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Analysis of *TAF90* Mutants Displaying Allele-Specific and Broad Defects in Transcription

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Yeast TAF90p is a component of at least two transcription regulatory complexes, the general transcription factor TFIID and the Spt-Ada-Gcn5 histone acetyltransferase complex (SAGA). Broad transcription defects have been observed in mutants of other TAF_{II}s shared by TFIID and SAGA but not in the only two *TAF90* mutants isolated to date. Given that the numbers of mutants analyzed thus far are small, we isolated and characterized 11 temperature-sensitive mutants of *TAF90* and analyzed their effects on transcription and integrity of the TFIID and SAGA complexes. We found that the mutants displayed a variety of allele-specific defects in their ability to support transcription and maintain the structure of the TFIID and SAGA complexes. Sequencing of the alleles revealed that all have mutations corresponding to the C terminus of the protein, with most clustering within the conserved WD40 repeats; thus, the C terminus of TAF90p is required for its incorporation into TFIID and function in SAGA. Significantly, inactivation of one allele, *taf90-20*, caused the dramatic reduction in the levels of total mRNA and most specific transcripts analyzed. Analysis of the structure and/or activity of both TAF90p-containing complexes revealed that this allele is the most disruptive of all. Our analysis defines the requirement for the WD40 repeats in preserving TFIID and SAGA function, demonstrates that the defects associated with distinct mutations in *TAF90* vary considerably, and indicates that *TAF90* can be classified as a gene required for the transcription of a large number of genes.

TFIID is one of several general initiation factors required for the reconstitution of RNA polymerase II (Pol II) transcription in vitro. In yeast, it is composed of the TATA-binding protein (TBP) and at least 14 associated factors collectively referred to as TAF_{II}s (23, 30, 34, 41, 42, 43, 46, 53). Individual TAF_{II} polypeptides contain distinct structural motifs and functional domains that are likely to be responsible for their specialized functions. For example, several functional domains have been identified in $hTAF_{II}250$, which include histone acetyltransferase (HAT), kinase, and ubiquitin-conjugating domains and bromodomains (8, 21, 32, 40). Many TAF_{II}s contain another specialized motif, the histone-like fold (11, 12, 13, 19, 36, 59). These include TAF60p, TAF17p, TAF68/61p, TAF40p, TAF19p, TAF47p, TAF25p, and TAF48p; for simplicity, only the yeast TAF_{II}s are mentioned, although their eukaryotic homologues also contain this motif (for reviews, see references 13, 19, and 36).

Perhaps the least understood motif found in TAF_{II}s is the WD40 repeats of TAF90/$hTAF_{II}100$/$dTAF_{II}80$ (9, 10, 42, 51, 52). The function of this domain is not known, but it is speculated to be involved in protein-protein interactions, as has been proposed for other WD40-containing proteins (49). Despite the highly conserved nature of this region of the protein, it has been reported that the WD40 repeat domain is not necessary for incorporation of $hTAF_{II}100$ into TFIID (9). Moreover, the *Schizosaccharomyces pombe* homologue of *TAF90*, *taf72*$^+$, cannot complement the function of *Saccharomyces cerevisiae TAF90*, but a chimera encoding the N terminus of TAF90p and the C terminus of Taf72p can do so (60), indicating that the N terminus of TAF90p provides the species specificity. Therefore, all of the available evidence suggests that the N terminus of TAF90p, and those of its homologues, is most important for its incorporation into TFIID. That is not to say that the C terminus is dispensable, as the only temperature-sensitive mutants characterized to date have mutations located within the C terminus (2).

There is an ongoing debate on what fraction of the genome is dependent upon certain TAF_{II} genes for its expression and on what accounts for the differences observed between laboratories and species (1, 17, 18). Mutation or depletion of certain TAF_{II} subunits results in restricted transcription defects (2, 20, 26, 33, 53), whereas mutation of other subunits reduces the transcription of many genes (3, 25, 31, 35, 37, 43, 45). The identification of certain TAF_{II}s besides TFIID in nuclear complexes (15, 22, 29, 39, 55) provided a possible explanation for the differences observed among TAF_{II} genes. For example, genome-wide studies demonstrated that *TAF145* and *GCN5* perform some redundant functions in transcription, as a double *taf145 gcn5* mutant shows defects in the transcription of a larger fraction of the genome than for the sum of the individual mutations (26). These observations suggest that certain TAF_{II}s are responsible for the transcription of a large number of genes because they are shared by TFIID and the Spt-Ada-Gcn5 HAT complex (SAGA). Nonetheless, an inconsistency in this theory is that mutation of *TAF90*, encoding a subunit of TFIID and SAGA, affects only a small number of genes (2, 26). Of the TAF_{II}s shared between TFIID and SAGA, TAF90p is the only one whose loss of function does not broadly affect transcription. However, the number of mutants characterized thus far has been small, and different mutant alleles might be expected to have diverse consequences for the structures of TFIID and

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SAGA. Another factor that complicates the interpretation of the results obtained using various TAF_{II} mutants is that the effects of these mutations on TFIID and SAGA integrity have not been thoroughly examined.

A comprehensive analysis of *TAF90* was performed in an attempt to understand its role in the function of TFIID and SAGA. Eleven temperature-sensitive mutants were isolated and were characterized in regard to their effects on transcription and on the structures of TFIID and SAGA. We found that all of the mutants contained amino acid substitutions within the conserved C terminus of the protein, particularly within the WD40 repeats. Mutations within this region caused defects in the ability of TAF90p to interact with TFIID and SAGA. While 10 of the 11 alleles displayed a variety of weak and selective transcription phenotypes, a single allele of *TAF90* was identified that caused a rapid reduction in the mRNA levels of a large number of genes. Our data indicate that the breadth and severity of the transcription defects of each mutant correlated strongly with their effects on TFIID structure but less so with their effects on SAGA activity. The allele-specific defects we report here illustrate the point that multiple alleles need to be examined to fully understand the role of TAF_{II}s in transcription.

MATERIALS AND METHODS

Yeast strains and genetic manipulations. The strains used in this study were as follows: YJR224 (*mat*α Δ*taf90*::*TRP1* [pRS316-*TAF90*] *ade2-101 his3*Δ*200 leu2-3,112 ura3-52 trp1-901 lys2-801 suc2*-Δ*9*), YJR226 (same as YJR224 except containing pRS313-HA-*TAF90* or pRS313-HA-*taf90-x* in place of pRS316-*TAF90,* where x is the allele number), YJR240 (*mat***a** Δ*taf90*::*HIS3* [pRS316-*TAF90*] *ade2-101 his3*Δ*200 leu2-*Δ*1 ura3-*Δ*99 lys2-801*), YJR241 (same as YJR240 except containing pRS415-HA-*TAF90* or pRS415-HA-*taf90-x*), YJR500 (*mat***a**/**α** *taf90*::*TRP1*/*TRP1* [pRS316-*TAF90*] *his4-917*δ/ *his4-917*δ *lys2-17R2*/*lys2-17R2 leu2*/*leu2 ura3-52*/*ura3-52*), YJR501 (*mat***a** *taf90*::*TRP1* [pRS316-*TAF90*] *his4-917*δ *lys2-17R2 leu2 ura3-52*), and YJR530 (same as YJR501 except containing pRS415-HA-*TAF90-x*).

Mutants were created by hydroxylamine mutagenesis of pRS313-HA-*TAF90* in vitro for 60, 90, and 120 min at 70°C. After purification of the plasmid, each pool was independently transformed into YJR224 and plated onto histidine dropout plates. The transformants were subsequently replica plated onto 5-fluoro-orotic acid (48) and placed at 24, 30, and 37°C. Temperature-sensitive mutants were identified based on growth at 24°C but inviability at 37°C. The mutants were verified, and the coding region was sequenced to identify the base substitutions. The mutants were transferred into pRS415 by gap repair, and the resulting plasmids were used to transfer the mutations into additional genetic backgrounds (YJR241 and YJR530). For the SPT analysis, strain FY632 was transformed with a *taf90*::*TRP1* DNA fragment generated by PCR (4), and the resulting transformants were selected for on synthetic complete medium without tryptophan. Individual colonies were screened by PCR and by Southern blotting to confirm the disruption of *TAF90*. YJR500 cells were transformed with pRS316-*TAF90*, and the transformants were sporulated on solid medium for 4 days. Tetrads were dissected, and genotypes of the resulting spores were identified by replica plating onto selective media and mating type testing. The resulting strain, YJR501, was transformed with pRS415 containing *TAF90* or its mutant derivatives. Next, transformants were selected for twice on 5-fluoro-orotic acid plates, and the temperature-sensitive mutants were confirmed by monitoring growth at 24 and 37°C.

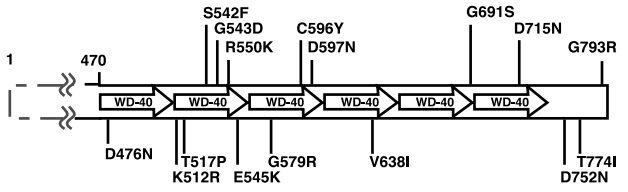
Analysis of RNA. To measure poly(A)$^+$ mRNA levels, total RNA was prepared by the acid-phenol extraction method, and 10 μg was applied to charged nylon using a dot blot manifold. The membrane was blocked and hybridized with a ^{32}P-labeled dT_{20} oligonucleotide (Pharmacia) as described previously (54). For Northern blotting (44) and S1 nuclease protection (7), total RNA was prepared by the glass bead disruption procedure (2, 53). For the genes *RPS5*, *TRX1*, *CLB2*, *CLN2*, *SUC2*, *HUG1*, and *scR1*, gel-purified PCR-generated probes were used. *ADH2* RNA was detected using an oligonucleotide probe specific for a region nonhomologous to *ADH1*.

Whole-cell extracts and immunoprecipitation (IP). Yeast cells were grown in 2% peptone–1% yeast extract–2% dextrose (YPD) supplemented with 20 μg of adenine sulfate per ml (YAPD) (500 ml) to an optical density at 600 nm (OD_{600}) of approximately 1.0 to 1.2. An equal volume of 24°C YPD (for permissive-temperature extracts) or 50°C YPD (for nonpermissive-temperature extracts) was added, and the cells were grown for an additional 3 h at 24 or 37°C, respectively. Whole-cell extracts were prepared as described previously (42, 58). One milliliter of whole-cell extract was adjusted to a total protein concentration of 1.5 mg/ml with 0.2 M buffer T (same as buffer B described by Reese et al. [42] but with 0.2 M potassium acetate), and the insoluble material was removed by centrifugation for 10 min at 14,000 rpm in a Microfuge. Two microliters of anti-TAF90p or anti-TAF145p polyclonal antibody (53) was added, and the mixture was incubated at 4°C for about 4 h. The tubes were centrifuged for 10 min at 14,000 rpm, and the supernatant was transferred to a new tube. Thirty microliters of a 50% slurry of protein A beads (Repligen) in 0.2 M buffer T was added, and the tubes were incubated overnight at 4°C with end-over-end mixing. The protein A beads were collected by low-speed centrifugation (500 rpm, 4°C, 5 min), and the supernatant was carefully removed. The beads were washed four times with 0.9 ml of ice-cold wash buffer (same as 0.2 M buffer T but containing 0.1% NP-40). After the final wash, the supernatant was carefully removed and the proteins were eluted by heating in 1.5× sodium dodecyl sulfate (SDS) loading buffer. Samples were fractionated on 8% (14- by 10-cm) and 12.5% (8- by 10-cm) SDS-polyacrylamide gels and transferred to nitrocellulose. For the inputs, 30 μg of extract was loaded onto similar gels.

Purification of SAGA. Six 500-ml cultures of yeast were grown at room temperature in YAPD until an OD_{600} of 1 to 2 was reached and then were diluted with an equal volume of prewarmed YAPD (50°C). SAGA was purified essentially as described by Grant et al. (14). Extracts containing an equal quantity of total protein (440 mg in about 40 to 43 ml) were mixed overnight at 4°C with 7.5 ml of Ni-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen). The slurry was poured into a 1.5- by 10-cm column (Kontes) at 4°C, washed with wash buffer (20 mM imidazole [pH 7.0], 100 mM NaCl, 0.1% Tween 20, 10% glycerol, 2 μg of pepstatin A per ml, 2 μg of leupeptin per ml, 5 μg of aprotinin per ml, and 1 mM phenylmethylsulfonyl fluoride [PMSF]), and eluted in same buffer containing 300 mM imidazole (pH 7.0). The Ni-NTA column eluate was loaded onto a Mono Q HR 5/5 column (Pharmacia), and the column was washed with 1 column volume of 100 mM Q-buffer (50 mM Tris-HCl [pH 8.0], 100 mM NaCl, 0.1% Tween 20, 10% glycerol, 2 μg of pepstatin A per ml, 2 μg of leupeptin per ml, 5 μg of aprotinin per ml, and 1 mM PMSF) and eluted using a linear gradient from 100 to 500 mM NaCl through a volume of 25 ml. The SAGA-containing fractions were pooled (about 3 ml) and concentrated to a final volume of about 400 μl using a Centricon YM-30 filtration unit (Millipore). A 250-μl portion was loaded onto a Superose 6 HR 10/30 column equilibrated with 40 mM HEPES (pH 7.5)–0.5 M NaCl–0.1% Tween 20–10% glycerol–1 mM PMSF and eluted at a flow rate of 0.2 ml/min using a Biologic HR system (Bio-Rad). Fractions (0.5 ml) were collected and frozen at −80°C after the addition of 50 μg of insulin.

RESULTS

Isolation and characterization of *TAF90* mutants. To study the function of *TAF90*, a screen for temperature-sensitive mutants was performed. Eleven mutants that failed to support growth at 37°C were isolated, and their mutations were identified by DNA sequencing (Fig. 1). All but two mutants (*taf90-8* and *-19*) have multiple amino acid substitutions. Strikingly, every mutant has at least one amino acid change within the C terminus, and 9 of the 11 mutants have substitutions within a WD40 repeat. Interestingly, 8 out of the 11 mutants have an amino acid substitution within the second WD40 repeat. Several of the mutants have mutations that lie close to the locations of those in two previously identified *TAF90* mutants, referred to as *taf90*$^{ts2\text{-}1}$ (S702D, G793R) and *taf90*$^{ts3\text{-}1}$ (G711E, G712S) by Apone et al. (2). In fact, *taf90-8* shares the exact amino acid substitution (G793R) as one of the two mutations contained in *taf90*$^{ts2\text{-}1}$. In no case did we isolate a mutant that contained changes exclusively within the N terminus of the protein. While we have not delineated the contributions of each of the substitutions in the mutants with multiple muta-



Strain	Mutation(s)
taf90-1	R154C, G543D
taf90-3	D476N, E545K, D715N
taf90-8	G793R
taf90-9	D476N, T517P, E545K, D715N
taf90-10	R75C, T774I
taf90-14	V457M, K512R
taf90-15	G306S, E396K, S542F
taf90-16	G43D, S139F, R550K, G579R, V638I, D715N, D752N
taf90-17	S542F, G691S
taf90-19	S542F
taf90-20	G115A, E434K, V438I, C596Y, D597N

FIG. 1. Isolation of *TAF90* mutants. A table of amino acid substitutions and the locations of those in the C terminus relative to the putative WD40 repeats are shown.

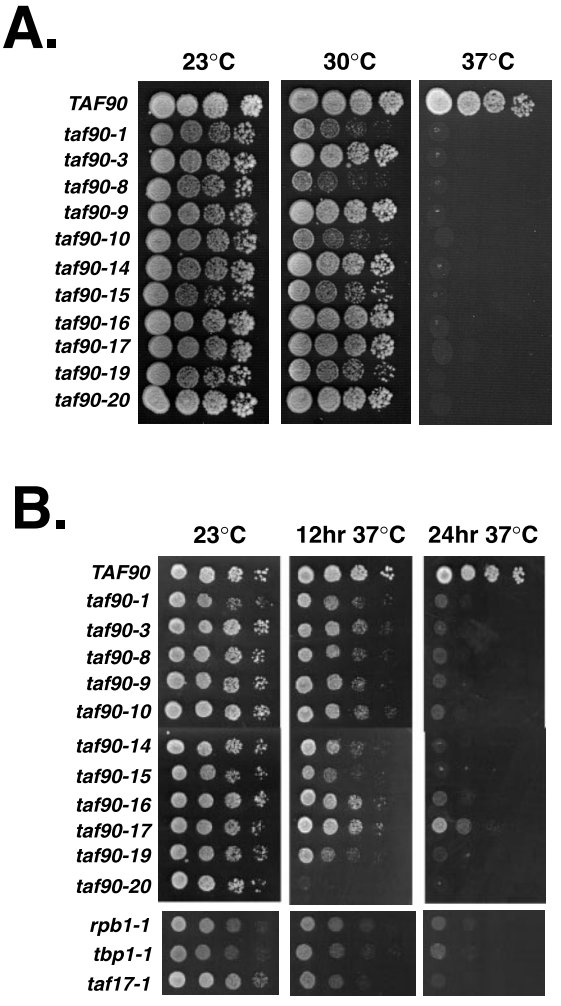


FIG. 2. Characterization of growth phenotypes. (A) Growth phenotypes on rich medium. Cultures were grown in rich medium to an OD_{600} of 1.0, and 2 μl of 10-fold serial dilutions was spotted onto agar plates. Plates were grown for 3 days at the temperatures indicated. (B) Recovery of *TAF90* mutants from exposure to the restrictive temperature. Cultures were grown in liquid rich medium to an OD_{600} of 1.0, and then 2 μl of 10-fold serial dilutions was spotted onto solid rich medium. Plates were grown either for 3 days at 23°C or for 12 or 24 h at 37°C and then for 3 days at 23°C. Since the cells were plated onto agar plates at room temperature, the exact time that the cells were exposed to a temperature of 37°C is not known.

tions, clearly the data as a whole suggest that mutations in the C terminus largely are responsible for the loss of function of these mutants.

The growth phenotypes of each allele were examined at 23, 30, and 37°C (Fig. 2A). As expected, none of the mutants grew at 37°C, consistent with their isolation as temperature-sensitive mutants. Differences in growth rates were observed among the mutants at 23, 30, and 34°C (not shown), suggesting a range of severity among the alleles. The *taf90-1*, *taf90-8*, and *taf90-10* mutants displayed a slight reduction in growth rate at 23°C, which was more prevalent at 30°C (Fig. 2A). In addition, a number of mutants grew significantly slower than wild-type cells at 23°C in liquid medium, such as the *taf90-1*, *taf90-3*, and *taf90-19* mutants, despite showing comparatively milder defects on solid medium at the same temperature (not shown).

It has been proposed that certain weak temperature-sensitive (leaky) alleles of TAF_{II} mutants fail to display true loss-of-function phenotypes because they have residual activity at 37°C, despite their apparent lack of growth in liquid culture (25, 31). For example, mutants with certain temperature-sensitive alleles of *TAF40* that display restricted transcription phenotypes do not lose viability even after 2 days at the nonpermissive temperature, whereas a mutant with another allele that displays broader transcription defects loses viability during that time (25). These observations necessitated that we analyze the ability of each mutant to recover from a transient exposure to the nonpermissive temperature, so as to judge the efficacy of the mutations in destroying *TAF90* function. In addition to the *TAF90* mutants, we analyzed three well-characterized temperature-sensitive mutants considered to be tight based on the rapid disruption of transcription that results from their transfer to 37°C. These mutants are those carrying the *rpb1-1* allele of the gene encoding the largest subunit of Pol II (20, 38), the *tbp*$^{ts-1}$ allele of the gene encoding TBP (7), and the *taf17*-L68P,E148G allele of the TBP-associated factor 17 (31). Serial dilutions of each culture were spotted onto rich medium and either were grown solely at the permissive temperature or were exposed to the restrictive temperature for 12 or 24 h before being returned to the permissive temperature to resume growth. The results indicate that most of the *TAF90* mutants showed a loss of viability similar to that of the *rpb1-1*, *tbp*$^{ts-1}$, and *taf17*-L68P,E148G mutants (Fig. 1B). The noted exceptions are the *taf90-20* and *taf90-17* alleles. The *taf90-20* mutant failed to recover from a 12-h exposure to 37°C and was by far the most severely affected among all the general transcription mutants examined here. This mutant was even more strongly affected than the *rpb1-1* and *tbp*$^{ts-1}$ strains. On the other hand, the *taf90-17* strain recovered even after a 24-h incubation at 37°C. Therefore, with the exception of the *taf90-17* mutant, all of the *TAF90* mutants described here were at least as defective

in their recovery from an exposure to the restrictive temperature as the *rpb1-1*, *tbp*$^{ts-1}$, and *taf17*-L68P,E148G mutants. This analysis uncovered differences in the growth phenotypes of the *taf90-17* and *taf90-19* mutants: the *taf90-19* allele is more deleterious to cell viability. Moreover, the *taf90-17* mutant grows well at 34°C, whereas the *taf90-19* mutant grows very poorly at this temperature (not shown). Therefore, based upon these two growth phenotypes, the additional amino acid substitution, G691S, contained in the *taf90-17* mutant is somewhat compensatory for the defects caused by the S542F mutation found in both mutants.

Mutations in certain SAGA subunits can suppress *Ty* insertions (SPT phenotype) and cause inositol and galactose auxotrophies (16, 56); therefore, we screened each mutant for these phenotypes. The *TAF90* mutants were reconstructed in a strain containing a *Ty* insertion at the *HIS4* locus, *his4-914* (56, 57), and plated onto medium lacking histidine. Whereas a strain containing a mutation in *spt3* grew on medium lacking histidine, indicating suppression, none of the *TAF90* mutants showed this phenotype (not shown). In addition, none of the mutants displayed obvious galactose or inositol auxotrophies under conditions that would support growth (not shown).

Allele-specific defects in Pol II-mediated transcription. To determine the effects of *TAF90* mutations on Pol II-mediated transcription, changes in mRNA levels were detected by probing a blot of total RNA with radiolabeled oligo(dT). This method has been used repeatedly to analyze transcription defects in TAF_{II} mutants (3, 25, 31, 46, 54). From the data presented in Fig. 3, it appears that shifting each mutant to 37°C had diverse consequences on the levels of total poly(A)$^+$ RNA. Based upon the magnitude and kinetics of RNA loss, the mutants can be roughly grouped into classes. The first category of mutants exhibited a reduction in mRNA at the permissive temperature, a small drop at the 1-h time point, and then maintenance of that level to 4 h. Examples of this class are the *taf90-1*, *taf90-15*, *taf90-17*, and *taf90-19* mutants. The next class showed slight, gradual reductions for the first 2 h and then a sharp decline at 4 h, such as the *taf90-8* mutant. Most mutants represent the third type, which exhibited a steady decrease immediately after the temperature shift. Examples of this class are the *taf90-3*, *taf90-10*, *taf90-14*, and *taf90-16* mutants and the previously described *taf90*$^{ts2-1}$ mutant (2, 54). Even within this group the kinetics and magnitude of RNA loss varied considerably, from the weaker phenotypes exhibited by the *taf90-3* and *taf90*$^{ts2-1}$ mutants to the stronger phenotype of the *taf90-14* mutant. Finally, there is one mutant, *taf90-20*, which is clearly unique. Shifting this mutant to 37°C resulted in a rapid, and significant, reduction in poly(A)$^+$ RNA levels (Fig. 3). Significantly, the kinetics and magnitude of mRNA loss observed in the *taf90-20* mutant were very similar to those of the Pol II mutant, *rpb1-1*. Specifically, the mRNA levels in the *taf90-20* mutant were reduced to 32% of the wild-type level by 1 h and to 7% by 4 h, while the quantities in the *rpb1-1* strain declined to 20 and 6%, respectively. In summary, it appears that a variety of transcription defects were observed, ranging from weakly affected, as in the case of *taf90-1* and *taf90-3*, to very severe, as seen in the *taf90-20* mutant.

Next, we wanted to determine if the changes in total poly(A)$^+$ RNA described above reflect the loss of a few very abundant RNAs or a reduction in the expression of many

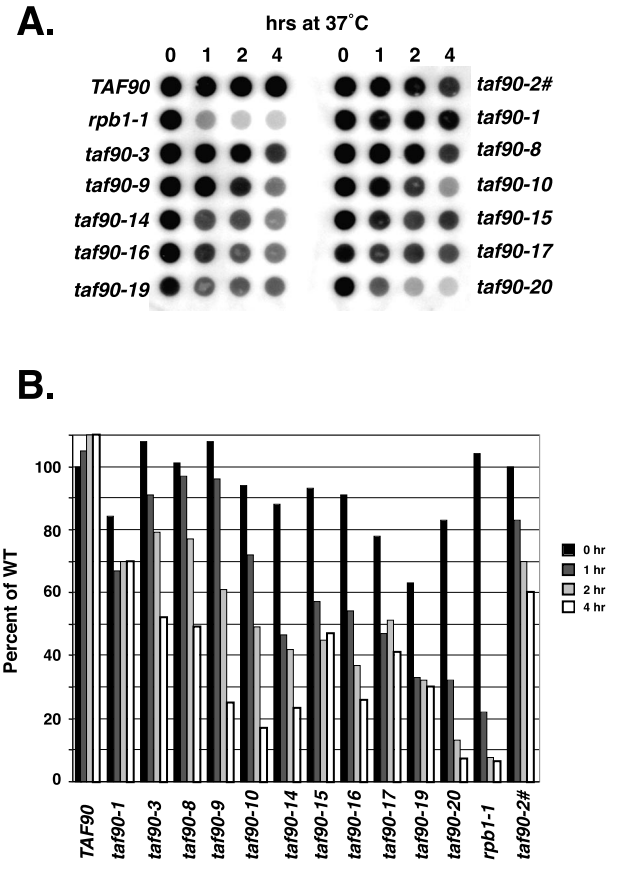


FIG. 3. Poly(A)$^+$ RNA levels. (A) Cultures were grown in YPAD to an OD_{600} of approximately 0.3 and then shifted to 37°C. Aliquots were removed prior to the shift (0 h) and after 1, 2, and 4 h at 37°C, and RNA was isolated by acid-phenol extraction. Ten micrograms of total RNA was spotted onto nitrocellulose, and poly(A)$^+$ RNA was detected by probing with a ^{32}P-labeled dT_{20} probe. (B) Quantification. Spots were analyzed using a phosphorimager. The percentage of wild-type (WT) levels was determined by comparing the counts in each spot with those in the wild-type spot from 0 h. The *taf90-2* strain (#) is a previously described noncogenic temperature-sensitive strain used as a control (2). The relative levels of mRNA in the mutants versus the wild type varied between 5 and 15% between experiments, but most points varied by less than 10%.

different RNAs. If the latter is true, we expect that the kinetics and magnitude of the reductions of specific transcripts will match those observed in the poly(A)$^+$ blots. Genes that have been routinely used to characterize other TAF_{II} mutants were selected (2, 3, 25, 35, 36, 37, 43, 46, 53). The steady-state levels of *TRP3*, *ACT1*, *DED1*, *TCM1*, and *MET19* RNAs were examined by S1 nuclease protection, and the transcripts of *CLB2*, *CLN2*, *RPS5*, and *TRX1* were detected by Northern blotting. Shifting cells to the restrictive temperature significantly affected the transcription of *TRP3*, *MET19*, *TCM1*, and *ACT1* in nearly every mutant (Fig. 4). The RNA levels of these genes showed a gradual decrease over time, and the severity of the defects varied among the mutants, similar to what was observed in the analysis of poly(A)$^+$ RNA quantities. For example, shifting the *taf90-1* mutant to 37°C only weakly affected the levels of total poly(A)$^+$ RNA up to 4 h, and similarly, nearly wild-type levels of mRNA for each of these individual genes

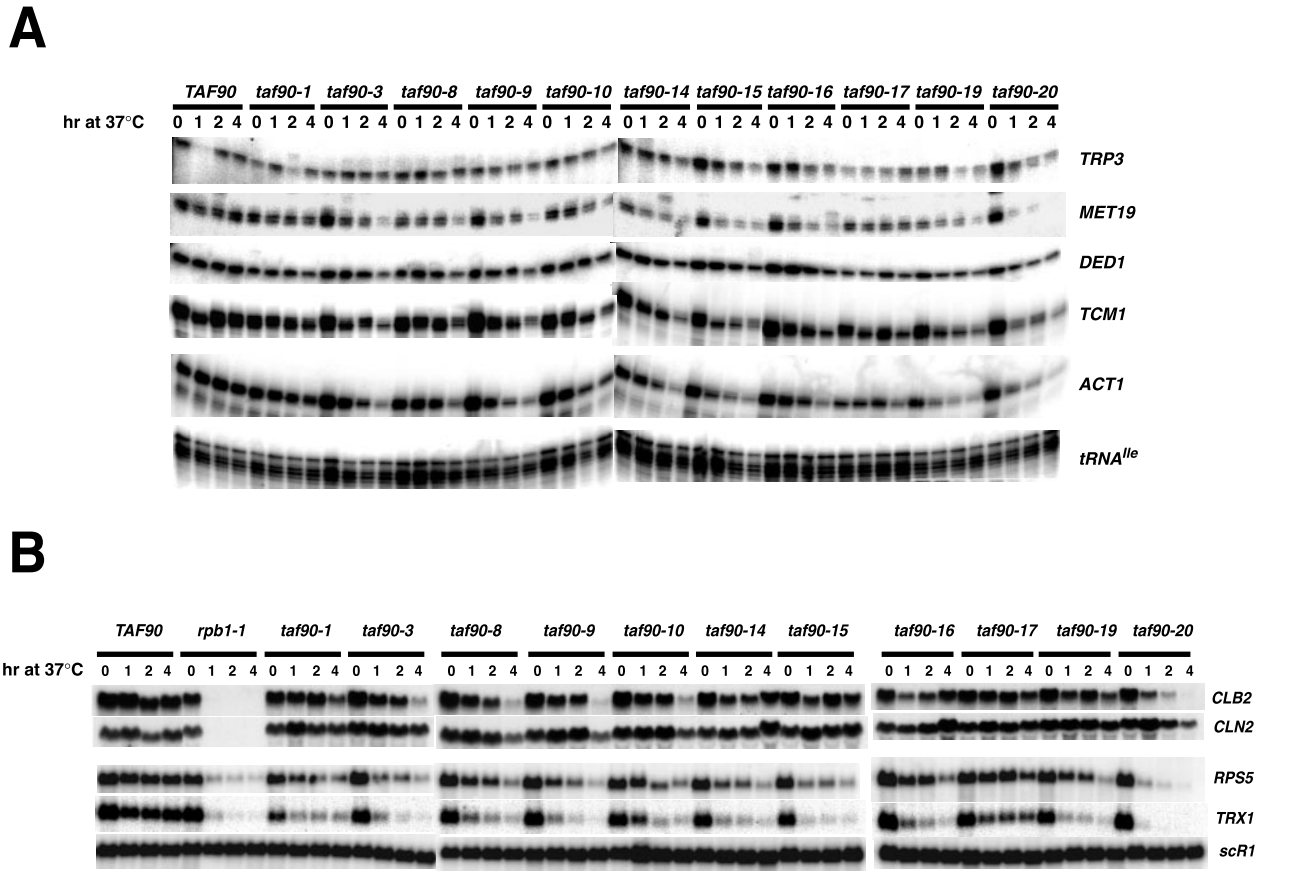


FIG. 4. Transcription analysis of specific messages. Cultures were grown, treated, and processed as described in the legend to Fig. 3. (A) S1 analysis of specific messages. Ten micrograms of total RNA was analyzed by S1 nuclease protection (7). $tRNA^{Ile}$, a Pol III transcript, was used as an RNA control. (B) Northern blotting. Fifteen micrograms of total RNA was fractionated on formaldehyde-containing gels and transferred to a membrane, and the specific transcripts were detected by hybridization with ^{32}P-labeled probes corresponding to the coding regions of *CLB2*, *CLN2*, *TRX1*, and *RPS5*. The Pol III-transcribed scR1 was used as a loading control.

were observed over the same time period (Fig. 4). Moreover, the *taf90-17* mutant displayed reduced total mRNA levels at 23°C, which were not strongly reduced further by the temperature shift, and likewise it displayed a reproducible reduction in *TRP3*, *MET19*, *DED1*, and *ACT1* RNA levels at 23°C that was not exacerbated by the temperature shift. Shifting the *taf90-20* mutant to 37°C caused rapid and dramatic losses of *TRP3*, *MET19*, *TCM1*, and *ACT1* mRNAs by 1 h, similar to what was observed for the levels of poly(A)$^+$ RNA. Therefore, the losses of poly(A)$^+$ RNA observed in each mutant closely correlated with the magnitude and kinetics of the losses of the individual transcripts examined here as well. In contrast to the case for these other genes, *DED1* transcription was only weakly affected in all mutants, including the *taf90-20* strain. Therefore, even with the strongest allele, some genes are not strongly affected by the inactivation of *TAF90*.

The messages for *TRX1*, *RPS5*, *CLN2*, and *CLB2* were analyzed because they are particularly sensitive to mutations in *TAF145* and other TAF_{II}s (3, 20, 25, 47, 54; J. C. Reese, unpublished data). The results presented in Fig. 4B indicate that the inactivation of most alleles, with the exception of *taf90-17*, strongly affected the transcription of *RPS5*, and all mutants displayed significant reductions in *TRX1* mRNA (Fig. 4B). Once again, inactivation of *taf90-20* had the most deleterious effect on the levels of *TRX1* and *RPS5* RNAs; their transcription is nearly abolished by 1 h at the restrictive temperature. This is the only allele that exhibited a reduction in these transcripts similar to that for the *rpb1-1* mutant. Even mutants such as *taf90-1*, *taf90-3*, and *taf90-8*, which displayed weaker reductions in total poly(A)$^+$ RNA (Fig. 3) and in the specific transcripts analyzed in Fig. 4A at up to 4 h, exhibited more rapid and larger reductions in the expression of these two genes. Thus, it appears that the expression of *RPS5* and *TRX1* is especially sensitive to *TAF90* mutations and is not as prone to allelic variations.

Cyclin expression is abolished in certain TAF_{II} mutants, and in some cases this may explain the cell cycle phenotypes observed in those mutants (20, 26, 28, 54). Our preliminary analysis revealed that only the *taf90-9* and *taf90-20* mutants displayed a convincing arrest in G_2/M at the restrictive temperature, whereas only very weak arrest phenotypes were observed in other mutants (J. C. Reese, A. K. Fisher, and T. A. Albright-Frey, unpublished data). The expression of the cyclins *CLN2* and *CLB2* was analyzed following a temperature shift, and with the exception of *taf90-20*, it appears that the alleles had a weak effect or no effect on *CLB2* and *CLN2* transcription (Fig. 4B).

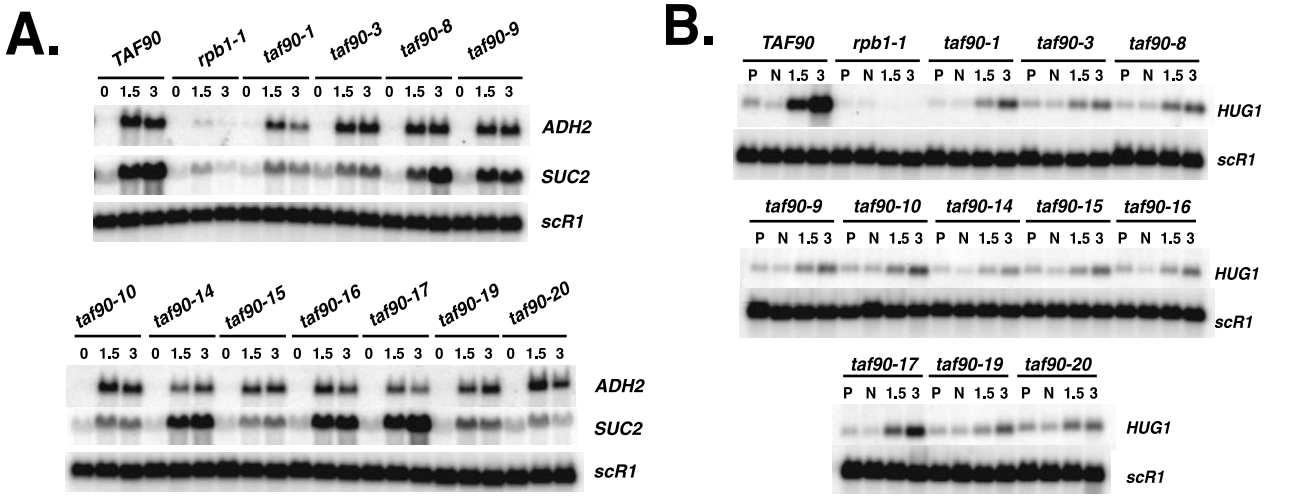


FIG. 5. Induced transcription. (A) Derepression of *SUC2* and *ADH2*. Cultures of YJR241-x were grown initially in YPAD at 23°C. Following the removal of an aliquot from each culture, cells were centrifuged, resuspended in YPA containing a low concentration of dextrose (0.05%) prewarmed to 37°C, and grown at the restrictive temperature. RNA was isolated from aliquots that were removed after 1.5 and 3 h at 37°C in low-dextrose medium. Transcripts were detected by Northern blotting. scR1 was used as a loading control. (B) Analysis of a DNA damage-induced gene. Cultures of YJR241-x were grown in rich medium and shifted to the nonpermissive temperature. Aliquots were removed (permissive [lanes P]) prior to the temperature shift. After 1 h at 37°C, MMS was added to 0.02%. Aliquots were taken at 1.5 and 3 h after treatment with MMS. A separate culture was maintained at 37°C but left untreated (nonpermissive [lanes N]) for 4 h. *HUG1* RNA was analyzed by Northern blotting. scR1 was used as a loading control.

Most mutants, such as *taf90-10*, showed a decrease in transcription only at the 4-h time point, a time when the decrease in transcription may result from decreased cell vitality.

Activated transcription in *TAF90* mutants. The genes analyzed thus far were actively transcribed at the moment the mutants were inactivated. We next examined the activation of genes when the mutants were exposed to the restrictive temperature. We first examined the derepression of catabolite-repressed genes, specifically, *ADH2* and *SUC2*. It has been reported that the depletion of TAF90p leads to a decrease in *ADH2* gene expression and that TFIID interacts with Ard1p, the specific activator of this gene. (24). *SUC2* was chosen for analysis because we have found that its derepression is severely impaired in certain TAF_{II} mutants (Reese, unpublished data). The wild-type strain and each of the mutants were grown to log phase in high-dextrose medium, shifted to 37°C, and then switched to prewarmed low-dextrose medium for 1.5 and 3 h. Although none of these mutants displayed abated *ADH2* transcription, mRNA levels were reduced in the *taf90-1, taf90-14*, and *taf90-17* mutants (Fig. 5A). Several alleles exhibited wild-type levels of *ADH2* RNA, reaffirming the allele-specific nature of these mutations. In addition, we found that the expression of *ADH2* is only weakly affected in the *taf90-20* mutant, which displayed the strongest transcription phenotype. We cannot account for the differences between our results and those of Komarnitsky et al. (24) with certainty. The previous study utilized a copper-inducible shutoff strategy that required significantly longer incubation times under the restrictive conditions, which may have resulted in secondary effects that adversely affected *ADH2* expression.

Several *TAF90* mutants showed large reductions in the levels of *SUC2* mRNA (Fig. 5A), including the *taf90-1* and *taf90-3* mutants, which we classify as having weaker transcription phenotypes. Interestingly, the *taf90-14* and *taf90-17* mutations appear to have no effect of *SUC2* transcription, whereas they were two that had a stronger effect on *ADH2* expression. Our analysis of these two catabolite-repressed genes highlights the allele- and gene-specific nature of the transcription defects in each mutant and clearly demonstrates the compensatory nature of the G691S substitution in the *taf90-17* mutant. The activation of *SUC2* is not strongly affected by the *taf90-17* allele, whereas it is drastically reduced by the *taf90-19* allele.

Depletion of TAF90p or inactivation of a temperature-sensitive allele strongly reduces the expression of DNA damage-induced genes (27). We therefore examined the expression of a representative of this class of genes, *HUG1,* in the mutants. Cells were shifted to 37°C and treated for 1.5 and 3 h with the DNA-damaging agent methyl methanesulfonate (MMS). A separate untreated aliquot of cells was maintained at 37°C for 3 h to detect changes in the uninduced level of transcription (Fig. 5B). The results presented in Fig. 5B indicate that inactivation of every allele affected the DNA damage-induced expression of *HUG1*. Expression was significantly reduced even in the *taf90-1*, *taf90-3*, and *taf90-17* mutants (although to a lesser extent), which display the most selective transcription defects of all of the mutants described here. In agreement with our previous analysis of the TAF_{II} dependence of DNA damage-inducible genes (27), these alleles appeared to specifically affect the derepression of this gene, as the uninduced level of RNA was not significantly reduced even after 3 h at 37°C (Fig. 5).

Some mutants of TAF_{II}s shared by TFIID and SAGA affect Gcn4p-activated transcription (3, 35, 37); therefore, we examined the expression of the Gcn4p-responsive gene *HIS3* in the mutants. *HIS3* contains several transcription start sites; a non-consensus TATA element (T_C) is responsible for constitutive transcription from position +1, while a consensus TATA element (T_R) is responsible for transcription from +13 (5).

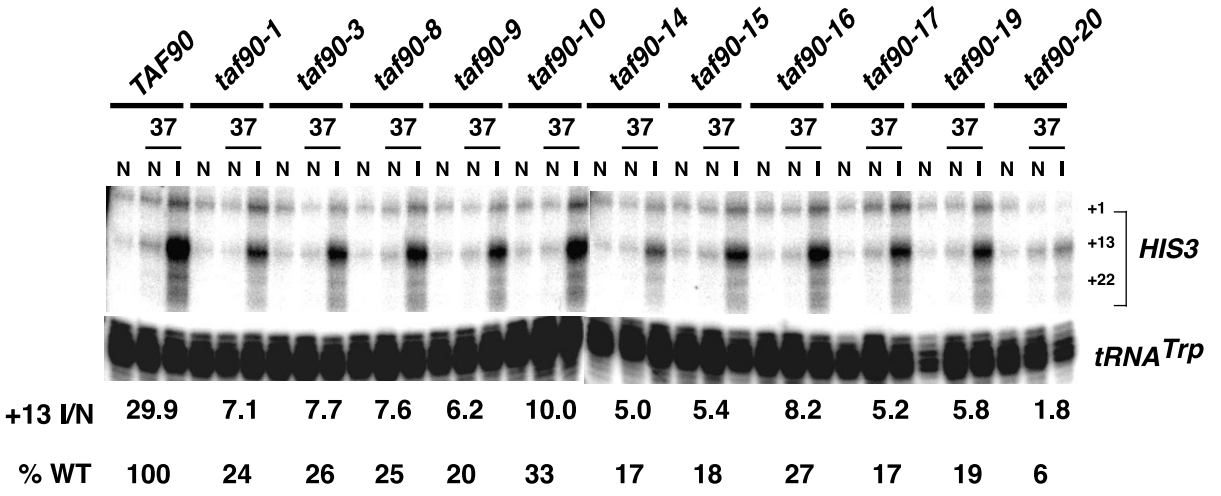


FIG. 6. Effects of *TAF90* mutations on Gcn4p-mediated transcription. Cells were grown under noninducing conditions in synthetic complete medium at 24°C (lanes N) and at 37°C for 3 h (lanes N, 37). A separate culture was grown in synthetic complete medium without histidine, shifted to 37°C for 1.5 h, and incubated for another 1.5 h in the presence of 30 mM 3-aminotriazole (lanes I, 37). Analysis of *HIS3* was performed by S1 nuclease protection. The induction level (+13 I/N) is the ratio of the +13 transcript from cells grown under inducing conditions at 37°C to that from cells grown under noninducing conditions at 37°C. The reduced tRNA signal in the *taf90-19* sample is a gel-loading artifact and is not reproducible. WT, wild type.

Gcn4p preferentially activates transcription from the +13 start site (5, 6). The cells were grown for 3 h at 37°C in complete medium (noninducing conditions) or in medium lacking histidine for 1.5 h at 37°C, followed by another 1.5 h in the presence of 30 mM 3-aminotriazole (inducing conditions). Comparing the levels of *HIS3* mRNA when the cells are grown under noninduced conditions at 23°C (permissive) versus 37°C (nonpermissive) gives an indication of the effect of each mutant on the basal transcription level. In all but one mutant, shifting the cells to 37°C for 3 h did not affect the uninduced levels of the +1 or +13 transcripts from *HIS3* (Fig. 6). A reduction in the +1 transcript was observed in the *taf90-20* mutant in this and other experiments. In contrast, the level of starvation-induced transcription from the +13 transcript was strongly reduced for every allele. The magnitudes of induction varied between 33 and 6% of the wild-type level, but most were reduced to approximately 20%. Not surprisingly, the *taf90-20* mutant displayed the largest reduction in induced transcription, to 6% of wild-type levels. Interestingly, unlike what was observed in the numerous examples presented above, the *taf90-19* and *taf90-17* alleles were equally defective for the activation of *HIS3* by Gcn4p. Thus, the compensatory nature of the G691S substitution is not universal and displays some gene specificity. These results demonstrate that *TAF90* is essential for the activation of *HIS3* by Gcn4, similar to the case for other TAF_{II}s found in both TFIID and SAGA.

Inactivation of TAF90 mutants reduces TFIID and SAGA subunit levels. Inactivation of certain TAF_{II} temperature-sensitive alleles, or depletion of its protein, results in the degradation of multiple TFIID and SAGA subunits (25, 31, 43, 46, 53). Codegradation of TAF_{II}s is a reasonable indicator of the disruption of complex structure; therefore, we monitored the levels of TAF_{II}s, TBP, and two SAGA components after shifting the cells to 37°C. In many mutants, the levels of TFIID subunits were not strongly reduced until 4 h at 37°C (Fig. 7). The clearest exception was the *taf90-20* mutant, which showed

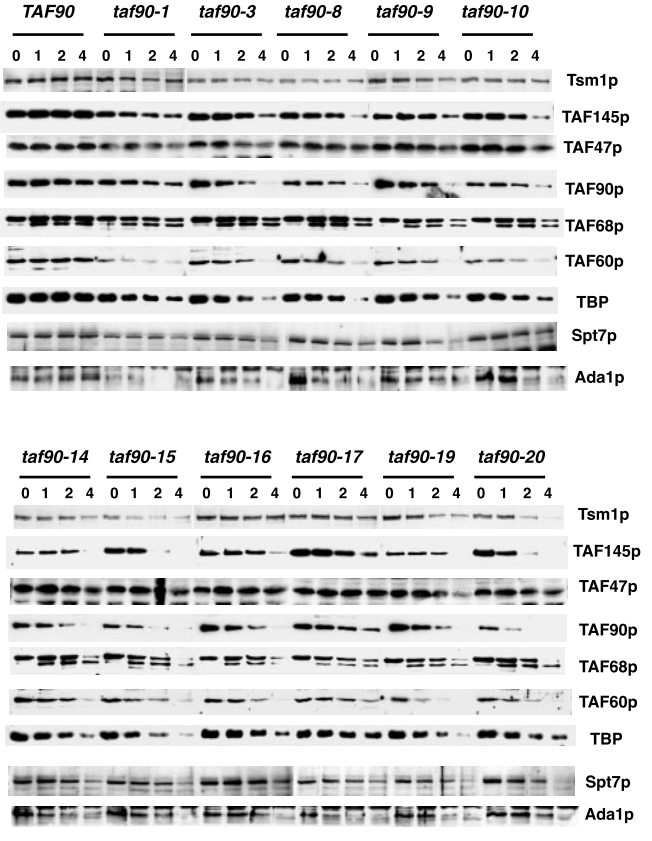


FIG. 7. Steady-state levels of TFIID and SAGA subunits following a temperature shift. Wild-type and mutant strains were grown at 24°C in YPAD to an OD_{600} of approximately 0.4, and after removal of an aliquot for the $t = 0$ time point, they were transferred to a 37°C shaking water bath. Aliquots of culture were removed after 1, 2, and 4 h at the nonpermissive temperature. Extracts prepared from these cells (20 μg of protein) were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose, and the specific proteins were detected by immunoblotting.

a steady decrease in TAF90p, TAF145p, TAF60p, and, to a lesser degree, TBP immediately upon the temperature shift. Thus, by this criterion the *taf90-20* allele is the most detrimental to the structure of the TFIID complex. Overall, the degree to which inactivation of these mutants reduced TAF_{II} levels correlated with their growth and transcriptional defects. The *taf90-20* and *taf90-17* mutants had the strongest and weakest effects on the levels of TFIID subunits, respectively, and likewise they had the most and least severe growth and transcriptional defects. Additionally, the *taf90-19* mutant displayed stronger growth and transcriptional defects than the *taf90-17* mutant (Fig. 2, 4, and 5) and likewise displayed more drastic reductions in TAF_{II}s. Finally, the *taf90-1* mutant, which showed significant growth defects at the permissive temperature (Fig. 2A and data not shown), had measurable reductions in TFIID and SAGA subunits, even when grown at 23°C. This is most obvious for TAF145p, TAF60p, TAF47p, TAF17p (see below), and Ada1p. The steady-state levels of two SAGA-specific components, Ada1 and Spt7, were also examined. Inactivation of each allele had very different consequences for these two proteins. Whereas Spt7p remained relatively stable in most mutants until the 4-h time point, the levels of Ada1p were significantly reduced earlier in the temperature shift. This is particularly apparent in the *taf90-8*, *taf90-14*, *taf90-17*, and *taf90-19* mutants, which showed noticeable reductions in Ada1p by 1 h. Interestingly, the *taf90-20* mutant, which had the largest reductions in TFIID subunits, exhibited relatively minor reductions in Spt7p and Ada1p up until the 4-h time point. Therefore, in regard to the degradation of these two SAGA subunits, this allele appears to be no more destructive than the other mutations.

Mutations in *TAF90* affect its ability to associate with TFIID and SAGA. Next, we examined the ability of each mutant to bind to TFIID and SAGA in extracts prepared from cells grown at the permissive temperature and after 3 h of growth at 37°C by co-IP using anti-TAF90p antisera. The ability of each mutant to incorporate into TFIID was judged by the IP efficiencies of Tsm1p, TAF145p, and TAF47p, since they are not subunits of SAGA (15). Likewise, immunoblotting for Spt7p, Ada3p, and Ada1p monitored their association with the SAGA complex. Unfortunately, the antibodies to Ada3p were not of sufficient quality to detect the levels of these proteins in whole-cell extracts; nonetheless, they could be used to analyze the immunopurified material, and these results are presented. The loads and IP material are presented in Fig. 8A and B, respectively. The quantity of TAF90p immunoprecipitated from each sample largely correlated with its relative abundance in the extracts (input samples) and in many cases was not significantly different for samples from cells grown at 23 and 37°C (Fig. 8B). Only the samples from the *taf90-16* and *taf90-20* mutants showed significantly reduced amounts of TAF90p in the IPs. Virtually every mutant displayed a temperature-dependent defect in its ability to interact with most TFIID subunits examined here. The two exceptions were the *taf90-1* mutant, which showed a temperature-independent reduction in its ability to interact with other TFIID subunits, and the *taf90-17* mutant, which showed only minor reductions in extracts from cells grown at 37°C (Fig. 8B). The *taf90-20* mutant showed the strongest defects. However, since the levels of TAF_{II}s were severely reduced in the extracts of this mutant, we can be

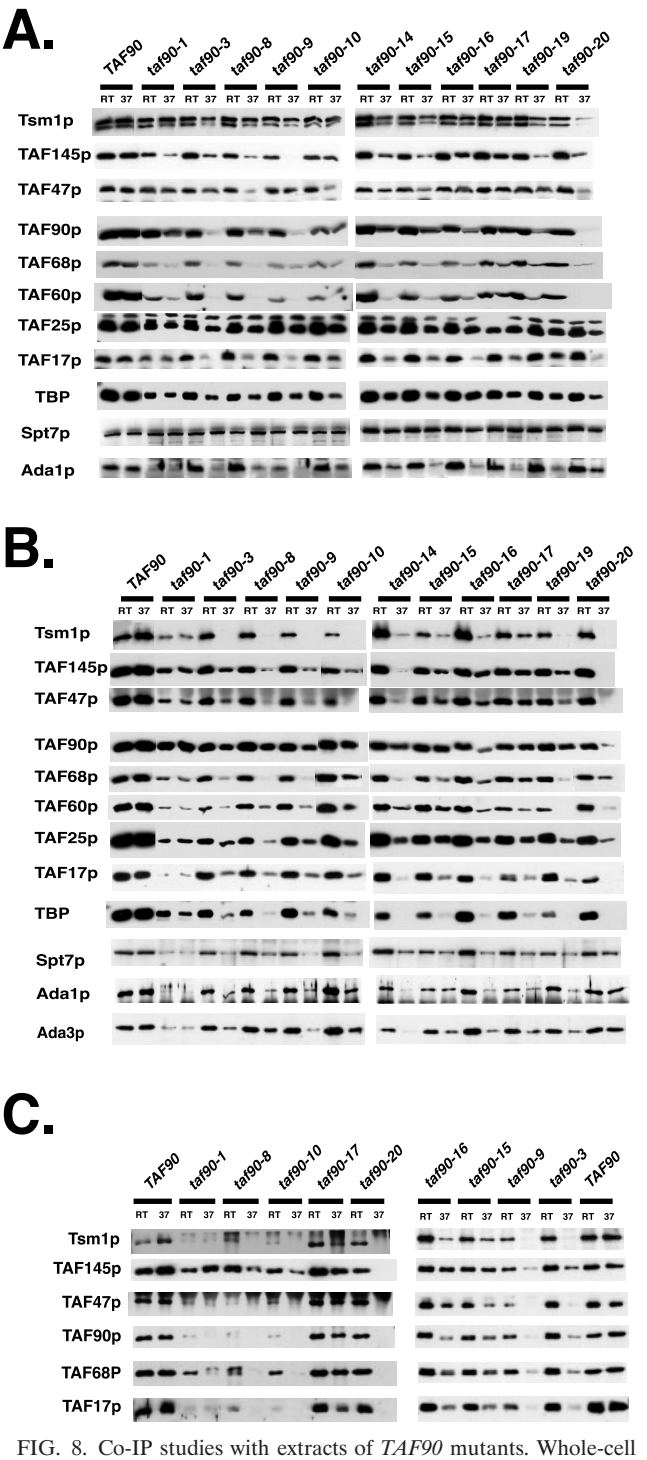


FIG. 8. Co-IP studies with extracts of *TAF90* mutants. Whole-cell extracts were prepared from cells grown at room temperature (approximately 24°C) or at 37°C for 3 h. Complexes containing TAF90p were immunoprecipitated from cell extracts using antibodies raised against TAF90p or TAF145p. Cell extracts and IPs were analyzed by Western blotting using the antibodies indicated. The input material (A) and complexes immunoprecipitated using antibody raised against TAF90p (B) or TAF145p (C) are shown.

certain only that the *taf90-20* allele is extremely disruptive to the TFIID complex. Small amounts of TAF90p, TAF60p, TAF68p, and TAF25p was detected in the IP material from the extracts of this mutant grown at 37°C, which may be attributed to TAF_{II}s contained in the SAGA complex (see below). This assay also highlights differences between the *taf90-17* and *taf90-19* alleles. In agreement with what was observed for these two alleles in regard to their effects on the steady-state levels of TAF_{II}s (Fig. 7), the binding of TFIID subunits to the taf90-19p mutant protein was reduced to a greater degree than that to the taf90-17p mutant protein.

Interestingly, while the levels of Tsm1p in the 37°C extracts were not significantly reduced in each of the mutants (Fig. 8A), very strong reductions in its ability to co-IP with the TAF90p mutants were observed (Fig. 8B). Moreover, the *taf90-3*, *taf90-16*, and *taf90-19* mutants appeared to be more strongly affected in their ability to co-IP Tsm1p versus either TAF145p or TAF47p, suggesting that these mutants somewhat selectively affect the interaction of Tsm1p with TFIID. The ability of TBP to co-IP with TAF90p was severely compromised in all but the *taf90-1* mutant when the cells were grown at 37°C (Fig. 8B). Therefore, the majority of the TBP in these extracts (Fig. 8A) likely represents free TBP or TBP distributed among other complexes. In a number of cases, the reduction of TBP in the IP material was disproportionately lower than that of TAF145p; therefore, the TAF145p-TBP interaction alone is not sufficient to maintain TBP in the TFIID complex. A similar result was also found in the analysis of a *TAF68/61* mutant (43).

The presence of Spt7p, Ada1p, and Ada3p in the immunoprecipitates was examined to ascertain each mutant's ability to incorporate into the SAGA complex. Most mutants, such as *taf90-3*, *taf90-8*, *taf90-9*, *taf90-10*, *taf90-14*, *taf90-16*, *taf90-17*, and *taf90-19*, displayed temperature-dependent interactions with Spt7p, Ada1p, and Ada3p, while the *taf90-1* mutant displayed a temperature-independent reduction in the IP efficiency of these three SAGA subunits. Thus, most of the mutants described here are defective for interacting with SAGA in a manner similar to that for TFIID. An interesting exception is the *taf90-20* mutant. This allele is the most disruptive to TFIID, but the quantities of these three SAGA subunits detected in the IPs were not significantly reduced. Therefore, the mutations contained in this allele do not affect its ability to interact with at least some SAGA components, and it is significantly more competent to bind to SAGA than most of the other mutants described here. Comparing the results for the *taf90-17* and *taf90-19* alleles revealed differences in the ability of these two mutants to interact with SAGA versus TFIID components. Whereas the additional amino acid substitution contained in the *taf90-17* mutant protein stabilized its interaction with multiple TFIID subunits (see above), it did not confer the same effect on SAGA components: the amounts of Spt7p, Ada1p, and Ada3p contained in the IPs from extracts of the *taf90-17* and *taf90-19* mutants were similar (Fig. 8B). Thus, it appears that the additional amino acid substitution is compensatory for TFIID but not for SAGA interactions. This hypothesis is consistent with the results indicating that both mutants are equally defective for *HIS3* activation (Fig. 6), a process heavily dependent upon SAGA function (for reviews, see references 16 and 56).

While the results of Fig. 7 suggest otherwise, it is a formal possibility that these mutations leave TFIID denuded of TAF90p but otherwise intact after a temperature shift. The assay shown in Fig. 8B measures the ability of TAF90p to interact with TFIID rather than the integrity of the complex; therefore, we repeated the IPs using antiserum to TAF145p (Fig. 8C). Overall, the results obtained using anti-TAF145p antibodies to co-IP TAF_{II}s confirmed those in Fig. 8B; that is, the quantities of TAF_{II}s coimmunoprecipitated from extracts of mutants grown at 37°C were significantly reduced. The noted difference is that less Tsm1p, TAF68p, and TAF47p were detected in the anti-TAF145 IPs from the extracts of the *taf90-1* and *taf90-10* mutants grown at the permissive temperature than in the anti-TAF90p IPs (compare Fig. 8B and C). In addition, no TFIID subunits were detected in the anti-TAF145p IPs from the 37°C extracts of the *taf90-20* mutant, even in overexposed blots (not shown); therefore, the small quantities of TAF90p, TAF68p, TAF60p, and TAF25p detected in the TAF90p IPs (Fig. 8B) likely originated from the SAGA complex. In conclusion, our results show that each mutation has different effects on TFIID structure, with *taf90-1* and *taf90-17* being the least severe and *taf90-20* being the most. In addition, comparison of the abilities of the taf90-17p, taf90-19p, and taf90-20p mutant proteins to copurify with TFIID and SAGA components suggests that TAF90p interacts with these two complexes differently.

TAF90 mutations reduce SAGA abundance and activity. The results described in Fig. 8 indicate that inactivation of most TAF90p mutants adversely affects SAGA subunit levels and/or their ability to interact with the complex. Nonetheless, these are indirect assays for SAGA complex activity, and the ability to co-IP SAGA subunits, as is observed in the *taf90-20* mutant, does not necessarily indicate that the mutant complex is functional. The preparation and fractionation of extracts from each of these 11 *TAF90* mutants to analyze SAGA, at least in duplicate, would be a significant undertaking. Therefore, three mutants were selected to more carefully determine their effect on the ability of the SAGA complex to acetylate nucleosomes. The *taf90-20* mutant was chosen since it completely disrupts TFIID structure and yet appears not to strongly affect its ability to copurify with some SAGA components (Fig. 8B). Thus, it is important to determine if SAGA, or a subcomplex, containing the mutant protein is active. The *taf90-17* mutant was chosen because it has the weakest effect on TFIID structure and transcription yet displayed measurable defects in its ability to bind SAGA subunits. Finally, *taf90-10* was chosen as a representative of a mutant that displayed defects in both complexes and exhibited a moderate transcription phenotype.

Extracts were prepared from cells grown at 37°C for 3 h, and HAT complexes were purified by sequential chromatography over Ni-NTA affinity and Mono Q ion-exchange columns (14). Aliquots of alternating Mono Q fractions were used in HAT assays and analyzed by Western blotting. Each of the four distinct HAT complexes (14) retained on Ni-NTA-agarose were resolved in the sample from wild-type cells, and their locations in the elution profile are indicated above Fig. 9A. Their identities were assigned by Western blotting for unique subunits (see below and data not shown) and by histone substrate specificity. In each of the mutants, four HAT activities were also resolved, but the amount contained in the fractions

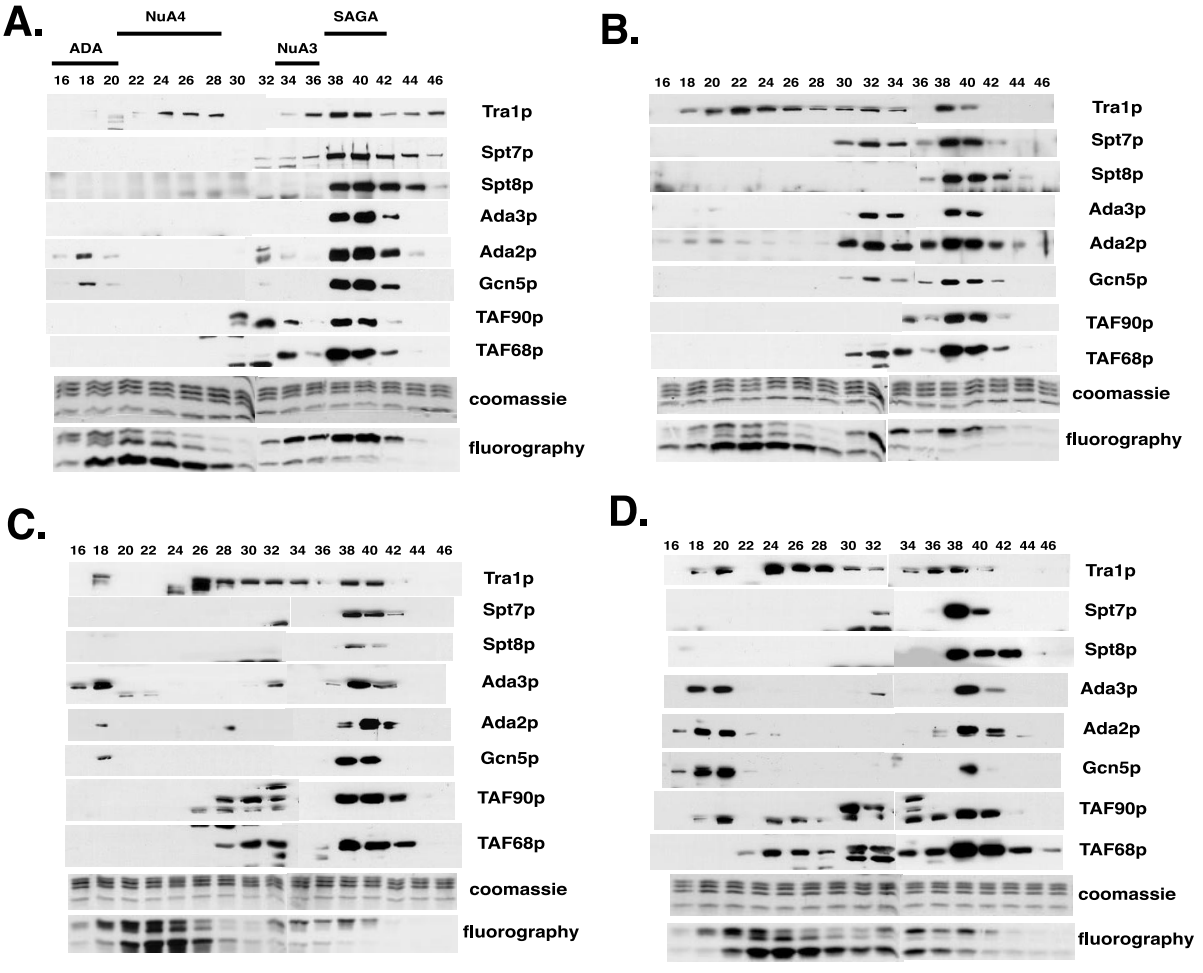


FIG. 9. Purification of HAT complexes from *TAF90* mutants. Extracts prepared from mutants grown at 37°C for 3 h were subjected to chromatographic analysis as described in Materials and Methods. Aliquots of the Mono Q column fractions were analyzed by Western blotting and HAT assays using nucleosomes as substrates. Elution profiles for the wild type (A) and the *taf90-20* (B), *taf90-17* (C), and *taf90-10* (D) mutants are shown. Note that the blots presented in panel D were deliberately exposed longer than those in panels A to C to detect the small amounts of SAGA subunits eluting in fractions 38 to 42.

where SAGA elutes (fractions 38 to 42) was significantly reduced (Fig. 9B to D). In contrast, the activities of the TAF90p-independent NuA4, ADA, and NuA3 complexes were comparable to those of the wild-type sample and were within the normal variation observed between different preparations (Fig. 9 and data not shown). Next, we examined the elution profiles of individual SAGA components by Western blotting. The Mono Q elution profile of the HAT complexes retained on Ni-agarose from wild-type extracts reveals that all of the SAGA subunits examined coeluted predominantly with their HAT activity in fractions 38 to 42, with the exception of those found in the ADA and NuA4 complexes (Fig. 9A). Western blotting of the fractions from each of the mutants revealed that significant amounts of most subunits were also in earlier fractions (Fig. 9B to D), indicating a breakdown of the mutant SAGA complexes. This is most obvious in the fractionation profile for the *taf90-20* mutant (Fig. 9B). Specifically, Tra1p broadly eluted in fractions 18 to 40, and significant amounts of Spt7p, Ada3p, Ada2p, Gcn5p, and TAF68p were detected in fractions 30 to 34 (Fig. 9B). It is not know if this represents a stable subcomplex of SAGA or free subunits that coincidentally coelute in these fractions. In addition to the appearance of breakdown products, the amounts of SAGA components eluting in fractions 38 to 42 were somewhat reduced in the *taf90-17* (Fig. 9C) and *taf90-20* (Fig. 9B) samples and were drastically reduced in the *taf90-8* (Fig. 9D) samples (for a more accurate estimation, see Fig. 10).

The reduced HAT activity observed in each *TAF90* mutant may be due to a reduction in the amount of SAGA, an altered complex with decreased catalytic activity, or both. Therefore, we directly compared the SAGA levels and activity in the peak Mono Q column fractions. Equal amounts (volumes) of the pooled fractions were used to measure the HAT activity using oligonucleosomes as substrates (Fig. 10A, left panel), and an aliquot was withdrawn and subjected to immunoblotting following the HAT assay (Fig. 10A, right panel). The results show that the levels of Gcn5p in the Mono Q fractions were reduced about 2.5- and 5-fold in the *taf90-20* and *taf90-17* mutants, respectively, whereas the level was reduced approximately 10-fold in the *taf90-10* mutant. The level of HAT activity in the *taf90-17* samples was roughly proportional to the levels of Gcn5p, but the *taf90-20* fractions contained an additional re-

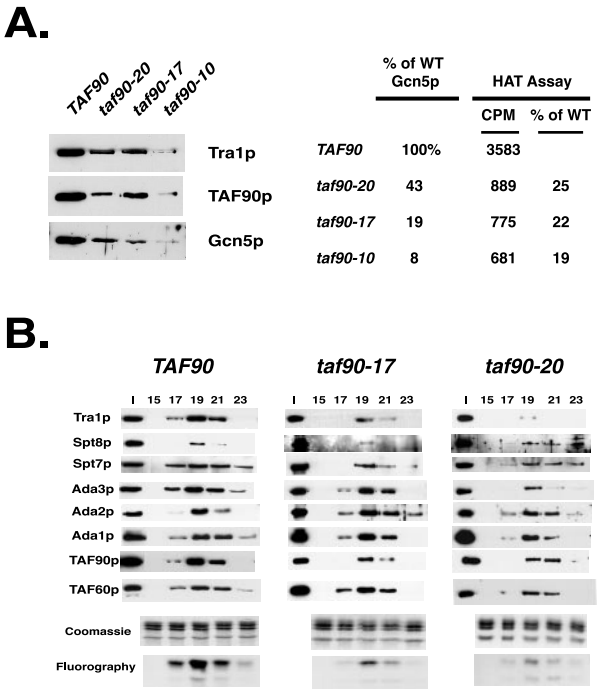


FIG. 10. Analysis of mutant SAGA complexes. (A) SAGA has reduced HAT activity in *TAF90* mutants. Fractions containing SAGA (fractions 38 to 42 from Fig. 9) were pooled and analyzed by Western blotting and HAT assays using oligonucleosomes as substrates. The relative levels of Gcn5p were determined from appropriately exposed blots using NIH Image software and are expressed as the percentage of the wild-type (WT) level. HAT activity was compared to that of SAGA isolated from wild-type cells, which was set at 100%. (B) Size exclusion chromatography. The pooled SAGA-containing fractions from the Mono Q chromatography (fractions 38 to 42 from Fig. 9) were concentrated by filtration and fractionated on a Superose 6 column. Each fraction was analyzed by Western blotting and HAT assays using nucleosomes as a substrate. The void volume corresponds to fraction 14. No HAT activity or SAGA components were detected in later-eluting fractions, and thus only fractions 15 to 23 are shown.

duction in activity that cannot be accounted for by reduced SAGA levels (Fig. 10A, right panel). For example, this mutant has 43% of wild-type Gcn5p levels but 25% of wild-type HAT activity (Fig. 10A). Thus, even though the SAGA complex from the *taf90-20* mutant is largely intact, its mutations reduce the activity of the complex. Higher HAT activity was detected in the *taf90-10* fractions compared to the level of Gcn5p. This is likely due to the contamination of these fractions with quantities of the NuA3 complex, which elutes near the SAGA complex (14) (Fig. 9A).

The integrity and activity of the mutant SAGA complexes from the *taf90-17* and *taf90-20* strains were analyzed by gel filtration chromatography. Unfortunately, the quantities of the SAGA complex from the *taf90-10* mutant were not sufficient for this analysis. The results presented in Fig. 10B show that the wild-type and mutant SAGA complexes eluted from the Superose 6 column with the expected mass of approximately 2 MDa (14, 15). While this analysis cannot rule out the possibility of a subtle change in structure or composition, it does suggest that the size of the mutant complexes is not significantly different from that of the wild type. In addition, we did not detect the presence of additional complexes eluting in later fractions by either HAT assays or Western blotting for SAGA subunits (data not shown). It is important to note that as observed in the Mono Q fractions, the nucleosomal HAT activity was significantly reduced (Fig. 10B). The HAT activities, measured in liquid assays, for the *taf90-20* and *taf90-17* mutants were approximately 19 and 15% of that for the wild type, respectively (not shown). We observed a slightly larger difference in HAT activities between the mutants and the wild-type complexes eluting from the sizing column versus that observed in the Mono Q fractions. This may be attributed to a further reduction in activity in the mutants during the processing of the samples for size exclusion chromatography or their separation from contaminating NuA3 complex. From these results, it appears that mutations in *TAF90*, like for *TAF68/61* (15), disturb the function of the SAGA complex by reducing the total amount of the SAGA complex present in the cell and impairing its ability to acetylate nucleosomes.

DISCUSSION

***TAF90* mutants display allele-specific and broad transcription defects.** The relatively thorough characterization of a large number of mutants has allowed us to make a number of observations regarding *TAF90* function. The first significant conclusion is that allele-specific defects in transcription can be observed among different mutants of the same gene, despite the fact that all alleles seem tight based upon their temperature-sensitive growth phenotypes. There were a number of clear examples where the expression of a gene was strongly reduced in one or more mutants but not in others. This argues that results obtained with any single temperature-sensitive allele may not reveal all of the functions of this gene. While we have largely concentrated our discussion on cases where the differences between alleles are very obvious, even the subtle differences in the magnitude and kinetics of RNA loss observed among all of the alleles described here could have consequences for the classification of certain genes as being *TAF90* dependent when a cutoff is applied. By no means does our study diminish the impact of the genome-wide studies performed to date. However, it already has been recognized that analyzing the genome-wide expression profile of any given mutant under a single growth condition does not constitute a comprehensive analysis of its function (for a review, see reference 61), and now it is clear that multiple alleles of the same gene may be necessary when conditional mutants are used.

The second important observation is that mutations in *TAF90* that cause a broad transcription defect can be isolated, as seen in mutants of other TAF_{II}s that are shared by TFIID and SAGA. Mutants of *TAF68/61*, *TAF60*, *TAF25*, and *TAF17* that satisfy this criterion had been described previously (3, 31, 37, 43, 46), but until this study such a mutant of *TAF90* had not been. The thermoinactivation of this mutant results in the rapid reduction in total poly(A)$^+$ RNA and in most of the specific messages analyzed here. Significantly, the magnitude and timing of these reductions were strikingly similar to those observed in the *rpb1-1* mutant, which is considered the standard for judging primary transcription defects (20, 26). This phenotype is unlikely to result from a reduction in only a small number of abundant messages, because we found that many

specific messages, some of which are of low to medium abundance, were reduced likewise. Therefore, mutations in *TAF90* that abolish transcription of many yeast genes can be isolated. We recognize that the analysis performed on this mutant thus far cannot accurately determine the exact fraction of the genome that is affected. Nonetheless, it is clear that this mutation does have much stronger effects on the expression of more genes than any of the other *TAF90* mutations described here or elsewhere. Equally clear is that inactivation of even the strongest mutant described here did not significantly affect the expression of all genes examined here, thus reinforcing the notion that TAF_{II}s are unlikely to be obligate transcription factors for the entire genome.

The cause of the broad transcription defects observed in certain TAF_{II} mutants is slowly being revealed. It has been proposed that redundancy between TFIID and SAGA explains this phenotype (26). While this explanation may account for an increase in the total number of genes affected in these mutants, it cannot solely account for the differences between these mutants and mutants of other TAF_{II}s found only in TFIID. The first evidence for this is that mutations in *TAF40* or depletion of TAF48p, two TAF_{II}s not in SAGA, cause reduced expression of many genes (25, 43). The second is the results presented here. Many of the *TAF90* mutants described here show significant reductions in their ability to interact with the SAGA components Ada3p, Ada1p, and Spt7p in co-IP experiments, yet they do not display the broad transcription phenotypes exhibited by the *taf90-20* mutant. Moreover, we found that the levels and activities of SAGA complex isolated from the *taf90-17*, *taf90-10*, and *taf90-8* (not shown) mutants were reduced at least as much as those of the *taf90-20* mutant. In light of these results, redundancy between SAGA and TFIID is at best an important contributing factor to the broad transcription defects observed in certain TAF_{II} mutants.

A reoccurring phenotype exhibited by most TAF_{II} mutants that show reduced expression of a large number of genes is that shifting them to the restrictive temperature results in dramatic reductions in the steady-state levels of most TFIID subunits (25, 31, 43, 46). This also is true of the *taf90-20* allele characterized here. It is striking how well the magnitudes of the transcription defects described here match the consequences of each mutant for the structure of TFIID. The *TAF90* mutants that display the most dramatic reductions in TAF_{II} levels when shifted to 37°C, and are the least competent to bind to TFIID in the co-IP studies, are also those that show the strongest transcription phenotypes. This is particularly apparent when comparing the weakest and strongest classes of mutants, *taf90-1* and *taf90-17* versus *taf90-20*, respectively. Therefore, our study argues that it is the rapid loss of many TFIID subunits that, when combined, results in broad transcription defects. This hypothesis is supported by a recent genome-wide analysis of transcription that showed that the combined effect of the loss of function of each individual TFIID subunit can account for the expression of greater than 70% of the genome (26). What now needs to be debated is what type of mutant, or depletion system, is most appropriate to assign functions to *TAF90*. Depletion strategies and mutants such as *taf90-20* are prone to secondary effects. It is likely that a comprehensive analysis of multiple alleles will be necessary.

The C terminus of TAF90p is required for TFIID and SAGA function. This study has established the importance of the WD40 repeats and the C terminus in maintaining the integrity of the TFIID and SAGA complexes. This result would not have been predicted from previous analysis of hTAF100, which suggested that the WD40 repeats are dispensable for its incorporation into TFIID or other TAF-containing complexes (9). Does this mean that human TFIID and yeast TFIID are different? It is more likely that these results are explained by the incorporation of the truncated hTAF100 into partial TFIID complexes or alternative TAF-containing complexes that had not been identified at the time of that study. We demonstrate that the C terminus of TAF90p is important for its association with TFIID and SAGA, but we cannot say that it is sufficient or that the N terminus does not make important contributions to this function as well. It is likely that regions across the entire length of the protein are required for TFIID and SAGA function. Interestingly, inactivation of nearly every mutant severely compromises its ability to co-IP with Tsm1p, TAF17p, and TBP, even though it retained some ability to copurify with other TAF_{II}s. This was even true for some of the alleles that have weaker effects on the co-IP of other TFIID subunits, such as TAF47p and TAF145p. This suggests that the interaction of Tsm1, TAF17p, and TBP is strictly dependent upon the integrity of the C terminus of TAF90p. This agrees very well with another analysis of $hTAF_{II}100$, which demonstrated that it directly interacts with $hTAF_{II}31$ (TAF17p homologue) and TBP (52). It is not known if Tsm1p, or its homologues, binds to the WD40 repeats of TAF90p.

Our results also indicate that *TAF90* is required for SAGA abundance and activity, as the complexes isolated from the *taf90-8* (not shown), *taf90-10*, *taf90-17*, and *taf90-20* mutants are significantly reduced in abundance and/or activity. Even though we did not purify SAGA from every mutant, essentially every allele showed impaired interactions with some SAGA subunits in co-IP studies, in particular Ada1p. Since Ada1 is essential for the structure and function of the SAGA complex (50), we can predict with a high degree of certainty that the activity of SAGA is compromised to some degree in each of the mutants described here. This prediction is supported by the inability of all mutants to support wild-type levels of starvation-induced transcription of *HIS3*, which is particularly sensitive to mutations in SAGA subunits. The C terminus of TAF90p is required for both SAGA and TFIID function, but it is likely that it binds to these two complexes differently. The first line of evidence for this is that the mutations contained in the *taf90-20* allele are the most disruptive to the integrity of TFIID and the least disruptive to that of SAGA. The second is the phenotypes of the *taf90-17* and *taf90-19* alleles. It is clear that the *taf90-19* allele has stronger effects on cell viability, on the integrity of the TFIID complex, and on transcription. However, both alleles are equally disruptive to the SAGA complex as judged by the co-IP studies (Fig. 8B) and are equally defective for the activation of *HIS3* (Fig. 6). Interestingly, both alleles contain the same S542F mutation, but *taf90-17* has an additional amino acid substitution that is compensatory in nature in regard to its interaction with TFIID but not SAGA. Molecular modeling of the WD40 repeat region of TAF90p, based on the known structure of another WD40 repeat protein, predicts that these mutations are on the same surface of the protein (not shown).

Whereas we did not isolate either a TFIID- or a SAGA-specific mutant in this screen, scoring for a temperature-sensitive phenotype may not be selective enough for the identification of such a mutant. Our discovery and analysis of the *taf90-17*, *taf90-19*, and *taf90-20* mutants suggest that isolating such a mutant is possible. Alternative screens that are more selective, such as an SPT phenotype for SAGA-specific mutants, may result in the identification of these types of mutants, which will further our understanding of the role of *TAF90* in these two important transcription regulatory complexes.

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