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Recruiting TATA-Binding Protein to a Promoter: Transcriptional Activation without an Upstream Activator

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The binding of TATA-binding protein (TBP) to the TATA element is the first step in the initiation of RNA polymerase II transcription from many promoters in vitro. It has been proposed that upstream activator proteins stimulate transcription by recruiting TBP to the promoter, thus facilitating the assembly of a transcription complex. However, the role of activator proteins acting at this step to stimulate transcription in vivo remains largely speculative. To test whether recruitment of TBP to the promoter is sufficient for transcriptional activation in vivo, we constructed a hybrid protein containing TBP of the yeast *Saccharomyces cerevisiae* fused to the DNA-binding domain of GAL4. Our results show that TBP recruited by the GAL4 DNA-binding domain to promoters bearing a GAL4-binding site can interact with the TATA element and direct high levels of transcription. This finding indicates that binding of TBP to promoters in *S. cerevisiae* is a major rate-limiting step accelerated by upstream activator proteins.

Promoter-specific transcription by RNA polymerase II in vivo is often dictated by upstream activator proteins (27). However, the mechanisms by which activator proteins stimulate transcription remain largely speculative (reviewed in reference 37). The activation domains of a number of activator proteins have been shown to interact directly with TATA-binding protein (TBP) or TBP-associated factors of the multisubunit TFIID (37). The ability of several activators to stimulate transcription correlates with their ability to interact with TBP (for example, see reference 19). These results suggest that activator proteins stimulate transcription by recruiting TBP to or facilitating its interaction with the TATA element (Fig. 1a). Once associated with the promoter, TBP may then nucleate assembly of a transcription-competent complex on the promoter (3, 23, 44). However, evidence for activator proteins functioning in vivo at a step of TBP recruitment is still lacking. A recent in vivo kinetic study showed that without an upstream activator, basal transcription from a marked promoter did not occur until 2 to 4 h after the expression of a correspondingly marked TBP, whereas activator-stimulated transcription could be detected within 30 min after expression of the marked TBP (22). However, in this study, the occupancy of TBP on the promoter proved to be technically difficult to measure (22). Therefore, the results did not rule out the possibility that a step(s) subsequent to the binding of TBP is accelerated by the upstream activator (6, 7).

In this study, we test whether recruitment of TBP to the promoter through its attachment to a promoter-bound heterologous DNA-binding domain would be sufficient for transcriptional activation. Our results support and extend studies recently reported by Chatterjee and Struhl (5) and Klages and Strubin (21). While the three studies are strikingly similar in their approaches and conclusions, they were designed and completed independently. Thus, these studies provide strong support for the model that the binding of TBP to promoters is a major rate-limiting step in vivo at least for some genes in *Saccharomyces cerevisiae* and that this step is accelerated by upstream activator proteins.

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MATERIALS AND METHODS

Media and strains. Media were prepared according to standard methods (1). The medium used for galactose induction was synthetic medium with 2% galactose, 2% ethanol, and 2% glycerol. Strain GGY1:171 (8) used for assays of GAL4 derivatives was obtained from Mark Ptashne's laboratory. This strain lacks the entire GAL4-coding sequence. Strain HXY1 is the same as GGY1:171 except that it lacks the *GAL1-lacZ* reporter. This strain was constructed by selecting segregants from GGY1:171 that lost the *GAL1-lacZ* reporter.

Site-directed mutagenesis and plasmid constructions. All DNA manipulations were carried out by using standard methods (31). The GAL4 derivatives were constructed in the yeast vector pHGX1, a single-copy plasmid that contains sequences encoding GAL4 residues 1 to 147 under the control of the yeast heat shock factor promoter (42).

The yeast TBP (yTBP) in GAL4-yTBP is a derivative in which the first 11 residues of yTBP were replaced with the first 45 residues of the *Drosophila* TBP. The GAL4 derivatives were constructed in the yeast vector pHGX1, a single-copy plasmid that contains sequences encoding GAL4 residues 1 to 147 under the control of the yeast heat shock factor gene promoter (42). GAL4-VP16 was described previously (41), and GAL4-CTF^R contains a derivative of the proline-rich domain of CTF/NF1 with its RNA polymerase II carboxy-terminal domain (CTD)-like motif replaced with three consensus CTD repeats (42).

The yeast plasmid GC1Z, which carries a GAL4-driven *CYC1-lacZ* reporter, was constructed as follows. Plasmid pLG669Z (12) was digested with restriction enzymes *Xho*I (see Fig. 4 for the sequence of the *CYC1* promoter region) and *Sma*I (within the 3′ noncoding region of *URA3*), and the *Xho*I sticky ends were filled in with the Klenow polymerase. A *Kpn*I linker, GGGGTACCCC, was then inserted by blunt-end ligation, which recreated the *Sma*I and *Xho*I sites. This yeast vector, yXL1, was digested with *Xho*I, and the sticky ends were filled in with Klenow polymerase. The oligonucleotide CGGAAGACTCTCCTCCG and its complement, which contain the GAL4-binding site, were then inserted into the filled-in *Xho*I site.

The *GAL1-lacZ* reporter was constructed by replacing the *CYC1* sequence in the yeast vector yXL1 with the *GAL1-10* promoter/leader region. An A-to-G substitution at the second position of the *GAL1* TATA element was introduced by site-directed mutagenesis.

Yeast transformation and β-galactosidase assays. Plasmid constructs were introduced into yeast strains as described previously (17). Transformed yeast cells were grown in selective medium to an A_{600} of 1.0 to 1.5. Cells were centrifuged in a 1.5-ml Eppendorf tube in a microcentrifuge for 2 min. The cell pellets were resuspended in β-galactosidase assay buffer (50 mM KPO_4 [pH 7.4], 1 mM $MgCl_2$) and were permeabilized with chloroform and sodium dodecyl sulfate as previously described (12). β-Galactosidase activity was determined by

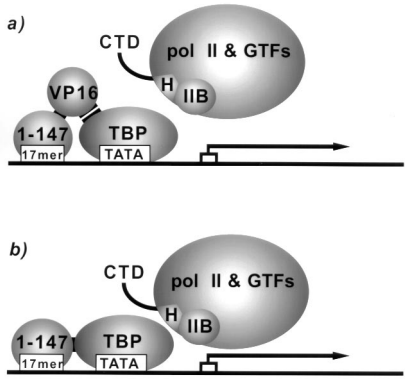


FIG. 1. A model of transcriptional activation by upstream activator proteins and by the hybrid protein GAL4-yTBP. (a) An upstream activator such as GAL4-VP16 interacts with TBP to recruit TBP to the promoter. (b) GAL4-yTBP, when bound to the 17-mer site, brings the attached TBP to the promoter. For simplicity, the TBP-associated factors or coactivators, or mediators, which facilitate the interaction between acidic activators and TBP (9, 16, 20, 23, 35, 36) are not shown. In both panels, TBP will interact with the TATA element. The bound TBP molecule is then recognized by the RNA polymerase II (pol II) holoenzyme complex (16, 23) through interactions of TBP with components of the holoenzyme complex, including general transcription factors (GTFs) such as TFIIB (IIB) and SRB proteins and multiple domains of the RNA polymerase such as the conserved H and CTD domains of the largest subunit, which can interact directly with TBP (13, 36, 38, 41, 42). The arrow indicate the start site(s) and direction of transcription.

using the substrate chlorophenol red-β-D-galactopyranoside (Boehringer Mannheim) as described previously (32).

Primer extension analysis of transcripts. Total yeast RNA preparation and primer extension were carried out by using standard methods (1).

RESULTS

The GAL4-yTBP hybrid protein activates transcription from the GAL1 promoter. To test whether recruitment of TBP to the promoter is sufficient to direct transcriptional activation in vivo, we constructed a hybrid protein containing yTBP fused to the DNA-binding domain of GAL4 (amino acids 1 to 147). Since GAL4 binds DNA even when packaged in nucleosomes (40), it will bring the covalently attached TBP to any promoter region that contains a GAL4-binding site. This attached TBP molecule could conceivably interact with the TATA element and direct the subsequent assembly of a transcription complex (Fig. 1b). We expressed the GAL4-yTBP hybrid protein in yeast cells and assayed the expression of genes bearing the GAL4-binding site. As shown in Fig. 2a, a *GAL1-lacZ* reporter gene is activated almost 1,000-fold in the presence of the GAL4-yTBP fusion protein. This level of activation by GAL4-yTBP is about 5 to 10% of those of the potent activators GAL4 and GAL4-VP16 and is even higher than that of GAL4-CTF^R, a proline-rich activator (42). Primer extension analysis of transcripts from the endogenous *GAL1* showed that GAL4-yTBP activates transcription of the native *GAL1* gene also to a level of 5 to 10% of those of the GAL4 and GAL4-VP16 activators (Fig. 2b). Furthermore, transcription activated by GAL4-yTBP (lane 3) initiates at the same major start sites as does transcription activated by wild-type GAL4 (lane 2) and GAL4-VP16 (lane 4), although transcription directed by GAL4-yTBP initiates also at a cluster of sites only about 70 bp downstream of the GAL4-binding site (compare the region between −188 and −253 of lanes 5 and 7 with that of lane 6 in Fig. 2c). These results suggest that recruitment of TBP to the promoter through its attachment to the DNA-binding domain of GAL4 is sufficient for transcriptional activation in vivo.

The GAL4-yTBP hybrid protein activates strongly and preferentially the TATA element closest to the GAL4-binding site in the *CYC1* promoter. To examine further the properties and generality of the GAL4-yTBP fusion protein in the activation of RNA polymerase II transcription in *S. cerevisiae*, we assayed transcriptional activation of a *CYC1* promoter that contains a GAL4-binding site in place of the native regulatory upstream activation sequences (UAS_C). As shown in Fig. 3a, the *CYC1-lacZ* reporter can also be activated by the GAL4-yTBP fusion protein. The level of activation by GAL4-yTBP is about 20% of that by GAL4-VP16. Since the *CYC1* promoter contains multiple TATA elements (15, 25), we examined whether GAL4-yTBP activated the same TATA element(s) as the native UAS_C and GAL4-VP16. As shown in Fig. 3b, the major start sites of transcription activated by GAL4-VP16 are located in a region from positions −25 to −60 (lane 2), which are essentially the same as those activated by the native UAS_C (lane 4). However, the major start sites of transcription activated by GAL4-yTBP are located at positions around −200 relative to the translational start site (lane 3). It appears that a cryptic TATA element, located at position −225, which is only 22 bp downstream of the GAL4-binding site (Fig. 4), is preferentially and strongly activated by GAL4-yTBP. In contrast, the two major, normally functional TATA elements at positions −177 and −121 (15, 25), which are 70 and 126 bp downstream of the GAL4-binding site, are preferentially activated by GAL4-VP16 and the native UAS_C (Fig. 4). Although the TBP attached to

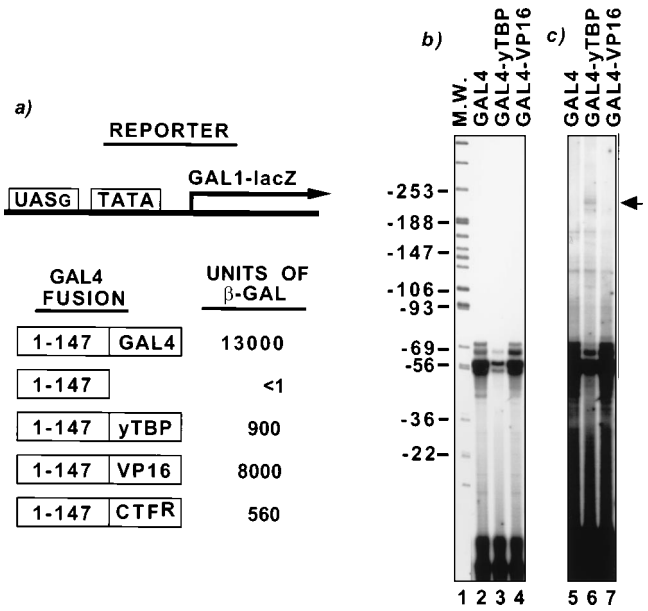


FIG. 2. High levels of transcriptional activation by GAL4-yTBP from the *GAL1* promoter. (a) β-Galactosidase assays of a *GAL1-lacZ* reporter gene activated by the wild-type GAL4 and its derivatives. Each value represents the average units of β-galactosidase (β-GAL) activity from two or three independent assays. The relative β-galactosidase activities varied within 20%. The induction of β-galactosidase activity from the *GAL1-lacZ* reporter was dependent on the GAL4-binding sites; a *GAL1-lacZ* reporter lacking GAL4-binding sites produced no detectable β-galactosidase activity above background levels (not shown). (b) Primer extension analysis of *GAL1* transcripts activated by wild-type GAL4 (lane 2), and its derivatives GAL4-yTBP (lane 3) and GAL4-VP16 (lane 4), with a primer starting at position +56 of *GAL1*. Molecular weight markers (M.W.; lane 1) are *Msp*I-digested pBR322 fragments. The indicated positions are based on those of the molecular weight markers and are relative to the A (designated position +1 in this study) in the translation initiation codon ATG of *GAL1*. Lanes 5 to 7 are the same as lanes 2 to 4, respectively, but with an about 10-fold-longer exposure. The region between −188 and −253 with additional sites of transcription initiation activated by GAL4-yTBP is indicated by an arrow.

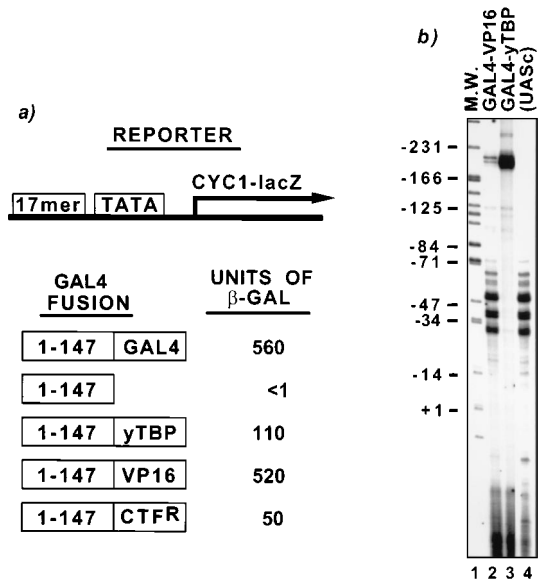


FIG. 3. High levels of transcriptional activation by GAL4-yTBP from a *CYC1* promoter bearing a GAL4-binding site. The GAL4 derivatives were cointroduced with GC1Z into *S. cerevisiae* HXY1 (see Materials and Methods). (a) β-Galactosidase assays of the *CYC1-lacZ* reporter activated by GAL4 derivatives. Each value represents the average units of β-galactosidase (β-GAL) activity from two or three independent assays. The relative β-galactosidase activities varied within 20%. The induction of β-galactosidase activity from the *CYC1-lacZ* reporter was also dependent on the GAL4-binding sites; a *CYC1-lacZ* reporter lacking GAL4-binding sites produced no detectable β-galactosidase activity above background levels (not shown). (b) Primer extension analysis of *CYC1-lacZ* transcripts activated by GAL4-VP16 (lane 2), GAL4-yTBP (lane 3), and the native UAS_C as in pLG669Z (lane 4), with a primer starting at position +78 of the reporter gene. The indicated positions are based on those of the molecular markers (M.W.; lane 1), which are *Msp*I-digested pBR322 fragments, and are relative to the A (designated position +1 in this study) in the initiation codon ATG of *CYC1*. Densitometry scanning showed that total signal from GAL4-yTBP (lane 3) is about 80% of that from GAL4-VP16 (lane 2).

the DNA-binding domain of GAL4 could conceivably function as a typical activation domain to act on other TBP molecules or other basal factors on the promoter, these results are most consistent with the idea that the TBP molecule attached to the DNA-binding domain of GAL4 interacts directly with a TATA element (Fig. 1b).

We noticed an inconsistency between the level of β-galactosidase activity and the level of transcripts from the *CYC1-lacZ* reporter when activated by GAL4-yTBP. Densitometry scanning of the autoradiogram presented in Fig. 3b showed that the level of transcripts activated by GAL4-yTBP is almost 80% of the level of transcripts activated by GAL4-VP16, which is in contrast to the 20% as assayed by β-galactosidase activity. This result can simply be explained by the fact that majority of the transcription activated by GAL4-yTBP initiates at sites upstream of the normal start sites, and these transcripts cannot be properly translated because of the presence of a number of AUG codons upstream of the normal AUG codon which are either out of frame of the normal AUG codon or run into stop codons before the normal AUG codon (Fig. 4). This may also explain the apparent lack of activation of the same *CYC1-lacZ* reporter by a LexA-TBP hybrid protein when assayed by β-galactosidase activity, as recently reported by Chatterjee and Struhl (5). In addition, the LexA operator site in the *CYC1-lacZ* reporter used by Chatterjee and Struhl (5) is 81 bp upstream of the first major, normally functional TATA element, whereas the 17-mer GAL4-binding site in our study is 70 bp upstream of the TATA element. We observed that increasing the distance between the GAL4-binding site and this TATA element to 80 bp results in a 5- to 10-fold reduction in the level of β-galactosidase activity when transcription is activated by GAL4-yTBP, although it has very little effect on the level of activation by GAL4-VP16 and GAL4-CTF^R (data not shown). Thus, when placed at similar distances from the TATA elements, both GAL4-TBP and LexA-TBP activate similar levels of β-galactosidase from the *CYC1-lacZ* reporter.

The GAL4-yTBP hybrid protein activates transcription from a promoter without a functional TATA element. To test whether a functional TATA element is required for transcriptional activation by the hybrid protein GAL4-yTBP, we tested the ability of GAL4-yTBP to stimulate transcription from a *GAL1-lacZ* reporter with a mutant TATA element. We introduced an A-to-G substitution at the second position of the TATA element in the *GAL1* promoter to change the sequence TATAAA to TGTAAA. This A-to-G substitution almost completely abolished the activation of the *GAL1-lacZ* reporter by GAL4-VP16 and GAL4-CTF^R (Fig. 5a to c). As shown in Fig. 5d, however, this mutant *GAL1* promoter could be activated by about 100-fold by GAL4-yTBP. These results show that GAL4-yTBP can direct significant levels of transcription from a promoter without any functional TATA element. The activation by GAL4-yTBP from this mutant promoter may reflect a more stable interaction of TBP with the mutant TATA element as a result of its attachment to the GAL4 DNA-binding domain that is already bound nearby.

The results presented in Fig. 5 also provide additional support for the idea that the TBP domain of the GAL4-yTBP hybrid protein does not act as a typical activation domain to recruit other TBP molecules to the promoter but interacts directly with the TATA element, as proposed in the model in Fig. 1b. As shown in Fig. 5a to c, in the presence of an altered-specificity mutant of TBP (TBP_{M3}) which recognizes the TGT AAA sequence (34), the mutant *GAL1* promoter could be activated by GAL4-VP16 and GAL4-CTF^R to a level of about

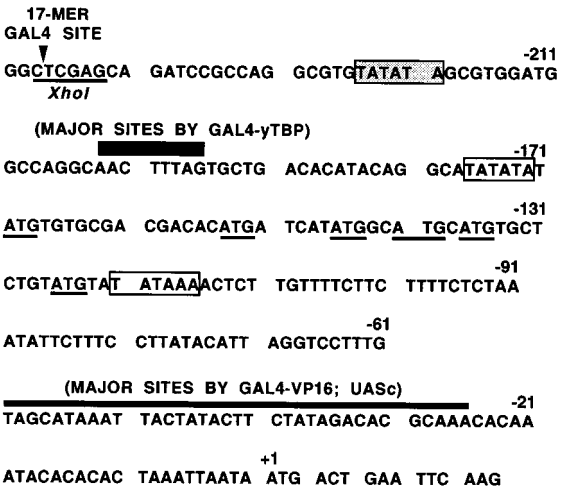
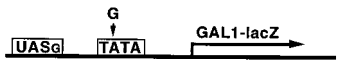


FIG. 4. Sequence of the *CYC1* promoter region and summary of the results presented in Fig. 3. The arrow indicates the position of insertion of the 17-mer GAL4-binding site. The shaded box indicates the potential TATA element identified in this study, which is located at position −225. Open boxes indicate the two major, previously characterized TATA elements, at positions −177 and −121, respectively (15, 25). Heavy lines above the sequence indicate regions with major start sites of transcription, with their thicknesses proportional to the relative levels of transcription. There are a number of minor start sites, which are not indicated (see to Fig. 3b for details). The ATG triplets upstream of the normal ATG are underlined.



	GAL4 FUSION	UNITS OF β-GAL TATA	TGTA	TGTA+TBP$_{M3}$
a	1-147	0.5	0.5	0.5
b	1-147 VP16	2800	3	580
c	1-147 CTF^R	230	1	43
d	1-147 yTBP	250	55	52

FIG. 5. Transcriptional activation by GAL4 derivatives from wild-type (TATA) and mutant (TGTA) promoters both in the presence and in the absence of TBP_{M3}. The *GAL1-lacZ* reporter was constructed by replacing the *CYC1* sequence in the yeast vector yXL1 (see the legend to Fig. 3) with the *GAL1-10* promoter/leader region in which the eighth codon of *lacZ* was fused to the third codon of *GAL1*. An A-to-G substitution at the second position of the *GAL1* TATA element was made by site-directed mutagenesis. Each of the GAL4 derivatives was introduced into yeast strain HXY1:TA/G1Z, which harbors the *GAL1-lacZ* reporter (TATA), yeast strain HXY1:TG/G1Z, which harbors the same *GAL1-lacZ* reporter but with the TATA-mutant promoter (TGTA), and yeast strain HXY1:TG/G1Z, carrying the altered-specificity mutant of yTBP (TGTA+TBP_{M3}). β-Galactosidase (β-GAL) activity was assayed as for Fig. 2.

20% of that of the wild-type promoter, which is consistent with levels seen previously on a similarly altered *HIS3* promoter (34). In contrast, there was no additional activation by GAL4-yTBP in the presence of TBP_{M3} (Fig. 5d). Chatterjee and Struhl have obtained similar results with a LexA-TBP hybrid on the HIS3 promoter (5).

DISCUSSION

This study and a number of other recent studies (2, 5, 21) have demonstrated that in *S. cerevisiae*, promoter-specific activation of transcription by RNA polymerase II can occur in the absence of conventional upstream activators. Recruitment of TBP to the promoter through its attachment to a heterologous DNA-binding domain is sufficient for transcriptional activation (this study and reference 5). The level of activation by GAL4-yTBP from a *CYC1-lacZ* reporter with a single GAL4-binding site can be up to 80% of that of the potent GAL4-VP16 (Fig. 3b). These results indicate that recruitment of TBP to and its stable interaction with the promoter can be a major in vivo rate-limiting step of transcriptional activation and support the idea that this step is accelerated by upstream activator proteins. The level of activation by GAL4-yTBP from the *GAL1* promoter, which has multiple GAL4-binding sites, is about 10% of that of the potent activator GAL4-VP16 (Fig. 2a). In this latter case, the much lower level of activation by GAL4-yTBP may reflect a lack of upstream activation domains acting at steps subsequent to the recruitment of TBP. These steps may involve the recruitment of other general transcription factors such as TFIIB (7, 26) and TFIIH (43) during the assembly of a transcription complex, or steps following the assembly of a transcription complex or initiation of transcription (28, 30, 41, 42), as postinitiation control is a common mechanism of transcriptional regulation in both prokaryotes and higher eukaryotes (11, 29).

It has been shown recently that an upstream activator can associate with an RNA polymerase II holoenzyme complex (16), suggesting that an upstream activator may activate transcription by directly recruiting the holoenzyme complex to the promoter. That the recruitment of the holoenzyme to the promoter may be a major mechanism of transcriptional activation by upstream activator proteins is further supported by the observation that fusion of the LexA DNA-binding domain to GAL11, another component of the holoenzyme complex, also results in transcriptional activation of promoters bearing the LexA DNA-binding site (2). Perhaps TBP is also a part of a larger RNA polymerase II holoenzyme complex, and fusion of TBP to the DNA-binding domain of GAL4 will direct this holoenzyme complex to promoters bearing a GAL4-binding site. This idea is consistent with the recent finding that TBP elutes through a gel filtration column with a profile similar to those of RNA polymerase II and other general transcription factors including a mediator subcomplex (35). Alternatively, TBP or the multisubunit TFIID may bind to the promoter independently and then assemble with the holoenzyme to form a transcription complex (Fig. 1; see also reference 16).

Our results show that optimal function of GAL4-yTBP to direct promoter-specific transcription requires two promoter elements, the GAL4-binding site and the TATA element. This suggests a functional similarity between the GAL4-yTBP hybrid protein and prokaryotic sigma factors in directing promoter-specific transcription. Sigma factors possess two conserved regions, region 2.4 and region 4.2, which make sequence-specific contacts with two promoter elements, the −10 and −35 elements, respectively (4, 24). TBP contains a region of sequence similarity to sigma factor region 2.4 (18). Interestingly, this region of TBP is important for its binding to the TATA element (14), a DNA sequence highly similar to the −10 promoter element contacted by σ^{70} region 2.4. TBP appears to be lacking a domain analogous to sigma factor region 4.2 and is dependent on upstream activators for promoter specificity. As we have demonstrated in this study (see also reference 5), this requirement for an upstream activator to confer promoter specificity can be bypassed by fusion of TBP to a heterologous DNA-binding domain. Why have the transcription apparatuses of eukaryotes evolved with upstream activator proteins separated from the TBP domain? One explanation is that recruitment of TBP or the multisubunit TFIID through its interaction with an upstream activation domain can provide a much greater flexibility for promoter arrangement, in particular for promoters under multiple, combinatorial controls.

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