Connecting a promoter-bound protein to TBP bypasses the need for a transcriptional activation domain

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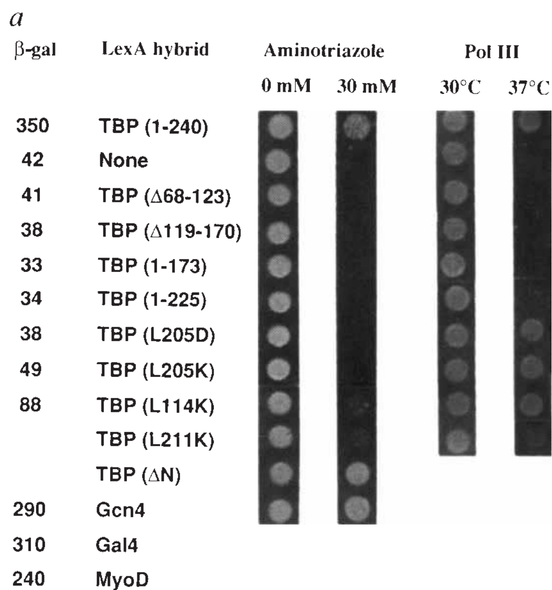
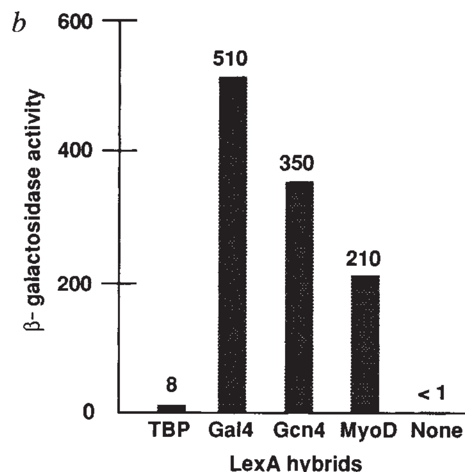
BIOCHEMICAL analyses have suggested potential targets for transcriptional activation domains, which include several components of the RNA polymerase II machinery[1–7], as well as the chromatin template[8–12]. Here we examine the mechanism of transcriptional activation in yeast cells by connecting a heterologous DNA-binding domain (LexA) to the TATA-binding protein (TBP). LexA–TBP efficiently activates transcription from a promoter containing a LexA operator upstream of a TATA element. Activation is promoter-specific and is sensitive to mutations on the DNA-binding surface of TBP; hence it is not due to a fortuitous activation domain on TBP. Thus a promoter-bound protein lacking an activation domain can stimulate transcription if it is directly connected to TBP. This suggests that recruitment of TBP to the promoter can be a rate-limiting step for transcription *in vivo*, and that interactions between activation domains and factors that function after TBP recruitment can be bypassed for activation.

LexA–TBP activates transcription from a promoter containing a single LexA operator 45 base pairs (bp) upstream of the *his3* TATA element and structural gene (Fig. 1*a*). Activation by LexA–TBP requires an intact TBP core domain; C-terminal and internal deletions at widely separated regions of the core domain are non-functional. LexA–TBP behaves comparably to LexA hybrids containing strong activation domains from yeast and mammalian proteins (Gal4, Gcn4, MyoD).

Because a wide variety of sequences can activate transcription in yeast[13,14], it was critical to exclude the possibility that TBP contains a sequence(s) that fortuitously behaves as a conventional activation domain. Such activation domains are typically localized to short regions of a protein, and they are remarkably insensitive to amino-acid substitutions or small deletions[13,15,16].





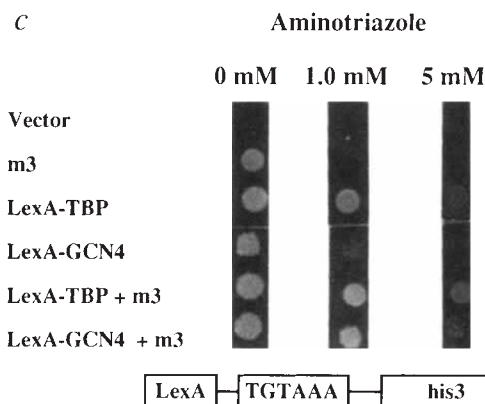


FIG. 1 Transcriptional activation by LexA–TBP and its derivatives. *a*, Strains containing the indicated LexA–TBP derivative and a promoter containing a LexA operator 45 bp upstream of the *his3* TATA element and structural gene[23] were tested for growth in aminotriazole (AT). The degree of AT resistance is directly related to the level of *his3* transcription[24,25]. A control strain containing a comparable promoter lacking the LexA operator does not grow in 1 mM AT in the presence or absence of LexA–TBP. Expression (β-gal) was directly measured with a *LacZ* derivative of the promoter on the *URA3* multicopy plasmid YEp356 (ref. 26). LexA–TBP derivatives were also tested for their ability to support RNA polymerase III (pol III) transcription by complementation of a temperature-sensitive, pol III-specific derivative of TBP (F155S) at 37 (ref. 27). *b*, Similar experiment except that strains contain YEp21-Sc3423 (ref. 13), a *LEU2* multicopy plasmid with the LexA operator located 81 and 135 bp upstream of the *cyc1* TATA elements and *lacZ* structural gene. *c*, Similar experiment except that the promoter differs by a single bp in the TATA element (TGTAAA), and strains are tested at lower AT concentrations. Where indicated, strains also contain a *URA3* centromeric plasmid expressing TBP[m3], a derivative that binds with increased affinity to TGTAAA[21].

METHODS. The centromeric *TRP1* expression vector YCp91 (ref. 23) containing LexA–TBP was generated by replacing the region encoding the HA1 epitope and the *CYC8* structural gene of YCp91-LexA-*CYC8* (ref. 23) with the TBP structural gene[28]. The resulting LexA–TBP hybrid contains 12 residues encoding the SV40 nuclear localization signal between the full-length LexA (1–202) an TBP (1–240) moieties. Deleted versions of LexA–TBP were generated by cleaving at the relevant *Pst*I, *Bgl*II, *Xba*I and *Hin*dIII sites. TBP derivatives lacking the non-conserved and non-essential N-terminal region[28] or containing amino-acid substitutions at residues on the DNA-binding surface of TBP[19,20] were converted into their LexA–TBP counterparts by replacing the relevant fragments. LexA-Gcn4 (ref. 13), LexA-Gal4 (ref. 29) and LexA-MyoD (ref. 30) have been described previously. The activities of β-galactosidase (average of 3–5 independent transformants) are normalized to the OD_{600} of cells at the time of collection and are accurate to ±20%. All yeast strains were derived from FT4 (ref. 23).

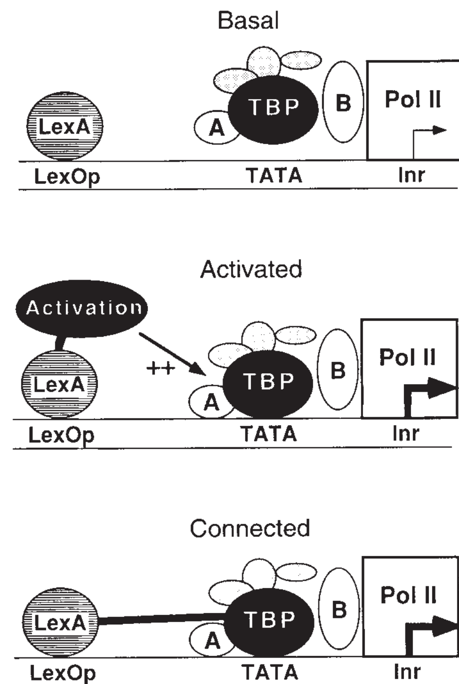


FIG. 2 Connecting a promoter-bound protein to TBP overrides the need for a transcriptional activation domain. Interactions of LexA hybrid proteins, TBP, TBP-associated factors (TAFs; shaded ovals), TFIIA, TFIIB and RNA polymease II at a promoter containing a LexA operator, TATA element and initiator (Inr). In basal transcription (LexA alone), the interaction between TBP and the TATA element is limiting (indicated by space between the two components), thereby leading to low levels of mRNA (thin arrow). Activation domains increase the recruitment or stability of TBP to the TATA element, thereby stimulating transcription (thick arrow). The target of the activation domain is not specified in the illustration (see text for discussion). A similar increase in transcription occurs when LexA is directly connected (thick line) to TBP. See text for details and limitations of this model.

We therefore examined LexA–TBP derivatives with single mutations at various positions on the DNA-binding surface of TBP[17,18] that affect TATA-element binding *in vitro*[19,20]. Such mutations are very unlikely to affect TBP structure or the activity of a conventional activation domain. However, if activation requires that a single LexA–TBP molecule interact both with a LexA operator and the adjacent TATA element, such mutated derivatives should be functionally impaired. Indeed, four such LexA–TBP derivatives show significantly reduced or non-detectable levels of expression (Fig. 1*a*). Moreover, these proteins are properly folded and otherwise functional *in vivo*, as indicated by their ability to support RNA polymerase III transcription. Thus activation by LexA–TBP requires binding of the TBP moiety to the TATA element, and it is highly sensitive to amino-acid substitutions at various locations on the DNA-binding surface.

Two additional observations provide independent evidence that LexA–TBP behaves differently from a conventional activator. First, LexA–TBP is inactive on a promoter containing a LexA operator upstream of the *cyc1* TATA elements, whereas LexA hybrids containing Gcn4, Gal4 or MyoD activation domains function efficiently (Fig. 1*b*). Thus, unlike a conventional activator, stimulation by LexA–TBP is strongly influenced by promoter context. Second, when tested on a promoter with a LexA operator upstream of a mutated *his3* TATA element (T̲GTAAA), LexA–TBP activates transcription to a modest extent whereas LexA–GCN4 activates very weakly (Fig. 1*c*). Introduction of TBP^{m3}, a derivative with increased affinity for T̲GTAAA sequences[21], does not significantly affect activation by LexA–TBP, whereas it clearly increases activation by LexA–GCN4 (and the native GCN4 and Gal4 activators[21]). Thus transcription by LexA–TBP, unlike LexA–GCN4, is not dependent on a physically unconnected molecule bound to the TATA element.

Taken together, the above results indicate that activation by LexA–TBP is due to the physical connection between the LexA and TBP domains that allows binding of a single molecule to the adjacent LexA operator and TATA element (Fig. 2). Because LexA and the physical connection are very unlikely to affect inherent TBP functions, activation by LexA–TBP almost certainly involves increased interaction of the TBP moiety with the TATA element as a result of its physical connection to a protein bound to a nearby site. This effect could be solely due to cooperative binding by physically connected domains, but it may also involve a local disruption of chromatin caused by LexA binding to its operator which increases binding of TBP to the TATA element. In either case, the results strongly suggest that interaction of TBP with the TATA element can be a rate-limiting step for transcription in yeast cells, and that activation domains can increases recruitment (or stabilize the interaction) of TBP to the promoter (Fig. 2). Independent evidence for this crucial role of activation domains has been obtained by measuring the rate at which TBP can productively access the chromatin template *in vivo*[22]. Although the mechanism for increased recruitment of TBP is unknown, an attractive hypothesis is that the artificial LexA–TBP connection mimics the interaction that normally occurs between the activation domain and TBP (or associated proteins).

LexA–TBP does not contain an activation domain, yet it stimulates transcription to a level comparable to native yeast activator proteins. This indicates that it is possible to generate circumstances in which interactions between activation domains and components of the basic machinery that function after TBP binding are not essential for transcriptional activation *in vivo*. However, our results do not preclude the importance of such interactions at native promoters *in vivo*. Such interactions are likely to be important for achieving maximal levels of transcription, particularly at promoters where the TBP–TATA element interaction is not the major limiting step.

Note added in proof: Our observation that LexA–TBP does not stimulate the *CYC1* promoter is consistent with the recent finding that TBP is bound to *CYC1* TATA elements *in vivo* in the absence of upstream activator proteins[31]. □

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Stimulation of RNA polymerase II transcription initiation by recruitment of TBP *in vivo*

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EUKARYOTIC transcriptional activators may stimulate RNA polymerase II activity by promoting assembly of preinitiation complexes on promoters through their interactions with one or more components of the basal machinery[1,2]. On the basis of its central role in initiating transcription-complex formation upon binding to the TATA box, the general transcription factor TFIID, which includes the TATA-binding protein (TBP) and several TBP-associated factors[3–5], has been implicated as a target for activators. Consistent with this idea, an increasing number of activators have been reported to bind directly to TBP[6–9]. To assess the functional importance of these *in vitro* interactions for transcriptional regulation *in vivo*, we made use of a novel strategy in yeast to show that a physical interaction with TBP is sufficient for a sequence-specific DNA-binding protein to increase initiation of transcription by RNA polymerase II. These results imply that binding of TFIID to promoter elements is a limiting step in transcription complex assembly *in vivo*.

The strategy consists of creating an interaction between TBP and RFX1[10], a human sequence-specific DNA-binding protein with no activation potential in yeast (see below), through their fusion to the complementary leucine-zipper motifs that specifically promote dimerization between the c-Myc oncoprotein and its partner Max[11] (Fig. 1*a*). The transcriptional effect of the dimerization-domain-mediated interaction between Myc–TBP and Max–RFX on the activity of a *his3* gene containing a single RFX-binding site upstream of the TATA box is assessed in yeast cells expressing either one or both of these hybrid proteins. Figure 1*b* and *d* shows that Max–RFX strongly activates *his3* expression only in cells containing Myc–TBP as a unique source of TATA-binding protein. Activation is strictly dependent on the presence of both an upstream RFX-binding site and a functional TATA element in the promoter of the *his3* gene (Fig. 1*c*). Surprisingly, whereas Max–RFX stimulates transcription from both +1 and +13 native *his3* initiation sites[12], VP16–RFX selectively increases the +13 transcript (Fig. 1*d*). Although the molecular basis for this difference is unknown, it suggests that VP16 may activate transcription by a mechanism distinct from the one involved in Max-mediated transactivation.

A critical issue is to exclude the possibility that Myc–TBP contains a fortuitous activation domain, and that it functions as a non-DNA-bound transactivator by being recruited to the *his3* promoter through its association with Max–RFX. To address this point, we investigated whether Myc–TBP behaves as an activator on a *his3* promoter containing the defective TATA element, TGTAAA, in cells expressing the TBP(m3) derivative that binds TGTAAA with increased affinity[13]. Figure 2*a* shows that TBP(m3) increases *his3* transcription in cells containing VP16–RFX, but not in cells expressing Max–RFX and Myc–TBP. Hence, Myc–TBP does not function as a conventional activation domain. The finding that Max–RFX and Myc–TBP stimulate transcription from the TGTAAA-containing promoter suggests that the fusion proteins bind DNA in a cooperative manner to allow a productive interaction between Myc–TBP and the TGTAAA promoter element.

To confirm that Myc–TBP is required as a general factor for RNA polymerase II (pol II) dependent *his3* transcription, derivatives specifically compromised in pol II functions were tested for Max–RFX transactivation. The mutants were selected by their ability to support cell growth only in conjunction with a human–yeast hybrid TBP (hy17) previously shown to function on pol II promoters, but to be defective for pol I and pol III transcription[14,15]. Complementation of hy17 requires the TBP mutants to function on pol I and pol III promoters, and their inability to support cell viability on their own would thus be indicative of a pol II specific defect. Two variants bearing mutations within regions known to mediate distinct RNA pol II functions were isolated. One derivative, Myc–TBP(m4), contains the double amino-acid change I194R, L205V which maps on the DNA-binding surface of TBP[13,16–18]. The second mutant, Myc-TBP(m5), carries a single I230K substitution in helix H2′. Interestingly, a pol II-specific temperature-sensitive mutant has been isolated that contains a single serine substitution at this position[19]. Figure 2*b* shows that, although they are unable to support cell viability on their own, both Myc–TBP mutants complement the growth defect of hy17. This phenotype is entirely consistent with the mutants being specifically defective for pol II transcription. The mutants were then tested, together with the Myc–hy17 chimaera, for their ability to respond to Max–RFX. Although Pol I and pol III-defective Myc–hy17 mediates normal Max–RFX transactivation, both mutants fail to do so (Fig. 2*b*). As the two derivatives bear amino-acid substitutions that are widely separated in the three-dimensional structure[16–18] and are therefore highly unlikely to both destroy a putative activation domain present in TBP, we conclude that Myc–TBP must bind DNA and function as a basal factor for pol II transcription to respond to Max–RFX. This identifies binding of TFIID on promoter DNA as a limiting step in the assembly of a functional preinitiation complex that may be subject to regulation by activators. By using a similar strategy, it should be possible to assess whether additional step(s) in the assembly pathway following template-binding of TFIID are also rate-limiting *in vivo*. □

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