Gene activation by recruitment of the RNA polymerase II holoenzyme

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The single amino acid “P” (potentiator) mutation in the holoenzyme component GAL11 creates an interaction between that protein and the dimerization region of GAL4. That interaction triggers strong gene activation when the GAL4 fragment is tethered to DNA. Here we show that, among a series of variants of the GAL4 dimerization region and different GAL11P alleles, the strength of the interaction as quantitated *in vitro* correlates with the degree of activation *in vivo*; swapping the protein fragments bearing the GAL4 dimerization region and the GAL11P mutation such that the latter is tethered to DNA and the former is attached to the holoenzyme does not diminish gene activation; gene activation in this system is squelched by overproduction of either a fragment bearing the GAL4 dimerization region or a fragment of GAL11 bearing a P mutation; and neither GAL11 nor GAL11P is a target of an acidic activating region. These results argue that the GAL4–GAL11P interaction triggers gene activation simply by recruiting the holoenzyme to DNA. Consistent with this view, we also show that fusion of LexA to another holoenzyme component, SRB2, creates an activator, and that an SRB2 mutant predicted on genetic grounds to interact especially efficiently with a holoenzyme containing a specific mutant form of polymerase also activates more efficiently when tethered to DNA.

[Key Words: RNA polymerase II holoenzyme; GAL4; GAL11; gene activation]

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Until recently it was generally accepted that the initiation of transcription of a typical gene by RNA polymerase II required the stepwise formation on DNA of a large protein complex that included as one of its components the polymerase itself (for review, see Conaway and Conaway 1993; Zawel and Reinberg 1995). In 1994 two groups reported that, in yeast, many of these components are preassembled in complexes found free of DNA (Kim et al. 1994; Koleske and Young 1994). The composition of these complexes varies somewhat depending at least in part on the isolation conditions. The “holoenzyme” described by Young and colleagues includes the polymerase, TFIIB, TFIIF, TFIIH, a group of SRB (suppressor of RNA pol B) proteins, GAL11, and, as most recently described, the SWI (switch)/SNF (sucrose nonfermenting) complex (Koleske and Young 1994; Wilson et al. 1996). Earlier experiments had suggested that TATA-binding protein (TBP) and associated proteins might also be associated loosely with the holoenzyme (Thompson et al. 1993). These findings suggested, in turn, a possible simplification in our understanding of how transcriptional activators might work. That is, rather than imagining these DNA-bound activators as catalyzing a multistep assembly process at the beginning of genes, it was

suggested that these activating regions contact the preassembled holoenzyme (Carey 1995; Koleske and Young 1995). Such interactions would trigger gene activation either by causing some crucial conformational change in the holoenzyme or simply by recruiting the complex to the gene. Assessment of the holoenzyme-contact model for gene activation is complicated by the finding that classic transcriptional-activating regions found on typical activators have been found to contact multiple putative targets *in vitro*, and the physiological relevance of any of those contacts has remained controversial (for example, see Zawel and Reinberg 1995).

Recently we analyzed a novel form of gene activation in yeast and suggested that a specific interaction between a DNA-tethered peptide and the holoenzyme can trigger gene activation (Barberis et al. 1995). We showed that a single amino acid change in the protein GAL11 created an interaction between that mutant protein (called GAL11P) and the dimerization region, not the activating region, of the transcriptional activator GAL4. As a result, a fragment of the GAL4 dimerization region (residues 58–97), tethered to DNA by fusion to a heterologous DNA-binding domain, functions as a strong activator specifically in GAL11P cells. GAL11 is a component of the holoenzyme (Kim et al. 1994; Barberis et al. 1995; Hengartner et al. 1995); indeed, all of the GAL11 protein in the cell appears to be in the holoenzyme (Barberis et al. 1995). Fusion of a DNA-binding domain (e.g.,

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that of LexA) directly to a carboxyl fragment of GAL11 (residues 799–1081), the portion of GAL11 that interacts with at least one other component of the holoenzyme (Barberis et al. 1995; A. Barberis and M. Ptashne, unpubl.), creates a particularly powerful transcriptional activator of genes bearing LexA-binding sites nearby (Himmelfarb et al. 1990; Barberis et al. 1995). The ordinary function of GAL11 is unknown, but that function is not restricted to the *GAL* genes; deletion of *GAL11* decreases transcription of many genes some 5- to 10-fold, presumably because GAL11 is required for full integrity of the holoenzyme (Suzuki et al. 1988; Barberis et al. 1995).

The experiments described herein first show that the strength of the interaction between the two components (GAL11P and the dimerization region of GAL4), as modified by mutation and as quantitated in vitro, predicts the degree of gene activation elicited in vivo. This interaction, we show, triggers gene activation even if the component parts are “swapped” such that a fragment of GAL11P is tethered to DNA and the GAL4 dimerization region is tethered to the holoenzyme. We show that gene activation mediated by the interaction between GAL11P and the GAL4 dimerization region is specifically inhibited (squashed) by overproduction of protein fragments bearing either interacting component. We also show that a classic-activating region binds neither to GAL11 nor to GAL11P, and that fusion of LexA to another holoenzyme component creates an activator. In the latter experiment, a variant of the holoenzyme component predicted on genetic grounds to interact with its partners more strongly also activates more strongly.

These results, taken with those of Barberis et al. (1995), argue that the interaction created by the P mutation between GAL11P and the GAL4 dimerization region is fortuitous, and that when (for example) the GAL4 fragment is tethered to DNA, it triggers gene activation by recruiting the holoenzyme. Accordingly, a special role is assigned neither to GAL11P nor to the GAL4 dimerization region in this process other than as part of a two-component interaction. These findings, taken with other results, suggest a model for how classical activating regions work and provide us with a tool for studying events correlated with gene activation (e.g., chromatin remodeling) in the absence of a classical activating region.

Results

Mutations affecting the interaction of GAL4(58–97) with GAL11P

Himmelfarb et al. (1990) described the original GAL11P allele as bearing isoleucine in place of the wild-type residue asparagine at position 342. A search for mutations elsewhere in the yeast genome or specifically in GAL11 (other than at position 342) that would yield the GAL11P phenotype failed (Barberis et al. 1995; data not shown). Randomization of the codon for position 342 of GAL11, however, yielded additional *GAL11P* alleles each of which bore a hydrophobic residue at that position (Bar-

beris et al. 1995). Table 1 shows that one of these GAL11P alleles, N342V, increased activation by LexA–GAL4(58–97) some twofold above that observed with the original GAL11P allele, and another, N342T, decreased activation some 10-fold. Nearly identical results were obtained with an activator bearing GAL4(58–97) in the context of GAL4(1–100); in the latter case the reporter bore GAL4-binding sites instead of LexA-binding sites. We measured the affinity of each of these three GAL11P alleles for GAL4(1–100) using surface plasmon resonance (SPR) and found a remarkable correlation with the results obtained in vivo; N342V bound about threefold more tightly than did N342I, whereas N342T bound ~10-fold more weakly.

We picked two variants of GAL4(58–97) that activated more strongly when tethered to DNA in a cell bearing the weak GAL11P allele N342T. Table 2 shows that these two mutants, both of which bore double changes, bound more efficiently to GAL11P N342T than the wild-type protein in vitro; F68S/R74Q, which activated more efficiently than did the other double mutant, also bound to GAL11P more tightly. Barberis et al. (1995) described two mutants of GAL4(58–97), R63G and Q66R, that when tethered to DNA activated less efficiently than the parent in cells bearing the original GAL11P allele. Table 2 shows that for these weaker-activating mutants as well as for the stronger-activating mutants just described the order of affinity for GAL11P as measured in vitro predicts the extent of gene activation in vivo.

The experiment of Table 3 describes another example of a correlation between affinity and activation. Thus, GAL4(1–100) activates more efficiently than does GAL4(1–147) in a cell bearing GAL11P N342I; in vitro

Table 1. The affinity of various *GAL11P* alleles for *GAL4*(1–100) correlates with the level of activation by *GAL4*(1–100) and LexA + *GAL4*(58–97) in yeast cells bearing the various *GAL11P* alleles

GAL11 allele	Activation by (β-gal. activity)		Affinity of GAL4(1–100) for GAL11P K _A (×10 ⁶ M ^{−1})
	GAL4(1–100)	LexA(1–202) + GAL4(58–97)	
N342 (WT)	<1	<1	<0.1
N342T	87	114	1
N342I	1251	950	10
N342V	1708	2060	33

Affinity measurements were made by surface plasmon resonance with GAL4(1–100) bound to DNA (see Materials and Methods). Activation by GAL4(1–100) was measured in NSY22, which is *gal4[−]* and *gal11[−]* and contains an integrated reporter with two GAL4 binding sites 50 base pairs upstream of the *GAL1* TATA box. Activation by LexA + GAL4(58–97) was measured in the yeast strain JPY42, which is *gal4[−]* and *gal11[−]* and bears an integrated *lacZ* reporter with two LexA binding sites 50 base pairs upstream of the *GAL1* TATA box. Both GAL(1–100) and LexA + GAL4(58–97) were carried on low-copy plasmids and expressed from the *ACT1* promoter. GAL11 and GAL11P alleles were expressed from the *GAL11* promoter on low-copy plasmids.

Table 2. Mutations in *GAL4*(58–97) that alter its affinity for *GAL11P* in vitro show correlated effects on activation in vivo

<i>GAL4</i> allele	<i>GAL11</i> allele	Activation by LexA(1–202) + <i>GAL4</i> (50–97) (β -gal. activity)	Affinity of <i>GAL4</i> (1–100) allele for <i>GAL11P</i> ($K_A \times 10^6 M^{-1}$)
WT	N342I	830	10
R63G	N342I	65	3.3
Q66R	N342I	4	1.4
WT	N342T	155	1.0
Q87R/K90E	N342T	570	18
F68S/R74Q	N342T	705	25

Protein binding experiments were performed as in Table 1 except that *GAL4*(1–100) mutants containing the indicated amino acid substitutions were bound to the sensorchip. The R63G and Q66R alleles were isolated from a screen for *GAL4* mutants that activated less well in a yeast strain containing a strong *GAL11P* allele (Barberis et al. 1995); the Q87R/K90E, F68S, and R74Q substitutions were found in a screen for *GAL4* mutants that activated strongly in yeast bearing a weak *GAL11P* allele. Activation was measured in JPY42 [see Table 1]. LexA + *GAL4*(50–97) was expressed from the *ACT1* promoter on a low-copy plasmid. The indicated *GAL11P* alleles were carried on a low-copy vector and expressed from the *GAL11* promoter.

the former binds to *GAL11P* more avidly than does the latter. Both of these *GAL4* molecules form stable DNA-binding dimers (Carey et al. 1989). We believe that residues 101–147 occlude partially the *GAL11P* recognition site.

The various affinity measurements reported in this paper were determined by SPR using a BIAcore instrument (Pharmacia) as described in Wu et al. (1996). In brief, the sensorchips bear biotinylated DNA fragments immobilized by interaction with streptavidin linked to the chip's dextran surface. *GAL4*(1–100), and various mutant derivatives thereof, were bound to the two *GAL4*-binding sites contained on the immobilized DNA. The *GAL11* fragment (residues 263–352) bearing the indicated substitution at position 342, expressed in and purified from *Escherichia coli* as a glutathione *S*-transferase (GST) fusion protein, was then passed over the chip.

We have also detected interactions of *GAL4*(1–100) with *GAL11P* using a gel mobility-shift assay. In this assay a DNA fragment bearing a single *GAL4*-binding site was subjected to gel electrophoresis in the presence of saturating *GAL4*(1–100) (or a mutant derivative) and increasing concentrations of one of the GST–*GAL11P* alleles. The GST–*GAL11P* alleles N342V and N342T were each found to cause mobility supershifts, indicative of interaction when tested with *GAL4*(1–100) and with the mutant *GAL4* derivatives Q87R/K90E and R63G. Wild-type *GAL11*(263–352) fused to GST did not interact in this assay with any of the *GAL4* derivatives, and none of the *GAL11* or *GAL11P* derivatives bound to DNA in the absence of a *GAL4* derivative. As estimated by the concentration of each *GAL11* derivative required for the supershift, the relative strengths of the various interactions reflect those quantitated by SPR (data not shown).

These mobility-shift assays also revealed that the various *GAL4* mutations shown in Table 2 diminished somewhat (~2- to 10-fold) the affinity of *GAL4*(1–100) for DNA. The effect is presumably attributable to a weakened formation of dimers, the DNA-binding species of *GAL4*. These deficiencies can compromise experiments with mutant *GAL4*(1–100) derivatives performed in vivo but are readily controlled for in vitro by measuring directly the amount of the *GAL4* derivative bound to DNA. Therefore, we measured activation by the *GAL4* mutants as LexA fusions; LexA(1–202) contains its own dimerization domain (Ruden et al. 1991), so these fusions need not rely on the crippled *GAL4* dimerization domain to bind DNA efficiently.

A domain swap experiment

Barberis et al. (1995) described a “mini” form of *GAL11* that comprises a fusion of two small fragments, presumably domains of the protein. One of these domains (residues 261–351) bears the site of the P mutation, and the other (residues 799–1081) activates transcription when tethered to DNA. This mini-*GAL11* complemented the defect in strains deleted for *GAL11* and, when bearing the N342I mutation, conferred the *GAL11P* phenotype. Figure 1A highlights these regions on a map of *GAL11* and outlines a “domain swap” experiment, the results of which are shown in Figure 1B. The experiment uses two fusion proteins: one bears the carboxyl fragment of *GAL11* attached to *GAL4*(1–100) and the other comprises the P fragment of *GAL11* fused to LexA. Thus, as shown in Figure 1B, the *GAL4* dimerization region [as part of *GAL4*(1–100)] is presumably attached to the holoenzyme, whereas the fragment of *GAL11* bearing the site of the P mutation is attached to a DNA-binding fragment.

Figure 1B shows that LexA + *GAL11*(263–352), bound to DNA, activates transcription if two conditions hold: (1) the DNA-tethered *GAL11* fragment bears a P mutation and (2) the cell expresses the *GAL4*(1–100) + *GAL11*(799–1081) fusion. Moreover, the degree of acti-

Table 3. *GAL4*(1–100) binds more tightly to *GAL11P* than does *GAL4*(1–147) and is a stronger activator in yeast

<i>GAL4</i> derivative	<i>GAL11</i> allele	Activation by <i>GAL4</i> derivative (β -gal. activity)	Affinity of <i>GAL4</i> derivative for <i>GAL11P</i> ($K_A \times 10^6 M^{-1}$)
<i>GAL4</i> (1–100)	N342I	1800	10
<i>GAL4</i> (1–147)	N342I	30	2.5

Affinity measurements were performed as in Table 1 except *GAL4*(1–100) or *GAL4*(1–147) was bound to the sensorchip. *GAL11P* N342I was expressed from its own promoter on a low-copy plasmid. Activation by these *GAL4* derivatives was measured in JPY16, a strain that is *gal4*[−] and *gal11*[−] and bears a *lacZ* reporter with UAS_G (which has four *GAL4* binding sites) at its native position upstream of the *GALI* TATA box.

Figure 1. A domain swap experiment shows that GAL4[58–97]-mediated activation in *GAL11P* cells does not require the structural integrity of GAL11P. (A) Two domains of GAL11P. The regions of GAL11P that interact with GAL4[58–97] and with the holoenzyme are shown. A fusion of these two domains creates a mini-GAL11P that works nearly as well as the full-length protein [Barberis et al. 1995]. (B) A domain swap experiment. GAL4[1–100] + GAL11[799–1081] and GAL11[799–1081] were expressed from the *ACT1* promoter on low-copy vectors. LexA[1–202] + GAL11[263–352] in the wild-type or P mutant form as indicated was expressed from the *ADH* promoter on a high-copy vector. The indicated plasmids were cotransformed into a derivative of JPY52, which is *gal4*[−] and *gal11*[−] and contains an integrated reporter with two LexA-binding sites 50 bp upstream of the *GAL1* TATA box.

vation elicited correlates with the strength of the P allele as determined in Table 1, that is, isoleucine at position 342 activates more weakly than valine at that position, and threonine at that position is weaker still.

GAL11 is not the target of a classic activating region

The experiment of Figure 2 shows that the addition of a classic activating region to GAL4[1–147] decreases the protein's affinity for GAL11P, but nevertheless greatly increases the transcriptional activating function of the molecule. Thus, the affinity of GAL4[1–147] for GAL11P is decreased some two- to threefold by addition of the 15-amino-acid activating peptide AH [Giniger and Ptashne 1987] (cf. panels 1 and 2, second column). Nevertheless, in a *GAL11P* cell GAL4[1–147] + AH activates some 60- to 70-fold more efficiently than does GAL4[1–147] (cf. panels 1 and 2, first column). GAL4[1–147] + AH binds undetectably to wild-type GAL11 (panel 3, second column) and activates some seven- to eightfold less well in wild-type *GAL11* cells than in *GAL11P* cells (cf. panels 2 and 3, first column). Thus, despite its negative effect on GAL11P binding, AH works synergistically with GAL4[58–97].

These experiments [and those of Barberis et al. (1995)] failed to reveal an interaction between AH and GAL11 or GAL11P in vitro. In contrast, we have shown by the same SPR methods that AH interacts with yeast TBP and yeast TFIIB with an affinity of $\sim 10^6 \text{ M}^{-1}$, whereas SH, a scrambled version of AH that does not activate in yeast [Giniger and Ptashne 1987], binds at least 10-fold less well (data not shown). We infer that AH contacts some components of the transcriptional machinery other than GAL11 or GAL11P (e.g., TBP or TFIIB, or both), and that this interaction, coupled with the GAL4 dimerization region–GAL11P interaction, activates transcription synergistically. A further demonstration that GAL11P does not interact with classical activating regions is implicit in the experiments of the following section.

Specific squelching

The holoenzyme diagrammed in Figure 3 bears GAL4[1–100] attached to a fragment of GAL11. We believe that

Figure 2. An acidic activating region that does not interact with GAL11P works synergistically with a domain that does. (Top) GAL4[1–147] activates weakly in *GAL11P* cells and binds with an affinity of $2.5 \times 10^6 \text{ M}^{-1}$ to GAL11P. (Middle) GAL4[1–147] + AH activates strongly in *GAL11P* cells although its affinity for GAL11P is reduced by the addition of an acidic activating region. (Bottom) GAL4[1–147] + AH activates to an intermediate level in wild-type *GAL11* cells but does not contact GAL11. In each case, affinity measurements were performed as in Tables 1–3 with the indicated GAL4 derivative bound to the sensorchip. Activation in vivo was measured in JPY16 (see Table 3); GAL4 derivatives were expressed from the *ACT1* promoter on low-copy plasmids. GAL11 and GAL11P N342I were provided on low-copy plasmids under the control of the *GAL11* promoter.

this holoenzyme could be recruited to DNA in three different ways: (1) by interaction with a DNA-tethered GAL11 fragment bearing a P mutation as in the experiment of Figure 1; (2) by interaction of a classical activator such as GCN4 with its natural targets; and (3) by binding directly to GAL4 sites on DNA. Figure 3 shows, consistent with these expectations, (1) that gene activation is elicited by DNA-tethered GAL11P (line 1); (2) by DNA-bound GCN4 (line 6); (3) or simply by the presence of GAL4-binding sites upstream of a reporter gene (line 10). The experiment also shows three examples of specific

Figure 3. Fragments of the GAL11P–GAL4 system and of acidic activators squelch specifically. (*Top panel*) Activation by LexA + GAL11P(263–352) is squelched by GAL4(50–97) and GAL11P(261–352) but not an acidic activator. The fusion protein expressing GAL4(1–100) + GAL11(799–1081) was the same as that described for Fig. 1B. LexA + GAL11P(263–352) bearing the N342I mutation was expressed from the *ADH* promoter on a low-copy plasmid. GAL4(50–97) and GAL11(261–352) bearing either the wild-type asparagine residue or valine substitution at position 342 were all expressed from the *ACT1* promoter on a high-copy vector. GAL4(74–881) contains the two acidic activating regions of GAL4 and was expressed from the *ADH* promoter on a high-copy plasmid. Activation was measured in the derivative of JPY52 described in Fig. 1B. (*Middle panel*) Activation by GCN4 is squelched by another acidic activator but not GAL4(50–97) or GAL11P(261–352). Activation by GCN4 was measured in a *gal4*[−] and *gal11*[−] derivative of yeast strain YT6 that bears a reporter with two GCN4-binding sites 156 bp upstream of the *GAL1* TATA box. GCN4 was provided by the endogenous chromosomal locus. The plasmids expressing the various squelchers were the same as those described in the *top panel*. (*Bottom panel*) Overexpression of GAL4(50–97) does not inhibit activation by GAL4(1–100) + GAL11(799–1081). Activation by the GAL4–GAL11 fusion protein was measured in NSY22, a *gal4*[−] and *gal11*[−] yeast strain that bears an integrated *lacZ* reporter with two GAL4-binding sites 50 bp upstream of the *GAL1* promoter. The plasmid expressing GAL4(1–100) + GAL11(799–1081) is the same as that described in Fig. 1B.

squelching (Gill and Ptashne 1988; Ptashne 1988). First, overexpression of the fragment GAL4(50–97) inhibited activation elicited by DNA-tethered GAL11P (line 2) but had no effect on activation elicited by GCN4 (line 7) nor on that mediated by binding to GAL4 sites (line 11). Second, overexpression of the fragment GAL11(261–352) bearing a P mutation inhibited activation elicited by DNA-tethered GAL11P but had no effect on GCN4-mediated activation (lines 4 and 8); the same fragment lacking a P mutation did not inhibit (line 3). Third, overexpression of GAL4(74–881), a molecule bearing a strong classical activating function, inhibited GCN4-elicited activation (line 9) but had no effect on activation by the DNA-tethered GAL11P fragment (line 5).

These results are consistent with the view that the interaction of GAL11P with the dimerization region of GAL4 is highly specific, and that classical activating regions (such as those found on GCN4 and on GAL4) interact neither with GAL11 nor with GAL11P.

LexA–SRB2: An activator

Koleske et al. (1992) showed that the conditional phenotype of a strain bearing an RNA polymerase II deleted for a portion of its largest subunit's carboxyl tail is suppressed by a mutant form of SRB2 (i.e., SRB2 P14H, also referred to as SRB2-1), and subsequently SRB2 was found to be part of the holoenzyme (Koleske and Young 1994). These results are plausibly interpreted as showing that the mutant form of SRB2 stabilizes the holoenzyme bearing the mutant RNA polymerase more efficiently than does the wild-type form of SRB2. Moreover SRB2-1 has no phenotype in wild-type cells (Koleske et al. 1992), suggesting that there is no difference in the efficiencies with which the two forms of SRB2 enter into the holoenzyme bearing wild-type RNA polymerase. The experiment of Figure 4 shows that fusion of SRB2 to LexA creates an activator. Moreover, as assayed as part of a LexA fusion protein, the mutant form of SRB2 (i.e., P14H) activates more efficiently than does the wild-type form in a strain bearing the mutant polymerase, whereas the two forms work about equally in a wild-type cell. Replacing proline with alanine at position 14 renders the SRB2 protein inactive in this assay. Mobility-shift assays show that SRB2, SRB2-1, and SRB2 P14A are expressed at indistinguishable levels in the strain bearing the mutant polymerase. Thus, the efficiencies with which SRB2 and SRB2-1 activate, as assayed when tethered to DNA, correlate well with genetic and biochemical observations (Koleske et al. 1992). Taken together, these results reinforce the notion that, as for GAL11, SRB2 does not contain a classic activating region; rather, it forms part of the holoenzyme and upon tethering to DNA it recruits the holoenzyme to the promoter.

A further correlation

A reasonably good correlation between activator-target affinity and the level of activation extends across two quite disparate systems of activation, namely that de-

Figure 4. SRB2, a component of the holoenzyme, activates transcription when tethered to DNA, as predicted by genetic results. SRB2-1, a mutant of SRB2 that rescues the defect caused by a shortened polymerase tail (CTD mutant), activates more strongly than wild-type SRB2 as a LexA fusion in the CTD mutant strain. LexA-SRB2 and mutants thereof were carried on low-copy vectors and expressed from the *ACT1* promoter. Activation elicited by LexA-SRB2 fusions was measured in two yeast strains, each of which bore an integrated reporter with two LexA-binding sites 50 bp upstream of the *GAL1* TATA box. The CTD mutant strain expresses, instead of wild-type RNA polymerase II, a mutant allele, *pRP1Δ104*, in which the polymerase tail is shortened.

scribed here and that described in Wu et al. (1996), which involves a classic-activating region excised from GAL4. Wu et al. (1996) showed (see Discussion) that GAL4 residues 840–881, which comprise part of the activating region II of GAL4, interact with yeast TBP and yeast TFIIB with a K_A of $\sim 10^7 \text{ M}^{-1}$ when tethered to DNA as a fusion with GAL4(1–100). As measured by SPR under identical conditions, the affinity of GAL4(1–100) for GAL11P N342I is within a factor of two of that value (Table 4). These affinities [yeast TBP for part of region II and GAL11P for GAL4(1–100)] have also been measured by tethering one component in each case to a nickel chip in the absence of DNA (Sigal et al. 1996); in both cases the measured affinities were within a factor of two of $K_A = 10^7 \text{ M}^{-1}$ (C. Bamdad and M. Ptashne, unpubl.). Table 4 also shows that on identical reporters, GAL4(1–100) activated in a *GAL11P* cell to a level nearly equivalent to that elicited by GAL4(1–100) + (840–881) in a wild-type cell. Various unknowns, for example, the number of GAL11 molecules in the holoenzyme, the possible existence of additional targets for GAL4(840–881), could complicate the interpretation of these results, but taken at face value they suggest a common mechanism of activation in these different systems.

Discussion

Our results provide strong confirmation of the idea that a specific protein–protein interaction between a DNA-tethered and a holoenzyme-tethered component can trigger gene activation efficiently. Thus, among four mu-

nants of the GAL4 dimerization region (residues 58–97) and three GAL11P alleles, the relative strength of the interaction measured in vitro using SPR predicted the relative degree of gene activation in vivo when GAL4(58–97) was tethered to DNA. Moreover, these interacting components could be rearranged without loss of function. In that experiment, a fragment of GAL11 bearing a P mutation (residues 263–352) fused to LexA activated transcription of a reporter bearing LexA sites specifically in a cell containing the GAL4 dimerization region [as part of GAL4(1–100)] tethered to the holoenzyme by fusion to the carboxyl part of GAL11 (residues 799–1081). Activation was observed only if the DNA-bound fragment bore a P mutation, and, as in the ordinary configuration, the affinity of the GAL11P allele for the GAL4 fragment paralleled the degree of activation. Thus, the effect of the P mutation does not depend on the structural integrity of GAL11P, a result contrary to that expected if, for example, GAL11P were to respond to GAL4(58–97) by undergoing some conformational change that would be necessary for gene activation. This conclusion is further reinforced by the following squelching experiments.

In the experiment of Figure 3, in which DNA-bound LexA + GAL11P(263–352) activated transcription, overexpression of either GAL4(50–97) or GAL11P(261–352) N342V specifically inhibited that activation; no inhibition was observed in the latter experiment if the GAL11 fragment bore the wild-type residue (asparagine) at position 342. A similar result was observed when GAL4(58–97) was overproduced in a *GAL11P* cell in which LexA + GAL4(58–97) activated a gene bearing LexA sites (data not shown). Thus, overproduction of peptides that titrate either the DNA-bound component or the relevant holoenzyme site squelches transcription. The fact that the holoenzyme-binding peptides inhibit rather than activate shows that some hypothetical conformational change induced in the holoenzyme by that interaction does not suffice for gene activation.

Mutations that create de novo a specific protein–protein interaction are evidently not common. Another ex-

Table 4. A correlation between activator–target affinity and level of activation extends across two disparate systems

Activator	Target	β-gal. activity	Affinity $K_A (\times 10^6 \text{ M}^{-1})$
GAL4(1–100)	GAL11P	425	10
GAL4(1–100) + (840–881)	TBP (TFIIB)	595	6 (4)

The affinity measurement in line 1 is taken from Tables 1–3 and those of line 2 are taken from Wu et al. (1996). β-galactosidase levels, taken as a measurement of gene activation, were determined in the yeast strain JPY37, which is *gal4⁻* and *gal11⁻* and bears a *lacZ* reporter with two GAL4 binding sites 50 base pairs upstream of the *GAL1* TATA box. GAL11 and GAL11P were provided from low-copy plasmids and expressed from the *GAL11* promoter. GAL4(1–100) and GAL4(1–100) + (840–881) were both expressed from the *ACT1* promoter on low-copy plasmids.

ample known to us is that in hemoglobin S, that mutation, like ours, introduces a hydrophobic residue in place of a nonhydrophobic residue (valine for glutamate), which in turn creates an interaction between hemoglobin molecules (Dean and Schechter 1978). The fact that GAL4(1–100) interacts with GAL11P significantly more tightly than does GAL4(1–147) suggests that such new interactions might be more frequently observed with protein fragments that present surface regions that are usually buried.

GAL11 is not a privileged site for interaction with the holoenzyme to elicit gene activation. Thus, fusion of LexA to SRB2 creates another activator. In this case a mutant form of SRB2 (SRB2-1) activates more efficiently than does wild-type in a similar fusion with LexA if the cell bears a polymerase partially deleted for its carboxy-terminal tail. The mutant SRB2 was identified by its ability to confer viability at low temperatures on such a strain (Koleske et al. 1992), plausibly because it more readily forms part of the holoenzyme with the defective polymerase. The results indicate a concordance between the efficiency with which a protein interacts with its holoenzyme partners and the efficiency with which that protein activates transcription when tethered to DNA.

GAL11P is not itself the target of a classic acidic activator. Thus, fusion of the short activating sequence AH to GAL4(1–147) modestly decreases the affinity of the protein for GAL11P and dramatically increases the activating ability of the GAL4 derivative. The results suggest that AH recognizes some component of the transcriptional machinery other than GAL11P. The fact that the fragment GAL11P(261–352) efficiently squelches GAL11P-mediated activation in the domain swap experiment, but has no effect on GCN4-activated transcription, reinforces the view that classical activators work through some target other than that seen by GAL11P. Perhaps as originally suggested, simultaneous contact with two components of the machinery activate transcription synergistically (Carey et al. 1990; Lin et al. 1990; Ptashne 1992). As discussed below we imagine, for example, that in *GAL11P* cells GAL4(1–147) + AH contacts, in addition to GAL11P (using the GAL4 dimerization region), either or both of the transcription factors TFIIB and TBP using AH.

A model

Several recent results, taken together, support a model for gene activation that invokes redundant activator-target interactions and recruitment (Struhl 1996). Thus, Wu et al. (1996) found that among a series of variants of a classical activating region excised from GAL4, the affinity for yeast TBP and for yeast TFIIB, as measured in vitro using SPR, predicted the efficiency with which each variant activated transcription in vivo. In an extension of that study, Y. Wu, C. Bamdad, Z. Zaman, and M. Ptashne (unpubl.) showed that this yeast-activating region bound more tightly to yeast TBP (and to yeast TFIIB) than to the corresponding human species, whereas the mammalian activator VP16 showed a small but op-

posite preference. In yeast the yeast activating region worked more efficiently with yeast TBP than with human TBP, whereas VP16 worked about equally well with either species of TBP. These results and those of others (Nerlov and Ziff 1995) implicate TBP as one target of classic-activating regions, a surmise consistent with the finding that DNA-tethered TBP works as an activator in yeast (Chatterjee and Struhl 1995; Klages and Strubin 1995; Xiao et al. 1995). The experiments reported in this paper and in Barberis et al. (1995) show that contact with the holoenzyme can trigger high levels of gene activation. We can rationalize these various observations by assuming that interaction of a DNA-bound activator with either TBP or with the holoenzyme recruits both components, the most efficient reaction being recruitment of the holoenzyme and cooperative binding of TBP and associated factors. Multiple bound activators would contact both components—TBP and TFIIB in the holoenzyme, and perhaps additional targets as well—and thereby ensure efficient recruitment of the protein complexes required for transcription (Hengartner et al. 1995; Wu et al. 1996). We note finally that the approximate order of magnitude of activator-target affinities we measure (10^7 M^{-1}) are several orders of magnitude higher than that of *E. coli* RNA polymerase for the activator protein CAP in the absence of DNA (R. Ebright, pers. comm.). Such differences are consistent with the fact that CAP requires close apposition to RNA polymerase on DNA to stimulate transcription, whereas a typical eukaryotic activator works when well-separated from its target on DNA.

Materials and methods

Genetic methods

Yeast transformations were performed by the method of Schiestl and Gietz (1989). Cells were grown and assayed for β -galactosidase activity as described by Rose et al. (1990). The standard deviation for cultures assayed in triplicate in at least two independent experiments was <20%. The isolation of GAL4 mutants bearing either the R63G or Q66R substitutions was described in Barberis et al. (1995). GAL4 mutants containing the F68S, R74Q, and Q87R/K90E mutations were isolated from a screen for mutants of LexA + GAL4(50–97) that activated more strongly in cells bearing the N342T allele of GAL11P. Mutant libraries were generated by PCR mutagenesis with *Taq* polymerase of GAL4 residues 50–97 (Zhou et al. 1991). SRB2 mutations were introduced by PCR with oligonucleotides containing the indicated changes. Details of plasmid and strain constructions are available upon request.

Protein purification

The *E. coli* expression plasmids producing derivatives of GAL11(263–352) as GST fusion proteins were constructed by cloning a PCR product encoding the indicated GAL11 or GAL11P allele into the plasmid pGEX-5X-1 (Pharmacia). The fusion protein was expressed in and purified from the *E. coli* strain BL21(DE3). Cells harboring the GST–GAL11 expression plasmid were grown at 37°C in Luria broth with 150 $\mu\text{g}/\text{ml}$ of ampicillin. When absorbance₆₀₀ reached ~0.6, the expression of the fusion protein was induced by the addition of IPTG to 1 mM.

Cells were harvested by centrifugation after an additional 3 hr of growth at 37°C and resuspended in an amount of PBS lysis buffer [140 mM NaCl, 2.7 mM KCl, 10.1 mM Na₂HPO₄, 1.8 mM KH₂PO₄ (pH 7.3), 20% glycerol, 1 mM PMSF, 1 µg/ml of benzamidine] equal to one-tenth of the original culture volume and lysed by sonication. NP-40 was then added to a final concentration of 0.1% followed by centrifugation at 15,000g. Glutathione–Sepharose beads (Pharmacia), prepared according to manufacturer's instructions in PBS (140 mM NaCl, 2.7 mM KCl, 10.1 mM Na₂HPO₄, 1.8 mM KH₂PO₄) were added to the extract in an amount equal to one-fifth of the supernatant volume and allowed to bind at 4°C for 1 hr. Bound Sepharose beads were then washed four times with PBS + 20% glycerol. The beads were eluted five times, each time with one bed volume of 10 mM reduced glutathione in 50 mM Tris (pH 8.0). Pooled fractions were dialyzed against 2000 volumes of PBS + 20% glycerol. Protein concentrations were determined by Bradford assay and confirmed by Coomassie staining of denaturing gels. GAL4 derivatives were purified as described in Reece and Ptashne (1993).

SPR measurement of protein–protein interactions

Sensorchip preparation was performed according to Wu et al. (1996) to produce chips that bear a DNA fragment containing two GAL4-binding sites. GAL4 derivatives were first passed over the DNA-bearing chip. Typically 10 µl of 0.01 mg/ml protein solution (~1 µM) in HBS [10 mM HEPES (pH 7.4), 150 mM NaCl, 0.0005% surfactant P20; Pharmacia] was injected at a flow rate of 5 µl/min, and the DNA was saturated by the GAL4 derivatives. Various GST–GAL11 or GAL11P fusions were then injected (typically 10 µl of 0.1 mg/ml solution in HBS at a flow rate of 5 µl/min). The DNA-bearing chip was then regenerated by washing with 10 µl of 0.1% SDS, a procedure that washes both proteins off the DNA, but leaves the DNA-bearing chip intact. For affinity measurements, GAL4 derivatives were again bound to the sensorchip followed by injection of a different concentration of GST–GAL11P. The apparent kinetic constants (k_{on} and k_{off}) of GST–GAL11P binding to various GAL4 derivatives were determined using BIAevaluation software (Pharmacia), and the apparent dissociation constants (K_D) and association constants (K_A) were calculated from k_{on} and k_{off} . The apparent association constant of each interaction was calculated from the results of six sensorgrams. In each sensorgram, 10 µl of an GAL4 derivative was first injected at a concentration of ~1 µM; subsequently 10 µl of GST–GAL11P was injected, followed by an injection of 10 µl 0.1% SDS to regenerate the sensorchip. The activator was injected at the same concentration in each sensorgram, but GAL11P was injected at six different concentrations in twofold serial increases. All of the injections were performed at a flow rate of 5 µl/min. A sensorgram of a blank buffer injection following the injection of the activator was subtracted from each of the six sensorgrams showing different concentrations of GST–GAL11P binding to the GAL4 derivative. The resulting sensorgrams corrected for the slow decay of the GAL4 derivative from the DNA. This correction in fact did not significantly change the calculated K_D s. The binding kinetics of all the interactions fit well to the first order kinetics model, and the k_{on} and k_{off} were solved using linear regression algorithm. The apparent equilibrium constant K_D was obtained by dividing k_{off} with k_{on} .

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