Transcriptional Activation in Yeast Cells Lacking Transcription Factor IIA

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ABSTRACT

The general transcription factor IIA (TFIIA) forms a complex with TFIID at the TATA promoter element, and it inhibits the function of several negative regulators of the TATA-binding protein (TBP) subunit of TFIID. Biochemical experiments suggest that TFIIA is important in the response to transcriptional activators because activation domains can interact with TFIIA, increase recruitment of TFIID and TFIIA to the promoter, and promote isomerization of the TFIID-TFIIA-TATA complex. Here, we describe a double-shut-off approach to deplete yeast cells of Toa1, the large subunit of TFIIA, to <1% of the wild-type level. Interestingly, such TFIIA-depleted cells are essentially unaffected for activation by heat shock factor, Ace1, and Gal4-VP16. However, depletion of TFIIA causes a general two- to threefold decrease of transcription from most yeast promoters and a specific cell-cycle arrest at the G2-M boundary. These results indicate that transcriptional activation *in vivo* can occur in the absence of TFIIA.

THE general transcription factor IIA (TFIIA) interacts with the TATA-binding protein (TBP) and with promoter DNA to form a TBP-TFIIA-DNA complex (reviewed in Orphanides *et al.* 1996; Roeder 1996). TFIIA stabilizes the association of TBP with the TATA element (Imbalzano *et al.* 1994), and it expands the TBP-DNA footprint both upstream and downstream of the TATA element (Lagrange *et al.* 1996). The two subunits of yeast TFIIA, Toa1 and Toa2, have distinct roles in the TBP-TFIIA-TATA complex (Geiger *et al.* 1996; Tan *et al.* 1996). Toa2 interacts with the aminoterminal stirrup of TBP, creating an expanded β-sheet upstream of the TATA element, whereas Toa1 contacts the phosphate backbone of DNA within and upstream of the TATA element. Because the regions of TFIIA contacting TBP and DNA are minimal, large, solvent-exposed surfaces remain available for interactions with other transcription factors at the promoter. In the context of the TBP-containing complex TFIID, TFIIA affects the interaction of several TAF subunits with promoter DNA (Oelgeschlager *et al.* 1996) and synergistic binding of TFIID to the TATA and initiator elements (Emami *et al.* 1997). Essentially, TFIIA stabilizes the binding of TFIID to the TATA box, and it expands the surface area at the core promoter for interactions with other proteins.

In most reactions *in vitro*, TFIIA increases the level of both basal and activated transcription (Orphanides *et al.* 1996; Roeder 1996). However, TFIIA is not absolutely required for accurate transcriptional initiation *in vitro*, and it is dispensable under certain experimental conditions. In some reactions reconstituted with the TFIID complex, TFIIA preferentially enhances the response to transcriptional activators (Ozer *et al.* 1994; Sun *et al.* 1994; Yokomori *et al.* 1994). The variable effect of TFIIA has been attributed in part to its ability to attenuate the repressive effects of certain factors (*e.g.*, Mot1, NC2, and HMG1) that destabilize the association of TBP with promoter DNA and with other components of the preinitiation complex (Auble *et al.* 1994; Ge and Roeder 1994; Goppelt *et al.* 1996; Mermelstein *et al.* 1996; Chicca *et al.* 1998). In the presence of these inhibitors of TBP function, TFIIA may stimulate transcription by functioning as an antirepressor. However, the biochemical properties of a TFIIA derivative indicate that antirepression is insufficient and, hence, unlikely to be the sole mechanism for transcriptional activation (Ma *et al.* 1996).

A more direct role for TFIIA in transcriptional activation is suggested by direct biochemical interactions between TFIIA and a set of diverse activation domains (Ozer *et al.* 1994; Kobayashi *et al.* 1995; Clemens *et al.* 1996; Damania *et al.* 1998). These activators stabilize a complex containing TFIID and TFIIA bound at the TATA element, suggesting that TFIIA can function as a cofactor in the recruitment of TFIID to the promoter (Lieberman and Berk 1994; Kobayashi *et al.* 1995; Damania *et al.* 1998). Activators may also accelerate a rate-limiting step in transcriptional activation by facilitating the isomerization of a preassembled TFIIA-TFIID-DNA complex to an open promoter state (Chi and Carey 1996). In the case of the ZEBRA activator, high-level-activated transcription involves both the recruitment of limiting components of the transcriptional machinery as well as this concentration-independent,

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activator-mediated isomerization of the TFIIA-TFIID-DNA complex. Moreover, this isomerization event appears to be limiting for activated transcription, as formation of an open TFIIA-TFIID-DNA complex permits activated transcription in the subsequent absence of the activator protein. Similarly, the nonhistone chromosomal protein HMG-2 potentiates activation at the level of the TFIID-TFIIA-TATA complex without increasing complex formation or altering the footprint on the promoter (Shykind *et al.* 1995). As with all biochemical studies, however, the physiological relevance of these observations is unclear.

Both subunits of yeast TFIIA are essential for cell growth, but the physiological role of TFIIA in transcription is unclear. Several studies have addressed this question using mutant derivatives of TBP or TFIIA that alter the TBP-TFIIA interface and severely inhibit TBP-TFIIA-TATA complex formation *in vitro*. First, a yeast TBP mutant defective for interacting with TFIIA specifically impairs the response to acidic activators, but does not generally affect Pol II transcription (Stargell and Struhl 1995). The phenotypes conferred by this yeast TBP derivative are largely suppressed by fusion to Toa2, indicating the importance of the TBP-TFIIA interaction; however, this TBP mutant does not address potential functions of TFIIA that are distinct from the TBP interaction. Second, human TBP mutants severely defective for interacting with TFIIA are transcriptionally incompetent in transiently transfected mammalian cells (Bryant *et al.* 1996). However, this inability to support transcription might not be due simply to a defective TBP-TFIIA interaction because the mutations radically alter exposed surfaces of TBP and, hence, may prevent interactions with other proteins. Third, yeast Toa2 mutants that weaken the interaction with TBP have selective transcriptional effects, and they partially inhibit cell-cycle progression at cytokinesis (Ozer *et al.* 1998a). However, these Toa2 mutants support cell viability, indicating that they retain a significant degree of TFIIA function.

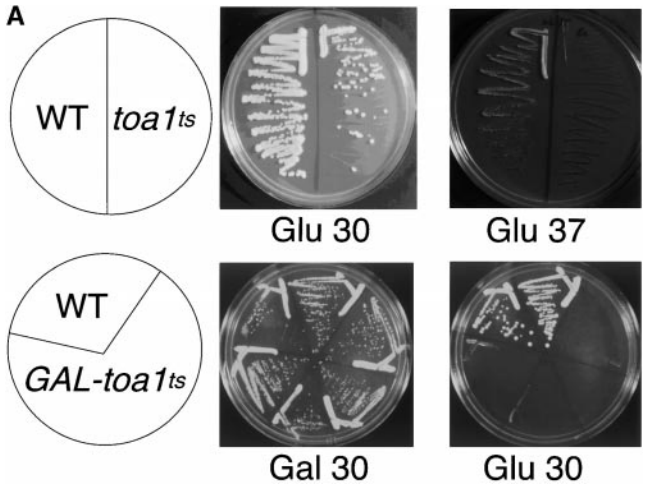
Because both subunits of TFIIA are essential for yeast cell viability, the most direct approach to analyze TFIIA function is to conditionally inactivate or deplete TFIIA in yeast cells. In this regard, it has been previously shown that a 10-fold reduction of TFIIA slows cell growth and produces only a modest 2-fold effect on transcription from a few promoters (Kang *et al.* 1995). The analyses in this report were limited, however, because of the partial nature of the conditional depletion, the limited set of genes analyzed, and the lack of experiments with activator-induced transcription after reduction of TFIIA levels. Here, we report a transcriptional analysis of cells that contain $<1\%$ of the wild-type level of TFIIA. Surprisingly, transcription of a variety of genes is generally reduced only 2- to 3-fold, and the response to a variety of transcriptional activators is largely unaffected. Nevertheless, TFIIA-depleted cells arrest specifically at the G2-M transition. Thus, TFIIA does not appear to be generally required for transcriptional activation, although it contributes to overall transcription levels and plays an important role at a subset of promoters.

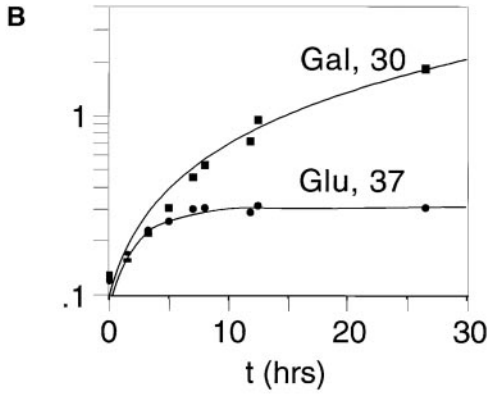
MATERIALS AND METHODS

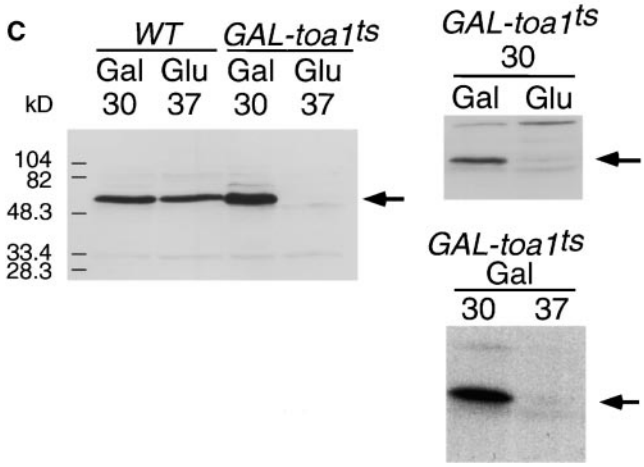
Generation of *toa1* temperature-sensitive allele: The template for PCR mutagenesis of the *TOA1* coding sequence pML3050 is a *TRP1* centromeric plasmid containing a *TOA1* allele with an engineered *Bam*HI site upstream of the start codon. PCR mutagenesis was performed under the following conditions: 10 ng template DNA, 50 pmol/primer, 10 mm Tris-HCl, pH 8.3, 50 mm KCl, 0.001% gelatin, 3 mm $MgCl_2$, 0.21 mm $MnCl_2$, 0.2 mm dATP, 0.2 mm dGTP, 1 mm dCTP, 1 mm dTTP and 5 units Taq polymerase (Boehringer Mannheim, Indianapolis) for 30 cycles of 94°, 30 sec; 40°, 1 min; 72°, 2 min. Mutagenic frequency in the resulting PCR product was 0.27% per nucleotide. The mutagenized PCR product was cotransformed with a *Bam*HI-*Nhe*I-cleaved *TRP1* centromeric plasmid bearing the *TOA1* locus cleaved (overlap of the PCR product with the gapped plasmid at the 5′ and 3′ ends was in excess of 20 bp on each side) into BY40, a yeast strain constructed by Brendan Cormack. BY40 is a derivative of KY320 (Chen and Struhl 1988) in which *TOA1* is present on a *URA3* multicopy plasmid and the chromosomal locus is disrupted by *ADE2*. Trp^+ transformants were tested for viable *TOA1* alleles by plasmid shuffling; cells growing on medium containing 5-fluoroorotic acid at 30° were then screened for conditional lethality at 37°. Four of the eight candidates identified reproduced the temperature-sensitive (ts) phenotype upon retransformation of the recovered plasmids, and the derivative with the strongest phenotype, designated *toa1*-ts, was chosen for further study.

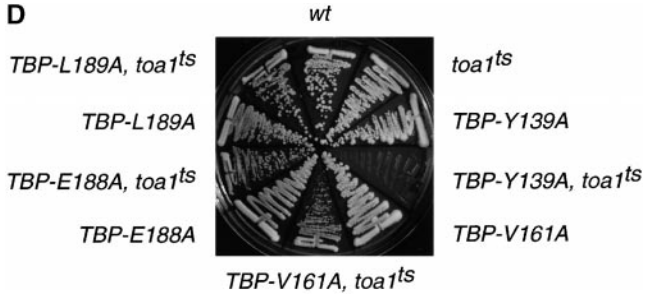
TFIIA depletion *in vivo*: The *toa1*-ts allele was transferred into a *TRP1* centromeric plasmid in which the FLAG epitope was engineered at the N terminus of Toa1, and the resulting FLAG-Toa1 DNA was then placed under the control of the *GAL1* promoter. Cells containing this *GAL1-toa1*-ts allele as the sole source of TFIIA were grown at 30° in YP or synthetic complete (SC) medium containing 2% galactose + 0.6% glucose (permissive conditions) to $A_{600} = 0.15$. Toa1 depletion was performed by shifting cells to conditions of growth in 2% glucose or 37° or both for ≥8 hr. To determine Toa1 levels, equal amounts of whole-cell protein extracts, as quantitated by Bradford assay, were separated by SDS-PAGE and transferred to an Immobilon P polyvinyldifluoride membrane (Millipore, Bedford, MA). FLAG-Toa1 was detected with an antibody (M5) against the FLAG epitope (IBI Biochemicals, New Haven, CT), using the Phototope-Star detection system (New England Biolabs, Beverly, MA) according to the manufacturers' instructions. In medium containing 2% galactose, Toa1 levels are much higher than are physiological (data not shown). Even under our permissive conditions of 2% galactose, 0.6% glucose, Toa1-ts levels are significantly higher than Toa1 levels in wild-type yeast strains. To analyze the terminal phenotype of TFIIA-depleted cells, wild-type and *GAL1-toa1*-ts cells were grown in double-shut-off conditions for 8 hr to $OD_{600} \sim 0.8$, and cells (10^7) were harvested, fixed, stained with 4′,6-diamidino-2-phenylindole (DAPI), and visualized by fluorescence microscopy as described previously (Trueheart *et al.* 1987).

To examine whether TBP derivatives containing mutations on surfaces responsible for interacting with DNA, TFIIA, or TFIIB are synthetically lethal under conditions of limiting TFIIA, we took advantage of the fact that levels of Toa1 expres-









sion in strains containing the *GAL1-toa1*-ts allele can be titrated downward by increasing the amount of glucose in galactose-containing medium. Specifically, TBP alleles on *LEU2* centromeric plasmids were introduced into yeast strain CC2, a derivative of BY40 that lacks the chromosomal copy of TBP but contains a *TRP1* centromeric plasmid expressing TBP. Trp^- segregants containing the mutated TBP derivatives as sole source of TBP were then transformed with a *TRP1* centromeric plasmid expressing Toa1-ts from the *GAL1* promoter, and the wild-type *TOA1* gene on a *URA3* centromeric plasmid was removed by plasmid shuffling.

Transcriptional analysis: For the initial experiments, cells were harvested after 8 hr in YP medium under double-shut-off conditions as described above. For analysis of Ppr1-dependent activation, cells were harvested following 8 hr under double-shut-off conditions in synthetic minimal medium containing 0.6% casamino acids with or without uracil. For analysis of heat shock factor (Hsf)-dependent activation, cells were grown in glucose-containing media at 30° for 12 hr and harvested following a 15-min heat shock at 37°. For analysis of Ace1-dependent activation, strains were grown in SC medium under double-shut-off conditions and induced with 100 or 400 μm $CuSO_4$ for 1 hr before harvesting. Gal4-VP16-dependent activation was assayed by growing strains expressing the activator from the *ADH1* promoter on a centromeric *URA3* plasmid, in synthetic minimal medium containing 0.6% casamino acids and lacking uracil, under double-shut-off conditions for 8 hr. Analysis of Ppr1- and Ace1-dependent activation of *his3* transcription was performed in derivatives of BY40 in which the *his3* locus is replaced by derivatives containing binding sites for the activators Ppr1 and Ace1 upstream of the *his3* TATA region (Iyer and Struhl 1995).

For most experiments, total RNA (40 μg, as quantitated by A_{260}) was hybridized to completion with a 10- to 100-fold excess of the appropriate ^{32}P-labeled oligonucleotides and was treated with S1 nuclease as described previously (Iyer and Struhl 1996). Transcript levels were quantitated with respect to the $tRNA^W$ internal control by PhosphorImage analysis. The *TRP3*, *rRNA*, *RPS4*, *SSA4*, *HSP104*, *CUP1*, *DED1*, *GAL1*, *HIS3*, and *HIS4* oligonucleotides have been described previously (Chen and Struhl 1988; Stargell and Struhl 1995; Iyer and Struhl 1996). For Northern blot analysis, 25 μg of total RNA was hybridized with the appropriate ^{32}P-labeled, randomly primed oligonucleotide probes.

RESULTS

Depletion of TFIIA *in vivo* using a double-shut-off strategy: Since both the Toa1 and Toa2 subunits of yeast TFIIA are essential for viability, analysis of TFIIA

Figure 1.—Characterization of a conditional allele of TOA1. (A) Growth of wild-type, *toa1*-ts, or *GAL1-toa1*-ts strains on medium containing glucose (glu) or galactose (gal) at 30° or 37°. (B) The *GAL1-toa1*-ts strain was grown in YP medium containing 2% galactose + 0.6% glucose medium at 30° and then either shifted (●) or unshifted (■) to medium containing 2% glucose at 37°; the zero time point corresponds to the time of the shift. (C) Western analysis of FLAG-Toa1 protein levels in wild-type or *GAL1-toa1*-ts strains grown in the indicated medium at the indicated temperatures. The panel on the left represents double-shut-off conditions, whereas the panels on the right indicate single-shut-off conditions. (D) Synthetic lethality. Strains containing the indicated derivatives of TBP and Toa1 were plated on glucose medium at 30°.

function *in vivo* requires a method for conditional depletion. Toward this end, we isolated a temperature-sensitive allele of *TOA1* (see materials and methods) that conferred normal growth at 30° while preventing colony formation at 37° (Figure 1A). Unfortunately, long incubation times (>12 hr) in liquid medium at 37° are required to fully deplete or inactivate Toa1, as assayed by both culture density and Western blot analysis of Toa1 levels (data not shown). Therefore, to produce a more efficient shut-off, the *toa1*-ts allele was placed under the control of the *GAL1* promoter. As anticipated, cell growth supported by this *GAL1-toa1*-ts allele is regulated by glucose on solid medium (Figure 1A) as well as in liquid culture (data not shown). Importantly, cell growth is most efficiently inhibited by shifting the culture to glucose medium at 37° (Figure 1B). The combined effect of glucose repression and protein thermolability results in depletion of Toa1 to <1% of the wild-type level (the limit of detection) within 8 hr. In contrast, either of the single-shut-off protocols (*i.e.*, glucose or temperature shift) results in detectable levels of Toa1 8 hr after glucose or temperature shift (Figure 1C), although depletion beyond the detection limit is achieved at later times.

We used the *toa1*-ts allele, a condition of low TFIIA activity, to examine whether TBP mutants with specific defects in preinitiation complex formation would display synthetic lethality. The TBP-Y139A mutation that causes a 100-fold reduced interaction with TFIIA (Stargell and Struhl 1995; Lee and Struhl 1997) is synthetically lethal with *toa1*-ts at 30°. In contrast, TBP mutations that weaken interaction with TFIIB (E188A and E189A; Lee and Struhl 1997) or DNA (V161A; Lee and Struhl 1995) are viable under these conditions (Figure 1D). Overexpression of TBP, TFIIB, Srb4 + Srb6 (coexpressed), TAF17, TAF90, TAF130, Ada1, Ada2, Ada3, or Gcn5 does not suppress the slow-growth phenotype caused by conditions of limiting TFIIA (data not shown). In fact, overexpression of TAF130 appears to exacerbate the slow growth caused by partial Toa1 depletion (data not shown), which is in accordance with a genetic interaction between these proteins (Kokubo *et al.* 1998; Ozer *et al.* 1998b).

Depletion of TFIIA causes a modest decrease of transcription from most Pol II promoters: Transcription of a variety of genes was examined in *GAL1-toa1* ts cells that were shifted to glucose medium at 37° for 8 hr to eliminate TFIIA (Figure 2). As expected, Pol III transcription, as exemplified by the gene encoding $tRNA^W$, is unaffected by TFIIA depletion. In contrast, TFIIA depletion causes a consistent two- to fourfold decrease in Pol II transcription from 9 of the 10 different promoters analyzed. In the exceptional case of the *ADH1* promoter, loss of TFIIA had no significant effect on transcription. The quantitatively modest but broad effect on Pol II transcription is in marked contrast to depletions of TBP (Cormack and Struhl 1992; Moqtaderi *et al.*

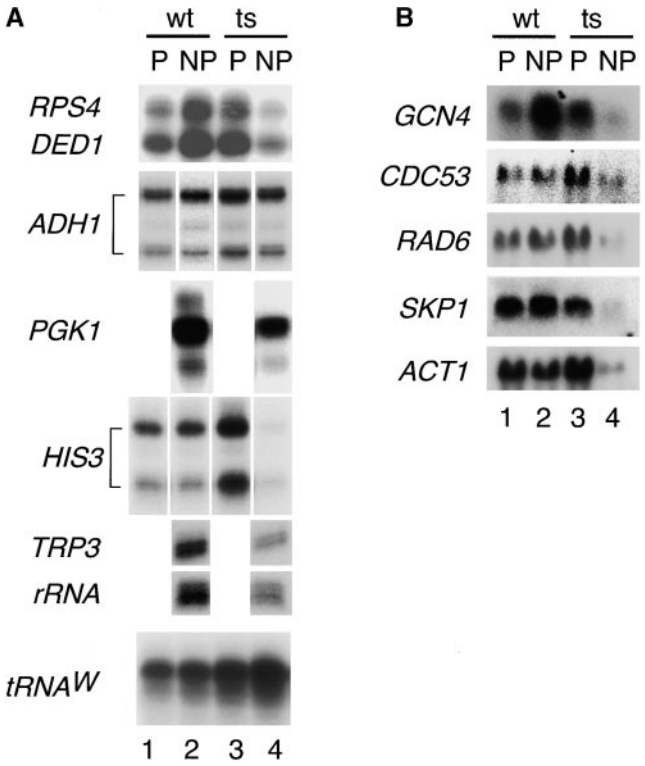


Figure 2.—TFIIA depletion broadly decreases transcription from Pol II promoters. Transcription from the indicated promoters was analyzed in wild-type (wt) and *GAL1-toa1*-ts (ts) strains grown for 8 hr in permissive (P) or double-shut-off (NP) conditions as follows: (A) S1 analysis and (B) Northern blotting.

1996), TFIIB (Moqtaderi *et al.* 1996), the Kin28 subunit of TFIIH (Cismowski *et al.* 1995; Valay *et al.* 1995), and the Srb4 subunit of Pol II holoenzyme (Thompson and Young 1995), all of which completely eliminate Pol II transcription. The TFIIA-depleted cells also show a small reduction in the levels of an rRNA precursor species, which is indicative of a slight defect in Pol I transcription. As general decreases in Pol II transcription indirectly reduce Pol I transcription (Nonet *et al.* 1987; Cormack and Struhl 1992), it seems likely that TFIIA is not directly involved in Pol I transcription, but rather influences rRNA synthesis indirectly through its effects on Pol II promoters. These observations indicate that TFIIA has a general but quantitatively modest effect on Pol II transcription in yeast cells.

Transcriptional activation by Ace1, Hsf, and Gal4-VP16 in the absence of TFIIA: If TFIIA is required for transcriptional activation, cells lacking TFIIA should be unable to induce high levels of transcription in response to activator proteins. In considering this question, we were concerned about the possibility that TFIIA at highly active promoters might be sufficiently stabilized so as to be relatively immune to the depletion methods. To circumvent this potential problem, most of the experiments were performed by first depleting cells of

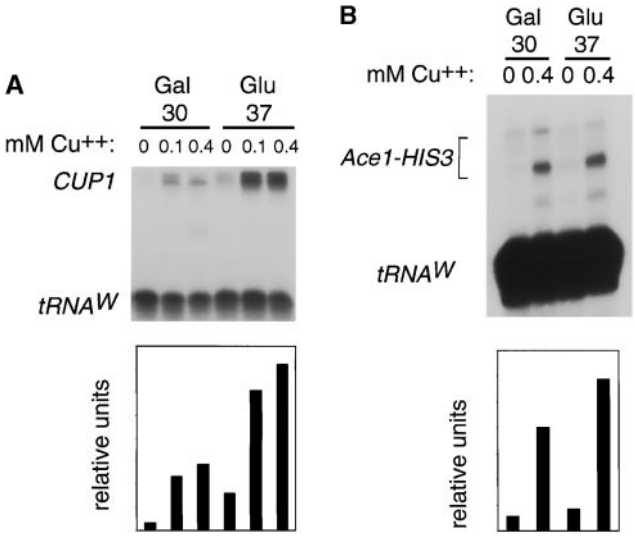


Figure 3.—Ace1-dependent activation is unimpaired under conditions of TFIIA depletion. *GAL1-toa1*-ts cells were grown under permissive (galactose medium at 30°) or nonpermissive (glucose medium at 37°) conditions for 8 hr, whereupon the cells were subjected to copper induction (concentrations indicated) for 1 hr. Transcription was analyzed from (A) the native *CUP1* promoter and from (B) an artificial promoter containing Ace1-binding sites upstream of the *his3* core promoter and structural gene. Quantitation relative to $tRNA^W$ was performed by PhosphorImage analysis.

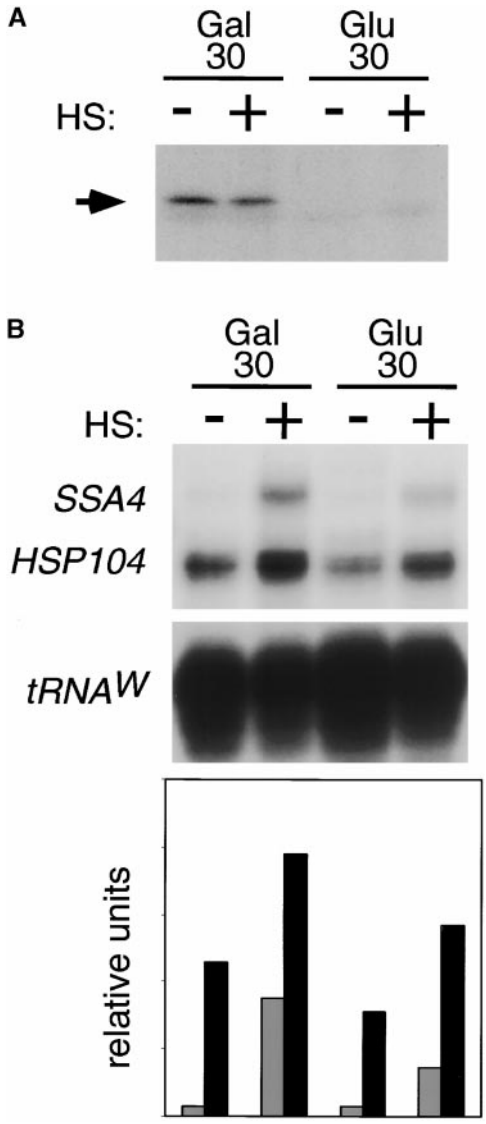


Figure 4.—TFIIA depletion does not affect Hsf-dependent activation. *GAL1-toa1*-ts cells grown in galactose or glucose medium (single-shut-off conditions) for 12 hr at 30° were subjected (+) or not subjected (−) to a 15-min heat shock at 39°, and were then analyzed for (A) FLAG-Toa1 levels by Western blotting or (B) *SSA4* and *HSP104* transcription by S1 analysis. Quantitation relative to $tRNA^W$ was performed by PhosphorImage analysis.

Toa1 and then inducing the function of a particular activator. In the case of the Ace1 activator, TFIIA-depleted cells were treated with copper, which is required for folding of the DNA-binding domain and subsequent activation. It should be noted that the uninduced level of *CUP1* transcription in Toa1-depleted cells is elevated relative to that in control cells, presumably reflecting the contribution of stress response elements in the *CUP1* promoter (Tamai *et al.* 1994). When normalized to uninduced levels of transcription, Ace1-dependent activation of *CUP1* expression is unaffected in the absence of TFIIA (Figure 3A). This phenomenon is not restricted to the *CUP1* promoter context, as TFIIA depletion does not alter the level of activated transcription of an Ace1-dependent *his3* allele (Figure 3B).

To extend this analysis to transcriptional activation by heat shock factor, we could not employ the double-shut-off method because the temperature shift induces the function of the Hsf activation domain. Thus, cells were depleted of TFIIA by glucose repression alone for 12 hr, a time at which Toa1 protein was reduced to undetectable levels (Figure 4A). When such TFIIA-depleted cells were subjected to a brief heat shock, Hsf-dependent activation of *SSA4* and *HSP104* was indistinguishable from that observed in cells grown in permissive conditions (Figure 4B). Thus, *de novo* activation by Hsf appears unaffected in cells previously depleted of TFIIA.

As a direct comparison with biochemical studies showing that the VP16 activation domain enhances TFIID-TFIIA-TATA complex formation (Kobayashi *et al.* 1995, 1998), we examined activation of the *GAL1* gene by Gal4-VP16 (Figure 5). Under double-shut-off conditions, activation of *GAL1* by Gal4-VP16 is unimpaired. Incidentally, *GAL1* transcription is not observed in TFIIA-depleted cells lacking Gal4-VP16, indicating that TFIIA is not required to maintain glucose repression of *GAL1*.

Taken together, these results indicate that TFIIA is not required or limiting for the function of the Ace1, Hsf, and Gal4-VP16 activators *in vivo*. Furthermore, in contrast to the vast majority of natural promoters whose

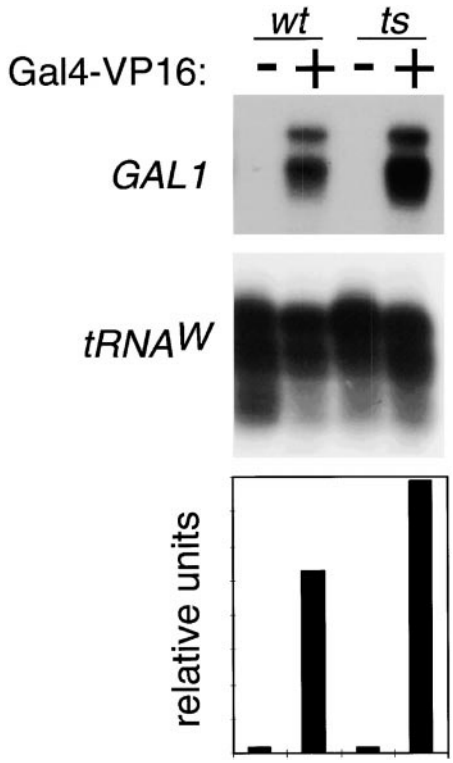


Figure 5.—Activation by Gal4-VP16 is unaffected in TFIIA-depleted cells. Wild-type (wt) and *GAL1-toa1*-ts (ts) cells that do (+) or do not (−) contain a plasmid expressing Gal4-VP16 were grown under double-shut-off conditions and analyzed for transcription of the native *GAL1* promoter by S1 analysis. Quantitation relative to $tRNA^W$ was performed by PhosphorImage analysis.

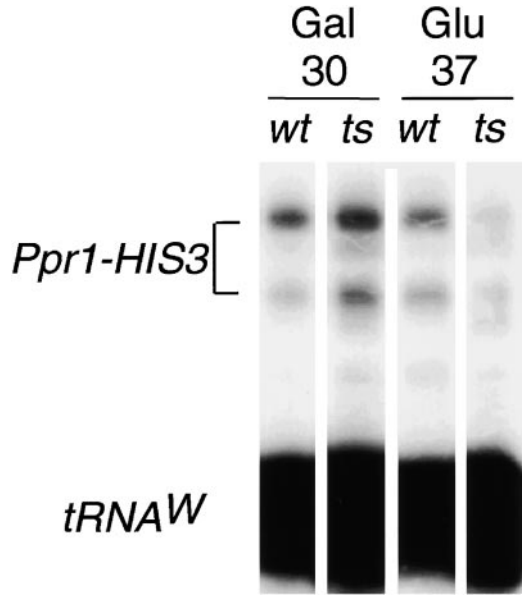


Figure 6.—Ppr1-dependent transcription of an artificial *his3* promoter is reduced under conditions of TFIIA depletion. Wild-type (wt) and *GAL1-toa1*-ts (ts) cells with a modified *his3* promoter containing a single Ppr1-binding site upstream of the *his3* TATA and initiator region were grown in synthetic minimal medium containing 0.6% casamino acids and lacking uracil under permissive and double-shut-off conditions. Under these conditions, *his3* transcription (assayed by S1 analysis) depends almost entirely on the activity of Ppr1.

function was reduced two- to fourfold upon loss of TFIIA (Figure 2), promoters responsive to these strong activators were essentially unaffected by TFIIA depletion. This observation suggests that strong activators such as Ace1, Hsf, and Gal4-VP16 can override the general limitation on transcription imposed under conditions lacking TFIIA.

Although promoters dependent on strong activators such as Ace1, HSF, and Gal4-VP16 appear unaffected by the loss of TFIIA, it is possible that promoters responsive to weaker activators might resemble typical yeast promoters and, hence, be sensitive to changes in TFIIA levels. We therefore analyzed a *his3* promoter derivative whose function completely depends on a binding site for the weak activator Ppr1 (Iyer and Struhl 1995). In TFIIA-depleted cells, Ppr1-dependent activation of *his3* transcription was reduced by a factor of three in comparison to control cells (Figure 6). As Ace1-dependent activation of the identical *his3* core promoter is unaffected (Figure 3B), this result suggests that the importance of TFIIA can depend on the activator. However, these results do not distinguish whether TFIIA depletion affects the function of the Ppr1 activator *per se*, or whether it affects an aspect of core promoter function that can be overridden by Ace1, but not Ppr1.

TFIIA-depleted cells arrest at the G2-M cell-cycle boundary: The morphology of TFIIA-depleted cells was examined by fluorescence microscopy with DAPI staining. A significant percentage (40–50%) of the TFIIA-depleted cells appear as large-budded cells with DNA concentrated at the septum, indicating arrest at or near the G2-M cell-cycle boundary (Figure 7). Under similar conditions, <10% of wild-type cells appear with large buds. This observation is consistent with and extends a previous observation that viable Toa2 mutants deficient in TBP interaction show an increased number of cells with a large-budded phenotype (Ozer *et al.* 1998a). In apparent contrast to this previous report, we did not observe cell clumping in our TFIIA-depleted cells; we do not know if this apparent conflict reflects differences in the TFIIA derivatives or in the yeast strain background. However, the specificity of the cell-cycle phenotype and the selective transcriptional effects conferred by the viable Toa2 mutants (Ozer *et al.* 1998a) indicate that TFIIA is particularly important for a subset of promoters.

DISCUSSION

Transcriptional activation can occur in the absence of TFIIA: *In vitro*, TFIIA can interact directly with activation domains (Ozer *et al.* 1994; Kobayashi *et al.* 1995; Clemens *et al.* 1996), and it is required for activator-

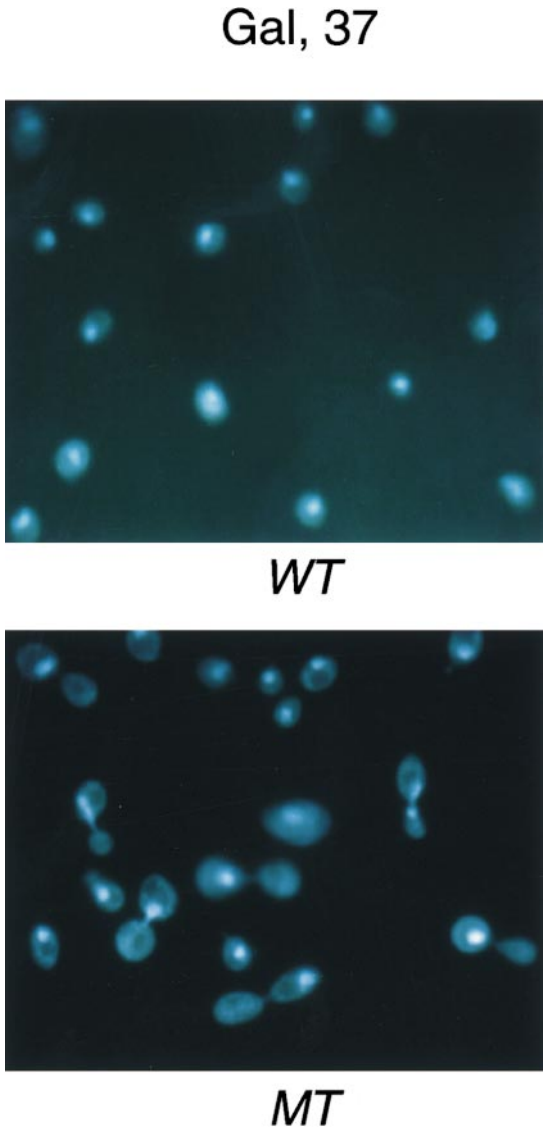


Figure 7.—TFIIA-depleted cells arrest at the G2-M cell-cycle transition. Fluorescence microscopy was performed on wild-type and *GAL1-toa1*-ts cells grown under double-shut-off conditions for 8 hr, which were then fixed and stained with DAPI.

dependent recruitment and isomerization of the TFIID-TATA complex (Lieberman and Berk 1994; Kobayashi *et al.* 1995; Chi and Carey 1996; Damania *et al.* 1998). Furthermore, the ability of the VP16 activation domain to interact with TFIIA and to stimulate assembly of the TFIID-TFIIA-TATA complex is strongly correlated with the level of transcriptional activation *in vitro* (Kobayashi *et al.* 1998). Here, we show that cells containing <1% wild-type levels of TFIIA are essentially unaffected for transcriptional activation by Ace1, Hsf, and Gal4-VP16. Moreover, these activators appear to override the two- to fourfold decrease in transcription in TFIIA-depleted cells that is typical of the majority of natural yeast promoters (see below). Thus, in apparent contrast to many biochemical results, TFIIA is not required for transcriptional activation by several activator proteins *in vivo*.

It is generally believed that activators stimulate transcription by directly interacting with components of the Pol II machinery and/or by recruiting chromatin-modifying activities to the promoter. Thus, activators that do not require TFIIA for transcriptional stimulation must be able to function via other targets. Some activators simply might not interact with TFIIA or affect the TFIID-TFIIA-TATA complex; hence, they would function exclusively through other targets. For activators that do interact with TFIIA and affect the TFIID-TFIIA-TATA complex (*e.g.*, VP16), these *in vitro* interactions might not occur under physiological conditions or they might be too weak to significantly contribute to transcriptional output *in vivo*. Alternatively, activator-TFIIA interactions might be physiologically significant but functionally redundant with activator interactions with other components of the Pol II machinery and/or chromatin-modifying complexes. Strong activators, such as Ace1, Hsf, and Gal4-VP16, might interact functionally with multiple targets *in vivo* such that the loss of any one target (*e.g.*, TFIIA) would not have significant transcriptional consequences. Of course, these considerations do not exclude the possibility that some activators require TFIIA for activation *in vivo*.

TFIIA is broadly but not absolutely required for transcription in yeast: TFIIA-depleted cells show a two- to fourfold decrease in the expression of 9 of 10 natural yeast genes examined, suggesting that TFIIA has an important function at most yeast promoters. However, TFIIA is not absolutely required for transcription *in vivo* because some promoters (*e.g.*, *ADH1* and those driven by strong activators) appear unaffected by the loss of TFIIA. Moreover, TFIIA does not behave as a general transcription factor because, unlike TBP (Cormack and Struhl 1992) or TFIIB (Moqtaderi *et al.* 1996), depletion does not abolish gene expression. In this regard, our results appear inconsistent with transcriptionally inactive mutants of human TBP that alter the TFIIA-interaction surface and eliminate the interaction with TFIIA (Bryant *et al.* 1996). We suspect that the human TBP mutants also affect the interaction with proteins other than TFIIA, particularly because of the radical nature of the amino acid substitutions.

As yeast promoters differ considerably in their upstream sequences, we suggest that the broad role of TFIIA in transcription *in vivo* most likely involves the core promoter region. The biochemical properties of TFIIA suggest two models relating to TBP and TATA elements. In one model, TFIIA directly stabilizes the association of TBP at promoters by virtue of its ability to form TBP-TFIIA-TATA complexes. In the alternative model, TFIIA indirectly affects TBP association with promoters by blocking general negative regulators of TBP function, such as Mot1 (Auble *et al.* 1994), NC2 (Gadbois *et al.* 1997; Kim *et al.* 1997), the N-terminal domain

of TAF130 (Kokubo *et al.* 1998; Ozer *et al.* 1998b), and perhaps the Not complex (Collart and Struhl 1994). We slightly prefer the latter model because negative regulators of TBP might have an effect similar to simply reducing the level of active TBP; this could account for why most promoters are affected in a quantitatively similar manner. On the other hand, the variability in the quality of TATA elements suggests that stabilization of the TBP-TATA complex by TFIIA might vary considerably depending on the promoter. These two models are not mutually exclusive and, in fact, may be functionally interrelated.

Although we favor the view that the broad role of TFIIA reflects a core promoter function, we cannot exclude the possibility that the general decrease in Pol II transcription is related to the response to activator proteins. *In vivo*, transcription requires activators because intact promoters are generally much more active than core promoter derivatives containing only the TATA and initiator elements. Thus, if TFIIA is a near-universal but not absolutely required target of activators, depletion of TFIIA could cause a wide reduction in Pol II transcription. In this regard, Srb4, a Pol II holoenzyme component, may be a direct activator target (Koh *et al.* 1998) even though it is generally required for transcription (Thompson and Young 1995).

Promoter specificity and functional similarities between TFIIA and TAFs: Although TFIIA broadly affects transcription, several lines of evidence suggest that TFIIA does not play an equivalent role in all genes. First, it seems unlikely that a nonspecific and quantitatively modest decrease in Pol II transcription, such as would occur in cells containing a partially defective Pol II subunit, would prevent cell growth. By this argument, the general two- to fourfold decrease in Pol II transcription in TFIIA-depleted cells would not explain why TFIIA is essential for cell viability. Second, TFIIA depletion does not affect transcription of *ADH1* or certain genes responsive to strong activators. Third, Toa1 and Toa2 mutations that partially inhibit TFIIA function selectively affect gene expression (Madison and Winston 1997; Ozer *et al.* 1998a). Fourth, depletion of TFIIA causes cells to arrest at a specific point in the cell cycle, the G2-M transition, suggesting that TFIIA is particularly important for transcription of some cell-cycle genes.

In several respects, the physiological role of TFIIA is reminiscent of that of the TAF subunits in the TFIID complex. As is the case for TFIIA, depletion or functional inactivation of such TAFs does not generally affect the response to transcriptional activators (Moqtaderi *et al.* 1996; Walker *et al.* 1996), but rather reduces transcription of selected genes (Moqtaderi *et al.* 1996; Shen and Green 1997; Walker *et al.* 1997). Moreover, TAF130 exerts its effects primarily at the level of the core promoter (Moqtaderi *et al.* 1996; Shen and Green 1997), and this also appears to be the case for other TAFs that are found in TFIID, but not in the SAGA complex (Moqtaderi *et al.* 1998). Finally, individual TAFs are important for progression through specific stages of the cell cycle (Apone *et al.* 1996; Walker *et al.* 1997), and loss of TFIIA or TAF90 blocks cell growth at the G2-M transition. These broad similarities are not surprising given that TFIIA and TAFs interact with TBP and promoter DNA; in this sense, TFIIA is a TAF-like component of the Pol II machinery that associates with but is not stably part of the TFIID complex. However, the transcriptional phenotypes of TFIIA- and TAF-depleted cells are clearly different, indicating that TFIIA and TAFs perform distinct physiological functions.

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LITERATURE CITED

Apone, L. M., C. A. Virbasius, J. C. Reese and M. R. Green, 1996 Yeast $TAF_{II}90$ is required for cell-cycle progression through G_2/M but not for general transcription activation. Genes Dev. **10:** 2368–2380.

Auble, D. T., K. E. Hansen, C. G. F. Mueller, W. S. Lane, J. Thorner *et al.*, 1994 Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. Genes Dev. **8:** 1920–1934.

Bryant, G. O., L. S. Martel, S. K. Burley and A. J. Berk, 1996 Radical mutations reveal TATA-box binding protein surfaces required for activated transcription *in vivo.* Genes Dev. **10:** 2491–2504.

Chen, W., and K. Struhl, 1988 Saturation mutagenesis of a yeast *his3* TATA element: genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. USA **85:** 2691–2695.

Chi, T., and M. Carey, 1996 Assembly of the isomerized TFIIA-TFIID-TATA ternary complex is necessary and sufficient for gene activation. Genes Dev. **10:** 2540–2550.

Chicca, J. J. N., D. T. Auble and B. F. Pugh, 1998 Cloning and biochemical characterization of TAF-172, a human homolog of yeast Mot1. Mol. Cell. Biol. **18:** 1701–1710.

Cismowski, M., G. Laff, M. Soloman and S. Reed, 1995 KIN28 encodes a C-terminal domain kinase that controls mRNA transcription in *Saccharomyces cerevisiae* but lacks cyclin-dependent kinase-activating kinase (CAK) activity. Mol. Cell. Biol. **15:** 2983–2992.

Clemens, K. E., G. Piras, M. F. Radonovich, K. S. Choi, J. F. Duvall *et al.*, 1996 Interaction of the human T-cell lymphotropic virus type 1 tax transactivator with transcription factor IIA. Mol. Cell. Biol. **16:** 4656–4664.

Collart, M. A., and K. Struhl, 1994 *NOT1(CDC39), NOT2(CDC36), NOT3*, and *NOT4* encode a global negative regulator of transcription that differentially affects TATA-element utilization. Genes Dev. **8:** 525–537.

Cormack, B. P., and K. Struhl, 1992 The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell **69:** 685–696.

Damania, B., P. Lieberman and J. C. Alwine, 1998 Simian virus 40 large T antigen stabilizes the TATA-binding protein-TFIIA complex on the TATA element. Mol. Cell. Biol. **18:** 3926–3935.

Emami, K. H., A. Jain and S. T. Smale, 1997 Mechanism of synergy between TATA and initiator: synergistic binding of TFIID following a putative TFIIA-induced isomerization. Genes Dev. **11:** 3007–3019.

Gadbois, E. L., D. M. Chao, J. C. Reese, M. R. Green and R. A. Young, 1997 Functional antagonism between RNA polymerase II holoenzyme and global negative regulator NC2 *in vivo.* Proc. Natl. Acad. Sci. USA **94:** 3145–3150.

Ge, H., and R. G. Roeder, 1994 The high mobility group protein HMG1 can reversibly inhibit class II gene transcription by interac-

tion with TATA-binding protein. J. Biol. Chem. **269:** 17136–17140.

Geiger, J. H., S. Hahn, S. Lee and P. B. Sigler, 1996 Crystal structure of the yeast TFIIA/TBP/DNA complex. Science **272:** 830–836.

Goppelt, A., G. Stelzer, F. Lottspeich and M. Meisterenst, 1996 A mechanism for repression of class II gene transcription through specific binding of NC2 to TBP-promoter complexes via heterodimeric histone fold domains. EMBO J. **15:** 3105–3116.

Imbalzano, A. N., K. S. Zaret and R. E. Kingston, 1994 Transcription factor (TF) IIB and TFIIA can independently increase the affinity of the TATA-binding protein for DNA. J. Biol. Chem. **269:** 8280–8286.

Iyer, V., and K. Struhl, 1995 Mechanism of differential utilization of the *his3* T_R and T_C TATA elements. Mol. Cell. Biol. **15:** 7059–7066.

Iyer, V., and K. Struhl, 1996 Absolute mRNA levels and transcriptional initiation rates in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **93:** 5208–5212.

Kang, J. J., D. T. Auble, J. A. Ranish and S. Hahn, 1995 Analysis of yeast transcription factor TFIIA: distinct functional regions and a polymerase II-specific role in basal and activated transcription. Mol. Cell. Biol. **15:** 1234–1243.

Kim, S., J. G. Na, M. Hampsey and D. Reinberg, 1997 The Dr1/DRAP1 heterodimer is a global repressor of transcription in vivo. Proc. Natl. Acad. Sci. USA **94:** 820–825.

Kobayashi, N., T. G. Boyer and A. J. Berk, 1995 A class of activation domains interacts directly with TFIIA and stimulates TFIIA-TFIID-promoter complex assembly. Mol. Cell. Biol. **15:** 6465–6473.

Kobayashi, N., P. J. Horn, S. M. Sullivan, S. J. Triezenberg, T. G. Boyer *et al.*, 1998 DA-complex assembly activity required for VP16C transcriptional activation. Mol. Cell. Biol. **18:** 4023–4031.

Koh, S. S., A. Z. Ansari, M. Ptashne and R. A. Young, 1998 An activator target in the RNA polymerase II holoenzyme. Mol. Cell **1:** 895–904.

Kokubo, T., M. J. Swanson, J. I. Nishikawa, A. G. Hinnebusch and Y. Nakatani, 1998 The yeast TAF145 inhibitory domain and TFIIA competitively bind to TATA-binding protein. Mol. Cell. Biol. **18:** 1003–1012.

Lagrange, T., T.-K. Kim, G. Orphanides, Y. W. Ebright, R. H. Ebright *et al.*, 1996 High-resolution mapping of nucleoprotein complexes by site-specific protein-DNA photocrosslinking: organization of the TBP-TFIIA-TFIIB-DNA quaternary complex. Proc. Natl. Acad. Sci. USA **93:** 10620–10625.

Lee, M., and K. Struhl, 1995 Mutations on the DNA-binding surface of TBP can specifically impair the response to acidic activators *in vivo.* Mol. Cell. Biol. **15:** 5461–5469.

Lee, M., and K. Struhl, 1997 A severely defective TATA-binding protein-TFIIB interaction does not preclude transcriptional activation *in vivo.* Mol. Cell. Biol. **17:** 1336–1345.

Lieberman, P. M., and A. J. Berk, 1994 A mechanism for TAFs in transcriptional activation: activation domain enhancement of TFIID-TFIIA-promoter DNA complex formation. Genes Dev. **8:** 995–1006.

Ma, D., I. Olave, A. Merino and D. Reinberg, 1996 Separation of the transcriptional coactivator and antirepression functions of transcription factor IIA. Proc. Natl. Acad. Sci. USA **93:** 6583–6588.

Madison, J. M., and F. Winston, 1997 Evidence that Spt3 functionally interacts with Mot1, TFIIA, and TATA-binding protein to confer promoter-specific transcriptional control in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **17:** 287–295.

Mermelstein, F., K. Yeung, J. Cao, J. A. Inostroza, H. Erdjument-Bromage *et al.*, 1996 Requirement of a corepressor for Dr1-mediated repression of transcription. Genes Dev. **10:** 1033–1048.

Moqtaderi, Z., Y. Bai, D. Poon, P. A. Weil and K. Struhl, 1996 TBP-associated factors are not generally required for transcriptional activation in yeast. Nature **382:** 188–191.

Moqtaderi, Z., M. Keaveney and K. Struhl, 1998 The histone H3-like TAF is broadly required for transcription in yeast. Mol. Cell **2:** 675–682.

Nonet, M., C. Scafe, J. Sexton and R. Young, 1987 Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Mol. Cell. Biol. **7:** 1602–1611.

Oelgeschlager, T., C. M. Chiang and R. G. Roeder, 1996 Topology and reorganization of a human TFIID-promoter complex. Nature **382:** 735–738.

Orphanides, G., T. Lagrange and D. Reinberg, 1996 The general initiation factors of RNA polymerase II. Genes Dev. **10:** 2657–2683.

Ozer, J., P. A. Moore, A. H. Bolden, A. Lee, C. A. Rosen *et al.*, 1994 Molecular cloning of the small (γ) subunit of human TFIIA reveals functions critical for activated transcription. Genes Dev. **8:** 2324–2335.

Ozer, J., L. E. Lezina, J. Ewing, S. Audi and P. M. Lieberman, 1998a Association of transcription factor IIA with TATA-binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **18:** 2559–2570.

Ozer, J., K. Mitsouras, D. Zerby, M. Carey and P. M. Lieberman, 1998b Transcription factor IIA derepresses TATA-binding protein (TBP)-associated factor inhibition of TBP-DNA binding. J. Biol. Chem. **273:** 14293–14300.

Roeder, R. G., 1996 The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem. Sci. **21:** 327–335.

Shen, W. C., and M. R. Green, 1997 Yeast $TAF_{II}145$ functions as a core promoter selectivity factor, not a general coactivator. Cell **90:** 615–624.

Shykind, B. M., J. Kim and P. A. Sharp, 1995 Activation of the TFIID-TFIIA complex with HMG-2. Genes Dev. **9:** 1354–1365.

Stargell, L. A., and K. Struhl, 1995 The TBP-TFIIA interaction in the response to acidic activators *in vivo.* Science **269:** 75–78.

Sun, X., D. Ma, M. Sheldon, K. Yeung and D. Reinberg, 1994 Reconstitution of human TFIIA activity from recombinant polypeptides: a role in TFIID-mediated transcription. Genes Dev. **8:** 2336–2348.

Tamai, K. T., X. Liu, P. Silar, T. Sosinowski and D. J. Thiele, 1994 Heat shock transcription factor activates yeast metallothionein gene expression in response to heat and glucose starvation via distinct signalling pathways. Mol. Cell. Biol. **14:** 8155–8165.

Tan, S., Y. Hunziker, D. F. Sargent and T. J. Richmond, 1996 Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature **381:** 127–134.

Thompson, C. M., and R. A. Young, 1995 General requirement for RNA polymerase II holoenzymes *in vivo.* Proc. Natl. Acad. Sci. USA **92:** 4587–4590.

Trueheart, J., J. D. Boeke and G. R. Fink, 1987 Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. Mol. Cell. Biol. **7:** 2316–2328.

Valay, J.-G., M. Simon, M.-F. Dubois, O. Bensaude, C. Facca *et al.*, 1995 The *KIN28* gene is required for RNA polymerase II-mediated transcription and phosphorylation of the Rpb1p CTD. J. Mol. Biol. **249:** 535–544.

Walker, S. S., J. C. Reese, L. M. Apone and M. R. Green, 1996 Transcription activation in cells lacking TAF_{II}s. Nature **382:** 185–188.

Walker, S. S., W. C. Shen, J. C. Reese, L. M. Apone and M. R. Green, 1997 Yeast $TAF_{II}145$ required for transcription of G1/S cyclin genes and regulated by cellular growth state. Cell **90:** 607–614.

Yokomori, K., M. P. Zeidler, J.-L. Chen, C. P. Verrijzer, M. Mlodzik *et al.*, 1994 *Drosophila* TFIIA directs cooperative DNA binding with TBP and mediates transcriptional activation. Genes Dev. **8:** 2313–2323.

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