Molecular Cell, Vol. 1, 917–924, May, 1998, Copyright ©1998 by Cell Press

Activator-Mediated Recruitment of the RNA Polymerase II Machinery Is the Predominant Mechanism for Transcriptional Activation in Yeast

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Summary

Eukaryotic transcriptional activators bind to enhancer elements and stimulate the RNA polymerase II (pol II) machinery via functionally autonomous activation domains. In yeast cells, the normal requirement for an activation domain can be bypassed by artificially connecting an enhancer-bound protein to a component of the pol II machinery. This observation suggests, but does not necessarily indicate, that the physiological role of activation domains is to recruit the pol II apparatus to promoters. Here, we show that transcriptional stimulation does not occur when the activation domain is physically disconnected from the enhancer-bound protein and transferred to components of the pol II machinery. The observation that autonomous activation domains are functional when connected to enhancer-bound proteins but not to components of the pol II machinery strongly argues that recruitment is the predominant mechanism for transcriptional activation in yeast.

Introduction

Eukaryotic RNA polymerase II (pol II) promoters typically contain enhancer, TATA, and initiator elements that are bound, respectively, by gene-specific activator proteins and the TFIID and pol II holoenzyme complexes (Struhl, 1995; Zawel and Reinberg, 1995). Of these, activator proteins show the greatest specificity and affinity for their cognate promoter elements. Moreover, many activator proteins can bind to their target sites in the context of nucleosomal templates, the physiologically relevant substrate (Kingston et al., 1996; Polach and Widom, 1996). In contrast, the TATA-binding protein (TBP) moiety of the TFIID complex displays less DNA sequence specificity than typical activator proteins, a property compounded by the nonoptimal TATA elements often found in natural promoters. More importantly, TBP is virtually unable to bind TATA elements in nucleosomal templates, although weak binding is observed when chromatin is disrupted by histone acetylation or by nucleosome remodeling (Imbalzano et al., 1994). Finally, the pol II holoenzyme does not appear to recognize specific DNA sequences, and its association with promoters presumably reflects protein–protein interactions with either TFIID or activators or both.

A fundamental question is how activator proteins stimulate transcription by the pol II machinery. Activators contain a DNA-binding domain that specifically recognizes enhancer elements and a physically separate activation domain that stimulates transcription of the target gene (Brent and Ptashne, 1985; Hope and Struhl, 1986; Ma and Ptashne, 1987a; Hope et al., 1988). Activation domains are functionally autonomous (i.e., they retain their functional activity when fused at different positions to a wide variety of heterologous DNA-binding domains [DBD] and when tethered at different positions in the promoter region). In fact, some natural activation domains (AD) (e.g., VP16) are brought to promoters by noncovalent interactions with DNA-binding proteins and not by direct tethering (Stern et al., 1989). In vitro, activation domains can interact with many components of the pol II machinery, but the physiological significance of these interactions is unclear.

Activation domains have been proposed to enhance transcription by a variety of mechanisms. These include simple recruitment of the pol II machinery to promoters (Klein and Struhl, 1994; Struhl, 1996; Ptashne and Gann, 1997), altering the conformation of components of the pol II machinery (Roberts and Green, 1994; Chi and Carey, 1996), modifying chromatin structure (Workman et al., 1991; Croston et al., 1992; Tsukiyama et al., 1994; Kingston et al., 1996), and affecting one or more steps after the transcriptional initiation event (Rougvie and Lis, 1990; Yankulov et al., 1994; Krumm et al., 1995). These possible mechanisms are not mutually exclusive, but their relative importance in vivo has yet to be established.

In the yeast *Saccharomyces cerevisiae*, the normal requirement for activation domains can be completely bypassed by artificial recruitment of pol II machinery to the promoter (Struhl, 1996; Ptashne and Gann, 1997) (Figure 1). Artificial recruitment is achieved by physically connecting an enhancer-bound protein to an individual component of the pol II machinery, such as TBP (Chatterjee and Struhl, 1995; Klages and Strubin, 1995; Xiao et al., 1995), TFIIB (Gonzalez-Couto et al., 1997; Lee and Struhl, 1997), TBP-associated factors (TAFs) (Apone et al., 1996; Gonzalez-Couto et al., 1997), and pol II holoenzyme subunits (Jiang and Stillman, 1992; Barberis et al., 1995; Farrell et al., 1996; Gaudreau et al., 1997). However, the relationship of these artificial recruitment experiments to the physiological mechanism by which activation domains enhance transcription by the pol II machinery is unclear. In particular, the direct and artificial connections between the enhancer-bound protein and the pol II machinery represent exceptionally strong protein–protein interactions in comparison to those typically observed with natural activation domains. As such, it is quite possible that the artificial recruitment experiments represent a bypass mechanism that is distinct from the physiological process that occurs with natural activators. Furthermore, artificial recruitment experiments are inherently unable to address other potential mechanisms utilized by activation domains because they are carried out in the absence of an activation domain.

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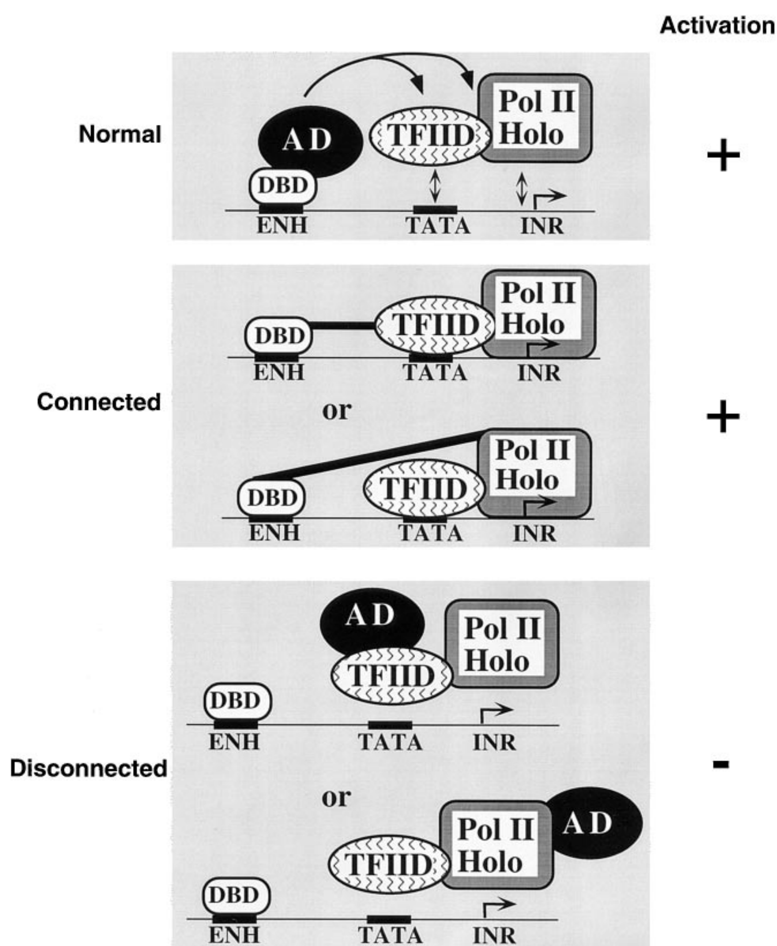


Figure 1. Normal, Connected, and Disconnected Situations Regarding the Organization of Transcriptional Activators and the Pol II Machinery at Promoters

(A) In the physiologically relevant situation, activator proteins bind enhancer elements via specific DNA-binding domains and stimulate transcription via functionally autonomous activation domains. Interactions between activation domains and the TFIID and/or the pol II holoenzyme complexes are indicated by arrows, although the direct targets within these complexes are not specified. Activators are shown as increasing recruitment of the pol II machinery to promoters (depicted by arrows between TFIID and the TATA element and the pol II holoenzyme and the mRNA initiation site) (see text).

(B) In the connected situation, transcriptional activation can be achieved in the complete absence of an activation domain by physically connecting (thick bold line) a component of either TFIID or the pol II holoenzyme to an enhancer-bound protein, thereby artificially recruiting the pol II machinery to promoters and bypassing the need for an activation domain.

(C) In the disconnected situation, transcriptional activation does not occur when the activation domain is transferred from its normal location on the enhancer-bound protein to a component of either TFIID or the pol II holoenzyme. As discussed in the text, it is presumed that the failure to activate transcription represents an inability of the pol II machinery to stably associate with promoters.

In this paper, we investigate the mechanism of transcriptional activation by transferring activation domains from their normal location on the enhancer-bound protein to a variety of components of the pol II machinery. In this situation, the enhancer-bound protein is disconnected from the pol II machinery, and transcriptional activation does not occur. The observation that autonomous activation domains are functional when connected to enhancer-bound proteins but not to components of the pol II machinery strongly argues that recruitment is the predominant mechanism for transcriptional activation in yeast.

Results

Experimental Design

We investigate the mechanism of transcriptional activation by asking the following question: can the presence of a strong activation domain in the preinitiation complex suffice for transcriptional enhancement in vivo? In principle, the presence of an activation domain within the pol II machinery should enhance transcription in vivo if the physiological role of activation involves one of the following mechanisms: (1) directly altering chromatin structure; (2) recruiting a chromatin-modifying activity to increase accessibility to the promoter; (3) inducing a conformational change in a component of the pol II machinery that increases transcriptional initiation; and (4) stimulating a step(s) after the initiation event. In contrast, if the physiological role of an activation domain is simply to recruit the pol II machinery to the promoter, the presence of an activation domain within the machinery itself would not be sufficient to trigger activation (i.e., the activation would not overcome the inherent inability of the pol II machinery to associate with promoters).

To test this idea, we transferred activation domains from their normal location on the enhancer-bound protein to a variety of components of the pol II machinery, thereby disconnecting the enhancer-bound protein from the pol II machinery (Figure 1). Two aspects of the experimental design are noteworthy. First, the transcription factors and protein domains in the disconnected situation are identical to those normally present at promoters under physiological conditions. Second, acidic activation domains are functionally autonomous, physically unconstrained, and stimulate transcription from virtually all of the positions tested previously. Moreover, other protein-interaction domains such as the helix-loop-helix of Myc and Max are functional when fused to components of the pol II machinery (Klages and Strubin, 1995; Gonzalez-Couto et al., 1997). Nevertheless, to further exclude potential artifacts associated with the protein fusions, we examined three different activation domains and five different components of the pol II machinery.

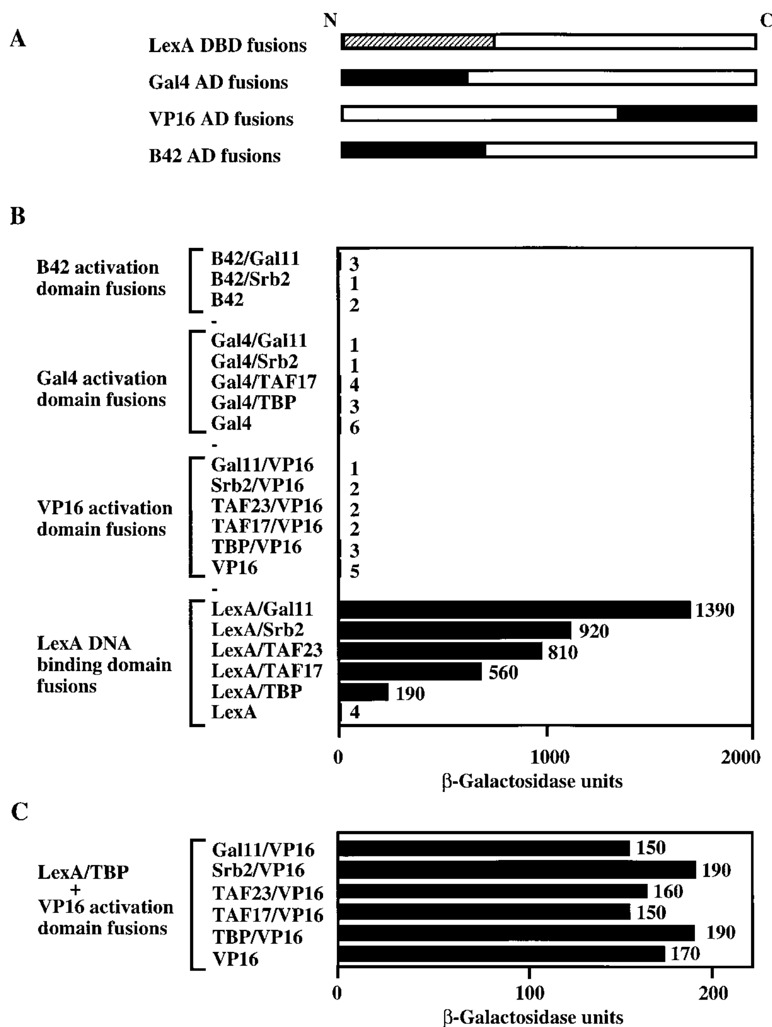


Figure 2. Activation Domains Stimulate Transcription When Connected to Enhancer-Bound Proteins but Not to Components of the Pol II Machinery

(A) Diagram of the fusion proteins with LexA DNA-binding domain (residues 1–202, hatched bar), activation domain (black bars), and TFIID or pol II holoenzyme component (full-length proteins, open bars) indicated.

(B and C) Transcriptional activation. β-galactosidase activities for yeast strains containing the indicated LexA DNA-binding domain and activation domain fusion proteins and the *LacZ* reporter JK103, which contains a promoter with four LexA operators upstream of the *GAL1* TATA and initiator elements. Values were normalized to A_{600} and represent the average of at least eight independent transformants. Similar results were obtained when the *LacZ* reporter was driven by a *his3*-derived promoter containing a single LexA operator in place of the normal Gcn4 binding site (Chatterjee and Struhl, 1995).

Activation Domains Do Not Stimulate Transcription When Transferred from Enhancer-Bound Proteins to Components of the Pol II Machinery

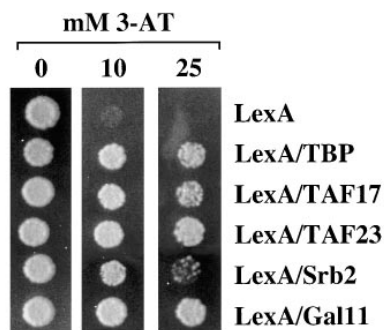
We connected activation domains to several components of the TFIID and pol II holoenzyme complexes (Figure 2A). The TFIID components include TBP as well as two TAFs, TAF17 and TAF23 (Moqtaderi et al., 1996). Yeast TAF17 is homologous to *Drosophila melanogaster* TAF40, which can interact in vitro with the VP16 and p53 activation domains (Goodrich et al., 1993; Thut et al., 1995), whereas yeast TAF23 is homologous to human TAF30, which interacts with the hormone-binding domain of the human estrogen receptor (Jacq et al., 1994). In contrast to these three TFIID subunits that are required for yeast cell viability, the two holoenzyme components used in this study, Srb2 and Gal11, are not essential for cell growth. In accord with previous artificial recruitment experiments, fusions of any of these TFIID or pol II holoenzyme components to a LexA DNA-binding domain result in high levels of transcription when assayed on the *gal1* (Figure 2B) or *his3* (Figure 3) promoters harboring LexA operators.

In contrast, when the above TFIID or holoenzyme components are fused to different activation domains (yeast Gal4, herpes virus VP16, or *Escherichia coli* B42), transcriptional activation of the *gal1* or *his3* promoters is not observed in any of the 11 cases tested (Figures 2B and 3). Furthermore, these activation domain fusions do not enhance the level of activation mediated by the LexA fusion proteins (i.e., strains containing both LexA and activation domain fusions behave indistinguishably from strains containing the LexA fusion alone) (Figure 2C and data not shown). Thus, the pol II machinery containing a functionally autonomous activation domain is unable to activate transcription from core promoters, and it cannot synergize with other activators to support higher levels of transcription. To put it another way, the presence of an activation domain within the pol II machinery does not bypass the need for a DNA-binding protein to interact with enhancer sequences.

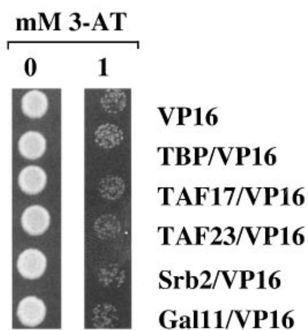
The Pol II Machinery Harboring a Strong Activation Domain Is Transcriptionally Competent and Supports Normal Cell Growth

This lack of transcriptional activation in the disconnected situation is not due to trivial technical reasons. First, Western blotting indicates that the activation domain fusion proteins are expressed at levels comparable

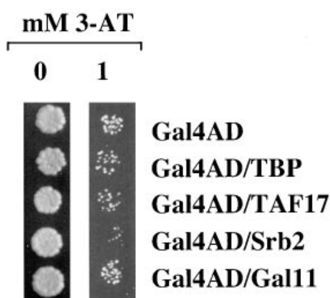
(A) LexA fusions



(B) VP16 fusions



(C) Gal4AD fusions



(D) B42 fusions

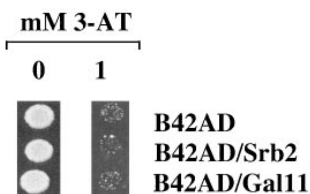


Figure 3. Transcriptional Activation of a Modified *his3* Promoter

The indicated proteins were transformed into the yeast strain L9FT4a, whose *his3* locus contains a single LexA operator upstream of the T_R TATA element (Tzamarias and Struhl, 1994). The resulting strains were assayed for *his3* expression by growth on SC media containing various amounts of 3-AT, a competitive inhibitor of the *his3* gene product. LexA fusions (A) support growth on 10 and 25 mM 3-AT and hence express *his3* at high levels, whereas the VP16 (B), Gal4 (C), or B42 (D) fusions grow extremely poorly on 1 mM 3-AT and indistinguishably from strains containing the activation domain alone and hence do not detectably express *his3* above background levels.

to that observed for the corresponding component of the pol II machinery for all of the cases tested (data not shown). Second and more importantly, all such fusion proteins are fully capable of complementing the phenotypic defects of the corresponding null mutant strains (Figure 4). This result indicates that fusions of the activation domain do not affect the biological function of the component of the pol II machinery, and that the fusion proteins are functionally integrated into the pol II machinery. Moreover, the complemented strains grow indistinguishably from the wild-type control strain, indicating that the fusion proteins do not detectably affect cell physiology. Third, the activation domain fusion proteins also fail to stimulate transcription even when examined in the corresponding null mutant strains (i.e., situations in which the fusion construct is the only copy of the gene in the cell) (Figure 5). In contrast, all of the LexA fusions support high levels of activation in the corresponding null strain backgrounds. This observation indicates that the failure of activation domain fusions to enhance transcription is not due to competition of the fusion proteins with the unfused component of the pol II machinery.

Discussion

The Yeast Pol II Machinery Is Inherently Unable to Activate Transcription In Vivo, Even When It Contains a Functional Activation Domain

We demonstrate that transcriptional enhancement does not occur when activation domains are disconnected from their normal location on an enhancer-bound protein and transferred to a component of the pol II machinery (Figure 1). It is extremely unlikely that the failure to activate is a negative result that is due to some peculiarity of the activation domain fusion proteins. The complementation assays (Figure 4) indicate that fusion of an activation domain does not detectably inhibit the function of the pol II machinery component. Conversely, activation domains are structurally and functionally autonomous. They invariably stimulate transcription when fused at various positions to numerous enhancer-bound proteins and when tethered at various positions in the promoter region; indeed, such fusion proteins are part of the standard repertoire for analyzing transcriptional regulation in eukaryotes. In striking contrast, all of the 11 cases tested here indicate that activation domains are not functional when fused to components of the pol II machinery despite the fact that other protein-interaction domains (e.g., HLH of Myc and Max) are functional when fused in the same manner to pol II machinery components (Klages and Strubin, 1995; Gonzalez-Couto et al., 1997).

While it is experimentally impossible to formally exclude a hypothetical structural or functional constraint on activation domains, there is no basis or plausible molecular mechanism for such an ad hoc constraint that is specifically limited to all of the 11 different situations involving a connection to the pol II machinery. On the contrary, a large body of experimental evidence is clearly inconsistent with such a functional constraint. For these reasons, the observation that activation domains function when connected to enhancer-bound proteins but not to components of the pol II machinery

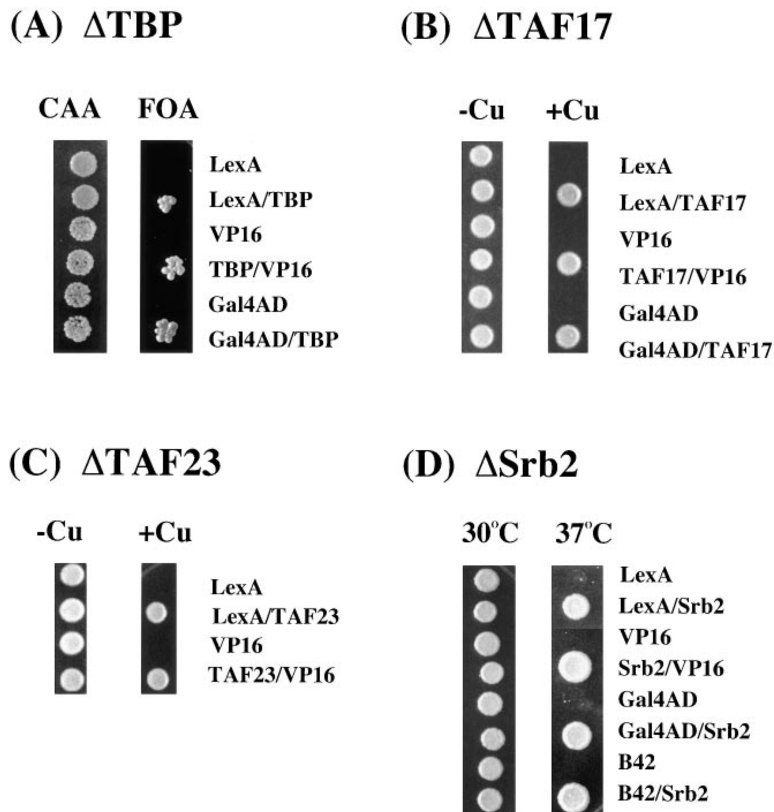


Figure 4. Complementation Assays

(A) TBP function was assayed in strain BYΔ2 by plasmid shuffling, as described previously (Cormack et al., 1991).

(B and C) TAF17 and TAF23 function was assayed in shutoff strains containing conditional TAF alleles that are expressed only in the absence of copper (Moqtaderi et al., 1996); thus, complementation assays were performed on SC media containing 500 μM copper.

(D) Srb2 function was assayed by complementation of the severe defect at 37°C conferred by an *srb2* deletion (Koleske et al., 1992).

reflects an inherent feature of the transcriptional activation mechanism. Thus, our results strongly argue that the yeast pol II machinery is inherently unable to activate transcription in vivo, even when it contains a functional activation domain.

Activator-Mediated Recruitment Is the Predominant Mechanism for Transcriptional Activation in Yeast

In comparing the normal and disconnected situations, the components of the pol II machinery, the domains of the activator, the promoter, and cell physiology are identical, yet transcriptional output is dramatically different (Figure 1). Given that all of the ingredients for transcriptional activation are available in the disconnected situation, the failure to activate almost certainly reflects an inability of the pol II machinery to interact with the promoter in vivo and not an inherent inactivity of the pol II machinery itself. Furthermore, our results indicate that unlike the activation domain, the requirement for the DNA-binding domain of the enhancer-bound protein cannot be bypassed even though there is considerable evidence that such DNA-binding domains are not directly involved in the transcriptional initiation process per se, other than bringing activation domains to promoters.

These considerations indicate that (1) efficient activation requires that the pol II machinery be firmly anchored at the promoter; (2) the pol II machinery is inherently unable to associate stably with the promoter, even if it carries an activation domain; (3) the DNA-binding domain provides the anchor that is required for the pol II machinery to associate stably with the promoter; and (4) the predominant role of the activation domain is to provide the connection between the anchor and the enzymatically active entity. Thus, the location of the activation domain is important because most enhancer-binding proteins can directly associate with nucleosomal templates, whereas TBP (and presumably TFIID) and the pol II holoenzyme cannot.

In accord with these ideas, it has been recently shown in vitro that high concentrations of pol II holoenzyme can bypass the need for an activator, whereas artificial connection to a holoenzyme component (Gal11) can activate transcription at low concentrations of pol II holoenzyme (Gaudreau et al., 1998 [this issue of *Mol. Cell*]). These (and most other) in vitro experiments are performed on naked DNA templates where there is no impediment to TBP/TFIID binding to TATA elements. Hence, activators can stimulate transcription in vitro only under conditions where TBP/TFIID and holoenzyme or both are limiting (or template concentration is low). In contrast, activators are required in vivo because they can directly bind nucleosomal templates; this permits recruitment of the pol II machinery that overcomes the inherent inaccessibility of TBP/TFIID and holoenzyme to promoters in the context of physiological chromatin.

Although we have directly analyzed transcription at only two artificial promoters, it is very likely that the recruitment mechanism described above is generally applicable for transcriptional activation in yeast. In particular, fusion of an activation domain to various components of the pol II machinery has no detectable effect on cellular physiology despite the fact that yeast contains numerous weak (or repressed) promoters with consensus TATA elements. If the pol II machinery containing an activation domain could artificially stimulate such

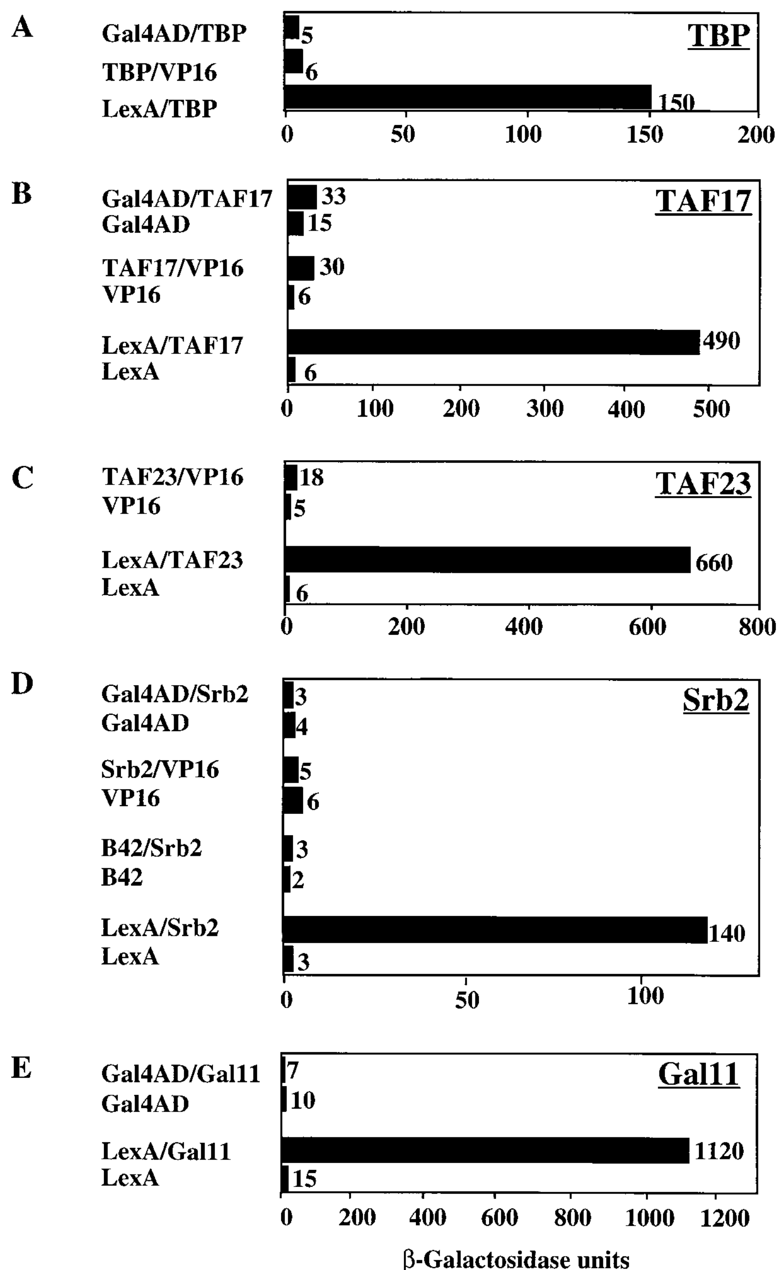


Figure 5. Transcriptional Activation of Hybrid Proteins in Corresponding Null Mutant Strains

Experiment was performed as described in Figure 2, except that molecules expressing the indicated hybrid proteins were transformed into the null strains for TBP, TAF17, TAF23, Srb2, and Gal11. Data is shown for the JK103 reporter, but similar results were obtained on the *his3-LacZ* reporter.

natural promoters, it almost certainly would cause general cellular mayhem. In this regard, TBP mutants that increase transcription from weak or repressed promoters are toxic and unable to support cell growth (Blair and Cullen, 1997; J. V. Geisberg and K. S., unpublished data). Thus, we conclude that the pol II machinery itself is unable to associate stably with most yeast promoters, and that the predominant function of activation domains is to recruit the machinery.

Previous evidence supporting a recruitment model for transcriptional activation in yeast relied on kinetic analysis of TBP function at the *his3* promoter (Klein and Struhl, 1994) and on artificial recruitment experiments (Struhl, 1996; Ptashne and Gann, 1997) performed on a limited number of promoters. In contrast to the artificial recruitment experiments, the experimental approach described here has several advantages; it involves the presence of an activation domain, avoids a bypass mechanism, and does not utilize artificial and covalent interactions between the enhancer-bound protein and the pol II machinery. As such, our results provide complementary and crucial evidence supporting a recruitment model for transcriptional activation, and they strongly suggest that activator-mediated recruitment is the predominant mechanism in yeast cells.

Additional Considerations

Our results do not address which components of the pol II machinery are targets of the activation domain under physiological conditions. It has been suggested

that activation domains represent a semispecific, sticky surface that can contact multiple targets such that no individual activator–target interaction is critical (Struhl, 1996). In addition, our results do not exclude alternative activation mechanisms such as stimulating promoter clearance or transcriptional elongation, although they suggest that such mechanisms are used for a minority of promoters in yeast. In higher eukaryotic organisms, such nonrecruitment activation mechanisms (Rougvie and Lis, 1990; Yankulov et al., 1994; Krumm et al., 1995) might be more prevalent.

Finally, the recruitment mechanism does not exclude a critical role for chromatin structure in the transcriptional activation process. On the contrary, chromatin-modifying activities can be components of the pol II machinery itself, or they can associate with the machinery. For example, TAF130 is a histone acetylase (Mizzen et al., 1996); the SAGA histone acetylase complex contains the TBP-interacting protein Spt3 (Grant et al., 1997); and there is conflicting evidence that the Swi/Snf nucleosome remodeling complex interacts with the pol II holoenzyme (Cairns et al., 1996; Wilson et al., 1996). Thus, recruitment of the pol II machinery is concomitant with the recruitment of chromatin-modifying activities to the promoter. We suggest that enhancer-bound activation domains overcome the inherent inability of the pol II machinery to productively interact with the nucleosomal template by simple recruitment (i.e., increasing local concentration) and by (directly or indirectly) recruiting associated chromatin-modifying activities that increase accessibility.

Experimental Procedures

DNAs

LexA hybrids were cloned in the YCp91 vector (Tzamarias and Struhl, 1994), which contains the *ADH1* promoter followed by an ATG codon, sequences encoding the SV40 nuclear localization signal, the HA1 epitope from influenza virus, and the *CYC8* termination region. The vector for expressing the hybrids containing the Gal4 activation domain (residues 768–881) or the B42 activation domain (Ma and Ptashne, 1987b) was similar to YCp91 except that it contains the *CYC1* terminator. The vector for expressing hybrids to the VP16 activation domain (residues 414–553) (Cress and Triezenberg, 1991) contains the *ADH1* promoter and the *CYC1* terminator, but it lacks the SV40 signal and HA1 epitope. The various expression cassettes were cloned into centromeric or 2 μ vectors marked with *TRP1* or *LEU2*. Further details on DNA construct generation are available on request.

Phenotypic Analysis

All yeast strains were transformants of L9FT4a (Tzamarias and Struhl, 1994) that contained three plasmids: the *LacZ* reporter JK103, which contains a promoter with four LexA operators upstream of the *GAL1* TATA and initiator elements (Kamens et al., 1990), on a multicopy *URA3* plasmid; the LexA hybrid proteins or the DNA-binding domain alone on a *LEU2* plasmid; and the activation domain hybrid proteins or the activation domain alone on a *TRP1* vector. β-galactosidase assays were performed on permeabilized cells, as described previously (Tzamarias and Struhl, 1994); values were normalized to A_{600} and represent the average of at least three independent experiments. The *his3* locus of the parental L9FT4 strain contains a single LexA operator upstream of the T_R TATA element (Tzamarias and Struhl, 1994). Yeast transformants were assayed for *his3* expression by growth on SC media containing various amounts of aminotriazole (3-AT), a competitive inhibitor of the *his3* gene product.

Complementations tests for the various protein fusions were performed as follows. TBP function was assayed in strain BYΔ1 by plasmid shuffling, as described previously (Cormack et al., 1991). TAF17 and TAF23 function was assayed in shutoff strains containing conditional TAF alleles that are expressed only in the absence of copper (Moqtaderi et al., 1996); thus, complementation assays were performed on SC media containing 500 μM copper. Srb2 function was assayed by complementation of the severe defect at 37°C conferred by an *srb2* deletion (Koleske et al., 1992). For the experiment shown in Figure 5, molecules expressing the indicated hybrid proteins along with the JK103 reporter were transformed into the null strains described above for TBP, TAF17 and TAF23, Srb2, or the *gal11* deletion strain (Himmelfarb et al., 1990) and assayed for β-galactosidase activity.

Acknowledgments

We thank Zarmik Moqtaderi, Mark Ptashne, Luc Gaudreau, Fred Winston, and Rick Young for strains and plasmids; Alcide Barberis, Mario Mencia, Zarmik Moqtaderi, and Laurie Stargell for useful advice and discussion; and Michel Strubin and Steve Buratowski for comments on this manuscript. This work was supported by a National Institutes of Health Postdoctoral Fellowship (GM17930) to M. K. and by grants from the National Institutes of Health (GM30186) and the Lucille Markey Trust to K. S.

Received February 25, 1998; revised March 26, 1998.

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