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.

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) was 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 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 gene promoter (41). GAL4-VP16 was described previously (41), and GAL4-CTF 350 contains a derivative of the proline-rich domain of TFII/D1 with its RNA polymerase II carboxy-terminal domain (CTD)-like motif replaced with three consensus CTD repeats (42).

The yeast plasmid GCIZ, which carries a GAL4-driven CYC1-lacZ reporter, was constructed as follows. Plasmid pLG669Z (12) was digested with restriction enzyme Xho I (see Fig. 4 for the sequence of the CYC1 promoter region) and Smal (within the 3' noncoding region of URA3), and the Xho I sticky ends were filled in with the Klenow polymerase. A Kpn I linker, GGGTTACC CCC, was then inserted by blunt-end ligation, which recreated the Smal and Xho I sites. This yeast vector, yXLI, was digested with Xho I, and the sticky ends were filled in with Klenov polymerase. The oligonucleotide CCGAAAGACTCTCCTCGG and its complement, which contain the GCIZ-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 yXLI 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...
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-CTF8, 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.

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

However, the level of transcripts activated by GAL4-yTBP is almost 80% of that from GAL4-VP16 (lane 2). 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).

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Struhl have obtained similar results with a LexA-TBP hybrid GAL4-yTBP from a of TBP to the promoter through its attachment to a heterologous activation of transcription by RNA polymerase II can occur in have demonstrated that in (TATA) and mutant (TGTA) promoters both in the presence and in the absence of TBPM3. The GAL1-lacZ reporter was constructed by replacing the CYC1 sequence in the yeast vector yX1 (see the legend to Fig. 3) with the GAL1-TBP 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:G1Z, which harbors the same GAL1-lacZ reporter but with the TATA-mutant promoter (TGT), and yeast strain HXY1:G1Z, carrying the altered-specificity mutant of yTBP (TGTA-TBP). 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 TBPM3 (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 TFIH (7, 26) and TFIH (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 TFIH 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 σ70 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 TFIH 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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