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TFIIA Has Activator-dependent and Core Promoter Functions *in Vivo**

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Laurie A. Stargell‡, Zarmik Moqtaderi§, David R. Dorris§, Ryan C. Ogg‡, and Kevin Struhl§¶

From the ‡Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado 80523 and the §Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

The physiological role of TFIIA was investigated by analyzing transcription in a yeast strain that contains a TATA-binding protein (TBP) mutant (N2–1) defective for interacting with TFIIA. In cells containing N2–1, transcription from a set of artificial *his3* promoters dependent on different activators is generally reduced by a similar extent, indicating that TFIIA function is largely nonselective for activators. In addition, TATA element utilization, a core promoter function, is altered at *his3* promoters dependent on weak activators. Genomic expression analysis reveals that 3% of the genes are preferentially affected by a factor of 4 or more. Chimeras of affected promoters indicate that the sensitivity to the TFIIA-TBP interaction can map either to the upstream or core promoter region. Unlike wild-type TBP or TFIIA, the N2–1 derivative does not activate transcription when artificially recruited to the promoter via a heterologous DNA binding domain, indicating that TFIIA is important for transcription even in the absence of an activation domain. Taken together, these results suggest that TFIIA plays an important role in both activator-dependent and core promoter functions *in vivo*. Further, they suggest that TFIIA function may not be strictly related to the recruitment of TBP to promoters but may also involve a step after TBP recruitment.

Initiation of RNA polymerase (pol)[1] II transcription requires the assembly of a large complex of proteins that must interact at the promoter in a productive manner (1, 2). Formation of this complex is accelerated by activators that bind to the promoter and aid in recruitment of the components in the complex. The first step in promoter recognition is binding of TFIID to the TATA element. TFIID is a multiprotein complex containing TATA-binding protein (TBP) and TBP-associated factors (3). TFIIA stabilizes the TBP-TATA interaction (4–7) by interacting directly with the TBP and DNA flanking the TATA element (8, 9). TFIIA also counteracts several negative regulators of transcription that specifically target TBP. It inhibits the abilities of Mot1 and NC2 to dissociate TBP from the TATA element (10–13), and it blocks the inhibition of TBP binding to the TATA element by the N-terminal domain of TBP-associated factor 130 (14). Thus, there are several mechanisms by which TFIIA functions at core promoters *in vitro*.

Although TFIIA is not required for *in vitro* transcription using highly purified components, activated transcription is often stimulated by TFIIA. This is in accord with the observations that TFIIA can interact directly with activation domains *in vitro* (15–17) and that TFIIA is required for activator-dependent stabilization of the TFIID·TATA complex (16, 18–20). A simple model is that the activator-dependent TFIID·TFIIA complex is formed rapidly and stably on the TATA element, thereby serving as an efficient scaffold for the remainder of the initiation complex. Alternatively, TFIIA could act as a coactivator, conveying information between the activator and TBP. In this regard, *in vitro* cross-linking of an activator to TBP bound at a promoter is inhibited by TFIIA (21), suggesting that TFIIA is positioned between the activator and TBP. However, other biochemical experiments suggest that simple recruitment of the TFIID·TFIIA complex is not sufficient for activated transcription (22, 23). TFIIA may alter the conformation of TFIID such that either TFIID or the TFIIA·TFIID complex is a target for the remainder of the initiation machinery. It should be noted that the functional interaction between an activator and TFIIA need not be direct, and biochemical studies have identified coactivator proteins that interact with the activator and TFIIA (24). The physiological relevance of these observations and implied mechanisms remains to be established.

Several studies have addressed the role of TFIIA *in vivo*, but the results do not establish whether the functions of TFIIA *in vivo* are related to the activator, the core promoter, or both. Previously, we showed that a yeast TBP mutant (termed N2–1) defective for interacting with TFIIA impairs the response to acidic activators but does not generally affect pol II transcription (25). However, human TBP mutants severely defective for interacting with TFIIA are generally incompetent for transcription in transiently transfected mammalian cells (26). On the other hand, mutants of the Toa2 subunit of yeast TFIIA that weaken TFIIA-TBP·TATA complex formation confer selective transcriptional effects (27). Interpretation of these results is complicated because the various mutations might differentially affect the quality of the TFIIA-TBP interaction and because potential functions of TFIIA that are unrelated to interactions with TBP are not addressed. In complementary experiments, reduction of intracellular TFIIA levels caused a broad, but quantitatively modest, effect on transcription (7), but these results were limited by the partial nature of the TFIIA depletion and the lack of experiments involving activator-dependent transcription.

In this report, we extend our analysis of the N2–1 derivative of TBP by systematically examining its ability to respond to a large number of activators, by determining the regions of promoters that are responsible for altered transcription in the N2–1 strain, and by performing artificial recruitment experi-

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¶ To whom correspondence should be addressed. Tel.: (617) 432-2104; Fax: (617) 432-2529; E-mail: kevin@hms.harvard.edu.

[1] The abbreviations used are: pol, polymerase; TBP, TATA-binding protein; GFP, green fluorescent protein.

ments. Our results indicate that TFIIA plays an important role in both activator-dependent and core promoter functions *in vivo*, and they suggest that TFIIA functions, at least in part, in a step after TBP is recruited to the promoter.

EXPERIMENTAL PROCEDURES

Analysis of his3 Promoter Derivatives—To analyze transcriptional stimulation by different yeast activators, we started with a set of 13 strains described previously (28) that contain derivatives of the *his3* promoter in which the natural enhancer region located upstream of the noncanonical (T_C) and canonical (T_R) *his3* TATA elements is replaced with a specific activator binding site. After transformation of these 13 strains with a *URA3* centromeric plasmid expressing wild-type TBP, the chromosomal TBP locus was replaced by a derivative in which the TBP protein coding sequence was replaced by the *LEU2* gene. Finally, derivatives of these strains expressing wild-type TBP or the N2–1 derivative as the sole source of TBP (on *TRP1* centromeric plasmids) were generated by plasmid shuffling. To analyze the ability of TBP and the N2–1 derivative to mediate the response to the various activators, cells were grown under appropriate conditions for the various activators, exactly as described previously (28). Levels of *his3* transcription were determined by quantitative S1 analyses using hybridization reactions containing 20–40 μg of RNA and *his3* and *ded1* ^{32}P-labeled oligonucleotide probes as described previously (29).

Expression Analysis Using Genome Microarrays—Yeast strains MMY101 and MMY102 (generated and kindly provided by Mario Mencia) were derived from ZMY117 (30) by *LEU2* disruption of the genomic copy of TBP in the presence of wild-type TBP on a *URA3* centromeric plasmid. *TRP1* centromeric plasmids expressing wild-type TBP (for MMY101) or N2–1 (for MMY102) were introduced into this background by plasmid shuffling. Cells of each strain were cultured in synthetic medium at 30 °C to an A_{600} of 1, collected by centrifugation, and frozen in liquid nitrogen. Total cellular RNA was isolated by hot acid phenol extraction (29), and poly(A)-containing RNA was purified using Qiagen oligotex resin. Oligo(dT)-primed double-stranded cDNA was derived from this poly(A)-containing RNA, and 1 μg of the product was transcribed *in vitro* to generate internally biotin-labeled complementary RNA (31). The biotin-labeled RNA probes were fragmented, hybridized to half a set of Affymetrix yeast gene chip arrays (chips C and D, representing ~3200 open reading frames from chromosomes IX to XVI), fluorescently labeled, and analyzed on a Molecular Dynamics confocal scanner (31).

The results for the two strains were analyzed and compared using Affymetrix GeneChip software. For the comparison of expression results from the two strains, the data were normalized by two different methods: in the first, the overall hybridization intensity of the N2–1 sample was set equal to that of the wild-type sample for a given chip; and in the second, the data were normalized to set the hybridization intensities of both samples to the actin open reading frame (present on both C and D chips) at parity. The two methods of normalization yielded similar results, although there were minor variations in the fold change, and thus the rank order, of the open reading frames exhibiting changed expression. The genome microarray results for several representative RNAs were confirmed by quantitative S1 nuclease protection assays.

Transcriptional Analyses of Promoter Chimeras—Based on the results of genome-wide analysis of transcription in the N2–1 strain, four genes were selected for analysis of their promoter sequences: *CTR1* and *PUT1*, which are down-regulated in the N2–1 background, and *ERG3* and *CYC1*, which are up-regulated in the N2–1 strain. All promoter fragments were produced by polymerase chain reaction using oligonucleotide primers that contain a restriction site at the 5′-end of the primer (an artificial *Bam*HI site, which adds 6 base pairs to each promoter between the upstream and core promoter regions). Upstream promoter fragments are 750 base pairs in length, and the core promoter regions span from the distal end of the TATA box to the +7 site relative to the A of the start codon. The hybrid promoter constructs were cloned in frame with the gene encoding a modified version of green fluorescent protein (GFP, a gift from Pam Silver). The resulting chimeras were linearized with *Eco*RV and integrated at the *ura3*-52 locus of the TBP or N2–1 strains used for the genome microarray analysis. Quantitative S1 nuclease protection assays were performed as described (29) on RNA harvested from each strain. The sequence of the 55 base GFP probe is CCGTATGTTGCATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCTAATT, and S1 nuclease digestion of the hybrid between yeast RNA and this probe yields a product of 50 bases.

Artificial Recruitment Experiments—LexA fusion constructs were tested in strain FT4, which contains a LexA operator 45 base pairs upstream of the *his3* TATA element and structural gene (32). To generate molecules expressing LexA-TBP derivatives, the region encoding Cyc8 of YCp91-LexA-*CYC8* (32) was replaced with the structural gene of wild-type TBP and the N2–1 derivative. The resulting molecule contains a 1.5-kilobase fragment of the *ADH* promoter driving expression of a hybrid protein consisting of the 202 amino acid coding sequence for LexA, the HA1 epitope, the SV40 nuclear localization signal, and the TBP derivative. YCp22 (the *TRP1* vector) and molecules expressing LexA and the LexA-TBP derivatives were transformed into strain FT4, and *his3* expression was monitored by spotting 10^4 cells on plates lacking histidine, containing either 0 or 20 mM aminotriazole, a competitive inhibitor of *his3*. LexA-TBP fusions were detected by immunoblot analyses of 100 μg of whole cell extracts using a polyclonal antibody to LexA and chemiluminescent detection.

Gal4 fusion constructs were tested in the strain MAV103, which contains the Gal4 UAS fused to the *his3* TATA element and structural gene (33). Polymerase chain reaction was used to amplify the open reading frames of Toa1 and TBP, and these were cloned into pPC97, which contains the Gal4 DNA binding domain (residues 1–147) on a CEN, *TRP1*-marked plasmid (33). Molecules expressing these Gal4 derivatives were transformed into strain MAV103, and *his3* expression was monitored by spotting 10^4 cells on plates lacking histidine, containing either 0 or 20 mM aminotriazole.

RESULTS

The TFIIA-TBP Interaction Is Important, but Largely Nonselective, for Activators to Stimulate Transcription from the his3 Promoter—In yeast and other eukaryotic cells, core promoters containing TATA and initiator elements are essentially inactive, indicating that transcription of essentially all genes requires activator proteins (34, 35). Previously, we showed that the N2–1 derivative of TBP was defective for transcription of genes responding to three different acidic activators, Gal4, Gcn4, and Ace1, and was defective *in vitro* for interaction with TFIIA (25). The growth and transcriptional phenotypes conferred by N2–1, but not by other TBP mutants, were suppressed by fusion to the Toa2 subunit of TFIIA, demonstrating that the defective TFIIA-TBP interaction is responsible for the phenotypes *in vivo* (25, 36). Although it is impossible to exclude the possibility that the N2–1 derivative might have other defects aside from its inability to interact with TFIIA, such additional defects do not account for the growth and transcriptional phenotypes. Interestingly, transcription of a number of other genes appeared unaffected in the N2–1 strain. This suggests that activators responsible for transcription of the unaffected genes should function normally in the N2–1 strain.

To test this hypothesis, we analyzed the role of the TFIIA-TBP interaction in 13 strains, each of which possesses a binding site for a different activator located upstream of the *his3* TATA and initiator elements (28). These sites include those recognized by acidic activators or by activators with unclassified activation domains, as well as a poly(dA·dT) element that stimulates transcription via its inherent effect on chromatin structure (37). Unexpectedly, in almost all cases, *his3* transcription is lower in the N2–1 strain than in the corresponding strain expressing wild-type TBP (Fig. 1). In the exceptional case of the activator Ppr1, transcriptional output is unaffected or slightly enhanced in the N2–1 strain. Thus, most of the activators tested are unable to support maximal levels of *his3* transcription in the N2–1 strain. Interestingly, the transcriptional defect is quantitatively similar (2–3-fold) in essentially all cases examined, indicating that the TFIIA-TBP interaction is largely nonselective for activator function.

The TFIIA-TBP Interaction Is Important for his3 TATA Element Utilization—The *his3* promoter contains a noncanonical TATA-like element (T_C) that is responsible for initiation at the +1 position and a consensus TATA element (T_R) that is responsible for initiation at +13 (28, 38–40). In accord with previous results (28), strains containing wild-type TBP show a clear

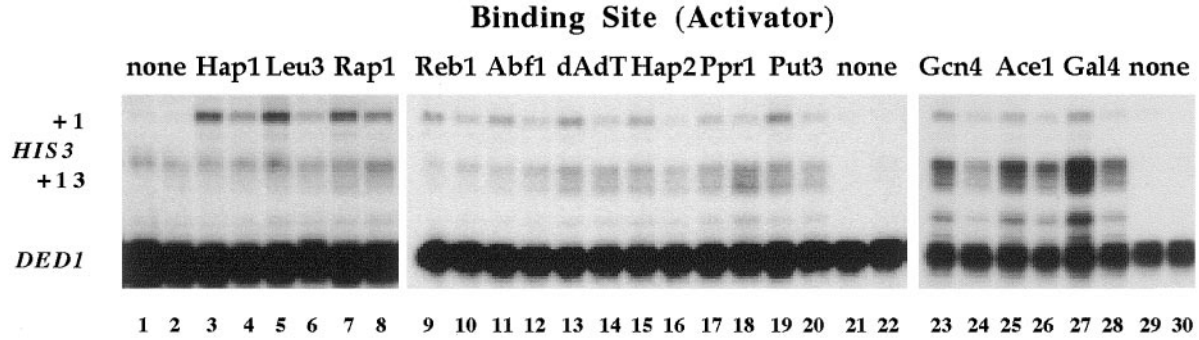

FIG. 1. **The TFIIA-TBP interaction is important, but largely nonselective, for activators to stimulate transcription from the *his3* promoter.** Stimulation of transcription by various yeast activators in strains containing either wild-type TBP (*odd numbered lanes*) or the TFIIA-defective N2–1 allele (*even numbered lanes*). RNA from strains containing *HIS3* promoters with binding sites for the indicated transcription factors or strains that lack a binding site (none) were subjected to quantitative S1 analysis; the positions of the *his3* (+1 and +13) and *ded1* transcripts are indicated. As the strengths of the activators vary, the three panels correspond to different exposures.

pattern of *his3* TATA utilization depending on the quality of activator (Fig. 1 and Table I). Specifically, as defined by the ratio of +13 to +1 transcripts, weak activators stimulate transcription predominantly through T_C, moderate activators stimulate transcription equally from T_C and T_R, and strong activators preferentially stimulate transcription through T_R. This pattern was interpreted in terms of functional saturation of T_C (and other weak TATA elements) at low to moderate levels of transcriptional stimulation (28).

As the wild-type pattern of *his3* TATA utilization is governed specifically by the core promoter region and not the activator (28), factors that alter this pattern do so by affecting core promoter function (30, 41–43). In this regard, the corresponding strains containing the N2–1 derivative display a different pattern of *his3* TATA element utilization (Table I). In promoters dependent on weak activators, a larger percentage of the total transcription is driven from T_R, producing a +13 to +1 ratio of nearly 1 or greater. A similarly increased preference for T_R and +13 initiation is also observed for moderate activators, including Ppr1. Importantly, increased utilization of T_R in strains containing N2–1 is observed even though the overall level of transcription is lower; this contrasts with the situation in wild-type cells where increased T_R utilization is associated with an overall increase in *his3* transcription levels (28). Taken together, these results suggest that T_C is saturating at a lower level of transcription in the N2–1 strain as compared with wild type strain and that noncanonical TATA elements depend on the TFIIA-TBP interaction to achieve maximal levels of transcription. Differential preference of *his3* TATA elements is not observed for potent activators (Gcn4, Ace1, and Gal4), suggesting that a strong activator can bypass at least part of the defect associated with a defective TFIIA-TBP interaction. The observation that the N2–1 derivative alters the wild-type rules of *his3* TATA element utilization provides evidence that TFIIA performs a function at core promoters.

A Defective TFIIA-TBP Interaction Delays the Induction of Gal4-dependent Activation—The modest defect in Gal4-dependent activation (Fig. 1) appears to conflict with our previous observation (25) that the N2–1 derivative was completely unable to mediate activation by Gal4. This apparent discrepancy was resolved by analyzing the kinetics of Gal4-dependent activation of *GAL1* transcription. The TBP-containing strain achieves maximal levels of activation by 30–60 min, with an increase in *GAL1* RNA observed after only 10 min in galactose (Fig. 2*A*; data not shown). In contrast, the N2–1 strain requires 6 h for detectable induction and 12 h to reach maximum output. Although there is a substantial growth difference between the TBP and N2–1 strains (doubling times 85 min and 6 h, respectively), the slow induction of *GAL1* transcription is not explained simply by the altered growth rates. For example, at a time corresponding to half a generation (45 min and 3 h, respectively), *GAL1* transcription is maximal in the strain con-

TABLE I
Differential usage of the two qualitatively distinct TATA elements

Activator	Ratio of +13/+1 Transcription[a]	
	Wild-type TBP	N2–1
Hap1	0.49	0.92
Leu3	0.50	1.3
Rap1	0.75	1.1
Reb1	0.58	1.4
Abf1	0.59	1.8
dAdT	1.2	3.5
Hap2–4	1.7	2.5
Ppr1	1.9	7.0
Put3	1.1	3.0
Gcn4	5.3	4.9
Ace1	5.9	6.1
Gal4	7.2	8.0

[a] The ratio of +13 transcription to +1 transcription, which reflects utilization of T_R or T_C, respectively. A value of 1 represents equal initiation from +1 and +13, values of less than 1 indicate preferential initiation from the +1 start site (driven by T_C), and values greater than 1 indicate preferential initiation from +13 (driven by T_R).

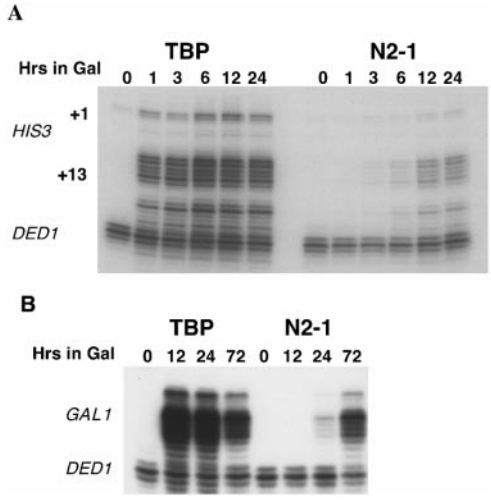

FIG. 2. **Kinetics of Gal4-dependent activation.** *A*, RNA was harvested from strains grown from 0 to 24 h in 2% galactose. Strains (FT4 background) contained the Gal4 DNA binding element driving expression of the *HIS3* gene with either wild-type (*TBP*) or the N2–1 allele. The positions of the *his3* (+1 and +13) and *ded1* transcripts are indicated. *B*, RNA was harvested galactose from strains (KY320 background) containing either wild-type (*TBP*) or the N2–1 allele that were grown in 2% galactose for 0 to 72 h. The positions of the *gal1* and *ded1* transcripts are indicated.

TABLE II
Genes that exhibit altered levels of expression in the N2–1 strain

Fold difference	Lower in N2–1	Higher in N2–1
>10	YNL157w	
	YNL144c	
	YNL195c	
	TIS11	
	YPR008w	
	PUT1	
6–7	YMR316c-b	RPL6B ex 1
	MSK1	YMR318c
	YPL136w	YNL057w
	YNR075w	YPL142c
		YJL118w
5	YPT53	YKL086w
	CTR1	YLR435w
	YOR071c	YLR376c
	YMR320w	RPS33B
	YLR126c	YPR064w
	YLR349w	YPL205c
	YMR040w	YPL148c
	BEM4	YPL238c
	YPL278c	
	YPL049c	
	YPL200w	
	YPR003c	
4	YLR267w	YMR269w
	YJR149w	CIN5
	YAP3	YMR303c
	YNL173c	YPR044c
	CYC2	YNR042w
	YOR019w	YOR246c
	YKL165c	RP23 cx 1
	YOR137c	YMR294w
	YOR387c	ZDS1
	MLS1	YLR265c
	YNL194c	YLL012w
	MPD1	DYN1
	YOR343c	YLR068w
	YNL054w	SPR40
	YOL031c	YKR077w
	ARE2	YJL222w
	YMR313c	CTK1
	YOR264w	YKR024c
	YNL148c	RPA12
	YPL222w	ERG3
	YMR085w	YKL082c
	YLR231c	CYC1
	YML132w	YML023c
	YOR070c	YJL148W
	YOR220w	ERG5
		YLR073C
		LTV1
		YMR095C
		YLR455w

taining wild-type TBP, whereas it is not detectable in the N2–1 strain. In the previously published experiment, which involved a different strain background, the doubling time for the N2–1 strain was 10 h, galactose induction was performed for 18 h, and *GAL1* transcription was not detected. However, when the original strain is cultured for longer times, a slight Gal4-dependent response is observed at 24 h, and a response corresponding to 20–30% of the wild-type level is observed at 72 h (Fig. 2*B*). Thus, in both the original and current strain backgrounds, the N2–1 derivative delays the induction of Gal4-dependent activation but only causes a mild (3–5 fold) defect in the maximal level of *GAL1* transcription.

Effect of a Defective TFIIA-TBP Interaction on Genome-wide Expression Patterns—To examine the global implications of an impaired TFIIA-TBP interaction, we compared the pattern of gene expression of N2–1 and wild-type cells using microarray technology (44). Analysis of ~3200 genes reveals that 42 (1.3%) are expressed at least 4-fold higher in the N2–1 strain than in the wild-type strain and that 47 (1.5%) are expressed at levels at least 4-fold lower in the N2–1 strain (Table II). If changes of at least 2-fold are considered, 9% of genes are expressed at higher levels in the N2–1 strain, and 8% are expressed at lower levels in the N2–1 strain relative to the strain containing wild-type TBP. This percentage of genes preferentially affected by at least 2-fold in the N2–1 strain is comparable to or higher than that caused by mutations in *GCN5* (5% affected), *SRB5* (16% affected), and *SWI2* (6% affected) (45). With the exception of increased expression of several ribosomal protein genes in the N2–1 strain, we detected no obvious patterns in the genes with changed expression levels. The wide range and apparent unrelated nature of genes preferentially affected by the N2–1 mutation are consistent with the relative lack of activator specificity observed with the modified *his3* promoters.

An important consideration in interpreting these genome-wide expression results is that equal amounts of wild-type and N2–1 mRNA samples were analyzed. For this reason, all changes caused by the defective TFIIA-TBP interaction (either positive or negative) are defined relative to the significant majority of "unaffected" genes. Overall changes in RNA levels, such as a possible broad reduction in the slowly growing N2–1 strain, are effectively normalized out and hence rendered imperceptible. Thus, we cannot distinguish whether the genes with changed RNA levels are indeed the only ones affected or whether they simply represent, within a globally changed background, an RNA population disproportionately sensitive (or abnormally insensitive) to a weakened TBP-TFIIA interaction. Finally, as the N2–1 strain is stable and viable, the transcriptional profile represents not just the primary effects of a sabotaged TBP-TFIIA interaction, but also all the secondary consequences that affect cell growth and perhaps even transcription by RNA polymerases I and III.

Sensitivity to the TFIIA-TBP Interaction Can Reside in Either the Upstream or the Core Promoter Region—To localize the dependence on the TFIIA-TBP interaction to the upstream or core promoter region, chimeric promoters were created among four genes specifically affected in the N2–1 strain (Fig. 3). *PUT1* and *CTR1* expression levels are decreased in the N2–1 strain as compared with wild-type TBP, whereas *ERG3* and *CYC1* RNA levels are increased (Table II and Fig. 4). One part of each chimeric promoter was derived from a gene whose expression is increased in the N2–1 strain, and the other part was derived from a gene whose expression was decreased. A complete set of reciprocal chimeras and control promoters with upstream and core regions from the same gene, were fused to the GFP structural gene and analyzed for transcriptional activity in wild-type and N2–1 strains. It should be noted that, with the exception of *CYC1*, the promoters utilized in this experiment have not been subjected to detailed analysis; hence, boundaries between core and upstream regions have been defined by the position of the likely TATA element.

For *PUT1*, decreased expression in the N2–1 strain clearly maps to the upstream region. Both chimeras containing the *PUT1* upstream region show significantly decreased expression in the N2–1 strain, whereas both chimeras containing the *PUT1* core region show increased transcription characteristic of the *CYC1* and *ERG3* genes. The transcriptional properties of these *PUT1* chimeras also suggest that the *CYC1* and *ERG3* upstream regions are important for the differential response to N2–1.

In the case of *ERG3* and *CTR1*, the upstream regions play the predominant role, but they do not completely account for the differential response to N2–1. Specifically, the *CTR1* upstream-*ERG3* core promoter chimera shows a decrease characteristic of *CTR1*, but the chimeric promoter is less affected than

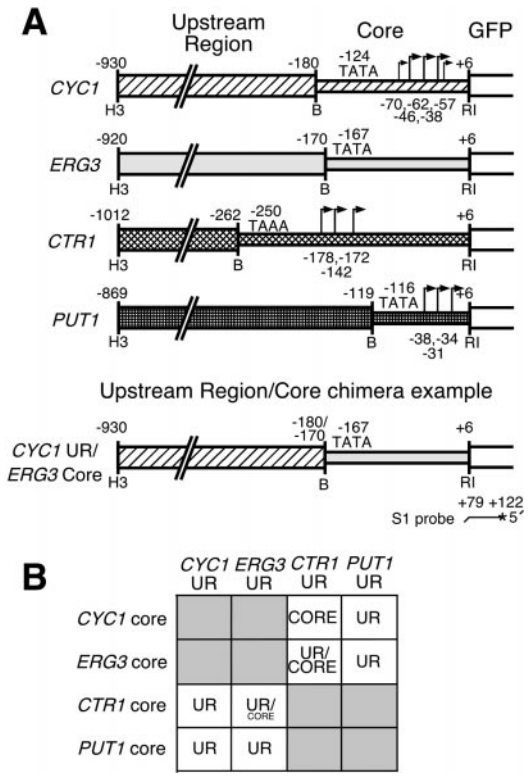

FIG. 3. **Generation of chimeric promoters derived from genes differentially affected by the TFIIA-TBP interaction.** *A*, diagrams of the *CYC1*, *ERG3*, *CTR1*, and *PUT1* promoters with the upstream regions (*thick boxes*), core regions (*thin boxes*), putative TATA elements (location defined with respect to the ATG codon at +1), and transcriptional initiation sites (*arrows* in cases where this information is known). The boundary between the upstream and core regions is an artificially generated *Bam*HI site, which was used to generate the chimeric promoters. All chimeric promoters were fused to the GFP structural gene and analyzed by S1 analysis using a probe corresponding to residues +79 to +122. *B*, transcriptional properties of the chimeric promoters as determined from data in Fig. 4. For each chimera, the region that determines the differential response in the N2–1 strain is indicated. In the *CTR1-ERG3* and *ERG3-CTR1* chimeras, both elements contribute with the upstream region playing a predominant role in latter case.

the natural promoter. Conversely, the reciprocal *ERG3-CTR1* chimera behaves more similarly to *ERG1* than to *CTR1*, but the chimeric promoter does not show increased expression in the N2–1 strain (it may even be very slightly reduced). Lastly, the core region of *CYC1* clearly contributes to the increased transcription in the N2–1 strain, because the *CTR1-CYC1* chimera behaves indistinguishably from the natural *CYC1* gene. Taken together, these results indicate that the function of both the upstream region and the core promoter can be sensitive to a defect in the TFIIA-TBP interaction.

The Defective TFIIA-TBP Interaction Cannot Be Overcome by Artificial Recruitment of TBP to the Promoter—When wild-type TBP is tethered to promoters via a DNA binding domain located upstream of a TATA element, transcriptional activation occurs in the absence of an activation domain (46–48). Under these artificial recruitment circumstances, interactions that require the activation domain are bypassed, and TBP and other general transcription factors can only perform core promoter functions. Conversely, LexA-TBP derivatives that fail to activate transcription upon artificial recruitment are defective in a core promoter function. By this criterion, the N2–1 derivative is defective in a core promoter function, because LexA-N2–1 does not activate transcription when artificially recruited to a promoter (Fig. 5*A*). LexA-N2–1 is expressed at a level equivalent to that of LexA-TBP (Fig. 5*B*), and it is capable of supporting cell growth (data not shown). These results indicate that the TFIIA-TBP interaction plays an important role in core promoter function *in vivo*.

Recruitment of TFIIA to a Promoter Efficiently Stimulates Transcription—In addition to TBP, artificial recruitment of TBP-associated factors (49–51), TFIIB (50, 52), and various subunits of the pol II holoenzyme (53–56) results in transcriptional activation. To examine whether TFIIA stimulates transcription when artificially recruited to a promoter, we examined the activity of a hybrid protein comprising the Gal4 DNA binding domain and the Toa1 subunit of TFIIA. As shown in Fig. 6, Gal4-Toa1 stimulates expression of a Gal4-dependent promoter to a level comparable to that observed with Gal4-TBP. Although we have not excluded the possibility of a fortuitous activation surface on Toa1 (57), this result suggests that tethering TFIIA to a promoter bypasses the need for activator-mediated recruitment of the pol II machinery. Although this observation does not necessarily indicate that TFIIA is a target of natural activators, it suggests that a hypothetical activator-TFIIA interaction will contribute to the level of transcriptional activation *in vivo*.

DISCUSSION

Transcriptional Defects Conferred by the N2–1 Derivative of TBP Are Relatively Nonspecific for the Activator—Our original studies of the N2–1 derivative indicated that transcription dependent on strong activators (Gcn4, Ace1, and Gal4) was severely defective, whereas constitutive expression from a broad range of pol II promoters was relatively unaffected (25). This led us to hypothesize that the N2–1 strain would be competent for the response to activators involved in constitutive transcription. In contrast to this expectation, analysis of *his3* promoters driven by a variety of activators reveals that N2–1 confers reduced (2–3-fold) levels of transcription in nearly every case (except Ppr1).

Our results force a revision to our original interpretation of the N2–1 derivative as being specifically defective in the response to acidic activators. Instead, the transcriptional defects of the artificial *his3* promoters are relatively nonspecific for the activator. In addition, microarray analysis indicates that the subset of genes preferentially affected in the N2–1 strain do not show a clear pattern suggestive of a response to specific activators. Finally, the relative lack of activator specificity is consistent with the observation that transcription of nearly all genes tested is reduced ~2–5-fold upon loss of TFIIA (7).

Why do TFIIA-depleted cells show a broad, but quantitatively modest, reduction in transcription (7), whereas N2–1 cells appear to display defects for a small subset of genes? One possibility is that the N2–1 derivative does not completely block the TFIIA-TBP interaction and hence confers a less severe effect than eliminating TFIIA. Alternatively, because TFIIA is present at normal levels in the N2–1 strain, it might be recruited to promoters by an alternative mechanism involving interactions with activators (15, 16), TBP-associated factors in the TFIID complex (58, 59), or TFIIE (60). Finally, the difference between N2–1 and TFIIA-depleted cells might be more apparent than real. A broad decrease in pol II transcription in N2–1 cells might be obscured (or normalized out) by the fact that equivalent amounts of RNA from wild-type and mutant cells are assayed even though the cells grow at different rates. Such a broad pol II defect could easily account for the slow growth of N2–1 strains, and slowly growing cells also have a reduced steady-state level of pol I and pol III transcription. In contrast, TFIIA-depleted cells are analyzed soon after the depletion, conditions in which indirect growth-dependent effects on pol I and pol III transcription should not be confounding,

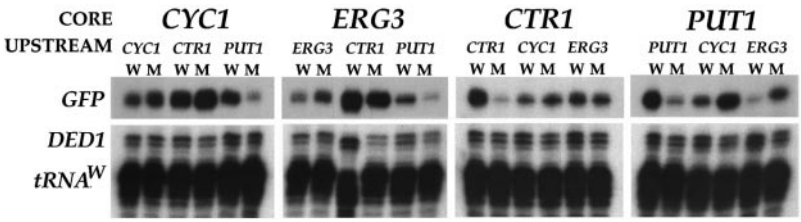

FIG. 4. **Transcriptional analysis of chimeric promoters.** RNAs from strains containing chimeric promoters with the indicated upstream and core promoter regions (see Fig. 3) were hybridized to completion with a mixture of oligonucleotide probes for *GFP*, *DED1*, and tryptophan tRNA, and the resulting products were treated with S1 nuclease. The three transcripts were analyzed on the same gel, but the autoradiograph was exposed for a longer time in the case of GFP.

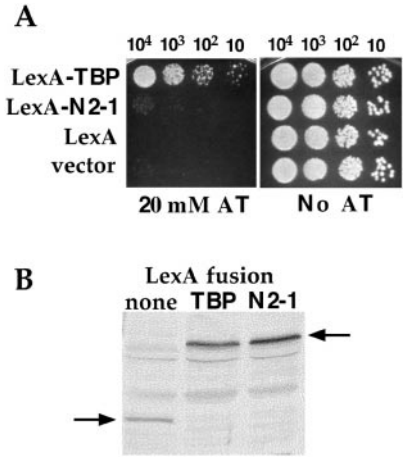

FIG. 5. **Artificial recruitment assay of LexA-TBP and LexA-N2–1.** *A*, strains containing LexA-TBP, LexA-N2–1, LexA, or vector and a promoter with a LexA operator 45 base pairs upstream of the *his3* TATA element and structural gene were tested for growth on 20 mM aminotriazole (*AT*). Plates were photographed after 3 days growth at 30 °C. The degree of AT resistance is directly related to the level of *his3* transcription. *B*, LexA-TBP and LexA-N2–1 proteins are produced at similar levels. Immunoblot analyses of 100 μg of whole cell extract from each of the indicated strains probed with anti-LexA antibody (a gift from R. Brent). The *arrows* indicate where the LexA-TBP fusions and LexA alone migrate.

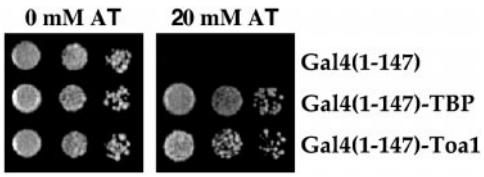

FIG. 6. **Transcriptional activation by artificial recruitment of TFIIA.** Strains expressing the Gal4 DNA binding domain (residues 1–147), Gal4-TBP, and Gal4-Toa1 and containing a promoter with the Gal4 enhancer fused to the *his3* TATA element and structural gene were tested for growth on 20 mM aminotriazole (*AT*). The degree of AT resistance is directly related to the level of *his3* transcription.

and the broad decrease in pol II transcription can be easily observed.

Role for TFIIA in Core Promoter Functions—Basal transcription from a core promoter using purified factors is a concept defined *in vitro*. *In vivo*, core promoters typically have very low levels of transcription (34), probably because of the repressive effects of chromatin (35, 61, 62). As such, it is likely that transcription in vivo represents some form of activated transcription. Consequently, it is often difficult to determine whether an observed transcriptional defect *in vivo* reflects a core promoter function or an activator-specific function. For example, a mutated TATA element reduces the level of activator-dependent transcription even though the TATA element is clearly involved in a core promoter function.

Three lines of evidence demonstrate that TFIIA is important for core promoter function(s) *in vivo*. First, the N2–1 strain shows differential utilization of the *his3* TATA elements, with T_C (the nonconsensus TATA element) saturating at a lower level of transcription in comparison to the wild-type strain. This suggests that, relative to T_R (the canonical TATA element), T_C is a weaker element in the N2–1 strain than in the wild-type strain. As the rules of *his3* TATA utilization are governed specifically by the core promoter region and not the activator (28), factors that alter these rules do so by affecting core promoter function (30, 41–43). Second, the N2–1 derivative does not stimulate transcription when artificially recruited to promoters. As artificial recruitment represents an experimental situation in which the normal activation process (*i.e.* dependent on activation domains) is completely bypassed, the defect of N2–1 must reflect a core promoter function. Third, analysis of chimeric promoters indicates that, in some cases, the core region can contribute to altered expression in the N2–1 strain.

Role for TFIIA in Activator-specific Functions—There are two basic ways to interpret the observation that the defect in activator-dependent *his3* transcription in the N2–1 strain is relatively nonspecific for the activator. In one interpretation, this defect may be because of the *his3* core promoter rather than the specific activator. In this regard, a defective core promoter function such as a weakened TBP-TATA interaction can cause the appearance of a defect in the response to transcriptional activators (28, 63, 64). In the alternative explanation, TFIIA could perform a common, but not absolutely required, function that is related to activators. For example, TFIIA could be a common target of activators or it could stabilize/alter the conformation of the direct activator target. In this regard, our observation that Gal4-Toa1 activates transcription suggests that an activator-TFIIA contact will contribute to transcriptional activity *in vivo*. These two basic explanations are not mutually exclusive, and they also apply to the related observation that depletion of TFIIA causes a general, and quantitatively similar, reduction in pol II transcription, even though individual promoters differ markedly in their upstream regions and hence activator binding sites.

Despite the difficulties of distinguishing between core and activator-specific functions in many situations, our analysis of chimeric promoters provides strong evidence that TFIIA does perform a function that is related to activators. In most cases tested, altered expression because of the defective TFIIA-TBP interaction is associated with the upstream region, not the core promoter. Although we cannot exclude the possibility that the dependence on the upstream region of the chimeric promoters is indirect (*i.e.* by altered expression of genes encoding proteins that interact with these upstream regions), these results strongly suggest that TFIIA displays some degree of specificity with respect to activators. Thus, our analysis of the N2–1 derivatives provides evidence that TFIIA has both activator-specific and core promoter functions *in vivo*.

Evidence That TFIIA Has a Role after Recruitment of TBP to

the TATA Element—A large body of biochemical evidence suggests that TFIIA stabilizes the TBP-TATA element interaction and stimulates recruitment of TBP to promoters. Our observation that artificial recruitment of TFIIA (via the Toa1 subunit) results in transcriptional stimulation is consistent with a role of TFIIA in TBP recruitment, because transcriptional activity is highly correlated with increased TBP occupancy *in vivo* (65, 66). However, by analogy with other TBP mutants (36), the observation that artificial recruitment of N2–1 does not bypass the defect in the TFIIA interaction suggests a role for TFIIA in a post-recruitment step for transcription *in vivo*. These results do not exclude a role of TFIIA in recruitment of TBP but rather suggest an additional function(s) of TFIIA after binding the TATA element. In accord with such a post-recruitment role, there are TFIIA mutants that are normal for formation of a TBP·TFIIA·TATA complex but defective for activated transcription *in vitro* (67). This suggests that formation of the stable complex is not sufficient to achieve activated transcription and that TFIIA is unlikely to function solely as a bridging factor between the activators and the general machinery. Although the nature of this post-recruitment step is unknown, TFIIA can induce conformational changes in TFIID at the promoter (59, 68), and it can physically interact with TFIIE (60), which functions late in the transcription process (1, 2). Thus, it is likely that TFIIA plays multiple roles in the transcriptional process *in vivo*.

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