Genome-wide Transcriptional Dependence on TAF1 Functional Domains

Received for publication, December 27, 2005 Published, JBC Papers in Press, January 2, 2006, DOI 10.1074/jbc.M513776200

Jordan D. Irvin1 and B. Franklin Pugh2

From the Department of Biochemistry and Molecular Biology, Center for Gene Regulation, The Pennsylvania State University, University Park, Pennsylvania 16802

TFIID is composed of the TATA-binding protein (TBP) and 14 TBP-associated factors (TAFs),4 of which all but one are essential for cell viability. Several of these TAFs, along with TBP, are also found in SAGA (4). Although TBP is widely regarded as responsible for delivering TFIID to promoters through interactions of TBP with the TATA box, TFIID largely functions at TATA-less promoters (3, 5–7). TATA-containing promoters tend to be TFIID-independent and instead prefer to load TBP via the SAGA assembly pathway (3). The vast majority of all yeast genes (80–90%) are regulated through a TFIID/TATA-less arrangement, whereas a smaller minority depend primarily on a SAGA/TATA arrangement. Strikingly, the latter class largely includes stress-induced genes. Thus transcription complex assembly via the SAGA pathway might provide a greater level of inducibility, which is characteristic of stress-induced responses (3, 6, 8). Under normal growth conditions, SAGA is not essential for cell viability (4, 9). In the absence of SAGA, expression of virtually the entire measurable yeast genome becomes TFIID-dependent. Thus, TFIID may be capable of setting up transcription complexes at all polymerase II-transcribed genes.

TAFs perform a variety of functions including interactions with transcriptional activators, other general transcription factors, and promoter DNA (10–15). Genome-wide studies using temperature-sensitive alleles of various TAFs indicate that some TAFs may be selective in the genes they activate (9, 10, 16). This suggests that distinct parts of TFIID might play important promoter-specific roles. Similarly, a variety of temperature-sensitive alleles located throughout TAF10 reveal potential promoter-selective roles for distinct regions of a single TAF (11).

TAF1 is considered to be a “hallmark” of TFIID in that it resides only in TFIID and not in SAGA, and it may serve as a scaffold upon which TBP and TAFs assemble, although other TAFs might also play a scaffolding role (12, 17–19). When the studies reported here were initiated, TAF1 had been systematically dissected into four functional domains: an N-terminal TBP-binding domain termed TAND, a TAF-TAF interaction domain, a putative histone acetyltransferase (HAT) domain, and a promoter recognition domain (12, 15, 18, 20–22). More recently, a fifth domain that interacts with TAF7 has been identified (18). In addition, the physiological significance of the yeast TAF1 HAT activity has come into question (23). Collectively, the potentially gene-specific roles of TAFs and the potential modularity of TAF1 and other TAFs led us to consider whether the various functional domains of TAF1 play gene-specific roles in vivo. Because TFIID contributes to the expression of nearly the entire yeast genome, a greater understanding of the yeast gene regulatory network might be achieved by assessing the contribution of each of the TFIID activities on genome-wide transcription.

Any investigation into the genome-wide function of TAF1 or any other essential factor is hampered by the fact that deleterious mutations

DNA binding sequence-specific activators regulate eukaryotic genes at many stages including the recruitment of chromatin remodeling factors that increase the accessibility of promoters to the transcription machinery. Activators also assist in the loading of the general transcription factors and RNA polymerase II at promoters to form a preinitiation complex that is capable of transcribing the gene. The transcription machinery assembles at promoters via two major pathways in yeast, one that involves TFIID and the other involving a compositionally related complex called SAGA (1–3).

Transcription factor IID (TFIID) plays a central role in regulating the expression of most eukaryotic genes. Of the 14 TBP-associated factor (TAF) subunits that compose TFIID, TAF1 is one of the largest and most functionally diverse. Yeast TAF1 can be divided into four regions including a putative histone acetyltransferase domain and TBP, TAF, and promoter binding domains. Establishing the importance of each region in gene expression through deletion analysis has been hampered by the cellular requirement of TAF1 for viability. To circumvent this limitation we introduced galactose-inducible deletion derivatives of previously defined functional regions of TAF1 into a temperature-sensitive taf1ts2 yeast strain. After galactose induction of the TAF1 mutants and temperature-induced elimination of the resident Taf1ts2 protein, we examined the properties and phenotypes of the mutants, including their impact on genome-wide transcription. Virtually all TAF1-dependent genes, which comprise ~90% of the yeast genome, displayed a strong dependence upon all regions of TAF1 that were tested. This finding might reflect the need for each region of TAF1 to stabilize TAF1 against degradation or may indicate that all TAF1-dependent genes require the many activities of TAF1. Paradoxically, deletion of the region of TAF1 that is important for promoter binding interfered with the expression of many genes that are normally TFIID-independent/SAGA (Spt-Ada-Gcn5-acetyltransferase)-dominated, suggesting that this region normally prevents TAF1 (TFIID) from interfering with the expression of SAGA-regulated genes.

This work was supported by National Institutes of Health Grant GM059055. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1Current address: Center for Cancer Research, NCI-Frederick, National Institutes of Health, Frederick, MD 21702-1201.

2To whom correspondence should be addressed: The Pennsylvania State University, Dept. of Biochemistry and Molecular Biology, 452 N. Frear Laboratory, University Park, PA 16802. Tel.: 814-863-8252; Fax: 814-863-8595; E-mail: bfp2@psu.edu.

3The abbreviations used are: TFIID, transcription factor IID; TBP, TATA box-binding protein; HAT, histone acetyltransferase; SAGA, Spt-Ada-Gcn5-acetyltransferase; TAF-TBP-associated factor; TAND, TAF1 N-terminal domain; HAT, histone acetyltransferase; gal, galactose; YPR, yeast extract, peptone, raffinose; CSM, complete synthetic medium; bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; ts, temperature-sensitive; HA, hemagglutinin.

4In this manuscript, we have followed the systematic TAF naming rules proposed by Tora (39).
block cell growth. Creation of temperature-sensitive alleles has been a powerful and productive means of dissecting essential functional regions. However, this approach can be biased and restrictive in that any mutation must knock out an essential function at the nonpermissive temperature but renders the protein functional at the permissive temperature. Because a large fraction of yeast genes are not essential for cell growth, this strategy could miss TAF1 mutations that are specifically defective in expression of many nonessential genes. As an alternative strategy, we employed a systematic targeted approach by disrupting known functional domains of TAF1. To minimize potential indirect effects caused by constitutive expression of the TAF1 mutants, we chose to express each mutant under an inducible promoter. Because TAF1 is essential, this approach necessitated the use of a functional copy of TAF1 to promote cell growth. However, when assaying for mutant function it was desirable to eliminate the functional TAF1 copy so that it would not obscure or suppress the deletion construct. This was achieved by using a temperature-sensitive TAF1 allele (3, 24).

Using this approach we first assayed for the ability of an induced version of wild type or mutant TAF1 to functionally complement the growth defect of a temperature-sensitive TAF1 allele. Next we characterized the expression, subcellular localization, TFIID integrity, and stability of the TAF1 mutants in order to better assess its potential to impact gene expression. Lastly, genome-wide expression studies were performed to evaluate the contribution of each functional domain to transcription.

**MATERIALS AND METHODS**

**Plasmids**

YCp50 (TAF1 WT, URA3), and pRS313 taf1ts2 HIS3 (25) were gifts from J. Reese of this department (Department of Biochemistry and Molecular Biology, Pennsylvania State University). pYN2 (TAF1WT TRPI) (15) was a gift from T. Kokubo (Yokohama City University, Japan), pII11 (pRS315 taf1ts2 LEU2) and pII12 (pRS315 TAF1 WT LEU2) were created by amplifying the TAF1 gene from pRS313 taf1ts2 or pYN2, respectively, with taf1ts2 oligonucleotides (Integrated DNA Technologies, supplemental Table S2) containing NotI and Sall restriction sites. The 4,456-bp PCR products were digested with NotI and Sall and ligated into digested pRS315 (5,953 bp) to create 10,396-bp plasmids pII11 and pII12. The PCR product contained 494 bp upstream of TAF1 open reading frame and 741 bp downstream. To confirm the taf1ts2 temperature-sensitive (ts) phenotype, pII11 and pII12 were transformed into Y13.2 (15) and plated on CSM-LEU. YCN1 (TAF1 WT URA3) was shuffled out of Y13.2 by plating cells on CSM-LEU + 5-FOA (Zymo Research). Cells were then grown at 23 or 37 °C for 3 days on CSM-LEU plates (yeast extract, peptone, dextrose). Cells were grown at 23 or 37 °C for 3 days on CSM-LEU (dextrose) plates to confirm the ts phenotype. The temperature-sensitive phenotype was confirmed independently in the taf1:KanMX null strain (yjdi381) on CSM-LEU (dextrose).

pCALF-T(PGK) (26) was converted to pCALF-FHT-T(PGK) 2.2 by inserting a 66-bp HIS-TEV oligo into the Ndel site. pUG6-FHT-T(PGK) (4,170 bp) was made by PCR-amplifying a 259-bp fragment containing the FHT sequence from pCALF-T(PGK) 2.2 plasmid. PCR product was digested with Sall, and 161 bp was ligated into Sall-digested pUG6 plasmid (4,009 bp) such that the orientation was FHT-loxP-KanMX-loxP.

**FHT-TAF1 Mutant Strains**

Saccharomyces cerevisiae strain BY4743 (27) (Invitrogen) was used as the parental strain. Initially, the strain was transformed with pSH47 (LIRA3) (28) encoding galactose inducible Cre recombinase. 70-mer oligonucleotides F4 and R2 (supplemental Table S2; regions of homology to TAF1 are in bold) were used to PCR-amplify 1991 bp of pA6a-His3MX6-PGAL1 containing the HIS3 gene and GAL1 promoter (29). The PCR product was transformed into BY4743 using a high efficiency lithium acetate method (28) to replace 550 bp of the endogenous TAF1 promoter with the GAL1 promoter, creating strain yLAC3. HIS3 homologous recombination transformants were selected on CSM-HIS-URA media and verified with colony PCR.

Regions of TAF1 were deleted by replacing coding sequences with an FHT tag. The FHT tag encodes three HA (Flu) repeats, a hexahistidine (Hi) sequence, and the TEV protease sequence (T). The kanamycin resistance region of pUG6-FHT-p was PCR-amplified with 68-mer oligonucleotides with 50-bp homology to distinct regions of TAF1 (supplemental Table S2, Fig. 1). 1,826-bp PCR products were transformed into yLAC3 and selected on CSM-HIS-URA (dextrose) plates containing 500 µg/ml G418 (Invitrogen). The kanamycin resistance cassette flanked by loxP sites was removed by induction of Cre recombine with 2% galactose for 4 h, leaving the FHT tag N-terminal to the mutation in TAF1. Kanamycin-sensitive colonies were identified by replica plating on media containing and lacking G418. Additionally, mutations were verified by colony PCR with primers specific to each mutation.

**Haploids**

Kanamycin-sensitive FHT-TAF1 strains (Table 1) were plated on CSM-HIS + 5-FOA to select cells having lost pSH47 (30) and verified by replica plating on CSM-HIS and CSM-HIS-URA. Strains were then transformed with YCp50 (TAF1 WT, URA3) and transformants selected on CSM-HIS-URA media. Strains were plated on pre-sporulation medium (1% yeast extract, 2% peptone, 10% dextrose) for 2 days at 30 °C. Cells were cultured in sporulation medium (0.3% potassium acetate, 0.02% raffinose) for 3 days at 30 °C. 200 µl of the culture was pelleted, resuspended in 1.2 m sorbitol, 10 m Tris, pH 7.4, and treated with 20 units of (1 mg/ml) zymolyase (MP Biomedicals) at room temperature for 20 min. Tetrads were dissected according to standard yeast techniques on YPD plates (yeast extract, peptone, dextrose). Spores were replica plated onto CSM-HIS-URA medium to select for the HIS3 gene (and therefore the GAL1 promoter). Mating types of the taf1 strains were confirmed with MATa and MATα sex tester strains. MATα leu− HIS+ LYS+ tetrads were selected. Strains were then transformed with pII11 (taf1ts2, LEU2) or pII12 (TAF1 WT, LEU2) and selected on CSM-LEU media. Cells that lost YCp50 (TAF1 WT, URA3) were selected by plating on CSM-LEU + 5-FOA.

**PCR**

1 × 25 mM MgCl2 buffer (Gene Choice), 2.5 units of Taq polymerase (Gene Choice), 0.002 units of Pfu polymerase (Stratagene), 0.4 mM dNTPs, and each primer at 0.2 µM was used per 50-µl reaction for 32 cycles.

**Cell Growth/Lethality/Dominant Toxicity Assays**

Viability—MATa haploid strains carrying YCp50 (TAF1 WT, URA3) were grown at 25 °C in YPD medium (yeast extract, peptone, dextrose). Cells were diluted into YPR (0.3% potassium acetate, 0.2% galactose and grown to mid-log phase. 0.5 A600 units of cells were removed, and 5 µl of 10-fold serial dilutions were plated. Cells having lost the YCp50 plasmid were selected by growing on CSM + 5-FOA with 2% dextrose or 2% galactose at 25 or 37 °C.

Toxicity—MATa haploid strains carrying pII12 (TAF1 WT LEU2) plasmid were grown in CSM-LEU (dextrose to mid-log phase. 0.5 A600 of cells were serially diluted 10-fold, and 5 µl was plated on CSM-
Genome-wide Functions of TAF1

TABLE 1

Yeast strains

| Strain | Promoter | TAF1 allele | Plasmid | MAT | Ref. |
|--------|----------|-------------|---------|-----|------|
| BY4743 | TAF1     | WT          | pSH47   | Diploid | 27   |
| yLAC3  | GAL1     | WT          | YCp50 TAF1 WT URA3 | a | This study |
| yji307 | TAF1     | WT          | YCp50 TAF1 WT URA3 | a | This study |
| yji302 | GAL1     | WT          | YCp50 TAF1 WT URA3 | a | This study |
| ydi277 | GAL1     | FHT-WT1     | YCp50 TAF1 WT URA3 | a | This study |
| ydi275 | GAL1     | FHT-TFI (Δ10–88) | YCp50 TAF1 WT URA3 | a | This study |
| ydi280 | GAL1     | FHT-TFI (Δ208–303) | YCp50 TAF1 WT URA3 | a | This study |
| ydi288 | GAL1     | FHT-HT4 (Δ645–768) | YCp50 TAF1 WT URA3 | a | This study |
| ydi295 | GAL1     | FHT-PB1 (Δ912–992) | pL12 TAF1 WT LEU2 | a | This study |
| ydi341 | GAL1     | FHT-PB1 (Δ912–992) | pL11 taf1ts2 LEU2 | a | This study |
| ydi352 | GAL1     | FHT-WT1     | pL11 taf1ts2 LEU2 | a | This study |
| ydi353 | GAL1     | FHT-WT1     | pL11 taf1ts2 LEU2 | a | This study |
| ydi354 | GAL1     | FHT-DT1 (Δ10–88) | pL12 TAF1 WT LEU2 | a | This study |
| ydi355 | GAL1     | FHT-DT1 (Δ10–88) | pL11 taf1ts2 LEU2 | a | This study |
| ydi356 | GAL1     | FHT-DT1 (Δ10–88) | pL11 taf1ts2 LEU2 | a | This study |
| ydi357 | GAL1     | FHT-DT1 (Δ208–303) | pL12 TAF1 WT LEU2 | a | This study |
| ydi358 | GAL1     | FHT-HT4 (Δ645–768) | pL12 TAF1 WT LEU2 | a | This study |
| ydi359 | GAL1     | FHT-HT4 (Δ645–768) | pL11 taf1ts2 LEU2 | a | This study |
| ydi360 | GAL1     | FHT-PB1 (Δ912–992) | pL12 TAF1 WT LEU2 | a | This study |
| ydi361 | GAL1     | FHT-PB1 (Δ912–992) | pL11 taf1ts2 LEU2 | a | This study |
| ydi362 | GAL1     | WT          | pL11 taf1ts2 LEU2 | a | This study |
| ydi363 | GAL1     | WT          | pL11 taf1ts2 LEU2 | a | This study |
| ydi366 | TAF1     | WT          | pL11 taf1ts2 LEU2 | a | This study |
| ydi367 | TAF1     | WT          | pL11 taf1ts2 LEU2 | a | This study |
| ydi375 | GAL1     | taf1ts2 KanMX | pL12 TAF1 WT LEU2 | a | This study |
| ydi379 | GAL1     | taf1ts2 KanMX | pL11 taf1ts2 LEU2 | a | This study |
| ydi381 | GAL1     | taf1ts2 KanMX | YCp50 TAF1 WT URA3 | a | This study |
| Y13.2  | TAF1     | ΔTAF1       | pYN1 TAF1 WT, URA3 | a | This study |

LEU + 2% dextrose or 2% galactose at 19, 25, and 37 °C. Photographs were taken after 96 h for 25 and 37 °C plates and after 120 h for 19 °C plates.

Galactose Shut-off

FHT-TAF1 strains were grown in YPR at 25 °C until the A600 was ~0.8. Galactose was added to 2%, and strains were incubated at 25 °C for 45 min. Dextrose was then added to 2%, and the cultures were placed in a 37 °C water bath. Equal-volume aliquots were removed at 15, 30, 45, 60, 120, and 180 min after the addition of dextrose. After Western blotting, quantitation was performed on four independent replicates using a densitometer and ImageQuant software from Amersham Biosciences. Exposure times were chosen so that all signals were in the linear range of detection. Local background was subtracted from the signal band at each time point. Intensity peaked at 15 min in dextrose (at 37 °C) for all strains. This value was set as 100%, and the percent of TAF1 remaining over time was plotted. Whole cells containing constitutively expressed HA-Bdfl were loaded in each lane of the immunoblots as an internal control for protein extraction, recovery, transfer, and immunodetection but were not included in the quantitation.

Immunofluorescence

Cultures were grown in YPR overnight at 30 °C, diluted to A600 = an A600 of 0.2, and grown for 3.5 h in YPR + 2% galactose at 30 °C. 2.0 A600 units were fixed in 3.7% formaldehyde, treated with zymolyase, and bound to polylysine-coated slides. FHT-tagged TAF1 proteins were visualized by incubating with anti-HA.11 monoclonal antibodies (1:1000, Babco) and then with goat anti-mouse IgG-Alexa Fluor 488 (Molecular Probes). 4',6-Diamidino-2-phenylindole was used to visualize nucleic acids (31). The method was adapted from Ref. 30. Samples were viewed on an Axioplan epifluorescence microscope (Carl Zeiss, Inc.). TIFF images were collected using a Spot2 cooled charge-coupled device digital camera (Diagnostic Instruments).

Immunoblotting

Cells were washed in 0.5 ml of 0.1 M sodium hydroxide for 5 min at room temperature, spin, resuspended in 2× protein sample buffer, and heated to 95 °C (32). HA-TAF1 mutants were electrophoresed in 7.8% Bis-acrylamide gels (PAGE) and transferred to nitrocellulose in 80% Tris-glycine-SDS/20% methanol for 120 min at 1.0 A. FHT-TAF1 mutants were detected with anti-HA (HA.11, Babco) and anti-mouse horseradish peroxidase antibodies (Amersham Biosciences) and exposed to Hyperfilm (Amersham Biosciences) with ECL (Amersham Biosciences).

Co-immunoprecipitation of TAFs

TAF1 strains were induced with 2% galactose in CSM-LEU media for 75 min at 25 °C. ~10 A600 equivalents of cellular lysate were immunoprecipitated with HA antibodies (Babco) and protein A-Sepharose (Amersham Biosciences) as described previously (19). Approximately 2.5 A600 of cells were loaded onto 4–12% Bis-Tris NuPAGE Novex gradient gels (Invitrogen) and transferred to polyvinylidene difluoride membrane in 80% Tris-glycine-SDS/20% methanol for 120 min at 1.0 A. Polyclonal TAF antibodies (gifts of P. A. Weil, Vanderbilt University), polyclonal HA antibodies (Rockland), polyclonal γTBP antibodies, and anti-rabbit horseradish peroxidase antibodies (Amersham Biosciences) were used to detect co-immunoprecipitated TAFs and TBP.

Galactose Addition Timing

For TAF1 production after galactose induction, cultures were grown in CSM-LEU raffinose at room temperature and induced with 2% galactose. 0.5 A600 aliquots were removed at 10-min intervals after the addition of galactose and immunoblotted. For TAF1 induction for use in microarray analysis, cultures were grown in CSM-LEU raffinose at room temperature until A600 ~ 0.8. Galactose was added to 2% at 0, 15, 30 or 60 min before shifting culture to 37 °C with warm CSM-LEU + 2% galactose. After shift to 37 °C, the cultures were incubated for 45 min before harvesting and use in expression profiling.

In generating histograms of log2 expression as a function of time in galactose prior to temperature shift, strain ydi375 (P GAL1 TAF1 WT + taf1ts2) was used as reference. Test strains were ydi363 (P GAL1-TAF1 WT + taf1ts2) and ydi381 (P GAL1-taf1ts2-KanMX + taf1ts2).
Microarray Analysis

Microarrays were performed essentially as described (3, 33). Briefly, cultures were grown at ~24 °C in CSM-LEU + 3% raffinose to a \( A_{600} \) of ~0.8. FHT-TAF1 mutants were induced by adding galactose to 2% at 30 min prior to temperature shift. Cultures were shifted to 37 °C by adding an equal volume of warm CSM-LEU + 2% galactose and placed in a 37 °C incubator for 45 min to inactivate taf1ts2. Cells were harvested by centrifugation at room temperature, washed in RNase-free (diethyl pyrocarbonate-treated) double distilled H\(_2\)O, and frozen in liquid nitrogen.

Total RNA and poly(A\(^+\)) mRNA purification, reverse transcription, and labeling with fluorescent dyes (Cy3 and Cy5 (Amersham Biosciences)), hybridization, and scanning were all performed as described (3, 33). 4 \( \mu \)g of mRNA was used for hybridizations instead of the conventional 2 \( \mu \)g. Slides were treated with Dye Saver\(^2\) (Genisphere) according to manufacturer’s instructions to preserve signal intensity. Raw data are accessible at GeneExpression Omnibus (www.ncbi.nlm.nih.gov/geo/; GenBank\(^TM\) accession numbers GSM65302 to GSM65315). Processed data are presented in supplemental Table S3. R software was used to mode-center replicates (dye swaps) (34).

Genes were filtered by several criteria to minimize false positives. 1) Genes were eliminated if their signal on the array was greater than 25% saturated. 2) The mean foreground signal minus the median background signal had to be greater than standard deviation of background signal. 3) Quality data were needed from both replicates of the dye swap. 4) The directional change of signal of the mutant (relative to reference) had to be equivalent in the replicates.
Genome-wide Functions of TAF1

K-Means clustering was performed using Cluster (35) on 2,103 genes that contained data in 80% of the experiments and had at least a change of 1.7-fold in one of the mutants. K was chosen to equal 5 clusters (K = 5). Clustering information was visualized using Treeview (35).

RESULTS AND DISCUSSION

Mutant Design—Four functional domains of TAF1, as previously demarcated, are illustrated in Fig. 1A. Because TAF1 is an essential gene, it cannot be constitutively replaced by taf1 mutants that knock out essential functions. However, transient replacement can be achieved by expressing TAF1 mutants under the control of an inducible promoter in a strain harboring a second allele of TAF1 that is temperature-sensitive (taf1ts2). To achieve this, a chromosomal deletion was performed using a PCR amplified cassette containing a kanMX site, loxP Cre recombinase sites. The cassette also contained coding sequences that allowed the deleted region to be replaced by an FHT epitope tag containing a triple HA tag, a hexahistidine tag, and a TEV protease cleavage site. After selection for recombinants on G418 plates, and subsequent excision of kanMX with the Cre recombinase, the deleted region contained the FHT tag and a single loxP site, both of which maintain an open reading frame through the deleted region. The location of each mutation (Fig. 1C) was verified by PCR (not shown). Immunoblot analysis revealed the presence of an appropriate size band that reacted with HA antibodies and was present in galactose-treated but not glucose-treated cells (Fig. 1D). Most mutants except “TF1” were expressed at levels that were comparable with endogenous TAF1 (Fig. 1D, left panel). High levels of expression of this mutant from the endogenous TAF1 promoter have been noted previously (12).

Growth Phenotypes—Diploid recombinants were transformed with a TAF1/URA3 plasmid and sporulated. Haploid spores were germinated, and the ability of the TAF1 mutants to support cell viability was measured by selecting for the loss of the TAF1/URA3 plasmid on media containing 5-FOA. As shown in Fig. 2A, none of the mutants or wild type TAF1 supported cell viability when TAF1 was placed under GAL1 control and cells were grown in dextrose, which confirms that TAF1 expression is under tight GAL1 control. In the presence of galactose, cells containing wild type, epitope-tagged wild type, or TAND-deleted (DT1) TAF1 grew at 25 °C. DT1 did not grow at 37 °C, confirming the previously reported temperature-sensitive phenotype of this mutant (15, 19, 33). All other mutants failed to grow at either temperature, reflecting the importance of the TAF interaction domain (TF1), putative HAT domain (HT4), and promoter-binding domain (PB1), as determined previously (19).

It is clear that TAF1 has a number of essential domains. Conceivably, destruction of any one domain could generate a dominant negative that is capable of engaging in certain essential interactions but not others and, as a result, competing out the wild type function. To test for this possibility, mutant TAF1 derivatives were expressed in the presence of wild type TAF1. As shown in Fig. 2B, mutant TF1 significantly inhibited growth at 19 and 25 °C, and PB1 displayed a more modest inhibitory effect. The latter is consistent with previous findings (12, 18). Not surprisingly, these results suggest that TAF-TAF interactions are important for TAF1 function. Without these interactions TAF1 might be able to engage other parts of transcription machinery, thereby blocking wild
type TAF1 function. In light of the observation that TF1 is particularly overexpressed compared with the other mutants, we cannot exclude the possibility that a large overproduction of TF1 is toxic to cells in a way that is unrelated to transcription. For the PB1 mutant, loss of the promoter binding region of TAF1 might allow other parts of TAF1 to engage the transcription machinery, thereby competing out wild type TAF1. Surprisingly, HT4 displayed little or no dominant negative behavior, despite having intact TAF-TAF and promoter-binding domains. Possibly this region might be important for overall TAF1 stability and/or function.

Subcellular Localization—Any potential defects in the function of TAF1 mutants might be attributable to subcellular mislocalization of the mutants. To address this possibility, immunofluorescence was conducted on the FHT epitope tag engineered into the TAF1 mutants. As shown in Fig. 3, all epitope-tagged wild type and mutant TAF1 proteins, except TF1, were properly localized to the nucleus as demarcated by staining with 4',6-diamidino-2-phenylindole. Cells lacking an epitope tag showed little or no staining. TF1 appeared throughout the cell, suggesting that its nuclear translocation signal was disrupted. Indeed, the amino acid sequence located between residues 230 and 246 corresponds to a potential nuclear localization signal (36, 37). The finding that TF1 is mislocalized to the cytoplasm does not exclude the possibility that some portion of TF1 enters the nucleus, where it could interfere with gene expression. This mutant has been shown to interact with TBP in vivo, indicating that at least a fraction is imported into the nucleus (12, 18, 19).

TFIID Complex Integrity—The ultimate goal of these studies is to transiently expose the cells to the TAF1 mutants while at the same time eliminate the functional copy of TAF1 that maintains cell viability. As the first step in this direction, we sought to assess whether the mutant TAF1 proteins could incorporate TBP and TAFs into a TFIID complex. As shown in Fig. 4, TAFs and TBP generally co-immunoprecipitated with HA antibodies and probed for the presence of FHT-tagged TAF1, other TAFs, and TBP.

Protein Stability—In the next step we sought to eliminate the endogenous TAF1 by employing a taf1ts2 strain. A shift in temperature to 37 °C causes degradation of the mutant Taf1ts2 protein, with it and TFIID being virtually eliminated by 45 min after the temperature shift (38) (data not shown). Next, we compared the expression level of the FHT-tagged TAF1 mutants at 37 °C. As shown in Fig. 5A, all HA-tagged mutants were inducible and present at 37 °C.

Using dextrose to shut off GAL1-driven expression, we examined the stability of the various TAF mutants at 37 °C (Fig. 5B, and quantitated in Fig. 5C). Epitope-tagged wild type TAF1 had a turnover half-life of ~60 min, whereas the TAF1 mutants, with the exception of TF1, had a significantly more rapid turnover with an apparent half-life of ~30 min. The actual half-life is expected to be somewhat shorter given that the approximate mRNA half-life is about 20 min (16). Thus, defects in regions of TAF1 that are required for interactions with TBP and promoters or contain the putative HAT domain lead to rapid TAF1 turnover.

The decay rate for TF1 mutant was biphasic, in which about half of the protein was degraded with a similar profile as in wild type TAF1 with the remaining half being very stable. Given that much of the TF1 mutant accumulates in the cytoplasm, unlike the other mutants, it might be less susceptible to protein degradation enzymes that target nuclear proteins. The fraction of TF1 that is degraded could be nuclear, although this is not known.

Genome-wide Expression—TFIID, including TAF1, contributes to the expression of ~90% of the yeast genome. Inasmuch as different TFIID subunits and different portions of TAF5 and TAF10 have been ascribed a gene-specific function (10, 11, 13, 14), we sought to examine, using microarray analysis, whether the four functional domains make gene-specific contributions to gene expression on a genome-wide scale.

To minimize potential indirect effects where the impact on the expression of certain genes like transcriptional regulators alters the expression of a large number of other genes, we sought a means of rapidly replacing the functional copy of TAF1. To do this we used a temperature-sensitive taf1ts2 allele as the functional copy (Fig. 1B). We developed a two-part strategy. First, GAL1-driven TAF1 mutants were induced with galactose, and then the taf1ts2 allele was inactivated by abruptly shifting the culture temperature to 37 °C for 45 min. The GAL1 promoter is TFIID-independent (3), and thus expression of the TAF1 mutants should continue when taf1ts2 is inactivated at the restrictive temperature. As a first step in this approach it was necessary to determine whether galactose-induced TAF1 could functionally replace the resident TAF1 on the short time scale of the experiment. This is crucial because TAF1 is part of the multi-subunit TFIID complex, and the degree of subunit exchange is not known. In Fig. 4, we demonstrate that many of the galactose-induced TAF1 mutants can be incorporated into TFIID. Second, to achieve maximum impact on a minimal time scale (to minimize indirect effects) we sought to determine the minimum amount of time needed to synthesize the TAF1 derivatives and have them functionally replace the resident functional copy of TAF1.

To determine the optimal timing for galactose induction, we first examined the kinetics of galactose-induced TAF1 expression, which as shown in Fig. 6A was achieved 45 min after the addition of galactose. This sets the minimum length of time over which expression profiling can be conducted. Next, we performed a series of galactose induction at varying times prior to heat inactivating the taf1ts2 allele, as illustrated in Fig. 6B. After 45 min at 37 °C, cells were harvested, and the level of isolated mRNA was measured for over 5000 yeast genes and compared with a noninducible wild type TAF1 strain subjected to the same conditions. Thus, a gene-by-gene impact of galactose-induced TAF1 (Fig. 6C, Pgal1 WT) versus a constitutively expressed TAF1 reference (WT)
was determined. These ratios were binned and plotted as a smoothed frequency distribution (Fig. 6C).

As a control representing complete replacement of the resident TAF1 with a galactose-induced TAF1, two independent reference experiments were compared (Fig. 6C, WT/WT). The shape, width, and peak location of this curve represents no change in expression and thus is diagnostic of complete substitution. At the other end of the spectrum, we compared a galactose-induced null TAF1 (again in a taf1ts2 strain) to a WT strain (Fig. 6C, null/WT, dashed line curve). This curve is diagnostic of no replacement and represents a maximal decrease in mRNA expression. As shown in Fig. 6C, induction of TAF1 either 30 or 60 min prior to heat shock resulted in no decrease in mRNA levels. However,
The article discusses the genome-wide functions of TAF1, a subunit of the transcriptional co-activator TFIID. The study involves the use of TAF1 mutants to analyze the role of TAF1 in gene expression.

**Figure 6** illustrates the replacement of endogenous TAF1 with galactose-induced TAF1. A, kinetic analysis of galactose induction of TAF1 under the control of the GAL1 promoter. Cultures grown in CSM-LEU raffinose medium at room temperature and induced with 2% galactose. Values were normalized to externally spiked B. subtilis controls based upon A600 cell density measurements.

**Figure 7** shows all domains of TAF1 are essential for nearly all TFIID-regulated genes. Strains harboring each of the indicated TAF1 mutants were induced with galactose 30 min prior to heat shock and then harvested 45 min later. mRNA was isolated and co-hybridized along with a galactose-induced untagged wild type reference (yjdi363). Data were normalized to externally spiked B. subtilis controls based upon A600 cell density measurements.

Each row corresponds to a gene and each column to a particular mutant (Fig. 7). Decreases in expression are indicated by green, increases by red, and no change by black. Data were clustered by K-means initially into five clusters. Because three of the clusters were visually similar, they were merged to form a single large cluster (Fig. 7, cluster 1). At nearly all genes, expression of epitope tagged wild type TAF1 (WT1) functionally replaced the taf1ts2 allele, yielding no changes in gene expression, as expected. Galactose induction of a null TAF1 allele lead to substantial decreases in expression at about 90% of all genes (Fig. 7, cluster 1), reflecting the previous determination that TFIID contributes to the expression of ~90% of the yeast genome (3). Similar drops in expression for these genes were observed for all other TAF1 mutants, suggesting that virtually all TAF1-dependent genes require all four functional domains of TAF1 for proper expression.

There are caveats to this conclusion. First, the TF1 mutant, which is defective in TAF-TAF interactions, is overexpressed and mislocalized to the cytoplasm, so it might not be available in the nucleus in sufficient quantities to rescue the taf1ts2 allele. Nevertheless, because most genes require one or more TAFs in order to function, the genome-wide expression profile derived from the TF1 mutant is expected to be no different from the taf1ts2 allele alone. The second caveat is that all nuclear-localized mutants degrade more rapidly than wild type TAF1. This rapid turnover could limit the amount of TAF1 mutants available for mediating gene expression, and thus the expression profile might be similar to the taf1ts2 allele. However, if the TAF1 functional regions demarcated by the DT1, HT4, and PB1 mutations play genes-specific roles, there should be sufficient levels of these mutants present in the nucleus to show gene-specific patterns, which, with one exception discussed below, was not observed. The third caveat is the possibility that deletion of each of these domains structurally destabilizes the entire
Genome-wide Functions of TAF1

protein thereby rendering it nonfunctional. Although this possibility cannot be excluded, previous studies have demonstrated that at least three of the functional domains (TAND, TAF-TAF interaction domain, and the HAT domain) are functional in the absence of the remainder of the protein, suggesting that each domain is not structurally dependent upon the others (12, 18).

Both clusters 2 and 3 consist largely of TAF-independent SAGA-dominated genes. Cluster 3 is quite small, being composed of 34 genes. This cluster of genes was slightly negatively regulated by TAF1, resulting in up-regulation (Fig. 7, red bars) in the taf1ts2 strain. Interestingly, in the presence of the HT4 mutant these genes were strongly up-regulated, indicating that the putative HAT domain of TAF1 might negatively regulate these genes.

Surprisingly, cluster 2 genes were down-regulated by the promoter-defective PB1 mutant, whereas all other mutations had minor effects on these genes. Apparently, TAF1, which lacks promoter recognition capability, interferes with the expression of genes that are normally regulated by SAGA rather than by TFIIID. The basis for this is unclear, but it raises the intriguing possibility that the promoter recognition activity of TAF1 not only helps target TFII D to TAF-dependent promoters as suggested previously (12) but also inhibits TFII D from interacting with TAF-independent/SAGA-dominated promoters. When this domain is removed, TAF1 might then bind to these promoters in a manner that interferes with TAF-independent/SAGA-regulated transcription. Alternatively, other intact domains in the PB1 mutant (e.g. TAND or the TAF interaction domain) could bind to and sequester components of the TAF-independent transcription pathway such as TBP.

Taken together the findings suggest that at least under 37 °C growth conditions, in which yeast grow normally, the four functional domains of TAF1 are essential at virtually all TAF1-regulated genes. Thus, in order for TFII D to function properly, TAF1 must interact with TBP, TAFs, and promoter DNA at all TAF1-dependent genes, and utilize the function(s) associated with the putative HAT region.

Acknowledgments—We thank J. Reese and T. Kokubo for the generous gifts of TAF1 plasmids, P. A. Weil for the TAF antibodies and TFII D, D. Ng and E. Spear for assistance with tetrad dissection and immunofluorescence, and J. Robinson and L. Clark for subcloning work. We are grateful to members of the Pugh laboratory for critical advice and suggestions.

REFERENCES

1. Naar, A. M., Lemon, B. D., and Tjian, R. (2001) Annu. Rev. Biochem. 70, 475–501
2. Green, M. R. (2000) Trends Biochem. Sci. 25, 59–63
3. Huisinga, K. L., and Pugh, B. F. (2004) Mol. Cell. 13, 573–585
4. Grant, P. A., Schieltz, D., Prye-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R., Ill, and Workman, J. L. (1998) Cell 94, 45–53
5. Basehoar, A. D., Zanton, S. J., and Pugh, B. F. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 16843–16848
6. Zanton, S. J., and Pugh, B. F. (2004) Mol. Cell. 10, 699–709
7. Zanton, S. J., and Pugh, B. F. (2004) Mol. Cell. 10, 101, 115–132
8. Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., Botstein, D., and Brown, P. O. (2000) Mol. Biol. Cell 11, 4241–4257
9. Lee, T. I., Causton, H. C., Hostege, F. C., Chen, S. L., Hennett, N., Jennings, E. G., Winston, F., Green, M. R., and Young, R. A. (2000) Nature 405, 701–704
10. Shen, W. C., Bhaumik, S. R., Causton, H. C., Simon, L., Zhu, X., Jennings, E. G., Wang, T. H., Young, R. A., and Green, M. R. (2003) EMBO J 22, 3395–3402
11. Kirschner, D. B., Chen, S. L., Tabin, C., Sanders, S. L., Ganoff, Y. G., Davidson, I., Weil, P. A., and Tora, L. (2002) Mol. Cell. Biol. 22, 3178–3193
12. Mencia, M., and Struhl, K. (2001) Mol. Cell. Biol. 21, 1145–1154
13. Kirschner, D. B., Tabin, C., Sanders, S. L., Klebanow, E., and Weil, P. A. (2001) Mol. Cell. Biol. 21, 6668–6680
14. Durso, R. J., Fisher, A. K., Albright-Frey, T. J., and Reese, J. C. (2001) Mol. Cell. Biol. 21, 7331–7344
15. Kokubo, T., Swanson, M. J., Nishikawa, J. I., Linnebusch, A. G., and Nakatani, Y. (1998) Mol. Cell. Biol. 18, 1003–1012
16. Holstege, F. C. P., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998) Cell 95, 717–728
17. Chen, J. L., Attardi, L. D., Verrizier, C. P., Yokomori, K., and Tjian, R. (1994) Cell 79, 93–105
18. Singh, M. V., Bland, C. E., and Weil, P. A. (2004) Mol. Cell. Biol. 24, 4929–4942
19. Bai, Y., Perez, G. M., Beechem, J. M., and Weil, P. A. (1997) Mol. Cell. Biol. 17, 3081–3093
20. Sanders, S. L., Garbett, K. A., and Weil, P. A. (2002) Mol. Cell. Biol. 22, 6000–6013
21. Mizzen, C. A., Yang, X. J., Kokubo, T., Brownell, J. E., Bannister, A. J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S. L., Kouzarides, T., Nakatani, Y., and Allis, C. D. (1996) Cell 87, 1261–1270
22. Kotani, T., Banno, K., Ikura, M., Linnebusch, A. G., Nakatani, Y., Kawai, M., and Kokubo, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7178–7183
23. Durant, M., and Pugh, B. F. (2006) Mol. Cell. Biol., in press
24. Walker, S. S., Shen, W. C., Reese, J. C., Apone, L. M., and Green, M. R. (1997) Cell 90, 607–614
25. Reese, J. C., Apone, L., Walker, S. S., Griffin, L. A., and Green, M. R. (1994) Nature 371, 523–527
26. Hou, Y., Irvin, J. D., Huisinga, K. L., Mitra, M., and Pugh, B. F. (2003) Mol. Cell. Biol. 23, 3186–3201
27. Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., and Boeke, J. D. (1998) Yeast 14, 115–132
28. Guldener, U., Heck, S., Fielder, T., Reinbacher, J., and Hagemann, J. H. (1996) Nucleic Acids Res. 24, 2519–2524
29. Longtine, M. S., McKenzie, A. III, Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J. R. (1998) Yeast 14, 953–961
30. Adams, A., Gottschling, D. E., Kaiser, C. A., and Stearns, T. (1997) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
31. Manzini, G., Barcellona, M. L., Avitabile, M., and Quadrifiglio, F. (1983) Nucleic Acids Res. 11, 8861–8876
32. Kushnirov, V. V. (2000) Yeast 16, 857–860
33. Chitikila, C., Huisinga, K. L., Irvin, J. D., Basehoar, A. D., and Pugh, B. F. (2002) Mol. Cell. Biol. 10, 871–882
34. Ihaka, R. G., R. (1996) J. Comp. Graph. Stat. 5, 299–314
35. Eisien, M. R., Spellman, P. T., Brown, P. O., and Botstein, D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14863–14868
36. Ruzzi, M., Marconi, A., Salim, M., Fabiani, L., Montebone, F., and Frontali, L. (1997) Yeast 13, 365–368
37. Dingwall, C., and Laskey, R. A. (1991) Trends Biochem. Sci. 16, 478–481
38. Walker, S. S., Reese, J. C., Apone, L. M., and Green, M. R. (1996) Nature 383, 185–188
39. Torl, M. (2002) Genes Dev. 16, 673–675