

Activation of Transcription In Vitro by Recruitment of the Yeast RNA Polymerase II Holoenzyme

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Summary

It has been argued that many transcriptional activators work by “recruitment,” that is, by helping the transcriptional machinery bind stably to DNA. We demonstrate here a realization of a strong prediction of this idea in an in vitro transcription reaction performed with purified yeast RNA polymerase II holoenzyme and a classical transcriptional activator. We show that the level of transcription reached by the activator working on low concentrations of holoenzyme can also be reached in the absence of activator by raising the holoenzyme concentration, and that under that condition the activator has no further stimulatory effect. We also show, in agreement with another prediction of the recruitment model, that in a reaction using a holoenzyme purified from cells bearing the “P” mutation, transcription is stimulated by a DNA-tethered peptide that binds the mutant holoenzyme component Gal11P but that lacks a classical activating region.

Introduction

Transcriptional activators that work by recruitment contact the transcriptional machinery and help it to bind stably to a nearby promoter. A different mechanism of activation involves interaction of the activator with the transcriptional machinery stably prebound to the promoter. We know of examples of both mechanisms in bacteria, and studies in vitro with those systems have described a characteristic difference between them: whereas the “activating” effect of activators that work by recruitment can be mimicked by simply raising the polymerase concentration, the effect of activators that work by the second mechanism cannot. This difference presumably reflects a fundamental difference in the nature of the activator–polymerase interactions in the two cases. That is, recruitment, in its simplest form, requires only a “noninstructional” interaction: one that provides binding energy so that the polymerase and the activator bind cooperatively. In contrast, the alternative mechanism evidently requires a more elaborate interaction, one that, for example, induces a conformational change in the prebound polymerase that triggers the initiation of transcription. Recruitment lends itself readily to synergistic activation by multiple activators and allows activators to be used in many different combinations to control transcription. A variety of experiments have led to the suggestion that many typical eukaryotic transcriptional activators work by recruitment. For a further discussion of these matters, see Ptashne and Gann (1997).

Here, we demonstrate holoenzyme recruitment in vitro using two forms of the yeast pol II holoenzyme and two different modes of activation. For the first, the holoenzyme is isolated from wild-type cells, and the activator (Ptashne and Gann, 1997) bears the DNA binding domain of Gal4 fused to the classical acidic activation domain of the yeast activator Hap4 (Wang et al., 1995). We find that the activator raises the level of transcription when the holoenzyme concentration is low; as predicted by the recruitment model, however, that level of transcription can also be obtained by raising the concentration of the holoenzyme in the absence of activator. Also as predicted, the activator has no stimulatory effect on transcription directed by the higher levels of holoenzyme.

For the second experiment, we use holoenzyme isolated from the Gal11P strain of yeast, analysis of which was crucial in formulating the recruitment model (Himmelfarb et al., 1991; Barberis et al., 1995; Farrell et al., 1996). The activator, which lacks a classical activating region, bears instead a peptide (a portion of the Gal4 dimerization region) that specifically binds to the mutant form of the holoenzyme component (Gal11) called Gal11P (Potentiator) (Barberis et al., 1995; Farrell et al., 1996; Ptashne and Gann, 1997). In vitro, the Gal4 dimerization region binds to purified Gal11P protein, but not to the wild-type form from which it differs by a single amino acid, and it activates transcription in Gal11P but not in wild-type cells. The strength of the interaction with a series of Gal11P mutants, as assayed in vitro, correlates with the degree of activation observed in vivo. These and other results indicated that the P mutation has created a novel interaction between an otherwise inert peptide and the holoenzyme, and that the binding energy of that interaction suffices—provided the peptide is tethered to DNA—to trigger gene activation by simply bringing the holoenzyme to the DNA. Here, we show that, as predicted, the Gal4 dimerization region, tethered to DNA, stimulates transcription directed by the Gal11P holoenzyme, but has no effect on wild-type holoenzyme.

Results

We purified RNA polymerase II holoenzyme from wild-type and from Gal11P cells according to Koleske et al. (1996). Holoenzyme purified in this way migrates on a sizing column with a molecular weight of roughly 2 million daltons (not shown), and protein immunoblot analysis confirms the presence of several known holoenzyme components (see Figure 1). Addition of TBP and TFIIE allows either holoenzyme to transcribe promoter-containing DNA, and the reaction can be stimulated by the addition of activators provided the DNA template also bears the appropriate activator binding sites (see Figure 2 and, for example, Koleske and Young, 1994; Kim et al., 1994; L. G., M. A., and M. P., unpublished data). The DNA template used in our experiments typically bears four Gal4 binding sites upstream of the adenovirus *E4*

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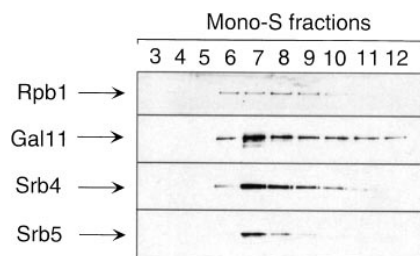


Figure 1. Purification of the Pol II Holoenzyme(s)

The fractions shown here were eluted from a Mono-S column, the last chromatographic step in our holoenzyme purification. The immunoblot analysis shows the presence of RNA polymerase II (Rpb1), Gal11 (or Gal11P in the case of a Gal11P holoenzyme), and Srb proteins (Srb4 and Srb5).

promoter; this template has previously been characterized in a yeast *in vitro* transcription system (Ohashi et al., 1994; Wu et al., 1996).

Figure 2 shows that, in an experiment performed with wild-type holoenzyme, the activator Gal4+Hap4 stimulates transcription markedly when the holoenzyme is present at a concentration arbitrarily numbered 1 (lanes 5 and 6). At twice that holoenzyme concentration, both the "basal" and the activated levels of transcription are higher but the degree of activation is less (lanes 3 and 4), and at a 4-fold higher holoenzyme concentration, the "basal" level of transcription is high and indistinguishable from that observed in the presence of activator (lanes 1 and 2).

Figure 3 shows that, in an experiment performed with holoenzyme isolated from a Gal11P strain, the dimerization region of Gal4 (residues 58–97), tethered to DNA in the form of the fragment Gal4(1–100), stimulates transcription. The figure also shows that Gal4(1–100), which lacks a classical activating region, did not stimulate transcription by wild-type holoenzyme. In experiments not

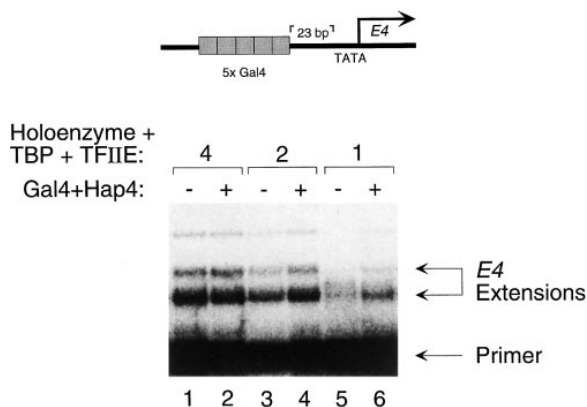


Figure 2. Stimulation of Transcription by a Classical Activator

For this *in vitro* transcription experiment, wild-type holoenzyme with TBP (1 pmol), TFIIE (0.5 pmol), and DNA template (0.3 pmol) was used with and without Gal4+Hap4 (1 pmol). Lanes 3 and 4 contain a 2-fold dilution of holoenzyme concentration used in lanes 1 and 2 (~0.5 pmol). Lanes 5 and 6 contain a further 2-fold dilution of that enzyme to lanes 3 and 4. The double arrow shows the *E4* extension products, and the single arrow shows the primer.

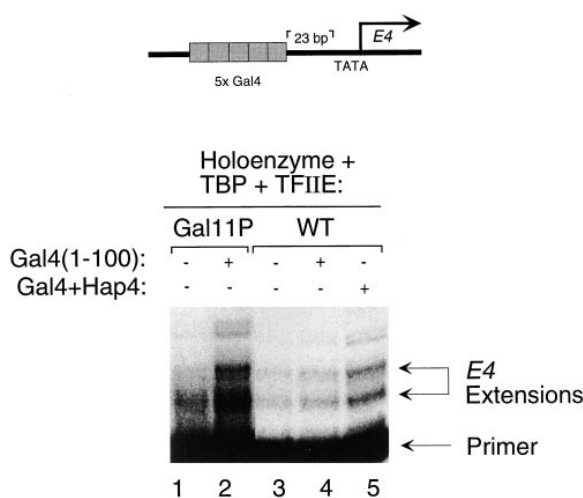


Figure 3. Stimulation of Transcription by Gal4(1–100)

(Lanes 1 and 2) *In vitro* transcription of holoenzyme isolated from Gal11P cells. Lane 1 is a transcription reaction in the absence of Gal4(1–100), and lane 2 is a reaction with 3 pmol of Gal4(1–100). (Lanes 3–5) The holoenzyme used was isolated from wild-type cells, and the activator was Gal4(1–100) (3 pmol; lane 4) or Gal4+Hap4 (1 pmol; lane 5). TBP and TFIIE were added as in Figure 2, and 50 fmol of DNA template was used with 0.11 pmol of nonspecific DNA (pGEM3Z; Promega). The double arrow shows the *E4* extension products, and the single arrow shows the primer.

shown, we found that, as expected, the Gal11P holoenzyme also responded to classical activators (e.g., VP16+Pho4).

Discussion

Our experiments show that, *in vitro*, increasing the concentration of the holoenzyme in the vicinity of the promoter—either by increasing the total concentration of wild-type holoenzyme or by interaction of the Gal11P-containing holoenzyme with a DNA-tethered peptide lacking a classical activating region—significantly increased the level of transcription. That level of transcription could also be achieved by a classical activator working on lower concentration of holoenzyme; at the higher concentrations, the activator had no effect. These results support the idea that simply bringing the holoenzyme to the DNA suffices, at least at certain genes, to trigger transcriptional activation, and that many transcriptional activators work by that mechanism. This surmise is consistent with a large series of "activator-bypass" experiments performed in yeast (Laurent et al., 1991; Jiang and Stillman, 1992; Barberis et al., 1995; Chatterjee and Struhl, 1995; Klages and Strubin, 1995; Kuchin et al., 1995; Xiao et al., 1995; Apone et al., 1996; Farrell et al., 1996; Gaudreau et al., 1997) and in bacteria in which high levels of transcription are achieved by artificially tethering the machinery near the promoter (Dove et al., 1997; Dove and Hochschild, 1998) or by raising the bacterial polymerase concentration *in vitro* (Meyer and Ptashne, 1980; Ptashne and Gann, 1997); see also Keaveney and Struhl (1998 [this issue of *Mol. Cell*]). The importance of this result lies in the implication

that no very specialized activator–transcriptional machinery interactions are required for gene activation, and that therefore activators can be mixed and matched in different combinations near genes where they work synergistically to control gene expression. As a result, regulatory networks are readily evolved in a fashion that would be more difficult were more specialized activator–transcriptional machinery interactions required. This perspective does not, of course, exclude the possibility that, in some cases at least, eukaryotic activators might perform functions other than or in addition to promoting cooperative binding of the machinery to DNA (see Ptashne and Gann, 1997; Ptashne, 1997). Our experiments measure only the hundred or so 5' residues of the transcript and therefore would not detect any effect of activation (or missing holoenzyme component[s]) required for further elongation; and, of course, the generality of our studies may be restricted by the fact that we have examined only a single reporter promoter.

Experimental Procedures

Protein Purification

Wild-type holoenzyme was purified through the Mono-S step as described in Koleske et al. (1996) using BJ2168 (*MAT α ura3-52 leu2 prb1-1122 pep4-3 prc1-407 gal2*; Ohashi et al., 1994). Gal11P holoenzyme was purified similarly from a *gal11* derivative of BJ2168 (JBY1; gift of J. Brickman) expressing Gal11P (N342V) from plasmid pSO32 (Farrell et al., 1996). Gal11P holoenzyme purified from the Mono-S step was applied to a Sepharose CL-4B gel filtration column (1.6 $\times$ 60 cm; Pharmacia) at a flow rate of 1 ml/min. Holoenzyme eluted in a peak corresponding to 25%–50% of the column volume, a result that is in accord with previously published data (Kim et al., 1994). This size-exclusion column was calibrated with blue dextran (2 MDa; Sigma). Peak fractions were then concentrated on a 1 ml Hitrap-Q column (Pharmacia). Gal4(1–100) and Gal4+Hap4 were generous gifts of N. Simkovich and S. Hahn, respectively. Recombinant yeast His-tagged TBP was purified using an *E. coli* expression plasmid (Stargell and Struhl, 1995), and both yeast TFIIIE subunits (Tfa1 and Tfa2) were purified from *E. coli* as described in Feaver et al. (1994).

Protein Immunoblot Analysis

All Western blots were carried out according to standard protocols (Harlow and Lane, 1988). Rpb1 polyclonal antibodies were obtained from Santa Cruz Biotechnology Inc. Rabbit polyclonal antibodies were used to detect Gal11/Gal11P, Srb4, and Srb5. Srb4 polyclonal sera was obtained from D. Chao. Gal11P (261–352; a fragment containing the P mutation), and the Srb5 protein used to generate the polyclonal antisera (Cocalico Biologicals) were purified from *E. coli* expression plasmids. Goat anti-rabbit IgG-HRP conjugate was used as a secondary antibody (Biorad) and detected using an ECL-plus chemiluminescent detection kit (Amersham).

In Vitro Transcription

Holoenzyme (1 μ g, which should correspond to approximately 0.5 pmol, or dilutions from that amount), TBP (1 pmol), and TFIIIE (0.5 pmol) were mixed in a total volume of 12.5 μ l and incubated at room temperature for 30 min. Separately, DNA (0.3 pmol for Figure 2 and 50 fmol for Figure 3), NTPs (1 mM each), and, in some cases, activator [1 pmol for Gal4+Hap4 and 3 pmol for Gal4(1–100)] were mixed in a separate volume of 12.5 μ l also for 30 min at room temperature. The two mixes were then added together and incubated for a further 30 min at room temperature. Buffer and salt conditions were as described (Koleske et al., 1996). Reactions were stopped and subjected to primer extension analysis (Carey et al., 1990). The cDNAs were then resolved on a 10% acrylamide gel and subsequently dried and exposed on Kodak Biomax-MS film.

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