

## Mapping and Functional Characterization of the TAF11 Interaction with TFIIA

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**TFIIA interacts with TFIID via association with TATA binding protein (TBP) and TBP-associated factor 11 (TAF11). We previously identified a mutation in the small subunit of TFIIA (toa2-I27K) that is defective for interaction with TAF11. To further explore the functional link between TFIIA and TAF11, the toa2-I27K allele was utilized in a genetic screen to isolate compensatory mutants in TAF11. Analysis of these compensatory mutants revealed that the interaction between TAF11 and TFIIA involves two distinct regions of TAF11: the highly conserved histone fold domain and the N-terminal region. Cells expressing a TAF11 allele defective for interaction with TFIIA exhibit conditional growth phenotypes and defects in transcription. Moreover, TAF11 imparts changes to both TFIIA-DNA and TBP-DNA contacts in the context of promoter DNA. These alterations appear to enhance the formation and stabilization of the TFIIA-TBP-DNA complex. Taken together, these studies provide essential information regarding the molecular organization of the TAF11-TFIIA interaction and define a mechanistic role for this association in the regulation of gene expression in vivo.**

Transcription by RNA polymerase II (Pol II) requires the cooperative interaction of multiple proteins to facilitate the assembly of a preinitiation complex (PIC) at the core promoter. The PIC comprises Pol II and the general transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (reviewed in reference 19). A fundamental and highly regulated step in PIC assembly is the recognition of the core promoter by the general transcription factor TFIID, which is a multiprotein complex comprised of TATA binding protein (TBP) and approximately 14 TBP-associated factors (TAFs) (1, 6, 18). TFIID provides multiple surfaces to mediate protein-protein interactions as well as interactions with the promoter DNA (48, 49). TBP binds the TATA element through sequence-specific contacts, and TAFs can moderate TBP affinity and specificity for core promoters through selective interactions with promoter sequences and/or other components of the transcription machinery (5, 6, 11, 28, 47, 56).

In *Saccharomyces cerevisiae*, promoters have been grouped into TAF-dependent and TAF-independent classes, based on their requirement for TAFs (29, 34). TAF-dependent promoters require TAFs for transcription, and TAFs are present at levels comparable to those of TBP on these promoters. In contrast, TAF-independent promoters do not require TAFs for activity, and TAF levels on these promoters are far below the level of TBP. Interestingly, approximately 84% of yeast genes are dependent upon one or more TAFs, whereas 16% of yeast genes are TAF independent (50). Taken together, these

results support the idea that individual TAFs play distinct roles and have selective functions in transcriptional processes.

The transcription factor TFIIA also influences steps critical for transcription initiation, but unlike TAFs, TFIIA is present at all promoters with an occupancy that correlates strongly with TBP (29). TFIIA enhances PIC assembly by increasing the affinity of TBP for DNA (4, 21, 24, 32) and stabilizing the TBP-TATA association through direct interactions with both DNA and TBP (16, 54). Mutational studies of both TFIIA and TBP clearly demonstrate the importance of the TFIIA-TBP interaction for transcription in vivo (24, 36, 43, 52). In addition to direct interaction with TBP, TFIIA can affect transcriptional processes via association with TFIID and/or TAFs (9, 26, 35, 41, 44, 48, 60), and TFIIA can stimulate the formation of the TFIID-DNA complex through interactions with regulatory proteins (10, 23, 25, 35, 51, 59, 60). Since not all promoters are TAF dependent but TFIIA is generally required, this suggests that the mechanism of action of TFIIA may be divided into TAF-related and TAF-unrelated functions.

TFIIA interacts directly with TAF11 (28). Here, we present biochemical and genetic evidence that further defines the functional interaction between TAF11 and TFIIA. Our studies demonstrate that two distinct regions of TAF11 are involved in the interaction with TFIIA. Both the structurally conserved histone fold domain and the N-terminal region of TAF11 play a role in associating with TFIIA in vivo. We show in vitro that TAF11 imparts changes to TFIIA-DNA and TBP-DNA interactions, which result in the stimulation of overall complex formation. Moreover, TAF11 promotes stable association of the TFIIA-TBP-DNA complex under nonoptimal conditions. Taken together, these results provide functional information about the unique role of TAF11 as a bridging factor that aids in stabilization of the TFIIA-TBP-DNA complex.

### MATERIALS AND METHODS

**Yeast strains.** All *Saccharomyces cerevisiae* strains used in the yeast two-hybrid assays for genetic selection of compensatory mutants and interaction studies were transformants of either MaV103 (57) or CG1945 (13). Both strains contain

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the *HIS3* reporter but in different promoter contexts (61). The compensatory interaction in vivo was assayed in a *TOA2* deletion strain expressing the *Toa2* derivative, *toa2-I27K*, described previously (28). The *toa2-I27K* strain was modified by integrating *taf11-E182G* at the chromosomal locus of *TAF11*. Viability testing, phenotypic characterization, and transcription analysis of *TAF11* mutant derivatives were conducted with YSB366, a derivative of YSB373, (relevant genotype, *MATa ura3-52 leu2 his3Δ200 taf11Δ::LEU2*) (27). Coimmunoprecipitation experiments were performed with YSB366, which was modified by chromosomal integration of sequences encoding the hemagglutination (HA) epitope at the 3' end of the coding sequence for *TAF1*. The HA tag was amplified from the pFA6a-3HA-TRP1 construct with corresponding primers, as previously described (37).

**DNA constructs.** Gal4 activation domain (AD) hybrids of *TAF11* site-directed mutant derivatives were constructed with primers designed to incorporate nucleotide changes and subcloned with in vivo recombination to the 2 μm *LEU2* marked vector pACT2.2-*LEU2* described previously (12, 28, 61). All PCR-derived AD plasmids were completely sequenced. The Gal4 DNA binding domain (DB) hybrid constructs used in this study, DB-*toa2-I27K*, DB-*Toa2*, and DB-*TAF13*, were constructed as previously described (28, 61) with the pPC97-*TRP1* vector (57). For *TAF11*-YCP22 constructs, *TAF11* derivatives were subcloned from AD constructs to the YCP plasmid containing the *TAF11* native promoter and terminator generated by PCR from genomic DNA. An *EcoRI* site was engineered at the ATG start codon and utilized for inserting three *myc* epitopes (GEOQLISEEDLN), creating *myc-TAF11*-YCP22.

**Isolation of *TAF11* compensatory alleles.** The *TAF11* gene was randomly mutagenized by PCR-based misincorporation as described previously (38). With the pACT2.2-*TAF11* (AD-*TAF11*) plasmid as the template, primers were used to amplify the entire *TAF11* open reading frame. The amplified products contained approximately 50 bp of 5' and 3' homologous sequence to allow for in vivo recombination to the pACT2.2-*LEU2* (AD) vector. The mutant library was cotransformed with the *NdeI*-*BamHI*-gapped pACT2.2-*LEU2* (AD) vector into the yeast strain MaV103 expressing the *Toa2* derivative, *toa2-I27K*, fused to the Gal4 DB (DB-*toa2-I27K*). Transformants were plated to synthetic complete media lacking tryptophan and leucine. Compensatory alleles were selected by replica plating to synthetic media containing 20 or 40 mM 3-aminotriazole (AT). AD plasmids from strains exhibiting growth on AT were recovered and retransformed into the strain expressing DB-*toa2-I27K* to confirm linkage of the plasmid to AT resistance.

**Two-hybrid assays and phenotypic studies.** Gal4 DB plasmids and Gal4 AD plasmids were transformed into yeast strains CG1945 or MaV103 by standard lithium acetate transformation. The resulting strains were spotted in 10-fold serial dilutions or streaked onto the appropriate selection medium that either contained or lacked AT and grown at 30°C for 4 to 7 days. For phenotypic studies, YCP22-*TAF11* derivatives were transformed to *TAF11* deletion strain YSB366. Viable strains were streaked to rich medium containing glucose (YPD) and incubated at either 30 or 38°C.

**In vitro interaction studies.** In vitro interactions with glutathione *S*-transferase (GST) fusion proteins were performed exactly as described previously (28). Proteins were detected by immunoblot with antibodies specific to the His tag (Santa Cruz) or GST (Sigma) and visualized by chemiluminescence (Pierce).

**Coimmunoprecipitation assays.** Coimmunoprecipitation experiments were essentially performed as described previously (14, 40) with a few modifications. *TAF11* deletion strain YSB366 was modified by integration of a PCR-derived HA tag to the C terminus of the chromosomal copy of *TAF1*. Modified *TAF11* deletion strains were transformed with *myc-TAF11*-YCP22 constructs. Strains expressing *myc*-tagged *TAF11* derivatives were grown in rich medium containing 2% glucose to an optical density at 600 nm, equal to approximately 1.0. Protein extracts were prepared immediately and precleared with 50 μl of protein A-Sepharose beads (Pharmacia) for 1 h at 4°C. Anti-TBP or anti-*myc* antibodies were coupled to protein A-Sepharose beads, and protein extracts were incubated with 50 μl of the antibody-coupled beads at room temperature for 2 h. Following six washes, the beads were boiled in loading buffer, and 15 μl was loaded for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting with antibodies directed at HA or *myc* epitopes.

**Transcription analyses.** Quantitative *S1* nuclease assays were conducted as previously described (22, 28) with approximately 25 μg of RNA. Total RNA was prepared by hot phenol extraction and quantitated spectrophotometrically at an optical density at 260 nm. Cells were cultured at 30°C in rich medium containing 2% glucose to mid-log phase and harvested. When a temperature shift was involved, cultures were made heat tolerant by heat shock incubation at 38°C for 15 min and then returned to 30°C for 1 h. After 1 h at 30°C, cultures were moved to 38°C and incubated for an additional hour. To activate transcription of the *HIS3* gene, 20 mM AT was added to cells in early log phase and cultures were

incubated for 1 h before harvest. Transcript levels were quantitated by phosphorimaging and normalized to the level of tRNA<sup>val</sup>, which is unaffected in *TAF11* derivative strains.

**Site specific protein-DNA photo-cross-linking.** Twelve radiolabeled *HIS3* templates containing a photoactivatable cross-linking agent at a specific site within the promoter were prepared as follows. Oligodeoxyribonucleotides containing a phosphorothioate at the third nucleotide from the 5' end were derivatized with *p*-azidophenacyl bromide as described previously (30). Derivatized oligodeoxyribonucleotide primers were phosphorylated with [ $\gamma$ -<sup>32</sup>P]ATP and annealed to a 76-bp complementary oligodeoxyribonucleotide encompassing the *HIS3* promoter. Double-stranded templates were prepared by primer extension, followed by ligation. Binding reaction mixtures contained 30 fmol of derivatized promoter fragment and purified, recombinant proteins as follows: TBP (0.5 nmol), TFIIA (1.1 nmol), and *TAF11/TAF13* (4.2 or 2.1 nmol) in 18 μl of 20 mM Tris (pH 7.5), 40 mM HEPES, 100 mM KCl, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 10% glycerol. Reaction mixtures were incubated with DNA templates for 30 min at 25°C. Irreversible protein-DNA cross-links were induced by irradiation for 4 min with UV light. Nucleoprotein complexes were digested with *S1* nuclease and electrophoresed on SDS-16% PAGE gels. Gels were dried, and protein-DNA cross-links were visualized by phosphorimaging.

**Electrophoretic mobility shift assays (EMSAs).** Binding reaction mixtures contained 0.1 pmol of a 23-bp <sup>32</sup>P-labeled *HIS3* promoter fragment containing the sequences 5' AATTCCTATAAAGTAATGTGGAG 3' or 5' AATTCCTATAAAGTAATGTGGAG 3' (53), purified recombinant yeast TBP (0.3 nmol), GST-tagged TFIIA (0.6 nmol), *TAF11/TAF13* (4.2 nmol), and 100 ng of poly-(dGdC). Reaction mixtures were incubated at 25°C for 30 min in 20 μl of 20 mM Tris (pH 7.5), 40 mM HEPES, 100 mM KCl, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 10% glycerol. Complexes were separated on a 5% acrylamide gel containing 50 mM Tris-borate and 1 mM EDTA and quantified by phosphorimaging. For stability studies, TFIIA-TBP-DNA and *TAF11/TAF13*-TFIIA-TBP-DNA complexes were allowed to reach equilibrium for 30 min and then challenged with 1,000-fold molar excesses of the specific competitor poly(dAdT) for the specified amount of time. Samples were resolved on 5% acrylamide gels, and complexes were examined by phosphorimaging.

## RESULTS

**Genetic selection for *TAF11* compensatory mutants that restore interaction with TFIIA.** Previously, we identified mutations in *TOA2* (which encodes the small subunit of TFIIA) that disrupt interaction with *TAF11* (28). Specifically, a *toa2* allele containing a lysine substitution at isoleucine 27 (*toa2-I27K*) is defective for interaction with *TAF11*. The location of the I27 residue within the four-helix bundle domain of *Toa2*, which is a region that projects away from the TBP-TFIIA-DNA interface, indicates that this region of TFIIA is important for interaction with *TAF11*. To define the surfaces of *TAF11* required for interaction with TFIIA, we employed the *toa2-I27K* allele in a genetic screen to isolate compensatory mutants in *TAF11*. The goal of the genetic selection was to identify mutations in *TAF11* that restore interaction with *toa2-I27K*. Analysis of the location of the compensatory mutations would reveal the surface(s) of *TAF11* required for interaction with TFIIA. We used a two-hybrid assay to exploit the loss of interaction exhibited by *toa2-I27K* to select for *TAF11* alleles that compensate for the *toa2-I27K* interaction defect. Although the artificial nature of the two-hybrid assay may magnify or diminish protein-protein interactions, the use of this assay allowed direct isolation of compensatory alleles. Moreover, this assay does not require compensatory alleles to support cell viability, since the two-hybrid assay is performed in strains expressing wild-type *TAF11* and TFIIA. Using the criterion of gain of function for the interaction between *toa2-I27K* and *TAF11* derivatives generated under mutagenic PCR conditions (see Materials and Methods), 20,000 transformants

TABLE 1. TAF11 compensatory mutant classes

Class	Interaction			Total
	toa2-I27K <sup>a</sup>	Toa2 <sup>b</sup>	TAF13 <sup>c</sup>	
WT	–	+	+	
I	+	+	+	54
II	+	–	+	3
III	+	+	–	18
Total				75

<sup>a</sup> Indicates interactions with strains expressing Gal4 DNA binding domain fusion to toa2-I27K.

<sup>b</sup> Indicates interactions with strains expressing Gal4 DNA binding domain fusion to Toa2.

<sup>c</sup> Indicates interactions with strains expressing Gal4 DNA binding domain fusion to TAF13.

were analyzed and a total of 75 independent compensatory alleles were selected.

**Compensatory mutations differentially effect TAF11 interactions with Toa2 and TAF13.** Gain-of-function mutations in TAF11 for interaction with toa2-I27K could potentially be detrimental to interactions with its dimerization partner TAF13 or with wild-type Toa2. As such, and to further categorize each of the 75 compensatory alleles, we assessed their ability to maintain wild-type interactions with TAF13 and Toa2 (Table 1). More than 70% of the alleles were competent for interaction with both TAF13 and Toa2 (class I). This indicates that certain compensatory mutations that can accommodate interaction with toa2-I27K have no significant effect on interactions with TAF13 or Toa2. Class II, representing 4% of the isolated alleles, contains mutations that disrupt interaction with Toa2 but have no effect on TAF11 interaction with TAF13. Conversely, 24% of the compensatory alleles (class III) exhibit loss of interaction with TAF13 but have no effect on interaction with Toa2. The differential interaction profile exhibited by these compensatory classes (and especially since none were defective for interaction with both Toa2 and TAF13) suggests that the TAF11 interaction with Toa2 is mediated by a separate surface than TAF13.

**A single substitution in TAF11 can confer compensatory interaction with toa2-I27K in vivo.** To identify the mutations in TAF11 that allow interaction with toa2-I27K, 30 of the 75 isolated alleles were sequenced. Each compensatory allele contained multiple amino acid substitutions randomly distributed throughout the open reading frame of TAF11 (Table 2). Despite this complexity, a few substitutions were common to many of the alleles in the collection. Approximately 60% of the alleles contained a mutation at the glutamic acid residue at position 182 (E182). Other amino acids frequently mutated were lysine 46 (K46), phenylalanine 171 (F171), and isoleucine 178 (I178). To determine if substitutions at these specific residues contribute to the compensatory interaction with toa2-I27K, site-directed point mutations were constructed, and each was independently assayed for interaction with toa2-I27K by the yeast two-hybrid assay. Mutation of E182 to alanine or glycine conferred a strong compensatory interaction with toa2-I27K (Fig. 1A). A TAF11 derivative with residue I178 mutated to methionine could also interact with toa2-I27K, although to a weaker extent. TAF11 derivatives harboring K46R or F171V were not competent for the compensatory interaction, indi-

cating that alleles containing these substitutions require additional mutations to interact with toa2-I27K. Each of the TAF11 derivatives was expressed at levels similar to those of full-length TAF11, as detected by immunoblotting with the HA tag (Fig. 1B).

To further confirm a direct interaction between the compensatory mutant E182G and TFIIA containing the toa2-I27K substitution in vitro, recombinant proteins were produced in bacteria, and interactions were examined by GST pull-down assays. Immunoblot analyses of recovered complexes demonstrated that the TAF11 protein derivative E182G had a gain of function for interaction with mutant TFIIA (GST-toa2-I27K), whereas wild-type TAF11 was defective for interaction with mutant TFIIA (Fig. 1C). In addition, E182G interacted with wild-type TFIIA (GST-Toa2). This result is consistent with further two-hybrid analyses shown below (Table 3).

We previously demonstrated that cells expressing toa2-I27K have a temperature-sensitive (TS) phenotype. Although TFIIA

TABLE 2. Amino acid substitutions in TAF11 compensatory alleles

Class	Allele	Mutations <sup>a</sup>
I	1	D9G F139S <u>E182G</u> Y322C
	2	I19T K29R <u>K46R</u> N59S K66R I90V I95T V141A <u>F171S</u> I178K N262D Y322C
	3	T2A <u>E182G</u> S236T L264C
	4	F26L I82T T145A <u>E182G</u> K212R F222L
	5	Q77H N83S K153R N161D V175A <u>E182V</u>
	6	K68E N192H
	7	K66R I90V K133E N227D
	8	N15S M31T Y67H N83S Q123L S157G Q278L S307C E317G N331S
	9	Q135R <u>E182G</u> D327G
	10	<u>K46R</u> K63R <u>I178V</u> T257A K268R K281E Q294R
	11	K84R <u>E182G</u>
	12	Q34H K71R K125R N130D <u>E182K</u> K287R F345L
	13	Y64C G181A <u>E182G</u> L290R L392S
II	14	V35A F142L <u>V170A</u> <u>F171V</u> <u>E182K</u> Q229R K255R D279G
	15	<u>Q40L</u> <u>I78M</u>
	16	K84I <u>E182G</u> S223P N227S Y230F
III	17	E122G F142L <u>V170A</u> <u>F171V</u> <u>E182V</u> Q232R E252K E339G F345L
	18	E38G K68E F124Y K133N S165G D250G
	19	I74V I82T Q97R L155Q <u>F171S</u> N227S D233G S238G D249G Q266R K281R N307D
	20	I74V L118P <u>F171S</u> Y179H E185V N192D K297E N307D T328A
	21	<u>K46R</u> I57N E87D F91L E122G V128A N130I L172R F222S
	22	<u>K46R</u> N109S E112G N161D N167D G176R K212E Q294L Y322C
	23	S56P E140G V152A <u>E182G</u> K205R N306D
	24	T28A I57T N62S <u>E182V</u> N227D Q337R
	25	Y64H S81P I95V <u>F171L</u> <u>E182G</u> L329C
	26	N148D <u>F171L</u> <u>E182V</u> Q337R
	27	I82T <u>F171L</u> <u>E182G</u> S223P D242G S273P
	28	F91A K125E V152D I224T
	29	F91S F142S T150A K177E <u>E182G</u> Q228R
	30	N88D Q135R V152G N192D L284P
	31	I19M <u>K46R</u> K71R L131S F142L S146P <u>E182G</u> I184T V239A E257G N307D N331V

<sup>a</sup> Substitutions are listed in the single-letter amino acid code. Wild-type amino acid residue numbers are followed by the mutant amino acid. Common amino acid substitutions are underlined.

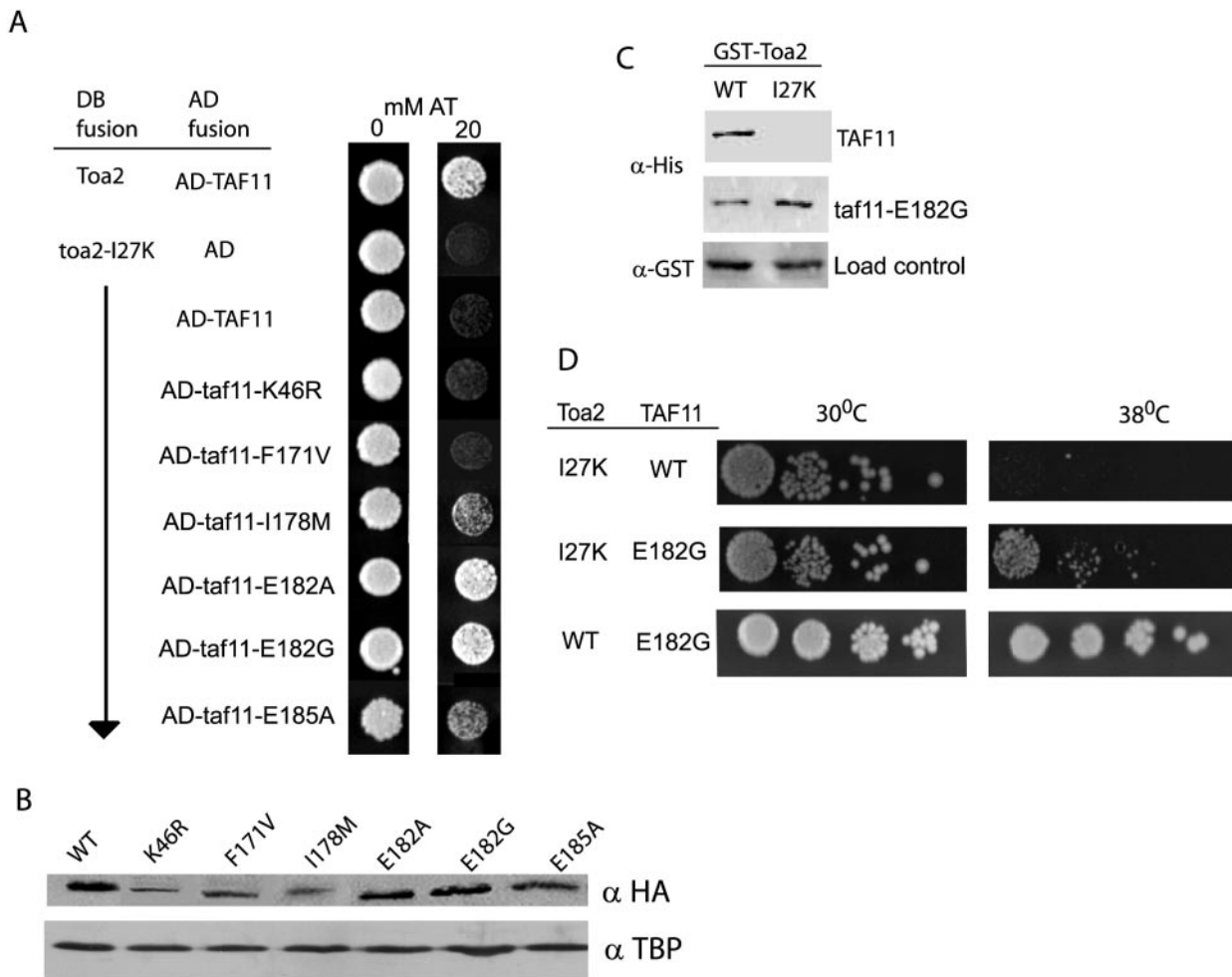


FIG. 1. Characterization of TAF11-Toa2 compensatory mutants. (A) Yeast two-hybrid assays showing representative TAF11 derivatives and their competency for interaction with *toa2*-I27K. Approximately  $10^4$  cells of each strain containing the indicated DB and AD fusion were spotted onto synthetic complete plates with 0 or 20 mM AT. Growth on AT is indicative of an interaction between the two-hybrid proteins. (B) Expression levels of AD-TAF11 fusions. Extracts were prepared from strains containing the indicated TAF11 derivative, and immunoblot analyses were performed with antibodies for HA to detect the AD fusion or TBP (load control). (C) The TAF11-Toa2 compensatory interaction is observed in vitro by a GST pull-down assay. Recombinant GST-TFIIA formed with GST-*toa2*-I27K or GST-*toa2* (wild type [WT]) were incubated with recombinant histidine-tagged wild-type TAF 11 or taf11-E182G. Protein complexes were isolated by incubation with GST, followed by washing. Samples were separated by SDS-PAGE and analyzed by immunoblotting with antibodies specific to the histidine tag of TAF11 or taf11-E182G. Immunoblotting with antibodies specific for GST was performed to assay the amount of GST fusion proteins recovered. (D) The TAF11-Toa2 compensatory interaction is observed out of the context of the yeast two-hybrid assay. Serial dilutions of strains expressing the indicated derivatives were spotted onto the appropriate selection medium and incubated at 30 or 38°C.

is likely to have TAF-dependent as well as TAF-independent functions, it is possible that the TS phenotype may be due to loss of interaction with TAF11. To determine if TAF11 compensatory mutations could function outside the context of the two-hybrid assay to suppress the TS defect, we integrated the mutation exhibiting the strongest compensatory interaction (E182G) at the chromosomal locus of TAF11 in a yeast strain expressing the *Toa2* derivative, *toa2*-I27K. Strains expressing *toa2*-I27K with wild-type TAF11, wild-type TOA2 with taf11-E182G, or both mutants (*toa2*-I27K with taf11-E182G) were spotted onto the appropriate selection medium and incubated at 30 or 38°C (Fig. 1D). All of the strains exhibited growth at 30°C. Consistent with our previous observations, the yeast strain expressing *toa2*-I27K did not grow at 38°C; however, when taf11-E182G was coexpressed with *toa2*-I27K, growth

was restored at 38°C. Thus, we conclude that the TAF11 mutation E182G confers a functional compensatory interaction with *toa2*-I27K in vivo, resulting in the suppression of the TS phenotype.

Strains expressing *toa2*-I27K are also unable to grow on galactose as an alternative carbon source (28). Although the taf11-E182G derivative suppresses the TS phenotype of *toa2*-I27K strains, it was unable to suppress the galactose phenotype (data not shown). This result is not entirely unexpected, since the *GAL* genes have been demonstrated to be TAF independent (34, 39). As such, transcriptional defects associated with *toa2*-I27K at these promoters are likely to reflect TAF-unrelated functions of TFIIA.

**Compensatory mutations map to a solvent exposed surface of TAF11.** Since TAF11 is highly conserved throughout evolu-

TABLE 3. TAF11 derivative summary

Mutant	Interaction <sup>a</sup>			Phenotype <sup>b</sup>
	I27K	Toa2	TAF13	
WT	-	+	+	WT
Q40L	-	-	+	WT
K46R	-	-	+	WT
V170A	-	-	+	WT
V170K	-	-	+	Lethal
F171V	-	-	+	WT
I178M	+	+	+	WT
E182G	++	+	+	WT
E185A	+	+	+	WT
Q40L F171V	-	-	+	WT
K46R F171V	-	-	+	WT
taf11-14	+	-	+	Slightly TS
taf11-15	+	-	+	WT
taf11-16	+	-	+	Slightly TS
taf11-ΔC	-	-	-	Lethal
taf11-ΔHFD	-	-	-	Lethal
taf11-ΔN	+	-	+	TS
taf11-ΔN E182G	-	-	+	Lethal
taf11-ΔN E182A	-	-	+	Lethal

<sup>a</sup> The indicated strains expressing Gal4 DNA binding domain fusions were tested for interaction with site-directed TAF11 mutants fused to Gal4 activation domain fusions by the two-hybrid assay. +, positive two-hybrid interaction; -, no two-hybrid interaction was detected.

<sup>b</sup> Yeast strains expressing the indicated TAF11 derivatives on a plasmid were expressed in a TAF11 deletion strain and assessed for conditional growth phenotypes at 15 and 38°C and on medium containing galactose, glycerol, polyethylene glycol, raffinose, inositol, sorbitol, diamide, benomyl, cadmium, and hydrogen peroxide. Lethal, TAF11 derivative did not support cell viability; TS, inability to grow at 38°C; WT, wild type.

tion, we used the three-dimensional structure of the human TAF11/TAF13 heterodimer (3) as a template to model the location of residues involved in the compensatory interaction. The residues E182 and I178 map to a solvent exposed surface within the  $\alpha$ -2 helix of the histone fold domain, a surface not involved in dimerization with TAF13 (Fig. 2). To further implicate the involvement of this surface, we tested whether a point mutant containing E185A, a residue whose location flanks that of E182, could confer compensatory interaction with *toa2*-I27K. As predicted, a mutant containing E185A interacted weakly with *toa2*-I27K, just as I178M does (Fig. 1A). Therefore, mutations in residues located on this surface can accommodate interaction with *toa2*-I27K.

If the TAF11 interaction with Toa2 requires a separate surface than that for TAF13, then we would predict that the compensatory mutations E182G, I178M, and E185A would have no effect on TAF13 interaction. To test this hypothesis, we assayed our panel of site-directed point mutants for interaction with TAF13 and Toa2 in the yeast two-hybrid assay (Table 3). Consistent with the prediction, all of the point mutants conferring compensatory mutations were competent for interaction with TAF13.

The analysis of overrepresented mutations also identified noncompensatory substitution F171V, which resulted in loss of interaction with wild-type Toa2 (with no change in TAF13 interaction) (Table 3). In addition, the substitution of alanine for valine at residue 170 (also represented in our allele collection) disrupted interaction with Toa2 without affecting TAF13 interaction (Table 3). This indicates that certain substitutions on the surface of the  $\alpha$ -2 helix are capable of disrupting

TAF11-TFIIA interaction specifically. Moreover, when lysine was substituted for valine at residue 170, the cells were nonviable. Based on these results, we conclude that the region encompassing residues V170, F171, I178, E182, and E185, which is within the  $\alpha$ -2 helix of the histone fold domain, defines a distinct region of TAF11 important for interaction with Toa2. Since this surface does not spatially overlap with the TAF13 interaction surface in the three-dimensional structure, these results also support the hypothesis that TAF11 may interact with TFIIA and TAF13 simultaneously.

**Analysis of TAF11 compensatory mutants reveals a second region required for interaction with TFIIA.** The analysis of the TAF11 compensatory mutations clearly implicate the  $\alpha$ -2 helix of the histone fold domain as an important interaction surface for the TAF11-TFIIA association; however, it is possible that other regions of TAF11 are required to facilitate the interaction. Since mutants that disrupt interaction with TFIIA may also reveal TAF11 interaction surfaces, we were specifically interested in the class II compensatory alleles, which are defective for interaction with Toa2 (Table 2). Analysis of the substitutions in these alleles allowed us to distinguish between mutations conferring compensatory interaction with *toa2*-I27K (gain of function) versus mutations that disrupt interaction with Toa2 (loss of function). One allele in particular, *taf11*-15, contained only two substitutions, Q40L and I178 M (Table 2). We previously demonstrated that the mutation I178 M confers a weak compensatory interaction with *toa2*-I27K without affecting interaction with wild-type Toa2 or TAF13 (Fig. 1A and Table 3). When we tested a TAF11 derivative with the Q40L substitution for interactions, we found that Q40L was not a compensatory mutation but resulted in a loss of interaction with Toa2 (Table 3). Thus, for the *taf11*-15 allele, the I178 M substitution contributed to the gain-of-function interaction with *toa2*-I27K, whereas the Q40L mutation resulted in loss of

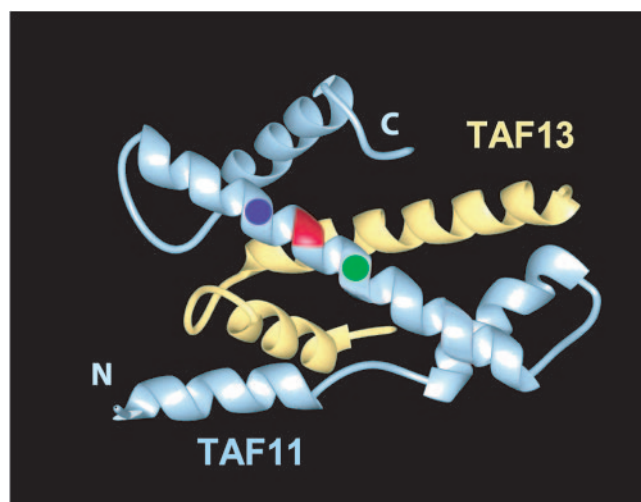


FIG. 2. Crystal structure of the human TAF11/TAF13 heterodimer. A ribbon diagram representation of the crystal structure of the histone fold domains of human TAF11 and TAF13. TAF11 is shown as a blue ribbon and TAF13 as a yellow ribbon. The locations of the homologous yeast residues involved in the compensatory interaction with *toa2*-I27K are denoted by E182 in red, I178M in green, and E185 in blue.

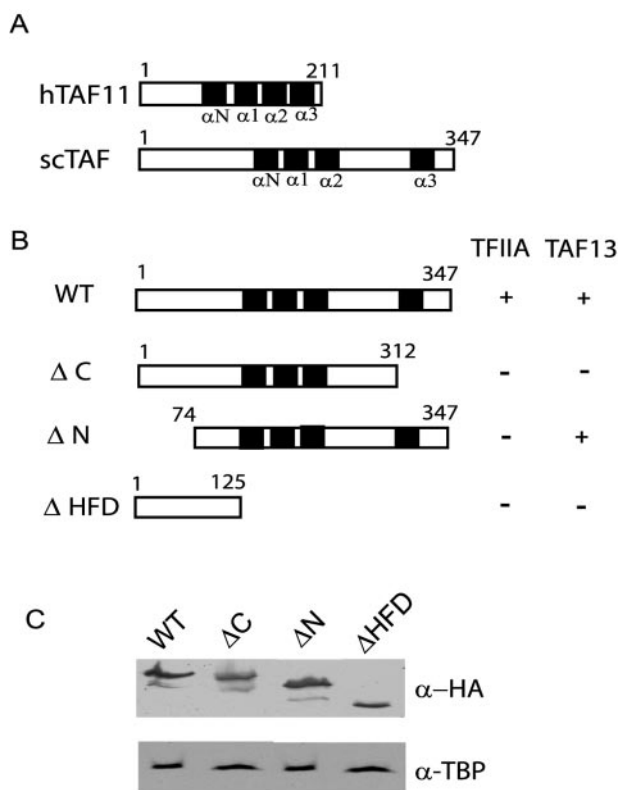


FIG. 3. Interaction with TFIIA requires both the N terminus and the histone fold domain of TAF11. (A) Sequence alignment of human and yeast TAF11 proteins. The conserved regions, which comprise the helices of the histone fold domain, are indicated by black boxes. (B) The N- and C-terminal residues are numbered for each deletion construct. +, Ability of deletion constructs to interact with TFIIA and TAF13 via the two-hybrid assay; -, no interaction. (C) Expression levels of AD-TAF11 deletions. Extracts were prepared from strains containing the indicated TAF11 derivative, and immunoblot analyses were performed with antibodies for HA (to detect the fusion) or TBP (load control).

function with Toa2. In addition to Q40L, a mutation at a nearby residue, K46R, was also present in our TAF11 allele collection. When we tested this derivative (K46R) for interaction with Toa2, it was also defective for the interaction (Table 3). Neither of these substitutions affected interaction with TAF13 (Table 3). The loss of interaction with Toa2 as a result of mutations at residues Q40 and K46, which reside in a region of TAF11 that is distinct from the histone fold domain, suggests that a second region of TAF11 may be important for interaction with TFIIA.

**The N terminus of TAF11 may play a role in the TFIIA interaction.** Analyses of two mutations in the N-terminal region of TAF11 (Q40L and K46R) suggest that interaction with TFIIA requires more than just the histone fold domain. To further investigate this hypothesis, a series of deletion derivatives encompassing distinct TAF11 domains was constructed for use in the yeast two-hybrid assay (Fig. 3A and B). All TAF11 derivatives were expressed at levels similar to those of full-length TAF11, as detected by immunoblotting with the HA tag (Fig. 3C). TAF11 deletion derivatives were then tested for interaction with TFIIA (Toa2) and TAF13 (Fig. 3B). As expected

from the TAF11-TAF13 structural studies (3), disruption of the histone fold domain by deletion of the  $\alpha$ -3 helix ( $\Delta$ C) was detrimental for TAF11 interaction with TAF13. In support of our findings regarding the location of compensatory mutations within this domain, the TAF11 derivative  $\Delta$ C was also defective for interaction with TFIIA. The TAF11 derivative  $\Delta$ N, which retains the histone fold domain but is missing the first 73 N-terminal amino acids, was functional for interaction with TAF13 but was not sufficient for interaction with TFIIA. These findings indicate that the N-terminal region of TAF11 is important for TFIIA association but is dispensable for interaction with TAF13. Taken together, we conclude that the histone fold domain is necessary and sufficient for TAF11-TAF13 dimerization, whereas the histone fold domain and the N-terminal domain both play a role in the TFIIA interaction.

**The N terminus of TAF11 is not required for interactions with TBP and TAF1.** Although deletion of the N-terminal domain of TAF11 does not affect interaction with TAF13 in the two-hybrid assay, it is possible that the N terminus is necessary for functional contacts within TFIID. To examine if other TAF11 contacts in TFIID were affected by this mutation, coimmunoprecipitation assays were performed in yeast strains expressing the wild type or the taf11- $\Delta$ N derivative. Association with TBP and with epitope-tagged TAF1 served as indicators of the ability of taf11- $\Delta$ N to maintain TFIID contacts (17, 48). Whole-cell extracts were prepared from strains expressing either wild-type TAF11 or taf11- $\Delta$ N (both of which are *myc* tagged). Polyclonal antibodies directed at TBP were used for immunoprecipitation, and the precipitated complexes were analyzed by immunoblotting for TAF11 (*myc*). As expected, wild-type TAF11 associated with TBP (Fig. 4). Moreover, taf11- $\Delta$ N associated with TBP at both 30°C and 38°C. In a reciprocal experiment, immunoprecipitation using monoclonal antibodies directed at the *myc* epitope (TAF11 or taf11- $\Delta$ N) resulted in coprecipitation of taf11- $\Delta$ N with TAF1 at both the permissive and nonpermissive temperatures. These results indicate that deletion of the N-terminal region of TAF11 does not appear to impact association with TBP or TAF1, suggesting that particular TFIID interactions are unaltered by the loss of the N-terminal region of TAF11.

**TAF11 mutants support cell viability and exhibit conditional phenotypes.** Thus far, the analysis has identified TAF11 derivatives that alter interaction with TFIIA. The collection includes derivatives that are defective for interaction with TFIIA by single mutations at specific residues (Q40L, K46R, V170A, and F171V), in the context of compensatory mutations (taf11-14, taf11-15, and taf11-16), and by deletion of the N terminus ( $\Delta$ N) and derivatives that confer compensatory interaction with the TFIIA mutant (I178 M, E182A, E182G, and E185). We next assessed whether these derivatives could support cell viability in a TAF11 deletion strain, and if they were viable, whether they conferred any mutant phenotypes on alternative carbon sources or at various temperatures. Each TAF11 derivative was cloned under the control of the endogenous TAF11 promoter and terminator and expressed in a strain in which the chromosomal copy of TAF11 had been deleted. All TAF11 mutant proteins were expressed to levels similar to those of wild-type TAF11, as detected by immunoblotting with the *myc*-tag (data not shown).

Strains expressing single mutations that disrupted interac-

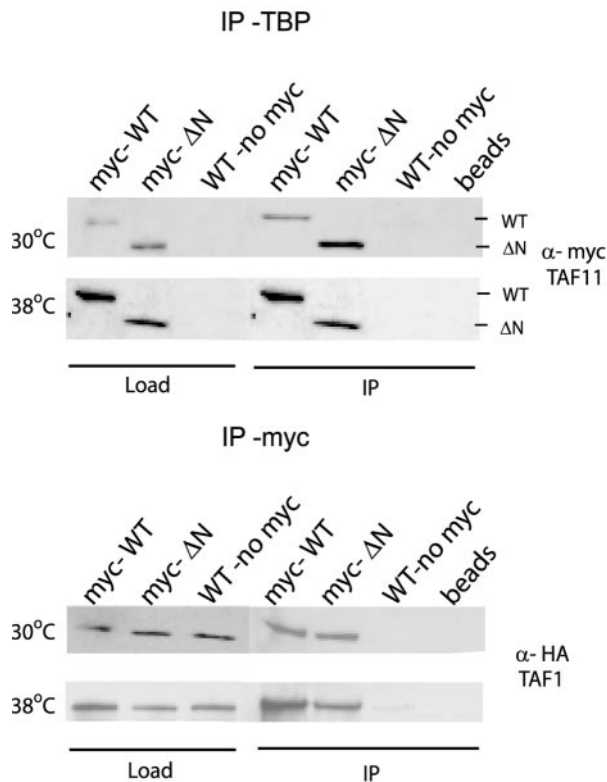


FIG. 4. TAF11 derivative taf11- $\Delta$ N associates with TBP and TAF1 in vivo. Coimmunoprecipitation was performed with cell extracts prepared from strains containing HA-TAF1 and either *myc*-tagged wild-type TAF11 (*myc*-WT), *myc*-tagged taf11- $\Delta$ N (*myc*- $\Delta$ N), or untagged wild-type TAF11 (WT-no *myc*). Cells were cultured at 30 and 38°C. Extracts (Load) were immunoprecipitated (IP) with TBP or *myc* antibodies, resolved by SDS-PAGE, and probed with monoclonal antibodies to either *myc* (top two panels) or HA (bottom two panels). A sample of protein A-Sepharose beads without conjugated antibody is shown as a control (beads).

tion with TFIIA, Q40L, K46R, V170A, or F171V supported cell viability and did not exhibit conditional growth phenotypes (Table 3). Two of the class II strains (Table 2), which possessed alleles defective for wild-type Toa2 interaction and were compensatory for *toa2*-I27K (*taf11*-14 and *taf11*-16), exhibited slow-growth phenotypes at 38°C (Table 3). Since these alleles contain several amino acid substitutions, it is likely that multiple mutations are necessary to confer a TS phenotype. This is consistent with observations from other studies with TS TAF11 and TAF13 derivatives, where multiple substitutions were also found (27, 33). As such, we constructed TAF11 derivatives containing the substitutions in both interaction regions, Q40L F171V and K46R F171V. However, these derivatives also supported cell viability and did not exhibit conditional growth phenotypes (Table 3). It may be that the loss of functional interaction observed in the two-hybrid assay can be compensated for by the redundancy of interactions that contribute to the formation of the TFIIA-TFIID complex in vivo and/or that more radical mutations are required to fully disrupt function.

To investigate more radically altered TAF11 alleles, cell viability was also examined for strains expressing TAF11 deletion derivatives (Fig. 3). We observed a strong correlation between cell viability and the ability of TAF11 to interact with

TAF13 and TFIIA (Table 3 and Fig. 3). That is, TAF11 deletions that disrupt interaction with both TAF13 and TFIIA were unable to support cell viability, underscoring the importance of these TAF11 interactions in vivo. Strains expressing the TAF11 deletion derivative taf11- $\Delta$ N (which lacks the first 73 amino acids) were viable but exhibited a TS phenotype. The taf11- $\Delta$ N derivative was functional for interaction with TAF13, as well as with TBP and TAF1, at permissive and restrictive temperatures (Fig. 4) but was not sufficient for interaction with TFIIA in the two-hybrid assay.

The compensatory alleles I178 M, E182A, E182G, and E185 each supported viability in a TAF11 deletion strain and exhibited wild-type growth phenotypes. Since these derivatives interact with wild-type TOA2 (both in vivo and in vitro), this is not unexpected. Interestingly, when these substitutions were expressed in combination with the  $\Delta$ N deletion derivative (*taf11*- $\Delta$ N E182G or *taf11*- $\Delta$ N E182A), the cells were nonviable. As such, interfering concomitantly with interactions in both domains compromises essential functions of TAF11.

**Certain TAF11 derivatives exhibit transcriptional defects at a subset of Pol II-transcribed genes.** To assess the functional consequences of a defect in the TAF11-TFIIA interaction at the molecular level, we compared wild-type TAF11 and our collection of TAF11 alleles for transcriptional competency. Constitutive expression of a subset of Pol II-transcribed genes, which have been shown to differ in their requirement for TAF function (29, 34, 50), was examined at 30 and 38°C by S1 nuclease protection assays (Fig. 5). There were significant reductions in transcription levels for the TAF-dependent genes *HTA2* and *RPS4* (three- and fivefold decreases, respectively) in the taf11- $\Delta$ N strain when compared to the wild-type strain. Similar decreases in transcript levels for these genes were observed when cells were grown at 38°C, indicating that the observed transcriptional defects are not conditional (Fig. 5). In contrast, strains expressing the TAF11 derivative Q40L exhibited slight defects for *HTA2* transcription only at the restrictive temperature (38°C). These differences could reflect the nature of the TAF11 mutations that disrupt interaction with TFIIA. Consistent with little effect on cell growth phenotypes, strains expressing single substitutions K46R and F171V and double substitutions Q40L F171V and K46R F171V did not exhibit transcription defects at either temperature. Similarly, transcription profiles for strains expressing taf11-14 and taf11-15 derivatives were comparable to those of wild-type strains. However, a strain expressing the compensatory allele taf11-16 exhibited a threefold decrease in *HTA2* transcription at 30°C. We also examined transcript levels in a strain expressing the compensatory mutation E182G to determine if this substitution had an effect on transcriptional competency. Interestingly, transcript levels at 30°C were comparable to those of the wild-type TAF11 strain; at 38°C, however, transcript levels for TAF-dependent genes *HTA2* and *RPS4* were reduced approximately twofold. These transcriptional defects may contribute to the inability of strains expressing E182G in the context of the  $\Delta$ N deletion to support cell viability. Taken together, TAF11 derivatives with altered interactions with TFIIA exhibit allele specific defects for transcription at a number of Pol II-transcribed genes.

We next tested the ability of TAF11 derivatives to mediate activated transcription. Gcn4-dependent activation of *HIS3*

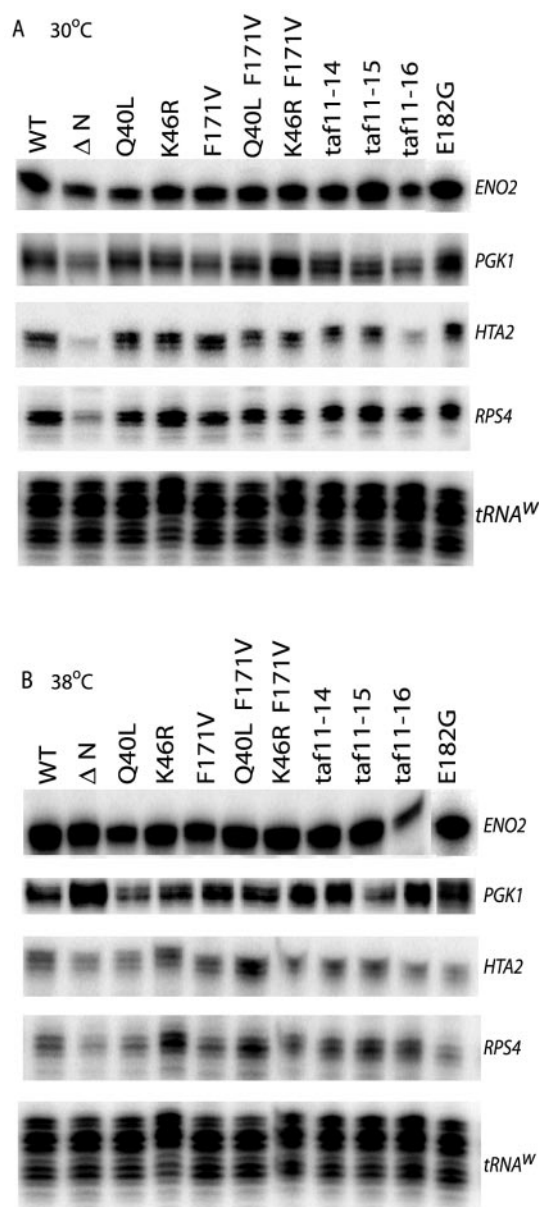


FIG. 5. Transcriptional analysis of *taf11*-ΔN for Pol II-transcribed genes. S1 nuclease protection analysis of specific Pol II messages. RNA was isolated from strains expressing the wild type (WT) or the indicated TAF11 derivative at 30°C (A) or at 38°C (B). Total RNA (25 μg) was hybridized with 100-fold excess of the indicated probe and treated with S1 nuclease. The Pol III transcript *tRNA<sup>W</sup>* was used as a load control.

transcription was assayed by growing the cells in AT, a competitive inhibitor of the *HIS3* gene product. At 30°C and in the absence of AT, constitutive *HIS3* transcription was diminished three- to sevenfold for both the +1 and +13 transcripts in all our TAF11 derivative strains (Fig. 6A). Defects for constitutive *HIS3* transcription in strains grown at 38°C were not as apparent, because strains expressing wild-type TAF11 produced very little of the +1 and +13 when grown at the elevated temperature (Fig. 6B). In contrast, in the presence of AT, activator-induced *HIS3* transcription in TAF11 derivative strains was indistinguishable from that of the wild type for both transcripts

for most of the strains expressing TAF11 derivatives at 30 and 38°C (Fig. 6C and 6D). Activated transcription was decreased twofold for the +1 transcript in strains expressing compensatory alleles *taf11-14*, *taf11-15*, and *taf11-16*. The absence of dramatic transcriptional defects for activator-induced *HIS3* transcription suggests either that loss of the TAF11-TFIIA interaction through mutations in TAF11 can be partially compensated for by a strong activator or that this interaction is not required for activation by a strong activator. Lack of a requirement for TAFs during activated transcription has been observed with yeast (39, 58) and higher eukaryotes (42). In contrast to subtle effects on transcriptional activation for the TAF mutant derivatives, strains expressing *toa2-127K* exhibit dramatic activation defects (28). This again implies that TFIIA possesses TAF-independent as well as TAF-dependent functions.

**TAF11 enhances TFIIA-TBP-DNA complex formation through changes to protein interactions with the DNA.** Having established that the TAF11-TFIIA interaction is important for transcription *in vivo*, it is critical to understand the mechanistic role of the TAF11-TFIIA interaction on the TBP-TFIIA-DNA complex. Previously, we found that TAF11 contributes to TFIIA and TBP associations at the promoter by specifically enhancing the formation of the TFIIA-TBP-DNA complex (28). To determine if TAF11 makes direct contacts with DNA, here we employed protein-DNA photo-cross-linking with the yeast *HIS3* promoter *in vitro*.

Twelve site-specific derivatized promoter fragments were constructed by incorporating *p*-azidophenacyl bromide (a pho-

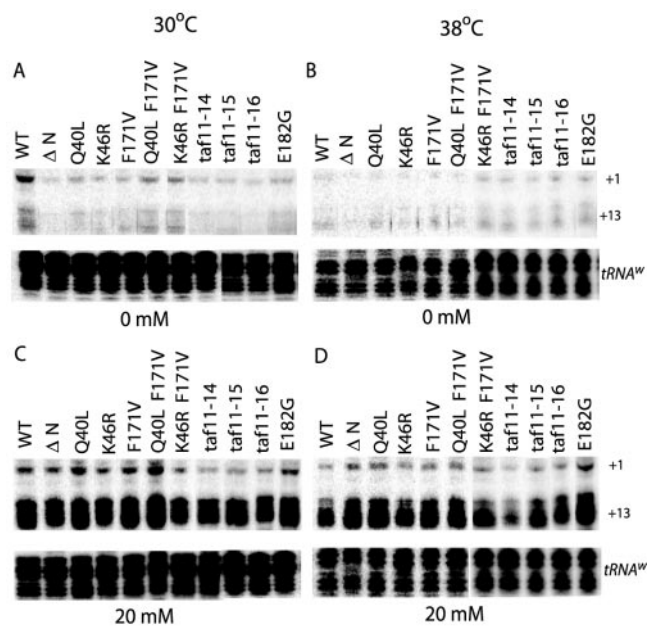


FIG. 6. Analysis of Gcn4-dependent activation of *HIS3* transcription. S1 nuclease protection analysis of constitutive *HIS3* transcription. Total RNA was isolated from strains expressing the wild type (WT) or the indicated TAF11 derivative grown at 30°C (A) and 38°C (B). The +1 and +13 start sites are indicated. Gcn4-activated *HIS3* transcription was measured by treating cells grown at 30°C (C) or 38°C (D) with 20 mM AT for 1 h prior to harvest and determining the level of *HIS3* transcripts.





at both the  $-3$  to  $-4$  and  $-6$  to  $-7$  positions (Fig. 7D and E). These small quantitative changes might reflect subtle conformational changes to TBP and TFIIA upon interaction with TAF11/TAF13 at the promoter. Since the imposed changes to TBP and TFIIA-DNA cross-link patterns were observed in the absence of substantial TAF11 or TAF13 DNA contacts, we conclude that alterations of TBP-TFIIA-DNA complex formation by TAF11 is predominantly mediated through protein-protein interactions between TAF11 and TBP and TFIIA.

**TFIIA-TBP-DNA complex formation is enhanced and stabilized by TAF11.** Thus far, we demonstrate that loss of interaction between TAF11 and TFIIA can impair transcription of certain genes, yet a strong activator such as Gcn4 can compensate for these transcription defects. It is possible that TAF11 interaction with TFIIA is specifically required when conditions for transcription initiation are suboptimal, such as at weak promoters where TBP-TFIIA-DNA complex formation is compromised. Our previous studies indicate that the sequence variant CATAAA substituted at the TATA element is not optimal for TBP and TFIIA complex formation because the TBP-TFIIA-DNA complex is not stable (53). As such, the CATAAA promoter provides us with a model to examine the effect of TAF11 at a weak promoter where TFIIA-TBP complex formation is not optimal. Using EMSA, we tested the hypothesis that TAF11-induced TFIIA-TBP-DNA complex enhancement would be more dramatic on a promoter containing the off-consensus sequence CATAAA than on a canonical promoter containing the sequence TATAAA. Recombinant TBP, TFIIA, and TAF11/TAF13 were incubated with radiolabeled promoter fragments, and complexes were resolved on 5% acrylamide gels (Fig. 8A). TBP and TFIIA formed a complex on both the TATAAA and CATAAA probes; however, at limiting concentrations of TFIIA, there was significantly less complex formed on CATAAA than on TATAAA. Upon addition of TAF11/TAF13, the amount of TBP-TFIIA-DNA complex formed increased and quantification revealed four-fold stimulation on the CATAAA promoter compared to only a twofold stimulation for the TATAAA promoter. Thus, TAF11 had a greater effect on complexes formed on the promoter containing the nonconsensus sequence, CATAAA compared to TATAAA. To determine if this stimulation was unique to CATAAA, we tested whether TAF11 could effect complex formation on a promoter containing the sequence TATAAG (another off-consensus element). As observed for CATAAA, complex formation on the TATAAG promoter was stimulated at a significantly higher level than for TATAAA by TAF11 (data not shown). Therefore, TAF11 has a greater enhancement effect at promoters that are compromised for TFIIA-TBP complex formation.

To test the hypothesis that an increase in complex formation is due to the ability of TAF11 to stabilize TFIIA and TBP interactions on the CATAAA promoter, complexes were formed on the CATAAA promoter in the presence and absence of TAF11/TAF13 and then challenged with a molar excess of specific competitor over time. Consistent with previous studies, the TFIIA-TBP complex on the CATAAA promoter was rapidly lost in the presence of competitor DNA. In contrast, the TAF11/TAF13-TFIIA-TBP-CATAAA complex remained stable for the entire 2-h time course, indicating that TAF11 dramatically stabilized TFIIA-TBP complexes formed

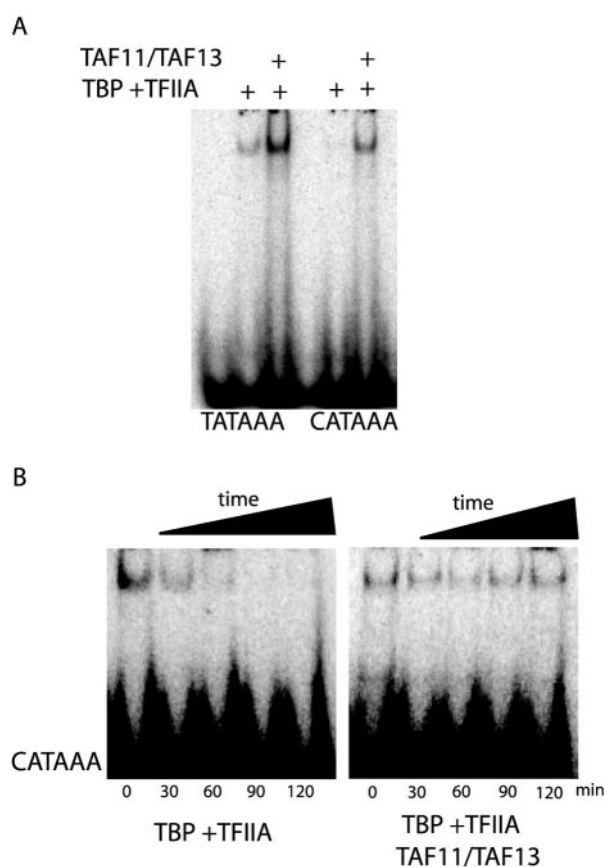


FIG. 8. TAF11 enhances and stabilizes TFIIA-TBP complexes on the nonconsensus CATAAA promoter. (A) An EMSA comparing the effect of TAF11 on TFIIA-TBP-DNA complex formation with TA TAAA or CATAAA. Approximately 0.1 pmol of radiolabeled DNA was incubated with TBP, TFIIA, and TAF11/TAF13 and incubated for 30 min. Protein-DNA complexes were resolved on 5% acrylamide gels. All reaction mixtures contained limiting amounts of TBP and TFIIA (0.3 and 0.5 nmol, respectively) and where indicated 4.2 nmol of TAF11/TAF13 heterodimer. (B) EMSA binding reaction mixtures were prepared as described in panel A, except that following a 30-min incubation, protein-DNA complexes were challenged with poly(dAdT) for the time period indicated and then resolved on 5% acrylamide gels.

on this nonconsensus promoter (Fig. 8B). Thus, TAF11 enhances protein interactions, leading to stable association of TBP and TFIIA at the promoter, a property that is particularly important for complexes formed on weak TATA elements.

## DISCUSSION

**TAF11 interacts with TFIIA via two distinct regions.** The isolation of compensatory mutants is the best genetic support for a direct interaction *in vivo*. Here, we describe TAF11 compensatory mutants for the *toa2-I27K* mutant of the small subunit of TFIIA. These results provide strong evidence that the interaction between TAF11 and TFIIA is essential for optimal transcription activity *in vivo*. Moreover, this approach defines the regions of both proteins critical for the interaction. We mapped the TAF11 interaction surface to the four-helix bundle domain of TFIIA, a surface not involved in interaction with TBP or DNA. Through analysis of overrepresented compensatory mutations in TAF11, we identified single substitutions

that restore interaction with *toa2-I27K* and function in vivo by suppressing the TS phenotype caused by loss of interaction between TFIIA and TAF11. The location of these compensatory mutations mapped to the surface of the structurally defined  $\alpha$ -2 helix within the histone fold domain of TAF11 (3), thus implicating the involvement of this region for interaction with TFIIA.

The mutated residues conferring compensatory interaction with *toa2-I27K* are located on the exposed surface of the histone fold domain opposite that involved with TAF13 interaction. In addition, certain mutations that confer either compensatory interactions or disrupt interactions with TFIIA have no effect on TAF13 interactions. These findings suggest that these associations require separate surfaces and that TAF11 interactions with TFIIA and TAF13 need not be mutually exclusive.

The predominance of histone fold domains in TAFs and the identification of TAF-TAF dimers have led to the proposal that these domains dictate dimerization specificity. Recently, we (61), and others (15) demonstrated that this domain provides an accessible surface for other TAF-TAF interactions, which are important for transcription. In this study, we show that the histone fold domain of TAF11 can mediate interactions with both TAF13 and TFIIA and provides the first evidence of an interaction between a histone fold domain of a TAF and a general transcription factor. Furthermore, our data suggest that the accessibility of the separate surfaces of the histone fold domains may allow for additional protein interactions required for efficient initiation complex assembly. These findings are further supported by studies of human TAF11, which show that mutations in residues on the solvent-exposed surface of the  $\alpha$ -2 helix abolish synergistic transcription activation by nuclear receptors (31). Interestingly, the residues implicated for this activity correspond to the same residues we identified as important for interaction with TFIIA (E182 and E185), indicating the functional importance of this interaction surface in higher eukaryotes.

In addition to the histone fold domain, TAF11 mutational analysis suggests that the N-terminal domain also provides an important region for TFIIA interaction. Mutations at specific N-terminal residues (Q40L and K46R) and deletion of the first 73 N-terminal residues (*taf11- $\Delta$ N*) result in loss of interaction with TFIIA. The deletion of the N terminus is particularly harmful to cells, as it results in a TS phenotype and defects in transcription. Since loss of the N-terminal residues does not alter TAF11 interactions within TFIID via TAF1 or TBP, these defects may reflect the loss of interaction with TFIIA. Unlike the histone fold domain, the N-terminal region of TAF11 is considerably less conserved throughout eukaryotes. However, residues Q40 and K46 are invariant in other yeast species and share similarity to the same residues in human TAF11, suggesting that specific residues within the N-terminal domain may be functionally conserved. Of interest is the lethality observed when the compensatory mutations E182G or E182A are expressed in the context of the N terminal deletion. This might reflect the functional interplay between these two interaction surfaces.

**TAF11 enhances TBP-TFIIA complex formation through protein-protein interactions.** While the binding of TBP to the TATA box is critical for the assembly of the transcription apparatus, other DNA-protein interactions also significantly

affect PIC formation. Several lines of evidence indicate that TAFs orient and stabilize the transcription machinery on the promoter, and DNA binding studies have revealed position dependent contacts between certain TAFs and DNA (5, 7, 41, 55). Further studies demonstrate that TAFs facilitate TBP recruitment and functional PIC assembly (29, 34, 50). Our previous studies show that TAF11 specifically enhances TFIIA-TBP-DNA complex formation (28). Here, we found by protein-DNA cross-linking assays that TAF11/TAF13 imparts very subtle but reproducible quantitative changes in TBP and TFIIA promoter interactions. Other studies have also shown that TFIIA interaction with TFIID induces changes to specific DNA contacts (41). We found no compelling evidence that TAF11 makes direct contacts with DNA on this promoter in vitro. The data presented here and in our previous studies suggest that TAF11/TAF13 is not stably associated with the TFIIA-TBP-DNA complex, since the addition of TAF11/TAF13 does not produce a supershifted complex in EMSAs (28). As such, we propose that the transient association of TAF11/TAF13 with the TFIIA-TBP-DNA complex is sufficient to result in a persistent conformational change, leading to an overall increase in TFIIA-TBP-DNA complex stability. In support of this model, weak interactions between TAFs and promoters have been reported at highly active TAF-dependent promoters in higher eukaryotes, suggesting that less stable binding by TAFs can be conducive to high transcriptional activity (8). Thus, through transient association with promoter bound proteins, TAFs may induce changes in protein conformations or promoter contacts that set the stage for more efficient function via increased stability and/or recruitment of other required transcription components.

**The TAF11-TFIIA interaction has critical core promoter functions.** The transcriptional analyses presented here and in other studies (27, 50) indicate that TAF11 is important for the transcription of a subset of genes transcribed by Pol II. What determines whether a promoter is dependent on TAF11 interactions? Several lines of evidence suggest that TAF11-dependent promoters may be those that exhibit suboptimal TBP-TFIIA-DNA complex formation. First, TFIIA-TBP-DNA complexes formed on a consensus TATA element were enhanced by TAF11 when concentrations of TBP and/or TFIIA are limiting (Fig. 7A and reference 28). TAF11 has very little effect on TFIIA-TBP-DNA complex formation when concentrations of TFIIA and TBP are in excess. Second, using a promoter fragment that contains the sequence CATAAA, which is a nonoptimal TATA element, we demonstrated that TAF11 can dramatically stabilize the TFIIA-TBP-DNA complex in vitro (Fig. 7B). Third, constitutive expression from the off-consensus element in the *HIS3* promoter is extremely sensitive to mutations in TAF11, as all of the TAF11 derivatives defective for interaction with TFIIA examined in this study exhibited defects for constitutive *HIS3* expression (Fig. 5B). Finally, the presence of a strong activator (Gcn4) can bypass these defects in the TAF11 mutant strains at the *HIS3* promoter, presumably by the mechanism of action of the activator, which is thought to increase rate-limiting steps in transcription (Fig. 5B).

The above results strongly suggest that a functional TAF11-TFIIA interaction may be essential when conditions for TFIIA-TBP-DNA complex formation are compromised. It is becom-

ing increasing clear from the analysis of vertebrate and yeast promoter sequences that a majority of genes contain TATA elements that are not perfect consensus elements (2, 20, 45, 46). In fact, in vertebrates a substitution at the first position with a C (CATAAA) is a very common occurrence, with 15% of the elements exhibiting this alteration. As such, the TAF11-TFIIA interaction may play a fundamental role in the efficient expression of a large class of genes.

Here, we show that a functional interaction between TAF11 and TFIIA has an essential mechanistic role during complex formation under nonoptimal conditions and that loss of interaction results in defects that alter cell growth and transcription in vivo. Our genetic studies have mapped the interacting surfaces on both factors and, combined with structural information presented in a number of studies, provide insight into the molecular architecture of the TAF11-TFIIA interaction. Taken together, these results firmly establish that TAF11 serves as a functional link between TFIIA and TFIID.

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