TAF25p, a Non-histone-like Subunit of TFIID and SAGA Complexes, Is Essential for Total mRNA Gene Transcription in Vivo*

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Steven L. Sanders‡, Edward R. Klebanow, and P. Anthony Weil§

From the Department of Molecular Physiology and Biophysics, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232-0615

We demonstrate, utilizing a temperature conditional mutant allele of the gene encoding TAF25p, that this non-histone-like TBP-associated factor, which is shared between the TFIID and SAGA complexes, is required for bulk mRNA gene transcription by RNA polymerase II in vivo. Immunoblotting experiments indicate that at the restrictive temperature, inactivation of TAF25p function results in a reduction of the levels of numerous TFIID and SAGA subunits, indicating its loss of function, like the histone-like TAFs, causes degradation of the constituents of these two multisubunit complexes. These data suggest that TAF25p plays a key structural role in maintaining TFIID and SAGA complex integrity. This is the first demonstration that a non-histone-like TAF is required for continuous, high level RNA polymerase II-mediated mRNA gene transcription in living yeast cells.

There is ongoing debate about the exact role that TAF18, a family of conserved integral protein subunits of TFIID, play in transcription by RNA polymerase II (see Refs. 1 and 2 for recent reviews). In contrast to initial studies, recently published reports from a number of laboratories indicate that at least a subset of TAF18s, the so-called histone-like TAF18 (3), are absolutely essential for ongoing high level total mRNA gene transcription in vivo (4–7). The histone-like TAF18s examined in these experiments are of particular interest, since it was also demonstrated recently that these TAF18s, TAF61p (histone H2B-like), TAF60p (histone H4-like), and TAF17p (histone H3-like), are present in both the TFIID and SAGA complexes (8).

The data from these studies has been used to formulate a model that postulates that the histone-like TAF18s form the structural “core” for both TFIID and SAGA complexes and when the integrity of these particular TAF18s is compromised, then the complexes dissociate and cease to function while the resulting free subunits are rapidly degraded. At present it is not yet clear whether the RNAP II transcription requirement for histone-like TAF18 function derives from the association of these TAF18s with the TFIID, the SAGA, or both complexes. Circumstantial evidence implicates the TFIID complex though, since all of the non-TAF18-encoding genes that comprise the SAGA complex (with the exception of TAF1) are non-essential genes, while (with but one exception, TAF30) all of the TFIID TAF18s are encoded by essential genes. This question remains to be formally tested though.

Having previously cloned and characterized TAF25, we decided to attempt to generate temperature-conditional alleles of the TAF25 gene, which we could use as a tool to examine the role of TAF25 in RNAP II transcription. We felt it important to test whether or not the non-histone-like TAF25, which is present in both TFIID and SAGA, is globally involved in total mRNA gene transcription by RNA polymerase II in vivo. We successfully generated temperature-conditional alleles of TAF25 and one particular mutant allele caused yeast cells to rapidly cease growth at the restrictive temperature. We characterized this taf25 mutant in detail with regards to its effects both upon specific and global mRNA gene transcription and TFIID/SAGA subunit protein integrity. Contrary to the TFIID functional and structural organization models recently described by others (reviewed in Ref. 2), we found that TAF25p function is continuously required for high level mRNA gene transcription in vivo. This is the first such demonstration that a non-histone-like TAF25 protein is continuously required for mRNA gene transcription in vivo. We discuss how these new data regarding TAF25p may be incorporated into current models of TFIID structural organization and function.

MATERIALS AND METHODS

Yeast and Bacterial Strains and Cell Cultivation—Escherichia coli strain XL-1-Blue (10) was used for routine plasmid propagation. E. coli KC8 (CLONTECH) was used for amplification of hydroxylamine mutagenized plasmid DNA. E. coli strain BL21 (11) was used for recombinant protein production (12). Yeast strain YEK16 (12), genotype MATA leu2 ura3 trp1 suc2 ade2 lys2 taf25 1::LEU2, was used for transformation and plasmid shuffle testing of the ability of mutant TAF25 alleles to grow at permissive and non-permissive temperatures (see details below). Yeast strains carrying rpb1-1, taf17-1, and tbf1-1 mutant alleles have been described previously (13–15). Yeast were grown in appropriate rich (YPAD) or selective (SC) medium as required (16).

Generation and Analysis of Temperature-sensitive Alleles of TAF25—Plasmid DNA was mutagenized following the general protocol of Sikorski and Boeke (17). Details available upon request. Seven plasmids containing taf25 alleles screened true through this protocol. The entire TAF25 ORF and ~200 bp of DNA both up- and downstream of the ORF were sequenced for these seven mutants.

Preparation and Analysis of RNA—Total yeast cell RNA was prepared using a hot phenol extraction method as detailed previously (18). RNAs were quantitated using UV absorbance and RiboGreen fluorescence (Molecular Probes), and equivalent amounts of RNA (12 µg) were analyzed by either slot blotting with a 32P-5’-end-labeled oligo(dT)20 probe (19) or by standard RNA (Northern) blotting of agarose gel fractionated total RNA (5 µg). RNA gel blots were probed using a PCR-amplified ORF of the gene indicated in the legend to Fig. 3 that had
been 32P-labeled by random priming. Hybridization and washing steps were performed as described (29). S1 nuclease protection assays were performed as described previously (15). Specific RNA signals were detected by autoradiography and PhosphorImaging (Molecular Dynamics).

Preparation of Yeast WCE and Immunodetection of Specific Proteins—Yeast WCEs for immunological detection of TFII D and SAGA constituents were prepared as described previously (12, 21, 22). Specific polyclonal antibodies recognizing TFII D and SAGA substituents were prepared in rabbits (Bethyl Laboratories, Montgomery, TX) using 1–2 mg of purified recombinant protein as immunogen for each rabbit. IgGs were affinity-purified using antigen coupled to Sepharose CL-4B as detailed previously (12, 21). Immunoblots were performed as described previously (12, 21) using optimal, empirically determined dilutions of specific polyclonal IgGs, typically 1/5000 to 1/10,000. In the case of HA3-TAF25p, monoclonal antibody 12CA5 (Boehringer Mannheim) was used as well.

RESULTS AND DISCUSSION

Hydroxylamine Efficiently Generated Temperature-sensitive Alleles of TAF25—By using hydroxylamine mutagenesis a number of distinct taf25 mutant alleles were generated. We obtained 7 out of ~20,000 yeast transformants that exhibited a readily scorable temperature-conditional growth phenotype. None of these mutants displayed a cold sensitive growth phenotype nor did they exhibit a cdc-like phenotype after extensive incubation at the non-permissive temperature.2 The locations of these mutations in TAF25 and the growth properties of these seven yeast strains are shown in Fig. 1A. Yeast strains YEK25.34 and YEK25.75 represent a missense mutation of codon glycine 101 to glutamic acid (GGG to GAG), while strains YEK25.19, YEK25.26, YEK25.59, and YEK25.92 were generated by missense mutation of glycine 101 to arginine (GGG to AGG). Yeast strain YEK25.76 carries a nonsense mutation at codon arginine 111 (CGA to UGA). All of these mutations are of the type expected for hydroxylamine mutagenesis, which induces C to T and G to A transition mutations (17). As shown in the lower part of Fig. 1A, none of these strains grow well at 37 °C. Strain YEK25.75 exhibited the greatest growth deficiency at 37 °C (Fig. 1 and data not shown) and was therefore selected for further analysis.

All of these mutations map to a portion of TAF25p that is highly conserved between disparate species (Schizosaccharomyces pombe, Arabidopsis thaliana, Drosophila melanogaster, Caenorhabditis elegans, mouse, human3). In fact, glycine 101 is absolutely conserved in seven of seven TAF25p orthologs, while arginine 111 is conserved in six out of seven (in S. pombe this residue is Lys rather than Arg). Additional mutagenesis studies performed in our laboratory3 indicate that residues in this highly conserved region of TAF25 are mutational sensitive and therefore, the high conservation of this amino acid sequence is functionally relevant. The region of homology among these TAF25p proteins extends from upstream of glycine 101 essentially to the C terminus of the molecule and sequences downstream of arginine 111 are essential for yeast cell viability.3 By rights then, if all these sequences are essential for TAF25p function, then YEK25.76 would be expected to be non-viable. We hypothesized that this strain can grow, at least at 22 °C, because the nonsense mutation at TAF25 ORF codon 111 is suppressed at a frequency sufficient to provide levels of TAF25p adequate for growth. To test this hypothesis we generated a yeast strain, which carried a version of TAF25 in which three consecutive stop codons were inserted at codons 111, 112, and 113. As would be expected if our suppression model is correct, this strain was inviable when the triply prematurely terminated TAF25 gene was the sole source of TAF25p in the cell.3

mRNA Synthesis Rapidly Ceases in Cells Carrying a taf25ts Allele at the Non-permissive Temperature—When cultures of YEK25.75 cells, which carry a taf25ts allele, are shifted from 22

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* S. Sanders, unpublished results.
* E. Klebanow, unpublished results.

**FIG. 1.** Hydroxylamine generates mutations in TAF25 that induce a temperature-sensitive growth phenotype. A, hydroxylamine was used to generate random mutations in TAF25. The NH2-terminally mutagenized TAF25 gene on a HIS3-marked* CEN/ARS plasmid (pRS413) was introduced into a yeast strain carrying a taf25 null deletion allele in the chromosome and a URA3-marked TAF25 gene on a CEN/ARS plasmid (pRS416). The chromosomal null mutant was uncovered by plasmid shuffle by plating on 5-FOA as detailed under “Material and Methods.” The ability of the mutagenized TAF25 gene to support growth was monitored by plating on YPAD and incubation at permissive (22 °C) and non-permissive (37 °C) temperatures. Growth of various taf25 mutant strains is indicated in the photograph of the resulting plates. The codon locations and the mutations in TAF25 that induce temperature-sensitive growth are shown. DNA sequencing of the complete TAF25 ORF indicated that these were the same mutations introduced into TAF25. Strain YEK25.75, marked with an asterisk, exhibited the most severe growth defect at 37 °C. B, yeast strains carrying either a WT TAF25 allele or the taf25ts allele were grown in YPAD liquid culture at the permissive temperature (22 °C). One-half of the culture was shifted to the non-permissive temperature (37 °C) at the point indicated by the arrow. Growth was monitored by measuring absorbance (light scattering) at 600 nm. These strains exhibited equivalent growth properties whether growth was monitored by A600 or cell counting (not shown). As controls, cultures of yeast strains separately carrying rpm1-1, bup1-1, and tem1-1 temperature-sensitive mutant genes were grown and manipulated similarly (not shown). □, TAF25 at 22 °C; □, TAF25 at 37 °C; ○, taf25 at 22 °C; ●, taf25 at 37 °C.
to 37 °C, their growth rate rapidly decreases and within 6 h ceases altogether (Fig. 1B). We examined RNA synthesis in TAF25 and taf25 ts cells grown at 22 and 37 °C for various lengths of time in order to assess the effects of this temperature shift upon mRNA synthesis. As controls, separate, independent cultures of cells carrying rpb1–1 (which encodes the largest subunit of RNA polymerase II), tsm1–1 (which encodes the TFIIID subunit TAF150p), or tbp ts–1 (which encodes TBP) mutant genes were also utilized and processed for RNA in parallel. Polymerase II-mediated mRNA gene transcription has been shown to be quite sensitive to loss of both Rpb1p (13) and TBP (15) function in vivo, while RNA polymerase II transcription in vivo appears insensitive to the loss of TAF150p (23).

The results of our analyses examining total poly(A) + mRNA are presented in Fig. 2A. It is clear from these data that total mRNA synthesis in cells bearing the taf25 ts mutation decreases almost as rapidly after a temperature shift as does mRNA biosynthesis in the yeast strain carrying the rpb1–1 mutation, a benchmark strain for such analyses. Total mRNA synthesis in tbp ts–1 cells is, as expected, also sensitive to a temperature shift while mRNA synthesis is insensitive to temperature in cells bearing either the wild type TAF25 gene or the tsm1–1 mutation. Importantly the decrease in total mRNA synthesis in the taf25 ts cells is nearly identical to the rate of loss of mRNA biosynthesis in the tbp ts–1 cells, indicating as direct of an involvement of TAF25p in RNA polymerase II-mediated transcription as that played by TBP. This loss of total mRNA synthesis in the taf25 ts cells is not due to general cell death as >95% of the cells remain viable after 4 h at 37 °C (data not shown).

The decrement in RNA synthesis in taf25 ts cells is restricted to mRNA biosynthesis as tRNA synthesis, which is catalyzed by RNA polymerase III, was unaffected as measured by S1 nuclease protection assays (Fig. 2B). As expected, tRNA synthesis decreases rapidly in tbp ts–1 cells (15). The rapid drop in total mRNA gene transcription observed in the slot blot analyses of poly(A)+ mRNA is also observed if the levels of specific mRNAs is examined by RNA (Northern) blotting (Fig. 2C). mRNA levels decrease specifically in taf25 ts cells shifted to the non-permissive temperature at rates roughly proportional to the half-lives of the individual mRNAs with six of the seven genes analyzed (compare the loss of mRNAs in rpb1–1 and taf25 ts cells, Fig. 2C). Only the expression of MET19 does not decrease at an equivalent rate in taf25 ts versus rpb1–1 cells. However, mRNA MET19 levels are reduced at later points (see 2 and 3 h, Fig. 2C), most likely an indirect effect due to loss of TBP or other TAF150p protein levels (see below). In total, these data clearly demonstrate that continued TAF25p function is acutely required for ongoing high level RNA polymerase II-mediated mRNA gene transcription in living yeast cells.

TFIID and SAGA Subunit Levels Are Differentially Degraded in taf25 ts Cells after a Shift to Non-permissive Temperature—As detailed in the Introduction, a model emanating from the recent studies of TAFII function in TFIID argues that the reason that the integrity of the histone-like TAF II μs, TAF61p, TAF60p, and TAF17p, is so crucial for TFIID activity is because these three TAF Ts form the “structural core” of the complex. Once the integrity of this TFIID core is compromised the complex dissociates and the free, non-complexed subunits are degraded. In order to test whether a comparable situation is operative in the case of the non-histone-like TAF25p, we examined the levels of various TFIID and SAGA constituents by immunoblotting SDS-PAGE fractionated WCEs prepared from both taf25 ts and TAF25 cells as a function of time after a shift to 37 °C. These analyses, shown in Fig. 3, were performed with aliquots of the same cells used for the RNA experiments of

Fig. 2 so that the results are directly comparable. Although such chemiluminescence experiments are difficult to quantify with great precision, it is clear from these immunoblots that all of the various proteins analyzed do specifically decrease with time in the taf25 ts cells. However, the rate of loss of the different proteins is variable, falling roughly into three classes. We have sorted these changes relative to the drop off in mRNA synthesis in the taf25 ts cells where we observed a decrease of ≥70–80% in the first hour after the temperature
free TAF_{13} subunits are then degraded if they are not stably incorporated into TFIID. This proposal is based upon our current data regarding the deleterious effects of TAF25p inactivation on RNA polymerase II transcription and TFIID subunit stability, our previously published data (12), which indicates that TAF25p can interact with high affinity with itself both in vitro and in vivo, and data that indicate TAF25p is present in superstoichiometric amounts in a highly purified TFIID fraction. This model can also be applied to SAGA structure, organization, and function. Our data does not allow us to definitively distinguish between loss of TFIID or SAGA function as the contributing factor to the loss of bulk mRNA synthesis in taf25ts cells. For reasons discussed above, however, it is likely that loss of TFIID function is the major contributing factor. Studies are currently in progress in our laboratory to directly investigate these and other aspects of TFIID structure and function using a combination of additional biochemical and genetic analyses. Such studies will prove crucial to dissecting the complex process of TFIID-mediated RNA polymerase II gene transcription.

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![Fig. 3. Characterization of TFIID and SAGA protein subunit levels in TAF25 and taf25ts cells at various times after a shift to non-permissive growth conditions.](image)

Cells taken from the cultures analyzed in the experiments of Fig. 2 were harvested and total WCE proteins prepared as detailed under "Materials and Methods." These protein samples were fractionated by SDS-PAGE, blotted to polyvinylidene difluoride membranes, and specific proteins, as indicated, were detected by immunostaining with affinity-purified polyclonal IgGs and chemiluminescence as detailed under "Materials and Methods." In the experiments of Fig. 2 were harvested and total WCE samples were fractionated by SDS-PAGE, blotted to polyvinylidene difluoride membranes, and specific proteins, as indicated, were detected by immunostaining with affinity-purified polyclonal IgGs and chemiluminescence as detailed under "Materials and Methods." In the case of certain TAFs, proteins (see TAF47p for example), protein levels were reduced at 0 h in taf25ts cells as compared with the 0 h point from TAF25 cells. shift. This is obviously the time interval most relevant, at least for initial discussion. TAF_{15}p 150p, 130p, 90p, 61p, 60p, 47p, 40p, 19p, and 17p all decrease by about 50% in the first hour after the temperature shift. TAF_{67}p 67p and 25p levels decrease more slowly, showing significant decreases only after 2 h at 37 °C. TAF25p, expressed from an HA_{3}-tagged gene (see "Materials and Methods"), was analyzed using both polyclonal antibodies to TAF_{25}p IgG and the 12CA5 anti-HA mAb with comparable results (data not shown). TBP (a TFIID subunit), Gcn5p (the acetylase subunit of the SAGA complex), and Ada2p (a SAGA subunit, not shown) behave similarly to TAF25p and TAF67p (Fig. 3). The levels of TAF30p, an apparent subunit of multiple distinct complexes in addition to TFIID (24, 25), do not significantly decrease over the time course analyzed. Clearly, inactivation of TAF25p via temperature shift results in the inactivation, through protein degradation, of the multiple constituents of both TFIID and SAGA complexes. This phenomenon has been seen by others in comparable analyses (4–7), although, as stated in the Introduction, this inactivation-directed protein degradation has been ascribed to abrogation of crucial structural interactions between the histone-like TAFs.

To incorporate these new data regarding the non-histone-like TAF, TAF25p, into current models of TFIID organization (see Introduction), we propose that, in addition to interactions between histone-like TAFs, TAF25p-TAF25p interactions are critical for maintaining TFIID structure, organization, and ultimately TFIID function. For example, when TAF25p-TAF25p interactions are disrupted in YEK25.75 cells, which carry the taf25ts mutant allele, overall TFIID structure is disrupted and...