported by NIH grant GM56884 to L.A.S. We are indebted to Kevin Lumb for the Fos AD DNA and for critical reading of the manuscript. We also thank Zarmik Moqtaderi for various TAF DNAs.

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