Molecular Cell, Vol. 2, 675–682, November, 1998, Copyright ©1998 by Cell Press

The Histone H3–like TAF Is Broadly Required for Transcription in Yeast

Zarmik Moqtaderi, Marie Keaveney, and Kevin Struhl*
Department of Biological Chemistry
and Molecular Pharmacology
Harvard Medical School
Boston, Massachusetts 02115

Summary

In yeast cells, independent depletion of TAFs (130, 67, 40, and 19) found specifically in TFIID results in selective effects on transcription, including a common effect on *his3* core promoter function. In contrast, depletion of TAF17, which is also present in the SAGA histone acetylase complex, causes a decrease in transcription of most genes. However, TAF17-depleted cells maintain Ace1-dependent activation, and they induce de novo activation by heat shock factor in a manner predominantly associated with the activator, not the core promoter. Thus, TAF17 is broadly, but not universally, required for transcription in yeast. TAF17 depletion and TAF130 depletion each disrupt TFIID integrity yet cause different transcriptional consequences, suggesting that the widespread influence of TAF17 might not be due solely to its function in TFIID.

Introduction

TFIID, a critical component of the eukaryotic RNA polymerase II (pol II) transcription machinery, is a multiprotein complex consisting of the TATA-binding protein (TBP) and approximately ten associated factors called TAFs (reviewed by Burley and Roeder, 1996; Verrijzer and Tjian, 1996). Within TFIID, the TBP subunit plays crucial roles in promoter recognition and assembly of an active transcription complex. TBP specifically binds TATA elements, which are found in most eukaryotic promoters. The resulting TBP–TATA complex has a novel structure that is specifically recognized by TFIIB, the factor primarily responsible for connecting TFIID to the pol II holoenzyme. Although TBP is sufficient to support accurate initiation from TATA-containing promoters in vitro, it is generally believed that TFIID (not TBP) is the functional entity that associates with promoters in vivo.

Biochemical analyses indicate that TAFs are important for core promoter function, particularly at promoters lacking TATA elements (Verrijzer et al., 1995; Burley and Roeder, 1996; Burke and Kadonaga, 1997). Transcription from such TATA-less promoters depends on initiator and downstream elements, and it is observed in reactions with TFIID, but not TBP. Moreover, TFIID specifically interacts with initiator and downstream elements via direct contacts with TAFs. Although these TAF–promoter interactions are weaker and less specific than the TBP–TATA interaction, they clearly provide part of the promoter recognition surface of TFIID. In addition to their promoter-binding properties, TAFs might also affect core promoter function by interacting with a general transcription factor(s).

A number of biochemical studies suggest that TAFs are important for the ability of transcriptional activator proteins to stimulate the pol II machinery. First, in many in vitro reactions, TFIID can support activated transcription, whereas TBP cannot (Burley and Roeder, 1996). Second, TAFs are required for certain activators to stimulate the association of TFIID and TFIIA to promoters (Lieberman and Berk, 1994; Chi et al., 1995), a step that can be rate limiting for transcription in vitro. Third, TAFs affect the formation/stability of an activator-dependent transcription complex at a step that occurs after recruitment of TFIID and TFIIB to the promoter (Choy and Green, 1993). Fourth, isolated TAFs can specifically interact with different classes of activation domains (Hoey et al., 1993; Chiang and Roeder, 1995; Caron et al., 1997; Uesugi et al., 1997), and the presence of the relevant TAF in a reconstituted TFIID subcomplex correlates with activator-dependent transcription (Chen et al., 1994). Fifth, multiple contacts between activation domains and TAFs can strongly increase TFIID binding to the TATA element and synergistically activate transcription (Sauer et al., 1995). From these observations, it has been proposed that individual TAFs within the TFIID complex function as direct and selective targets of activation domains (Verrijzer and Tjian, 1996).

Although the observations above suggest that TAFs play some role in the activation process, efficient activation can occur in the absence of TAFs in vitro (Kim et al., 1994; Koleske and Young, 1994; Oelgeschlager et al., 1998). This indicates that TAFs are not essential for activation and that other components of the pol II machinery suffice as targets. Moreover, there is no evidence that activation domains interact with TAFs in the TFIID complex; hence, interactions with isolated TAFs might involve protein surfaces that are inaccessible in the context of intact TFIID. Finally, TFIID-dependent activation in vitro requires coactivators (e.g., PC2 and PC4) that are distinct from the general factors required for basal transcription (Burley and Roeder, 1996). Thus, a convincing case for TAFs as direct targets of activators has yet to be made, and it is entirely possible that TAFs play an indirect role in the activation process.

The physiological functions of TAFs have been assessed primarily in yeast. TAFs are essential for cell growth, but individual depletion of various TAFs does not significantly affect transcriptional activation of the vast majority of genes including those responsive to well-characterized activators such as Gcn4, Gal4, Ace1, and Hsf1 (Apone et al., 1996; Moqtaderi et al., 1996a; Walker et al., 1996). However, TAF depletion selectively affects the transcription of a small subset of genes. Depletion of TAF130(145) or TAF19 differentially affects *his3* TATA element utilization and reduces *trp3* transcription, suggesting that these TAFs might be important at certain promoters containing weak TATA elements (Moqtaderi et al., 1996a). Depletion of TAF130

*To whom correspondence should be addressed (e-mail: kevin@hms.harvard.edu).

also affects transcription of certain cell-cycle and ribosomal protein genes, and analysis of hybrid promoters indicates that TAF function is associated with the core promoter region, not the enhancer (Shen and Green, 1997; Walker et al., 1997). TAF mutations in *Drosophila* (Sauer et al., 1996) or hamster cells (Wang and Tjian, 1994; Suzuki-Yagawa et al., 1997) selectively affect gene expression, although the molecular bases of these effects are not well understood. Thus, in general accord with the biochemical studies, TAFs have important physiological roles in core promoter function, although the precise relationship between in vitro and in vivo studies remains to be determined. At present, there is no evidence for activator-specific effects of TAFs in vivo. However, TAFs might be physiological targets for a limited subset of activators, and/or activator–TAF interactions might be redundant with other activator–pol II machinery interactions and hence nonessential for activation in vivo (Struhl, 1996).

The above conclusions regarding physiological functions of TAFs are limited to those TAFs that have been examined in vivo. Here, we use our previously described copper-inducible double shutoff technique (Moqtaderi et al., 1996a) to examine the functions of additional yeast TAFs. We were particularly interested in TAF17 (Moqtaderi et al., 1996b), the histone H3-like TAF (Xie et al., 1996), because its *Drosophila* homolog interacts with TFIIB and the VP16 activation domain in vitro (Goodrich et al., 1993). Surprisingly, and in contrast with our observations on other TAFs, we find that depletion of TAF17 results in a decrease in transcription of most, but not all, pol II genes. A subset of TAFs including the histone-like TAFs have recently been shown to be present in the yeast SAGA and human PCAF histone acetylase complexes (Grant et al., 1998a; Ogryzko et al., 1998; Struhl and Moqtaderi, 1998), and our results suggest the possibility that the presence of TAF17 in SAGA (or some other complex) might contribute to its broad transcriptional function.

Results

Creation of TAF Depletion Strains

In previous work (Moqtaderi et al., 1996a), we described a copper-inducible, double-shutoff method to generate conditional alleles of essential genes such as TAFs, TBP, and TFIIB. In this method, the addition of copper causes the repression of TAF transcription and the targeting of preexisting TAF protein for ubiquitin-mediated degradation. Because this procedure results in the direct elimination of the desired protein, it is advantageous over the use of temperature-sensitive mutant proteins, which often retain specific or partial functions upon shift to the restrictive temperature.

Using this method, we generated yeast strains harboring conditional alleles of yeast *TAF17*, *TAF40*, and *TAF67* (Moqtaderi et al., 1996b). As expected, these strains grow normally in the absence of copper, but they stop growing within 6 hr upon the addition of copper sulfate to 500 μM (Figure 1). The kinetics of growth inhibition upon copper addition are comparable to those observed previously when using the same method to deplete other TAFs, TBP, or TFIIB (Moqtaderi et al., 1996a).

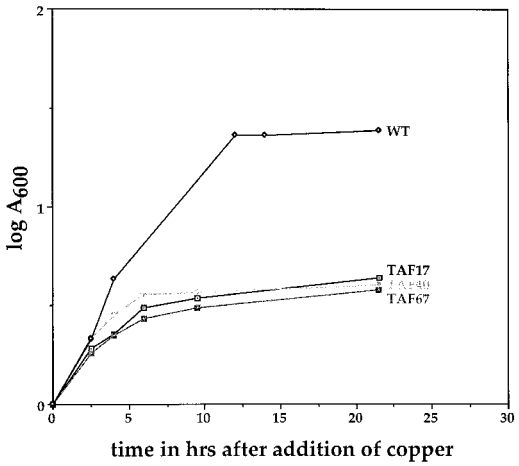

Figure 1. Growth Curves of the TAF Depletion Strains

Strains harboring either the *URA3* centromeric vector YCplac33 or conditional alleles of the indicated TAFs were cultured in synthetic complete medium lacking uracil, and copper sulfate was added to 500 μM at time 0.

Depletion of TAF40 or TAF67 Differentially Affects *his3* TATA Element Utilization

To assess the transcriptional roles of the TAFs, strains containing the conditional alleles were grown in permissive conditions, TAF shutoff was initiated by the addition of copper sulfate, and transcription was assayed by S1 nuclease protection at various times after the shift. As an initial characterization, we analyzed transcription of *ded1*, which contains a typical TATA-containing promoter (Struhl, 1985), *his3*, which contains nonconsensus and consensus TATA elements that, respectively, direct initiation from the +1 and +13 sites (Struhl, 1986; Chen and Struhl, 1988; Mahadevan and Struhl, 1990; Iyer and Struhl, 1995), and *trp3*, whose promoter contains a nonconsensus TATA element (Martens and Brandl, 1994). In accord with all our previous TAF depletions (Moqtaderi et al., 1996a), loss of TAF40 or TAF67 does not affect *ded1* or +13 *his3* transcription (Figure 2). However, depletion of either TAF40 or TAF67 causes a significant decrease in the level of the *his3* +1 and *trp3* transcripts, a phenotype previously observed upon conditional knockout of TAF19 or TAF130 (Moqtaderi et al., 1996a). Thus, TAF40 and TAF67 (as well as TAF19 and TAF130) differentially affect *his3* TATA element utilization and *trp3* transcription and hence are important for certain promoters with weak TATA elements.

Depletion of TAF17 Broadly Affects Pol II Transcription

In striking contrast to the results with other TAFs described above and previously, depletion of TAF17 causes a significant overall decrease in the transcription of both *his3* (from both +1 and +13 TATA elements) and *ded1* (Figure 2). It is very unlikely that the deleterious effects of TAF17 depletion on *his3* are merely a more extreme manifestation of the selective +1 defect observed above and that the +1 defect is a harbinger of overall transcriptional loss. In the TAF17 shutoff, as with a shutoff of the general transcription factors TBP or TFIIB (Cormack and

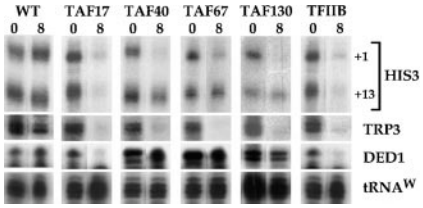

Figure 2. Promoter-Specific Effects of TAF Depletion

RNA from strains harboring either a vector (WT) or the indicated conditional TAF allele was harvested before (0) and 8 hr after the addition of 500 μM copper sulfate. RNA levels were determined by S1 nuclease protection assay with probes for the transcripts shown.

Struhl, 1992; Moqtaderi et al., 1996a), the *his3* +1 and +13 transcripts disappear with indistinguishable kinetics; thus, the effects of TAF17 depletion on *his3* transcription are both quantitatively and qualitatively distinct from the effects of depleting other TAFs.

Analysis of several genes that vary in their overall mRNA level and in TATA element quality (Figure 3) indicates that, in all cases tested, depletion of TAF17 significantly reduces the level of transcription. This decrease in pol II transcription mirrors that seen upon depletion of the general transcription factor TFIIB, but with slightly slower kinetics. We doubt that this slight kinetic difference is functionally significant; it likely reflects a difference in the rate of Ubr1-dependent proteolysis of the TAF17 and TFIIB derivatives and/or a difference in the amounts of TAF17 and TFIIB that are in excess over that needed for normal transcription. Furthermore, our observations are in accord with the results of Michel et al.

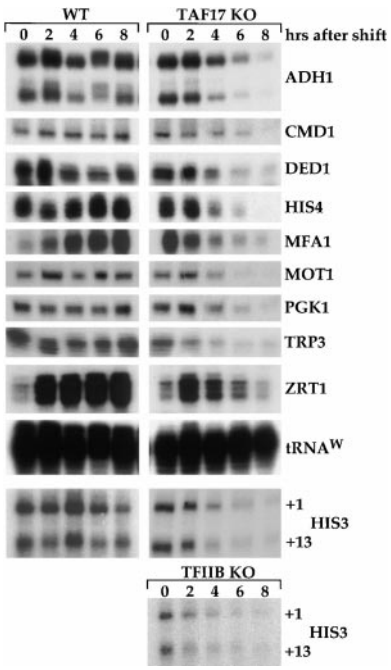

Figure 3. Decreased Levels of Many RNAs after TAF17 Depletion

RNA isolated from parental (WT) or TAF17 conditional knockout strains at the indicated timepoints after copper addition was analyzed by S1 nuclease protection assay, using probes for the RNAs shown at right.

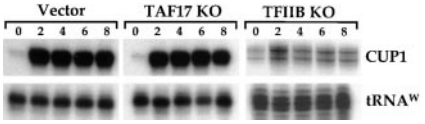

Figure 4. Ace1 Activation Is Maintained during TAF17 Depletion

RNA from strains harboring a vector or a conditional allele of TAF17 or TFIIB was isolated at successive time points after copper addition. CUP1 transcription was analyzed by S1 nuclease protection assay.

(1998) and Apone et al. (1998) (both in this issue of *Molecular Cell*), who have inactivated TAF17 using thermosensitive alleles. Thus, TAF17 broadly affects pol II transcription, a result in notable contrast to the far more limited and gene-specific effects mediated by other TAFs we have tested by the same method (TAFs 130, 90, 60, 19, and Tsm1).

Ace1-Dependent Activation of *CUP1* Transcription Is Insensitive to TAF17 Depletion

Although depletion of TAF17 causes a broad decrease in transcription, it does not affect all genes. Copper-inducible (i.e., Ace1-dependent) transcription of *cup1*, which was begun concomitantly with the copper-induced initiation of TAF depletion, remains unaffected even 8 hr after the shift (Figure 4), conditions in which most cells are dead and transcription of other genes is significantly reduced. In contrast, cells depleted of TFIIB not only exhibit a general decrease in pol II transcription, but they fail to maintain activated levels of *cup1* transcription (Moqtaderi et al., 1996a). Thus, TAF17 behaves in a manner that is distinct from a general transcription factor in that it is not universally required for all pol II transcription in vivo.

In the course of these experiments, we found that *ZRT1*, which encodes a high-affinity zinc transporter (Zhao and Eide, 1996), is inducible by copper (Figure 3). Although *cup1* transcription appears unaffected by TAF17 depletion, copper-inducible transcription of *zrt1* is reduced over time as TAF17 is depleted. Copper induction of *zrt1* is unlikely to involve Ace1 or a direct effect of copper. Instead, the addition of copper probably causes zinc limitation, a condition that stimulates *zrt1* transcription in a manner that depends on the Zap1 activator protein (Zhao and Eide, 1997). Thus, these results suggest that TAF17 is important for Zap1-dependent activation but is not essential for Ace1-dependent activation.

TAF17-Depleted Cells Can Mediate De Novo Transcriptional Activation by Heat Shock Factor

We also examined TAF17-depleted cells for the ability to induce transcription of heat shock genes de novo (Figure 5). In this experiment, cells were treated with copper for 8 hr, conditions under which transcription of most genes has already been significantly reduced, and then subjected to a short (14 min) heat shock. Analysis of the *hsp104* and *ssa4* transcripts shows that, despite the widespread decrease in pol II transcription, the TAF17-depleted strain is able to mount a normal heat shock activation response. In contrast and as expected,

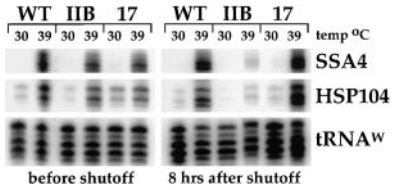

Figure 5. Activation by Heat Shock Factor after TAF Shutoff

Cultures of the parental strain, TFIIB shutoff strain (IIB), or TAF17 shutoff strain (17) before or 8 hr after shutoff by copper addition were split, with half being subjected to a heat shock. Activation of the heat shock genes *SSA4* and *HSP104* was assayed by S1 nuclease protection.

cells depleted of TFIIB do not activate *hsp104* or *ssa4* transcription after a brief heat shock. We conclude from this, taken together with the results on *cup1*, that the pol II transcription machinery is not fundamentally crippled in TAF17-depleted cells, as it is in cells depleted of a basal transcription factor.

The fact that *hsp104* and *ssa4* mRNA levels are rapidly induced by a brief heat shock treatment strongly argues for de novo transcriptional activation by heat shock factor under conditions of TAF17 depletion. However, it is formally possible that the immunity of the heat shock response to the deleterious effects of TAF17 depletion is due to some special property of the heat shock transcripts, rather than the heat shock activator itself. To distinguish the contribution of the activator itself from the effects of the promoter context, we examined transcription of a modified *his3* gene in which the upstream promoter region was replaced by a binding site for heat shock factor (Figure 6). As expected, this HSE-containing *his3* gene is inducible by heat shock, and depletion of TFIIB abolishes the ability of a 14 min heat shock to induce transcription. In contrast, TAF17-depleted cells strongly induce *his3* transcription upon heat shock to a level only 2-fold below that observed in wild-type cells. As the half-life of *his3* mRNA (10 min at 30°C; Iyer and Struhl, 1996) is short and unaffected by heat shock (Cormack and Struhl, 1992), these results indicate that the immunity of the heat shock response to TAF17 depletion is significantly determined by the HSE and the heat shock transcriptional activator.

TAF17 and Other TAFs Are Required for the Integrity of the TFIID Complex

To investigate the molecular functions of TAF17, we determined the composition of the TFIID complex upon depletion of TAF17 and other selected TAFs. Previous analyses of whole-cell extracts indicated that depletion of a single TAF can cause a decrease in the intracellular levels of other TAFs (Walker et al., 1996), but these studies did not directly address the fate of TFIID. To determine the TAF composition specifically within the TFIID complex, we immunoprecipitated TBP and its associated proteins at various times after depletion of a particular TAF, and then analyzed the immunoprecipitated complexes by Western blotting using antibodies to TAFs 130, 90, 60, and 30.

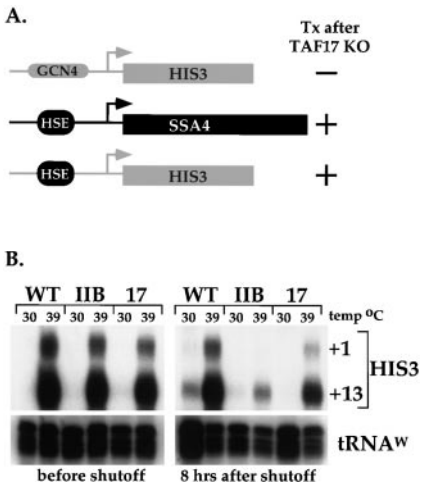

Figure 6. Activation of a Heat Shock–Inducible *HIS3* Allele after TAF Shutoff

(A) Schematic representation of the transcriptional phenotypes of wild-type HIS3, wild-type SSA4, and a heat shock–inducible HIS3. (B) Heat shock–inducible HIS3 transcription was assayed by S1 nuclease protection assay after heat shock as in Figure 5.

As expected, the control strain maintains normal levels of TAFs 130, 90, 60, and 30 after 8 hr of growth in copper-containing media (Figure 7). In contrast, depletion of any individual TAF analyzed by this means (i.e., TAFs 130, 60, and 17) results in dissociation of the TFIID complex. While the kinetics of TFIID disintegration were slightly faster under TAF17 shutoff conditions compared to the TAF130 and TAF60 depletion strains, the amount of any TAF associated with TBP in all strains 8 hr after depletion was $<10\%$ of that present before depletion. This observation suggests that the distinct transcriptional differences observed in the TAF17 depletion strain are not simply a result of a more rapid or complete TFIID breakdown. Furthermore, Western blot analyses of the same whole-cell extracts indicate that the levels of TBP and various TAFs decrease minimally over time, indicating that their presence in the cell cannot be correlated with an association with TBP. Additionally, the overall yield of protein and distribution of total cellular protein (as determined by Ponceau S staining of filter-bound protein after electrophoresis and transfer) in the conditional strains remain globally normal. Thus, the broad transcriptional defects observed upon TAF17, but not TAF130 or TAF60, depletion are not due to major differences in the integrity of the TFIID complex.

Discussion

TFIID-Specific TAFs Are Important for a Common Core Promoter Function

Our results indicate that TAF40 and TAF67 share the previously published transcriptional profile of TAF130 or TAF19 (Moqtaderi et al., 1996a); that is, each is important for *his3* +1 and *trp3* transcription but not for *his3* +13 and *ded1* transcription. It thus appears that a subset of TAFs comprising TAFs 19, 40, 67, and 130 is important for a core promoter function of TFIID. Strikingly, no member of this TAF subset is present in the

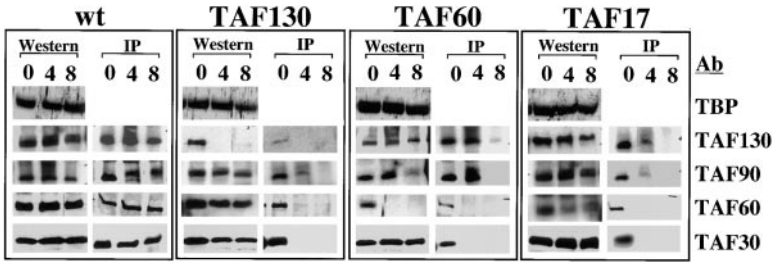

Figure 7. Dissociation of the TFIID Complex upon Individual TAF Depletion

Whole-cell extracts from wild-type, TAF130, TAF60, and TAF17 depletion strains were prepared before and at time points 4 and 8 hr after copper addition. The results from Western blot and immunoprecipitation analyses are shown on the left and right sides of each panel, respectively. For Western blotting, 30 μg of protein was electrophoresed, transferred to membrane, and probed with the antibodies shown on the right side of the figure.

To determine the integrity of the TFIID complex upon individual TAF depletion, TBP and its associated proteins were immunoprecipitated from the same extracts with α-TBP-coupled beads, and the precipitated complexes were blotted and probed with the TAF antibodies shown on the right.

SAGA histone acetylase complex, which has recently been shown to include TAFs 90, 61, 60, 25, and 17 (Grant et al., 1998a). Conversely, none of the SAGA TAFs we have tested (TAFs 90, 60, 17) match the shared transcriptional profile of TAFs 19, 40, 67, and 130.

Depletion of TAFs 19, 40, 67, or 130 can be viewed as a first step in disentangling TAF function in TFIID from broader TAF roles in multiple complexes. We speculate that the core promoter defects seen after depletion of TAF130, when immunoprecipitations show TBP to be virtually denuded of TAFs (Figure 7), as well as the similar defects seen upon depletion of TAFs 19, 40, and 67, might represent the transcriptional output by the isolated TBP subunit, rather than TFIID. Interestingly, the transcriptional profile in these TAF-depleted strains is remarkably similar to the one observed in yeast cells containing human TBP, which presumably interacts poorly with yeast TAFs (Cormack et al., 1994).

TAF17 Is Important for the Transcription of Most Genes

Unlike previously studied TAFs, which are required for transcription of relatively few genes, we find that TAF17 is important for the transcription of most genes. Although the kinetics of the broad transcriptional defects after TAF17 depletion are slightly slower than those observed upon depletion of the general factor TFIIB, they are nonetheless too rapid to be accounted for as the indirect result of the specific transcriptional shutoff of some crucial gene. A model invoking such an indirect effect would have to posit not only that TAF17 exerts highly specialized transcriptional control over a few critical genes, but also that the proteins encoded by these affected genes are very short lived. In fact, the Ponceau S staining profile of total cellular protein remains normal after TAF17 shutoff, indicating that most proteins, whatever the transcriptional status of the genes encoding them, are stable over the time course of the experiment. Furthermore, extracts prepared from transcriptionally inactive cells (due to thermal inactivation of TBP) are fully competent for basal transcription in vitro, indicating that the remaining basic components of the pol II machinery are relatively stable (Schultz et al., 1992). Most convincingly, the ability of TAF17-depleted cells to mount a de novo heat shock response implies that all the proteins involved in the basic aspects of pol II transcription remain present after TAF17 depletion, even though the genes encoding them may no longer be transcribed. Thus, we conclude that the adverse transcriptional effects of TAF17 depletion directly reflect the primary function(s) of TAF17. In accord with this conclusion, independent shutoff experiments using thermosensitive alleles of TAF17 have also demonstrated the broad influence of TAF17 in pol II transcription (Apone et al, 1998; Michel et al., 1998).

Evidence that TAF17 Has an Important Transcriptional Role Outside of TFIID and Probably in the SAGA Histone Acetylase Complex

Examination of TFIID integrity indicates that loss of any one of several TAFs, including TAF17, leads to demolition of the TFIID complex. Thus, TAF17 does not appear to be a special linchpin of TFIID, and the broad pol II transcriptional defects seen upon TAF17 depletion are unlikely to be attributable to some uniquely deleterious effect of TAF17 depletion on TFIID integrity. Depletion of other TAFs, such as TAF130, disrupts TFIID integrity without causing broad transcriptional defects. These observations suggest that the broad importance of TAF17 might also involve a function(s) exercised outside of TFIID.

The recent discovery that TAF17 is a member of the SAGA histone acetylase complex (Grant et al., 1998a) provides an obvious context for non-TFIID functions of TAF17. The SAGA complex is generally thought to be nonessential, because deletion of Gcn5 histone acetylase or several of the Ada or Spt components selectively affects transcription of a small subset of genes (Roberts and Winston, 1997; Grant et al., 1998b). SAGA may have a more significant role, because mutations of the Ada1 or Ada5/Spt20 components cause more severe phenotypes, including slow cell growth (Marcus et al., 1996; Roberts and Winston, 1996; Horiuchi et al., 1997), but it seems unlikely that the broad effects of TAF17 could be entirely due to its presence in SAGA. Instead, we suggest that the broad transcriptional role of TAF17 might be due to its presence in both the TFIID and SAGA complexes such that eliminating TAF17 from only one complex has only a selective effect on transcription. In addition, TAF17 might be present in yet other complexes that could contribute to its widespread effects on transcription.

Because the histone H3-like TAF17 and the histone

H4-like TAF60 interact to form a histone-like tetramer (Xie et al., 1996), it might be expected that depletion of either TAF should result in similar transcriptional effects. However, we previously reported that depletion of TAF60 does not have a dramatic effect on transcription, although a small and general effect was observed at later time points (Moqtaderi et al., 1996a). Although this TAF60 depletion dramatically decreases TAF60 protein levels and TFIID integrity, it is of course possible that our depletion of TAF60 is less efficient than the depletion of TAF17 and that a low amount of TAF60 (and hence TFIID) might be sufficient for many transcriptional events. This could reflect a difference in relative sensitivity to Ubr1-dependent proteolysis of the TAF17 and TAF60 derivatives, which could occur in the context of TFIID, SAGA, or the uncomplexed protein. In this regard, recent experiments using thermosensitive alleles suggest that TAF60 and the histone H2B-like TAF61 (which we have not tested) have broad effects on transcription comparable to those mediated by TAF17 (Michel et al., 1998).

Models for TAF17 Function

There are three classes of models to account for the broad, but not universal, effects of TAF17. First, in the SAGA and/or TFIID context, TAF17 (or a closely associated factor such as the other histone-like TAFs) might act as a near-universal target of activators that serves to directly recruit the pol II machinery to promoters. This model assumes that transcription in vivo requires activator proteins, an assumption strongly supported by the general observation that intact promoters are much more active than core promoter derivatives containing only the TATA and initiator elements. The possibility of almost universal targets for activators is supported by experiments suggesting that Srb4, a pol II holoenzyme component, is a direct activator target (Koh et al., 1998). Our results with TAF17 are strikingly similar to those obtained for Srb4 in that these proteins have broad transcriptional consequences but minimal effects on activation by Ace1 or Hsf (Thompson and Young, 1995; Lee and Lis, 1998).

Second, rather than being a common target of activators, TAF17 might be required for pol II transcription in much the same way as a basal factor. Alternatively, TAF17 could be important for the proper structure or stability of a general factor such as TFIIB, especially as the *Drosophila* homolog of TAF17 interacts with TFIIB in vitro (Goodrich et al., 1993). This model seems less likely in light of the ability of TAF17-depleted cells to maintain Ace1-dependent activation and to activate heat shock genes, both of which indicate that the basic pol II machinery is essentially intact.

Third, the broad role of TAF17 might not reflect specific functions of this TAF but rather would result from redundant functions of TFIID and SAGA for some critical aspect of transcription. In this view, each complex can provide this function for most genes, but few genes are active in the absence of both complexes. A specific version of this model is that the common function is histone acetylation given that both TFIID (Mizzen et al., 1996) and SAGA (Grant et al., 1997) are histone acetylases; that is, if both complexes are functionally inactivated, the genomic chromatin is largely deacetylated and, hence, transcriptionally repressed.

In light of these models, it is interesting to consider why cells depleted for TAF17 or Srb4 retain the ability to mediate activation by Ace1 and heat shock factor, even though transcription of most genes is strongly impaired. At present, it is unclear whether this results from the raw strength or special properties of these activators. In the context of the activator-target model, certain strong activators might efficiently use any of several targets and thus be less strictly dependent on any one. In contrast, a typical activator might be entirely reliant on the presence of a particular target, or it might need the presence of multiple targets to generate a significant transcriptional response. In terms of the other models for TAF17 function, Ace1 and heat shock factor might override the effects of a defective (but not completely inactive) pol II machinery, or they might interact with the pol II machinery with sufficient affinity to recruit it to promoters even in the context of repressed chromatin.

Experimental Procedures

DNAs and Strains

TAF depletion strains were constructed using the double shutoff parent strain ZMY60 as previously described (Moqtaderi et al., 1996a) or ZMY117 (isogenic except for *leu2::PET56*). In the parent strain, the transcriptional repressor Rox1 and the Ubr1 protein, involved in ubiquitin-meditated protein degradation, are expressed under the control of a copper-inducible promoter. In the context of this parent strain, one introduces the desired TAF allele expressed under the control of the *ANB1* promoter, a natural target of Rox1, and fused to an N end recognition signal for ubiquitin-mediated degradation. Thus, in the absence of copper, Rox1 and Ubr1 are not expressed, and the TAF is expressed normally. With the addition of copper, both Rox1 and Ubr1 are highly induced and can function, respectively, to repress further TAF transcription and target preexisting TAF protein for degradation. To construct the conditional knockout alleles of TAFs 17, 40, and 67, sequence encoding amino acids 1–108 of TAF17, 1–157 of TAF40, or 1–194 of TAF67 was fused in-frame to an *ANB1*-driven ubiquitin-arginine-lacI-HA N-terminal cassette as described (Moqtaderi et al., 1996a). The resulting *URA3* integrating constructs were linearized within the TAF sequence and introduced into ZMY117 by lithium acetate transformation, thereby introducing copper-inducible TAF alleles at their normal genetic loci. The strain harboring a heat shock–driven allele of *his3* was created by two-step gene replacement in the double shutoff parent strain with the HSE-*his3* molecule described previously (Iyer and Struhl, 1995).

Transcriptional Analysis

TAF depletion and control strains were cultured at 30°C in synthetic complete medium lacking uracil to an OD_{600} of 0.3–0.5. Copper sulfate was then added to 500 μM to initiate the depletion of the conditionally expressed TAF. At various times after the addition of copper, total cellular RNA was isolated, and RNA levels of various genes were determined by S1 nuclease protection assay using 20–40 μg of RNA per sample and oligonucleotide probes (Iyer and Struhl, 1996). For the heat shock experiments, strains were incubated with copper for 8 hr, at which point both cell growth and pol II transcription have decreased substantially in the TAF shutoff strains. The culture was then split in two, with half subjected to a 14 min heat shock at 39°C and half left at 30°C.

The sequences of the previously unpublished S1 oligonucleotide probes used are as follows: *ADH1*, CCTTTTTGAGTTTCTGGGATAG ACATTGTATATGAGATAGTTGATTGTATGCTTGGTATAGCTTGACT; *CMD1*, CGCCTGATCCATCACTAACCTCTCTTAGCATATCATCTAC

TTCGGCCAACA; *MFA1*, GCTGGGTCCCAGAAGACACCTTTGATAA TATAGTTGTCCTTCTTTTCACTGCACCAG; *MOT1*, GGGTGCTGTTT GGCCAAGTCTCCCATTTGATCGGCAGCCATGTTCCGGACTACTT GGGATTCC; *PGK1*, CCTTGGCACCACCTAAGATGGCCAAGAATG GTCTGGTTGGGTTCTCCAAAGCCACTAA; and *ZRT1*, CACCCAAA CCGATGGCCACACAGATTGGTGTGGTTAACCCATACGCAACACA TAGGGCCCATGGCCTGGTAG.

Analysis of the TFIID Complex

Whole-cell extracts from wild-type and TAF depletion strains were prepared before and at 4 and 8 hr after copper induction. Cells were lysed with glass beads in buffer containing 150 mM Tris acetate (pH 7.8), 50 mM potassium acetate, 20% glycerol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT. For Western blot detection, 30 μg of protein (unless otherwise specified) was electrophoresed on 10% or 12% SDS-polyacrylamide gels and electroblotted to nitrocellulose membranes. Antibody probing and washing was performed according to standard techniques, and proteins were visualized using the Supersignal or Ultrasupersignal system as recommended by the manufacturers (Pierce). For purposes of quantitation, serial dilutions of the sample from time 0 were analyzed in parallel to generate a standard curve.

Immunoprecipitations were performed in a 1 ml reaction volume in buffer A (20 mM HEPES [pH 7.6], 1 mM DTT, 1 mM EDTA, 20% glycerol, 125 mM potassium acetate, 1% Nonidet-P40) using 300 μg of whole-cell extract prepared as above. Protein extracts were precleared by incubation for 2 hr at 4°C with 50 μl of preswollen protein A–Sepharose beads. The extracts were then incubated with 25 μl of α-TBP-coupled protein A–Sepharose beads for 12 hr at 4°C on a rotator apparatus. The beads were subsequently washed six times with 1 ml vol of buffer A. Samples were resuspended in Laemmli buffer, boiled for 4 min, and the complexes were resolved on SDS-polyacrylamide gels. Western blotting was performed as described above.

Acknowledgments

Z. M. dedicates this paper to Katalin Szabo on the occasion of her retirement from the Hackley biology department. We thank Michael Green for TAF30, 60, 90, and 130 antibodies; Laurie Stargell and Stephen Buratowski for TBP antibodies; and Mario Mencia, Bertha Michel, Stephen Buratowski, and Michael Green for discussion. This work was supported by an NIH postdoctoral fellowship (GM17930) to M. K. and by an NIH research grant (GM30186) to K. S.

Received July 28, 1998; revised September 9, 1998.

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