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Distinguishing between Mechanisms of Eukaryotic Transcriptional Activation with Bacteriophage T7 RNA Polymerase

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Summary

To distinguish between mechanisms of eukaryotic transcriptional activation, we tested whether yeast upstream promoter elements can stimulate transcription by a heterologous transcription machinery, bacteriophage T7 RNA polymerase. The *gal* enhancer-like element recognized by GAL4 protein or the *ded1* poly(dA-dT) element was placed upstream of the T7 promoter and *his3* structural gene, and T7 RNA polymerase was produced in yeast cells. Under conditions where the *gal* element would normally be either activating or nonactivating, *his3* transcription by T7 RNA polymerase was not stimulated above the level observed in the absence of any upstream element. In contrast, the *ded1* poly(dA-dT) element stimulated transcription 7-fold, similar to the enhancement observed on the native *ded1* promoter. Activation by the *ded1* element thus may involve effects on the chromatin template that facilitate entry of the transcription machinery, whereas activation by the *gal* element may involve specific contacts between GAL4 and the transcriptional machinery.

Introduction

Numerous models for how enhancer elements and upstream activating sequences (UASs) stimulate transcription by eukaryotic RNA polymerase II can be classified into two kinds, "chromatin accessibility" and "protein–protein contact" (Figure 1). In chromatin accessibility models, enhancer elements (in association with specific DNA-binding proteins or by virtue of an unusual property inherent in their DNA sequences) cause changes of local chromatin structure such that other proteins involved in transcriptional initiation gain access to the chromatin template. In protein–protein contact models, enhancer-binding proteins activate transcription by interacting directly with other proteins of the transcription machinery. Although various specific versions have been proposed, the distinction between these two classes of models is that in chromatin accessibility mechanisms, there are no direct interactions between enhancer-binding proteins and the transcription machinery.

Chromatin accessibility models have been proposed because DNA in chromatin, the natural template in vivo, is relatively inaccessible to proteins. Initial support for such models came from observations that the chromatin structure of active genes differs from that of inactive genes, particularly around the promoter region (Weintraub and Groudine, 1976; Wu, 1980; Weisbrod, 1982). Actively transcribed genes have an increased sensitivity to DNAase I, and promoter regions are associated with DNAase I–hypersensitive sites. For example, the SV40 enhancer, which usually is associated with a stretch of nucleosome-free DNA several hundred base pairs in length, alters the pattern of DNAase I hypersensitivity in adjacent DNA (Varshavsky et al., 1979; Saragosti et al., 1980). Topoisomerase II cleavage sites are located predominately in or near the SV40 enhancer, suggesting that the structural change is due to local supercoiling (Yang et al., 1985).

Protein–protein contact mechanisms are analogous to the mechanism proposed for positive control of transcription in bacteriophage λ, for which it is believed that the cI protein binds adjacent to the promoter and activates transcription by contacting the host RNA polymerase (Hochschild et al., 1983). To account for the fact that eukaryotic enhancer elements can function at long and variable distances from promoters and in either orientation, several models have been proposed. Current evidence favors a "looping" model in which enhancer-binding proteins interact with the transcription machinery bound at the promoter region through bending of the intervening DNA (reviewed by Ptashne, 1986). For example, enhancer activity is influenced by the helical relationship between the enhancer and other promoter elements (Takahashi et al., 1986), much like the situations involved in repression of the bacterial *araBAD* promoter (Dunn et al., 1984) and in cooperative interactions between two molecules of λ repressor (Hochschild and Ptashne, 1986). Such loops in DNA mediated by prokaryotic DNA-binding proteins have been directly visualized by electron microscopy (Griffith et al., 1986).

Characterizaton of two yeast UAS-binding proteins, GAL4 and GCN4, provides a clue for how these eukaryotic activator proteins exert their functions (Brent and Ptashne, 1985; Keegan et al., 1986; Hope and Struhl, 1986). Analyses of deletion and hybrid proteins indicate that GAL4 and GCN4 are organized into separate functional domains for DNA binding and transcriptional activation. In both cases, short acidic regions are sufficient for the activation function (Hope and Struhl, 1986; Ma and Ptashne, 1987), suggesting that these regions do not encode catalytic activities such as topoisomerases, nucleases, and methylases. Instead, it has been proposed (Hope and Struhl, 1986) that these acidic regions represent surfaces that are used for interactions with other proteins. However, these observations do not distinguish between the two classes of models described above because the acidic regions could interact directly with the transcription machinery, or they could interact with chromatin structural proteins such as histones, which are highly basic.

In addition to upstream regulatory elements that interact with known activator proteins, there are naturally occurring poly(dA-dT) sequences that act as upstream elements for constitutive expression (Struhl, 1985a). Detailed analysis of the divergently transcribed *his3* and *pet56* genes sug-

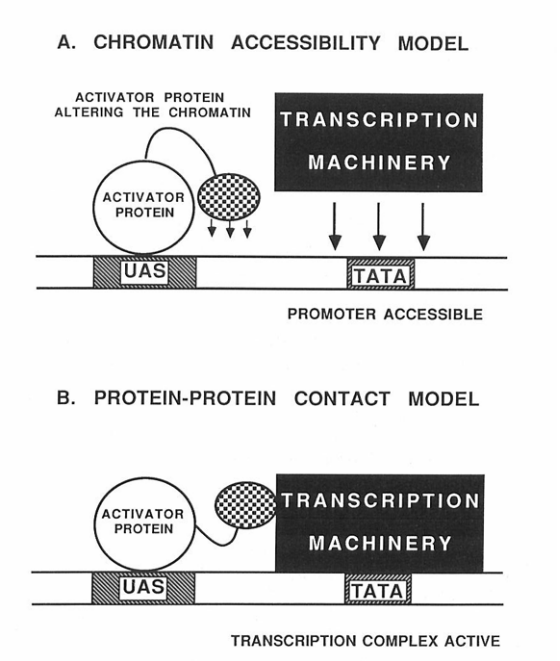

Figure 1. Two Models for Transcriptional Activation by Upstream Activating Elements

(A) Chromatin accessibility models. Activator proteins interact with a UAS to cause a change in local chromatin structure such that the transcription machinery gains access to the chromatin template. (B) Protein–protein contact models. Activator proteins interact directly with other proteins of the transcription machinery to form a functional transcription complex. Activator proteins contain a DNA-binding domain (open circle) and a transcriptional activation region (checkered circle).

gests that constitutive promoters utilizing poly(dA-dT) sequences have properties different from those of inducible promoters involving specific activator proteins (Struhl, 1986). From the unusual structural features of poly(dA-dT) sequences and from the observation that longer sequences promote higher levels of transcription, it has been proposed that poly(dA-dT) sequences might function by providing an entry site for the transcription machinery, perhaps by excluding nucleosomes (Struhl, 1985a).

A major problem in distinguishing between chromatin accessibility and protein–protein contact models is that the eukaryotic transcription machinery is composed of at least 10 proteins that are subunits of RNA polymerase II as well as several (possibly many) other proteins that are less well characterized. However, the two classes of models can be distinguished in the following way. Models that depend on accessibility to the chromatin template and not on specific interactions predict that activation should not depend on the particular transcription machinery. In contrast, protein–protein contact models predict that activation should be specific to the normal RNA polymerase II transcription machinery, because eukaryotic activator proteins should not activate a heterologous transcription machinery with which they could not make specific contacts.

Here we determine whether yeast upstream promoter elements are able to activate transcription by a heterologous and well-defined transcription machinery, bacteriophage T7 RNA polymerase. T7 RNA polymerase, a single polypeptide of 98,000 daltons, initiates transcription from T7 promoters with extremely high specificity and efficiency without requiring any other cofactors (Chamberlin and Ryan, 1982). We find that the poly(dA-dT) element necessary for constitutive *ded1* expression stimulates transcription by T7 RNA polymerase, whereas the enhancer-like element recognized by GAL4 protein does not. These results suggest that yeast cells use both chromatin accessibility and protein–protein contact mechanisms to activate transcription.

Results

Experimental Design

To determine whether yeast upstream elements enhance transcription by T7 RNA polymerase, we introduced two kinds of DNA molecules into yeast cells. The first set of molecules were capable of producing T7 RNA polymerase in yeast. The second set were target molecules in which the yeast upstream element of interest was fused upstream of the T7 promoter, which itself was fused directly to the *his3* mRNA coding sequences. The target molecules, which contain either the enhancer-like element recognized by GAL4 protein or the constitutive poly(dA-dT) element from the *ded1* gene, are similar to previously described *gal-his3* (Struhl, 1984) or *ded1-his3* (Chen and Struhl, 1985) fusions except that the yeast TATA element has been replaced by the T7 promoter. In this way, the yeast (TATA-dependent) transcription machinery was effectively replaced with T7 RNA polymerase. Thus, by comparing levels of *his3* RNA initiated from target or control molecules, it is possible to determine whether transcription by T7 RNA polymerase is enhanced by yeast upstream elements.

Introducing T7 RNA Polymerase into Yeast Cells

We wanted to produce T7 RNA polymerase in yeast cells at various levels for three reasons. First, we were concerned that making too much T7 RNA polymerase would be detrimental to cell growth because overproduction of the enzyme in the presence of the T7 promoter can be lethal to Escherichia coli cells (Tabor and Richardson, 1985; Studier and Moffatt, 1986). Second, since it seemed unlikely that T7 RNA polymerase would contain a signal for nuclear localization in yeast, we wished to make enough of the enzyme to increase the chances for its entry into nucleus. Third and most important, we wanted to achieve an appropriate level of T7 RNA polymerase so that transcription by this enzyme would be rate-limiting.

Based on these considerations, the gene encoding T7 RNA polymerase was cloned into three different expression vectors (Figure 2). Yeast cells containing these plasmids were lysed and assayed for T7 RNA polymerase activity. It was found that they produced T7 RNA polymerase at levels of 0.01%, 1%, and 4% of total cellular protein. The cells that were making the highest amount of T7 RNA polymerase grew a little more slowly than normal. These three plasmids were then introduced into cells containing the T7 promoters in various configurations on the chromosome.

Plasmids	DNA elements for expressing T7 RNA polymerase	DNA elements for propagating plasmids	T7 RNA Polymerase	
			Glucose	Galactose
YCp86	—	ars1,cen3	<2	<2
YCp86-Sc3694	P_{Ded1} → RNApol (H R)	ars1,cen3	24	50
YCp86-Sc3695	P_{Gpdh} → RNApol → T_{Gpdh} (S K H B)	ars1,cen3	2500	2200
AB701	—	2μ origin	<2	<2
AB701-Sc3696	P_{Gpdh} → RNApol → T_{Gpdh} (S K H B)	2μ origin	9500	5300

Figure 2. Plasmids for Expression of T7 RNA Polymerase in Yeast

Vectors for expressing T7 RNA polymerase contain the *ura3* gene and the following genetic elements: *ded1* promoter (gray bar); *gpdh* promoter (striped bar); *gpdh* terminator region (black bar); *ars1*, a yeast chromosomal sequence that permits autonomous replication; 2μ, the replication origin of the native yeast plasmid; and *cen3*, the centromere sequence of chromosome III. Restriction sites used for cloning are abbreviated as H (HindIII), R (EcoRI), S (SalI), K (KpnI), and B (BamHI). The specific activities of T7 RNA polymerase in plasmid-containing strains grown in glucose or galactose medium are measured in units per mg protein. Purified T7 RNA polymerase has a specific activity of 255,000.

Transcription from the T7 Promoter in Yeast Is Dependent on T7 RNA Polymerase

A 56 bp EcoRI fragment containing the T7 promoter was inserted in either orientation between UAS_G, the galactose-dependent enhancer-like element, and the *his3* structural gene (Figure 3A). The resulting DNA molecules were introduced into yeast strain KY320 such that they replaced the *his3* locus. Cells containing these T7 promoter derivatives were transformed with plasmids containing the T7 RNA polymerase gene. The resulting strains were grown in the medium containing galactose, and the levels of *his3* RNA were quantitated by S1 nuclease analysis using the level of *ded1* RNA as an internal control.

As expected, *his3* transcription was observed only when the T7 promoter was oriented correctly with respect to the *his3* structural gene (Figure 3B, lane 7). *his3* transcripts initiated by T7 RNA polymerase were not observed when the T7 promoter was absent (Figure 3B, lane 3) or in the reversed orientation (lane 5), nor were they observed

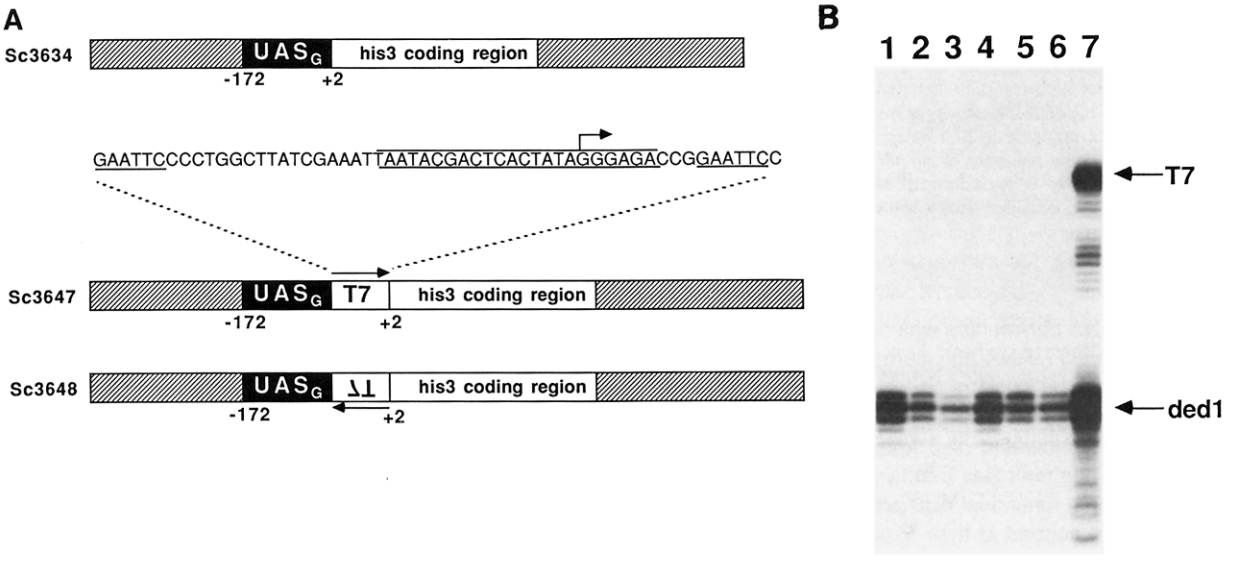

Figure 3. his3 Transcription by T7 RNA Polymerase

(A) DNA structures. The shaded bar represents a 6.1 kb segment containing the *pet56*, *his3*, and *ded1* genes. The black bar indicates the 365 bp DNA segment containing UAS_G, replacing the region between −172 and +2 of the *his3* gene (Struhl, 1984). The nucleotide sequence of the 56 bp EcoRI fragment containing the T7 promoter is shown. The 23 bp T7 promoter is overlined and underlined, EcoRI sites are underlined, and the arrow indicates the site used for transcription initiation by T7 RNA polymerase. The T7 promoter fragment was inserted in either orientation at the EcoRI site at position +2.

(B) RNA analysis. RNAs from cells containing the T7 promoter and/or T7 RNA polymerase, grown in galactose medium, were hybridized to an excess of the *his3* and *ded1* oligonucleotide probes (see Experimental Procedures) and treated with S1 nuclease. Lane 1, untransformed strain KY320 (deletion of the *his3* structural gene); lanes 2, 4, and 6 contain Sc3634 (no T7 promoter), Sc3648 (T7 promoter in reversed orientation), and Sc3647 (T7 promoter in correct orientation), respectively, in the absence of T7 RNA polymerase; lanes 3, 5, and 7 contain Sc3634, Sc3648, and Sc3647, respectively, in the presence of high levels of T7 RNA polymerase (AB701-Sc3696). The positions of the bands corresponding to the *ded1* and T7-initiated *his3* RNAs are indicated. (Because of the +2 break point of the *his3* alleles, the band corresponding to the T7-initiated transcript is 67 nucleotides). The specfic activity of the *his3* probe is about 5-fold lower than that of the *ded1* probe.

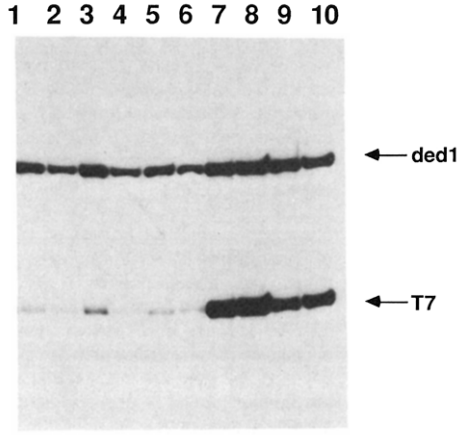

Figure 4. Effect of UAS_G on Transcription by T7 RNA Polymerase

Strains containing Sc3647 (where UAS_G is upstream of the T7 promoter; see Figure 3A) and plasmids capable of synthesizing various amounts of T7 RNA polymerase (see Figure 2) were grown in glucose (odd lanes) or galactose (even lanes) medium. RNAs from such cells were hybridized to an excess of strand-separated *his3* and *ded1* probes (see Experimental Procedures) and treated with S1 nuclease. Lanes 1 and 2, YCp86 (control plasmid lacking the T7 RNA polymerase gene). Lanes 3 and 4, AB701 (control plasmid lacking T7 RNA polymerase gene). Lanes 5 and 6, YCp86-Sc3694 (expresses 24 and 50 units/mg of T7 RNA polymerase); lanes 7 and 8, AB701-Sc3696 (expresses 9,500 and 5,300 units/mg of T7 RNA polymerase); lanes 9 and 10, YCp86-Sc3695 (expresses 2,500 and 2,200 units/mg of T7 RNA polymerase) (see Figure 2). The faint bands at the position marked T7 in lanes 1–6 are probably due to low levels of read-through *his3* transcription. (Because of the nature of the *his3* probe and the +2 break point of Sc3647, *his3* transcripts initiated at the T7 promoter or at any position upstream of the +2 site cannot be distinguished.) These bands are not observed in Figures 3B and 5B because in this experiment the specific activity of the *his3* probe is about 3-fold higher than that of the *ded1* probe, and the autoradiographic exposure is longer. The relatively high specific activity of the *his3* probe also accounts for why the bands representing T7-initiated transcripts appear somewhat more intense in the presence of the *gal* element (lanes 7–10) than in its absence (Figure 5B, lanes 5 and 6); when correctly normalized, the amount of *his3* transcription relative to the *ded1* control is similar within experimental error.

when T7 RNA polymerase was not produced (lanes 2, 4, and 6). Thus T7 RNA polymerase produced in yeast is able to enter the nucleus and can faithfully initiate transcription from its own promoter. Interestingly, strains producing the T7-initiated *his3* transcripts were phenotypically His^-; this indicates that these transcripts were not translated into functional *his3* protein, probably because they are not capped at their 5′ ends.

UAS_G Does Not Affect Transcription by T7 RNA Polymerase in Yeast

The UAS_G element in these experiments is the 365 bp fragment located between the divergently transcribed *GAL1* and *GAL10* genes (St. John and Davis, 1981; Guarente et al., 1982). It is responsible for the 500-fold induction of transcription of these genes that occurs when cells are grown in the medium containing galactose. UAS_G has the properties of a eukaryotic enhancer element in that it activates transcription when placed at long and variable distances from a promoter and in either orientation (Struhl, 1984). Binding of GAL4 protein to this sequence, which occurs only in galactose medium (Giniger et al., 1985), leads to transcriptional activation. Thus we can determine whether UAS_G has any activation effect on T7 transcription by comparing levels of *his3* transcripts in cells grown in galactose versus glucose medium.

The results of these experiments are shown in Figure 4. First, the level of *his3* RNA is roughly proportional to the amount of T7 RNA polymerase that is produced, indicating that transcription by T7 RNA polymerase is rate-limiting. Second, at any of the three different levels of T7 RNA polymerase, there is no detectable difference in the level of transcription in cells grown in glucose versus galactose medium, showing that UAS_G has no transcription-activating effect on the T7 promoter. The level of *his3* transcription observed in glucose or galactose medium is similar to that observed in a control experiment in which the UAS_G element was removed. (When normalized to the amount of *ded1* RNA and corrected for the specific activities of the probes, the difference is less than a factor of 2; see Figure 5B.) Thus, although GAL4 protein bound to UAS_G greatly stimulates transcription by yeast RNA polymerase II, it is incapable of stimulating transcription by T7 RNA polymerase under conditions where enhanced levels could have been observed (i.e., nonsaturating enzyme concentrations).

A Poly(dA-dT) Upstream Element Enhances Transcription by T7 RNA Polymerase

To determine whether poly(dA-dT) sequences could affect T7-mediated transcription, we constructed a molecule in which UAS_G was replaced by the upstream element that is responsible for the constitutive transcription of the *ded1* gene. The *ded1* upstream element contains a stretch of 34 bp with 28 dT residues on the coding strand (Figure 5A). As a control, we constructed a molecule lacking both the *ded1* upstream element and UAS_G. As described above, these derivatives were introduced into KY320 to replace the *his3* locus. The resulting strains were transformed with the set of plasmids containing the T7 RNA polymerase gene and were subsequently assayed by S1 analysis for their levels of *his3* transcription.

In striking contrast to the results obtained with UAS_G, the *ded1* poly(dA-dT) sequence significantly enhanced transcription by T7 RNA polymerase from the T7 promoter (Figure 5B). Densitometric scanning using an internal control showed that when T7 RNA polymerase was 1% of cellular protein, *his3* transcription in cells having this poly(dA-dT) sequence upstream of the T7 promoter was 7.5-fold higher than in cells without this sequence (compare lanes 5 and 10 in Figure 5B). The enhancement was slightly lower, 4.2-fold, when T7 RNA polymerase was 4% of the cellular protein (compare lanes 6 and 11 in Figure 5B), possibly because the level of T7 RNA polymerase was approaching saturation.

These enhancement effects due to poly(dA-dT) sequences were only observed in vivo. Transcription in vitro by T7 RNA polymerase of purified YIp55-Sc3684 DNA, which contains the *ded1* poly(dA-dT) element, or YIp55-Sc3680 DNA, which lacks this element, produced similar

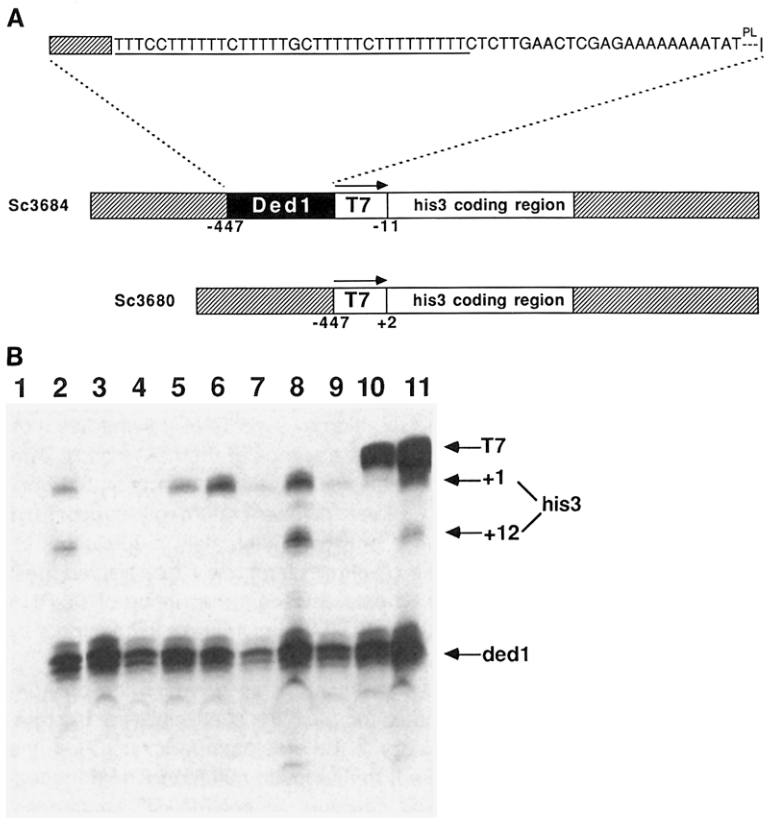

Figure 5. Effect of the *ded1* Poly(dA-dT) Element on Transcription by T7 RNA Polymerase

(A) DNA structures. The shaded bar represents a 6.1 kb segment containing the *pet56*, *his3*, and *ded1* genes. For Sc3684, the black bar indicates the DNA segment containing the *ded1* poly(dA-dT) element, which replaces the region between −447 and −11 of the *his3* gene (Struhl, 1985a). The T7 promoter was inserted in the correct orientation at the EcoRI site at −11. The nucleotide sequence of the coding strand near the fusion point includes the poly(dA-dT) element (underlined), the TAT nucleotides of the *ded1* TATA element (last three nucleotides), and 15 bp of the mp18 polylinker (sequence not shown). The control molecule, Sc3680, is essentially the same as Sc3684 except that it lacks the *ded1* promoter fragment, and the break point in the *his3* mRNA coding region is at position +2.

(B) RNAs from strains grown in glucose medium, containing Sc3684 (where the *ded1* element is upstream of the T7 promoter; lanes 7–11) or Sc3680 (lacking the *ded1* element; lanes 3–6) and either plasmids capable of synthesizing T7 RNA polymerase (see Figure 2) at 25 units/mg (YCp86-Sc3694; lanes 4 and 9), 2400 units/mg (YCp86-Sc3695; lanes 5 and 10), or 9500 units/mg (AB701-Sc3696; lanes 6 and 11) or control plasmids (YCp86, lane 7; AB701, lanes 3 and 8), were hybridized to an excess of *his3* and *ded1* oligonucleotide probes (see Experimental Procedures and Figure 3B) and treated with S1 nuclease. Because of the nature of Sc3684, the T7-initiated RNAs protect the entire oligonucleotide probe and hence produce hybridization products of 76 nucleotides (T7 arrow). The positions of the normal +1 and +12 *his3* transcripts (corresponding to bands of 68 and 57 nucleotides) and the *ded1* internal control are also indicated. For strains containing Sc3680, the T7-initiated RNAs produce a band of 67 nucleotides, which is indistinguishable from transcripts representing initiation from the normal +1 site; however, this band is clearly due to T7-initiated transcripts since at this exposure it is not observed in the absence of T7 RNA polymerase (lane 3). Although the *his3* fusion points of Sc3680 and Sc3684 are not identical, other experiments indicate that varying the fusion point does not affect the level of T7-initiated transcripts (data not shown). The apparently high levels of the *his3* +1 and +12 transcripts in lane 8 simply reflect a 3-fold overloading of RNA (as shown by the intensity of the *ded1* transcript). The specific activities of the *his3* and *ded1* probes are similar.

levels of RNA when the templates were linear (Figure 6) or supercoiled (data not shown). Moreover, templates with or without the poly(dA-dT) element supported similar levels of transcription in vitro over a range of enzyme concentrations, salt concentrations, and temperature (data not shown). Thus the enhancement effect by the *ded1* poly(dA-dT) element observed in vitro reflects properties of the chromatin template, not inherent properties of the enzyme.

The 7-fold enhancement effect of the *ded1* element on transcription by T7 RNA polymerase is very similar to its enhancement of transcription by yeast RNA polymerase II. Deletion of the poly(dA-dT) element from the intact *ded1* promoter reduces transcription by a factor of 8 (S. Kanazawa and K. Struhl, unpublished results). Moreover, as shown in Figure 5B, the *ded1* poly(dA-dT) element also increased the level of *his3* transcription by RNA polymerase II even when the promoter lacked a TATA element. Transcripts initiated at the normal +1 and +12 sites, which are presumably synthesized by RNA polymerase II, are observed in the presence of the *ded1* poly(dA-dT) element (Figure 5B, lanes 7–11), whereas at this autoradiographic exposure they are not seen in the absence of this element (Figure 5B, lanes 3–6; transcription initiated at +1 cannot be assessed in lanes 5 and 6 because of comigration of the transcript initiated by T7 RNA polymerase). An overexposure of the experiment shown in Figure 5B indicates that the poly(dA-dT) element causes a 5–20-fold increase in level of normal *his3* transcription (precise quantitation is difficult due to the low levels). As expected, the levels of these RNA polymerase II transcripts were 5–10-fold lower than the levels observed in analogous *ded1-his3* fusion promoters containing the *ded1* TATA element (Chen and Struhl, 1985).

Discussion

Different Upstream Elements Activate Transcription by Distinct Mechanisms

The experiments in this study were designed to distinguish between chromatin accessibility and protein–protein contact models for the mechanism of transcriptional activation by eukaryotic upstream elements. The two classes of models predict different outcomes for whether upstream elements can stimulate transcription by T7 RNA polymerase in yeast cells. Chromatin accessibility models predict that activation should not depend on the particular

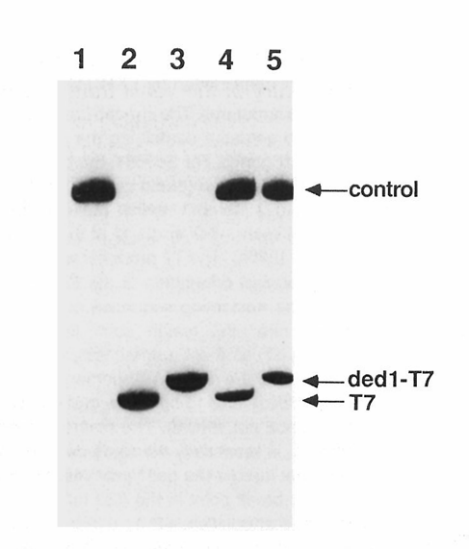

Figure 6. Transcription In Vitro by T7 RNA Polymerase

YIp55-Sc3684, which contains the *ded1* poly(dA-dT) element, and YIp55-Sc3680, which lacks the *ded1* element (see Figure 5A), were digested with HindIII such that transcription by T7 RNA polymerase would generate run-off transcripts of 339 nucleotides (for Sc3684) or 327 nucleotides (for Sc3680). A control plasmid containing a T7 promoter was similarly cleaved such that transcription would generate a 500 nucleotide RNA species. Lane 1, control plasmid; lane 2, T7 plasmid (Sc3680); lane 3, *ded1*-T7 plasmid (Sc3684); lane 4, equimolar mixture of control and T7 plasmids; lane 5, equimolar mixture of control and *ded1*-T7 plasmids.

transcription machinery; hence, upstream elements that operate by this mechanism should also stimulate T7 transcription. In contrast, elements that work by protein–protein contact should not stimulate transcription by T7 RNA polymerase because it is very unlikely that yeast activator proteins could make the required specific contacts with this prokaryotic enzyme.

The observation that transcription by T7 RNA polymerase is stimulated by the *ded1* poly(dA-dT) element but not by UAS_G strongly suggests that these upstream elements mediate their effects by different molecular mechanisms. Moreover, since enhancement of transcription by T7 RNA polymerase can be observed in one case, a failure to detect such enhancement in another case cannot be explained simply as an artifact of the experimental design. In terms of the models shown in Figure 1, the results are consistent with the view that the *ded1* poly(dA-dT) element enhances transcription by increasing chromatin accessibility, whereas the enhancer-like element UAS_G activates transcription through bound GAL4 protein contacting other components of the yeast transcription machinery. Potential mechanisms for activation by the *ded1* element or UAS_G will be discussed below.

Poly(dA-dT) Sequences Enhance Transcription by Increasing Chromatin Accessibility

The fact that the *ded1* poly(dA-dT) upstream element enhances transcription by T7 RNA polymerase in yeast cells but not in vitro on purified DNA indicates that this enhancement reflects properties of the transcriptional activation process in vivo, rather than some unusual property of T7 RNA polymerase. Moreover, supercoiled or linear DNA templates containing or lacking the poly(dA-dT) element are transcribed in vitro by T7 RNA polymerase with equal efficiency under a variety of conditions. This suggests that the transcriptional enhancement in vivo is not due to special buffer or ionic conditions in the intact cell, nor is it due to effects on supercoiling. Thus, since it seems unlikely that T7 RNA polymerase has any specific interactions with yeast proteins, the most plausible mechanism for transcriptional activation is increased accessibility to the chromatin template due to the poly(dA-dT) element.

The 7-fold enhancement of transcription by T7 RNA polymerase is similar in magnitude to the enhancement observed for transcription by yeast RNA polymerase II either in the presence or absence of the native *ded1* TATA element. These similar enhancement effects on two very different transcription machineries strongly support the view that the *ded1* poly(dA-dT) element mediates its effects through the chromatin template. They also suggest that the *ded1* element activates transcription of T7 RNA polymerase or the yeast RNA polymerase II machinery by a similar mechanism.

There are several mechanisms by which the poly(dA-dT) sequence could increase the accessibility of the transcription machinery to the chromatin template. First, the RNA polymerase II transcription machinery might recognize the unusual structure of poly(dA-dT) sequences. These sequences deviate from the standard B-DNA helix in that they have a helix repeat of 10.0 bp instead of the normal 10.6 bp (Peck and Wang, 1981; Rhodes and Klug, 1981), contain a distinctively narrow minor groove (Alexeev et al., 1987), and are associated with kinks or bends in DNA (Koo et al., 1986). This possibility seems unlikely because there is no evidence that T7 RNA polymerase recognizes such a structure. Second, the transcription machinery could enter the template because poly(dA-dT) sequences might be relatively free of nucleosomes in vivo; such sequences are known to inhibit nucleosome formation in vitro (Kunkel and Martinson, 1981; Prunell, 1982). Third, poly(dA-dT) sequences might be recognized and bound by chromatin-associated nonhistone proteins. For example, α protein from mouse binds to any DNA sequence that is at least 6 bp in length and contains only dA and dT residues; interestingly, it contains an acidic C terminus (Solomon et al., 1986). Perhaps proteins like this could bind poly(dA-dT) sequences and interact with the basic histones, thereby perturbing the chromatin structure.

Comments on the Mechanism Involving GAL4 and Other Activator Proteins

Since transcription by T7 RNA polymerase can be stimulated by the *ded1* poly(dA-dT) element, the failure of UAS_G to enhance transcription argues against a simple chromatin accessibility model in which GAL4 alters a relatively inert chromatin template into a form that is more accessible to the transcription machinery. By this argument, we think it unlikely that the short acidic regions of GAL4 involved in transcriptional activation open the chromatin structure by interacting with histones. However, since T7

and yeast RNA polymerases almost certainly initiate transcription by different mechanisms and probably have different rate-limiting steps, it is possible that GAL4 directly or indirectly alters the chromatin template in a particular way that strongly stimulates transcription by yeast RNA polymerase II but is irrelevant to transcription mediated by T7 RNA polymerase. Alternatively, GAL4 might require an additional factor, such as a protein that binds to yeast TATA elements, to alter chromatin structure. (This model also implies an interaction between GAL4 and the putative TATA-binding protein; see below.)

Although specific chromatin accessibility models cannot be excluded, we believe that our results are more compatible with the view that activation by GAL4 requires protein–protein contacts with the yeast transcription machinery, and that the failure to activate transcription by T7 RNA polymerase reflects an inability to form the correct contacts. In this sense, our experiments provide independent evidence for previous suggestions that the short acidic regions of GAL4 and GCN4 sufficient for the transcriptional activation function do not encode catalytic activities but instead represent surfaces that interact with other proteins (Hope and Struhl, 1986; Ma and Ptashne, 1987; Struhl, 1987). In principle, the interaction between upstream activator proteins and the yeast RNA polymerase II machinery could stimulate transcription by creating an "active transcription complex" and/or by permitting cooperative DNA binding of the relevant components. Although it is unknown which protein(s) of the yeast transcriptional machinery might be contacted, the fact that GAL4 and GCN4 activation of *his3* transcription occurs with only one of the two *his3* TATA elements suggests that these (and presumably other) yeast activator proteins interact with a protein that binds to the relevant TATA element (Struhl, 1986, 1987). Presumably RNA polymerase II would recognize the complex between GAL4 and the putative TATA-binding protein, although the molecular mechanism for the interaction(s) involved is unknown.

Why Are Two Apparently Distinct Mechanisms Used to Activate Transcription?

The protein–protein contact mechanism makes it possible to regulate gene expression in several ways. Different classes of genes can be distinguished simply by virtue of their promoters being recognized by different activator proteins. Coordinate induction can be achieved by having a single activator protein recognize common UAS elements in the promoters of genes whose transcription is to be controlled. For a given gene or set of genes, the rate of transcriptional initiation can be altered by environmental or developmental cofactors that affect the activity or synthesis of the relevant activator proteins. In addition, potential interactions between upstream activator proteins and TATA proteins might be restricted such that transcriptional activation could only occur with certain combinations.

On the other hand, the chromatin accessibility mechanism involving poly(dA-dT) sequences provides a simple way to transcribe genes at a constant rate. Instead of requiring specific protein factors, transcription can be activated simply by poly(dA-dT) sequences creating active chromatin windows for entry of the yeast transcriptional machinery. Moreover, the level of constitutive transcription for a given gene can be set by adjusting the length and/or quality of the poly(dA-dT) sequences. In this way the constitutive transcription of a variety of "unrelated" yeast genes can be "controlled" by a common mechanism.

Experimental Procedures

DNA Manipulations

DNA molecules were generated by standard techniques, and their structures were verified by sequencing double-stranded molecules by the chain-termination method (Chen and Seeburg, 1985). The plasmids used for producing T7 RNA polymerase in yeast cells were constructed as follows.

YCp86-Sc3694 was generated by first inserting a 290 bp Asp718–EcoRI fragment containing the *ded1* upstream and TATA elements (positions 646 to 931 as described by Struhl [1985b]) between the HindIII and EcoRI sites of YCp86, a centromere vector containing the bacterial origin of replication and ampicillin resistance gene from pUC18 for propagation in E. coli, as well as *ura3*, *ars1*, and *cen3* for selection and maintenance of the plasmid in yeast (K. Struhl, unpublished). Then, a 2.7 kb EcoRI fragment containing the T7 RNA polymerase gene (from 38 bp upstream of the AUG initiation codon to 14 bp after the termination codon) was cloned into the EcoRI site. This EcoRI fragment was generated by cloning the 2.7 kb BglII–BamHI fragment of pGP1-8, a derivative of pGP1-2 (Tabor and Richardson, 1985), into pUC7 (Vieira and Messing, 1982) and recutting with EcoRI.

Plasmid AB701-Sc3696 was constructed by introducing the T7 RNA polymerase gene between the KpnI and BamHI sites of AB701, a high-level expression vector obtained from Genetics Institute that contains the promoter and terminator region of the *gpdh* gene, the *gpdh* translational initiation region (the first G of the KpnI site is part of the ATG initiation codon), and a 2μ origin of replication. This was accomplished by ligating a 33 bp synthetic KpnI–XmnI oligonucleotide (containing six additional nucleotides after the ATG codon and the first 25 bp of the T7 RNA polymerase coding region up to the XmnI site), the XmnI (partially cut)–HindIII fragment containing the remainder of the T7 RNA polymerase gene from mGp1-2 (Tabor, unpublished), and KpnI- and HindIII-cut AB701. As a consequence of the construction, two additional amino acids, valine and proline, were added to the N terminus of T7 RNA polymerase. YCp86-Sc3695 was constructed by transferring the SalI–BamHI fragment from AB701-Sc3696, containing the *gpdh* promoter and terminator and T7 RNA polymerase coding region, into YCp86.

All DNA molecules harboring the T7 promoter contain a 6.1 kb segment of yeast chromosomal DNA with the entire *pet56-his3-ded1* gene region (Struhl, 1984, 1985b) cloned in the *ura3*$^+$ vector YIp55 (Struhl, 1986). The T7 promoter was derived from the φ10 promoter of bacteriophage T7 and cloned as a 56 bp EcoRI fragment (Tabor and Richardson, 1985). Molecules containing the UAS_G element are similar to previously described *gal-his3* fusions (Struhl, 1984) except that the 365 bp UAS_G fragment was fused to position +2 with regard to *his3* mRNA. The T7 promoter fragment was inserted at the EcoRI site at the boundary of UAS_G and at position +2 of *his3* mRNA.

YIp55-Sc3684, which contains the *ded1* poly(dA-dT) element upstream of the T7 promoter, was generated by first replacing a BamHI–EcoRI fragment containing the *his3* promoter region (from −447 to −11) by a BclI–EcoRI fragment containing the *ded1* promoter region (nucleotides +572 to +897 as defined by Struhl [1985b]). The *ded1* fragment, which was produced by Jennifer Macke as part of a Bal31-generated deletion series, contains the entire upstream poly(dA-dT) element but lacks a functional TATA element (it contains only TAT of the *ded1* TATA element). The T7 promoter fragment was inserted at the EcoRI site that defines the boundary between the *ded1* element and *his3* mRNA coding sequences. YIp55-Sc3680, which contains the T7 promoter but lacks any yeast upstream element, was constructed similarly except that an EcoRI linker replaced the *his3* promoter region from −447 to +2.

Introducing DNA into Yeast

KY320, the yeast strain used throughout this study, is similar to KY117 (*a, ura3-52, lys2-801, ade2-101, trp1-Δ1, his3-Δ200, GAL⁺*) (Struhl, 1984) except that part of the *leu2* locus (the region between the EcoRI and BstEII sites in the structural gene) has been replaced with the intact *pet56* locus (details will be published elsewhere). DNA molecules containing the T7 promoter (and appropriate control molecules) were introduced into KY320 such that they replaced the *his3* locus (Hill et al., 1986). The resulting strains were then transformed by the set of plasmids capable of producing T7 RNA polymerase by using the *ura3* marker on the expression vectors. For biochemical analysis, yeast transformants were grown in medium that contained 0.6% casamino acids, 50 mg per liter each of tryptophan and adenine, and 2% of either glucose or galactose. This medium lacks uracil and hence is selective for cells containing the *ura3⁺* plasmids producing T7 RNA polymerase.

T7 RNA Polymerase Assay

Fifteen milliliters of each transformed cell was grown to an A_{600} of 1 in either glucose or galactose medium as described above, washed twice with sterile water, and dissolved in 0.4 ml of 1 M sorbitol, 50 mM Tris, 14 mM β-mercaptoethanol. Cells were treated with 40 units of lyticase for 5 min at 30°C and then frozen in dry ice. For making cell extracts, the frozen cells were thawed on ice for 15 min, vortexed four times with 0.7 g of acid-washed glass beads for 45 sec, and spun for 30 min in a microcentrifuge. The specific activity of T7 RNA polymerase was determined as described by Tabor and Richardson (1985).

Analysis of *his3* RNA

The procedures for isolation of total RNA, preparation of ^{32}P-5′-end-labeled probes, DNA–RNA hybridization, nuclease S1 treatment, and product analysis by gel electrophoresis were performed with minor modifications of previous procedures (Chen and Struhl, 1985; Struhl, 1985a, 1985b). RNA preparations were hybridized to completion with an excess of single-stranded *his3* and *ded1* probes; in this way, levels of *his3* RNA can be normalized to the *ded1* internal control. For the experiment shown in Figure 4, the probes were prepared by labeling the 5′ ends of appropriate *his3* and *ded1* restriction fragments and then separating the strands on a 4% native acrylamide gel as described previously (Struhl, 1985b). For the experiments shown in Figures 3 and 5, the *his3* and *ded1* probes were prepared by phosphorylation of synthetic oligonucleotides with T4 polynucleotide kinase. The *his3* oligonucleotide, 76 bases in length, extended from positions +68 to −8 of the native *his3* gene. The *ded1* oligonucleotide, 45 bases in length, contained 41 bases complementary to *ded1* mRNA sequences (nucleotides +161 to +201) and 4 noncomplementary bases at the 3′ terminus; in this way, products due to hybridization of *ded1* RNA (41 nucleotides) can be distinguished from undigested probe (45 nucleotides). In experiments involving the oligonucleotide probes, the hybridization temperature was reduced to 55°C. The levels of *his3* and *ded1* RNAs were quantitated by scanning the autoradiograms with a Beckman DU-6 spectrophotometer. For each determination, the intensity of the *his3* band(s) was normalized to that of the internal *ded1* control. In addition, the relative specific activities of the *his3* and *ded1* probes were determined using a control of yeast RNA from wild-type cells. (With probes of equal specific activity, the ratio of *ded1*:*his3* RNA is 5.) This correction for the relative specific activities of the probes makes it possible to compare *his3* RNA levels that were determined in separate hybridization experiments.

Transcription In Vitro

Plasmid DNAs were prepared by CsCl gradient centrifugation, digested with HindIII, phenol-extracted, and ethanol-precipitated. Transcription reactions were performed with 200 μg of linear DNA(s) and 50 units of purified T7 RNA polymerase in 45 μl reaction mixtures containing 40 mM Tris (pH 7.5), 10 mM $MgCl_2$, 5 mM dithiothreitol, 300 mM each of GTP, ATP, and UTP, and 30 mM [α-^{32}P]CTP (10^8 cpm/nmol) (Tabor and Richardson, 1985). After 15 min, the reaction mixtures were extracted with phenol and chloroform and ethanol-precipitated. About 30% of each reaction product was loaded onto a 6% polyacrylamide gel containing 7 M urea. After electrophoresis, the gel was dried for autoradiography.

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