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A Eukaryotic Transcriptional Activator Bearing the DNA Specificity of a Prokaryotic Repressor

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Summary

We describe a new protein that binds to DNA and activates gene transcription in yeast. This protein, LexA-GAL4, is a hybrid of LexA, an Escherichia coli repressor protein, and GAL4, a Saccharomyces cerevisiae transcriptional activator. The hybrid protein, synthesized in yeast, activates transcription of a gene if and only if a *lexA* operator is present near the transcription start site. Thus, the DNA binding function of GAL4 can be replaced with that of a prokaryotic repressor without loss of the transcriptional activation function. These results suggest that DNA-bound LexA-GAL4 and DNA-bound GAL4 activate transcription by contacting other proteins.

Introduction

In Saccharomyces cerevisiae, the protein GAL4 turns on transcription of the *GAL1* gene when bound to an upstream region called UAS_G. This region contains four 17 bp sites of related sequence, a near-consensus of which (the "17-mer") mediates GAL4 activity in vivo and binds GAL4 in vitro (Giniger et al., 1985; Keegan, personal communication). Both UAS_G and a single 17-mer function when placed at several positions within a region between 40 and 600 nucleotides from the *GAL1* transcription start site, or when placed upstream of a different gene, *CYC1* (Guarente et al., 1982b; West et al., 1984; Giniger et al., 1985). GAL4 is active in wild-type strains only when cells are grown on medium containing galactose, because, it is thought, growth on this medium leads to dissociation of GAL4 from an inhibitory protein, GAL80 (Oshima, 1982). GAL4 activity is reduced when cells are grown on medium that contains glucose and galactose (Oshima, 1982; Yocum et al., 1984), at least partly because GAL4 binds UAS_G inefficiently under these conditions (Giniger et al., 1985).

Upstream activation sites (UASs) have been found upstream of all RNA polymerase II-dependent yeast genes the regulatory regions of which have been carefully studied. For example, upstream of *CYC1* are two sites called UAS_{C1} and UAS_{C2}. Cellular gene products, probably encoded by the *HAP1*, *HAP2*, and *HAP3* genes (Guarente et al., 1984), presumably interact with these sites. If UAS_G is inserted upstream of *CYC1* in place of UAS_{C1} and UAS_{C2}, *CYC1* transcription becomes dependent on GAL4 and is regulated like *GAL1* transcription. Although the properties of UASs are similar in other respects to those of the enhancer sequences found in higher eukaryotes, UAS_G and the two UAS_Cs have been reported to be inactive when positioned downstream of the transcription start point of a gene (Struhl, 1984; Guarente and Hoar, 1984).

The current investigation was prompted by a consideration of two mechanisms by which GAL4 might turn on transcription. According to the first, GAL4 would bind to DNA in some way that would stabilize an unusual DNA structure (eg., left-handed DNA), and the perturbed structure would then somehow be transmitted down the helix, where it would help proteins bind near the transcription start. According to the second idea, GAL4 would contact DNA without greatly perturbing the structure of the DNA around the binding site, and activation of transcription would occur when GAL4 touches other proteins. In Escherichia coli, lambda repressor acts as a positive regulator (of its own gene) by the second mechanism; repressor binds to a site adjacent to the RNA polymerase binding site and touches RNA polymerase. One line of evidence that led to this picture was the isolation of lambda repressor mutants called *pc* (for Positive Control) that bind DNA but fail to activate transcription (Guarente et al., 1982a). The amino acids changed in *pc* mutants are clustered in a region on the surface of the lambda repressor molecule (Hochschild et al., 1983) that is thought on the basis of other experiments to be that portion of the molecule that touches RNA polymerase.

Consideration of the lambda experiments led us to try to separate the ability of GAL4 to bind DNA from its ability to stimulate transcription. However, instead of seeking to preserve GAL4's DNA binding while eliminating its ability to activate transcription, we sought to confer a new DNA binding specificity on GAL4 while preserving its ability to stimulate transcription. To this end, we constructed a new protein called LexA-GAL4, the DNA binding specificity of which came from an E. coli repressor called LexA.

In E. coli, LexA represses many genes. Like the repressors of lambda-like phages, LexA probably binds as a dimer to its operators (R. Brent, Ph.D. thesis, Harvard University, Cambridge, Massachusetts, 1982). Moreover, the LexA monomer seems to have an overall organization similar to that of the phage repressors; an amino terminal domain that binds operator DNA and contains weak dimerization contacts, a carboxy-terminal domain that contains stronger dimer contacts, and a flexible hinge region that connects the two (Brent and Ptashne, 1981; R. Brent, Ph.D. thesis 1982; Little and Hill, 1985; Shnarr et al., 1985). The first 87 amino acids of LexA contain the information necessary for specific binding to the LexA operator (Brent, unpublished) and 16 amino acids of the putative hinge region (Little and Hill, 1985). If the cellular DNA is damaged, RecA protein and amino acids within the C-terminus of LexA catalyze cleavage of LexA within the hinge region.

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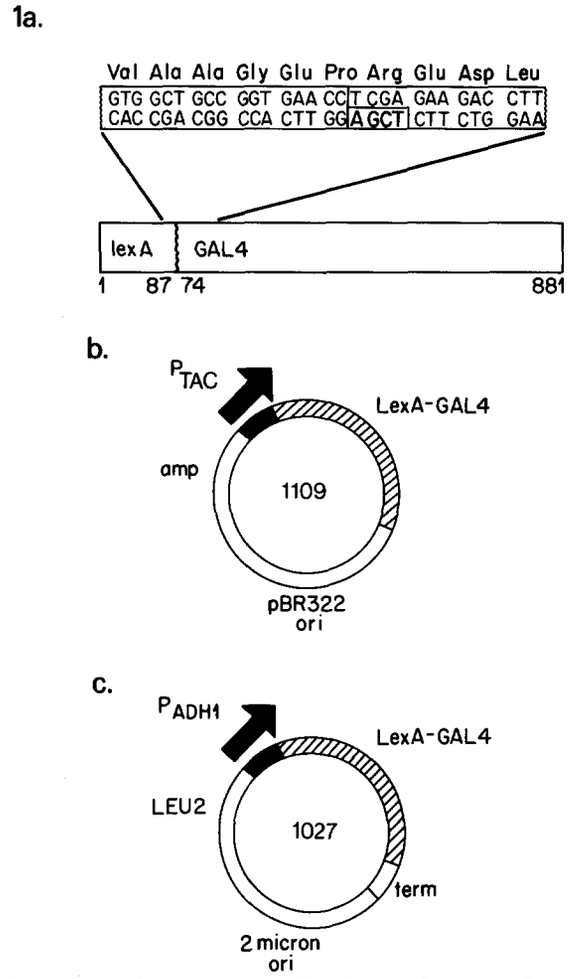

Figure 1. LexA-GAL4 and Plasmids That Direct Its Synthesis in Bacteria and in Yeast

(A) Sequence of LexA-GAL4 fusion junction. *lexA* coding sequence extending to the Xmn I site within the hinge region (vertical line), was abutted to a filled-in Xho I site in *GAL4*, as described in Experimental Procedures. Bases of the filled-in site are shown in boldface. This ligation recreated the Xho I site. Amino acid sequence of the protein near the fusion junction is shown above the line. (B) Plasmid 1109, which directs synthesis of LexA-GAL4 in E. coli. (C) Plasmid 1027, which directs synthesis of LexA-GAL4 in yeast.

Because it is unable to dimerize efficiently, the proteolytic amino-terminal fragment binds operator with greatly reduced efficiency, and transcription of LexA-repressed genes is induced (reviewed in Walker, 1984; and Little and Mount, 1982).

We have recently reported the synthesis of LexA in yeast. If a *lexA* operator is inserted upstream of *GAL1* between UAS_G and the transcription start, LexA represses *GAL1* transcription. We inferred from this that LexA enters the yeast nucleus and binds to the *lexA* operators upstream of *GAL1* (Brent and Ptashne, 1984). In this paper we describe the construction of a gene that encodes a hybrid protein, LexA-GAL4. When LexA-GAL4 is synthesized in E. coli, it binds to *lexA* operators. When LexA-GAL4 is synthesized in yeast, it activates transcription, if and only if a *lexA* operator is present near the start point of transcription.

Table 1. LexA-GAL4 Represses *lexA* Transcription

Plasmid	Regulatory Protein Produced	Units of β-galactosidase
pBR322	none	2000
pKB280	Lambda repressor	1800
PRB451	LexA	120
1109	LexA-GAL4	140

LexA-GAL4 represses transcription in E. coli. The bacterial strain used, RB1003, lacks functional LexA, but contains a *lacZ* gene, the transcription of which is directed by a *lexA* promoter. It was transformed separately with the plasmids below, which encoded regulatory proteins under the control of the lac or tac promoters. In these experiments, IPTG was added to the culture medium to inactivate lac repressor and induce synthesis of the respective proteins. Cells were grown to a density of 1×10^8 cells/ml in LB medium that contained 60 μg/ml carbenicillin. At this point IPTG was added to the culture to a concentration of 5×10^{-4} M. Growth was resumed for 4 hr, and β-galactosidase was assayed, and numbers refer to units of β-galactosidase activity, computed as described in Miller, 1972, in different cultures.

Results

The gene that encodes LexA-GAL4 was derived from two DNA fragments, one encoding the amino-terminal 87 residues of LexA, and the other encoding the carboxy-terminal 807 amino acids of GAL4. These fragments were ligated and were inserted into plasmids that directed synthesis of LexA-GAL4, in one case in bacteria under the control of the tac promoter, and in the other case in yeast under the control of the *ADH1* promoter (see Experimental Procedures and Figure 1).

LexA-GAL4 Recognizes *lexA* Operators in E. coli

Two experiments show that, in E. coli, LexA-GAL4 recognizes *lexA* operators. The first test exploits the fact that LexA represses transcription of its own gene (Brent and Ptashne, 1980). In the bacterial strain used in this experiment, the *lacZ* gene was fused to the *lexA* promoter, so that the amount of β-galactosidase in the strain was a measure of transcription from that promoter. The strain also carried a mutation that inactivated the chromosomal *lexA* gene. Table 1 shows that LexA-GAL4 repressed transcription from the LexA promoter by 16-fold. The second test is based on the fact that derepression of certain LexA-repressed genes is necessary for recovery from DNA damage. Cells that contain a mutant form of LexA, which recognizes operators but cannot be proteolysed, are especially sensitive to the lethal effect of ultraviolet irradiation (UV) (reviewed in Walker, 1984; and Little and Mount, 1982). Proteolysis of LexA in vivo is catalyzed by RecA, which apparently recognizes, in part, amino acids in the C terminus of LexA (Little, 1984). Since LexA-GAL4 lacks the C terminus of LexA, we expected that otherwise wild-type E. coli containing LexA-GAL4 would be UV-sensitive. Figure 2 shows that, as expected, cells containing LexA-GAL4 were profoundly UV-sensitive (Figure 2).

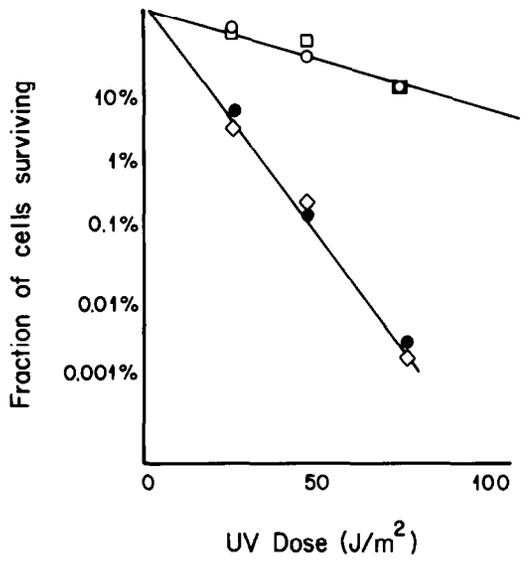

Figure 2. Effect of LexA-GAL4 Synthesis on Sensitivity of E. coli to Killing by UV Irradiation

The top line shows UV sensitivity of cells transformed with a plasmid that contains a promoter but does not direct the synthesis of a regulatory protein (open squares), or with a plasmid that directs the synthesis of lambda repressor (open circles). The bottom line shows the sensitivity of cells transformed with plasmids that direct the synthesis of LexA (closed circles) or of LexA-GAL4 (diamonds).

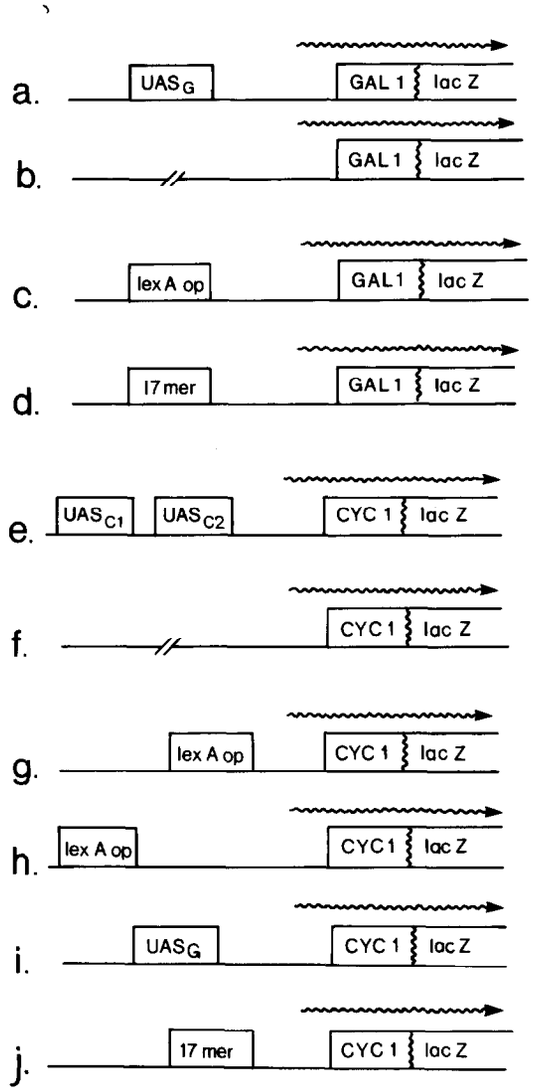

Figure 3. DNA Constructions Used in These Experiments

In each construction, the name of the plasmid, and the distance from the primary or most upstream transcription start site (for the *GAL1* and *CYC1* constructions, respectively) to the upstream element is indicated. The methods used to make these constructions are described in Experimental Procedures. (a) LR1Δ20B, 128 nucleotides; (b) LR1Δ1 lacks an upstream element; (c) 1145, 167 nucleotides; (d) pSV15, 128 nucleotides; (e) pLG669-Z, about 190 nucleotides; (f) pLG570Z lacks an upstream element; (g) 1155, 178 nucleotides; (h) 1057, 577 nucleotides; (i) pLGSD5, about 190 nucleotides; (j) pSV14, 178 nucleotides.

LexA-GAL4 Activates Transcription in Yeast when Bound to a *lexA* Operator

We transformed yeast with a plasmid that directs the synthesis of LexA-GAL4 (Figure 1) and separately transformed cells with a plasmid that directs synthesis of native LexA (Brent and Ptashne, 1984). In addition, we transformed these strains with plasmids that carried the constructs shown in Figure 3. As shown in the figure, these plasmids carry part of the *GAL1* or *CYC1* gene fused to *lacZ*. Upstream of the fusion gene the plasmids contained one of the following: UAS$_G$, the 17-mer, UAS$_{C1}$ and UAS$_{C2}$, a *lexA* operator, or none of these elements. To determine the amount of transcription of the *lacZ* fusion genes, we measured the amount of β-galactosidase activity in cultures of these doubly transformed cells.

LexA-GAL4 stimulated production of β-galactosidase directed by the *CYC1-lacZ* fusion gene if and only if the plasmid contained a *lexA* operator (Table 2). When the *lexA* operator was located 590 nucleotides upstream of the nearest *CYC1* transcription start site, β-galactosidase production was two-thirds that obtained when the *lexA* operator was positioned 178 nucleotides upstream. Compared with the amount obtained when cells were grown in medium that contained galactose as the only carbon source, the amount of β-galactosidase directed by LexA-GAL4 in cells grown in glucose medium was diminished by about a factor of three. Native LexA did not stimulate β-galactosidase production from these plasmids.

LexA-GAL4 also stimulated production of β-galactosidase from a plasmid that contained the *GAL1-lacZ* fusion gene if and only if the plasmid contained a *lexA* operator (Table 3). Table 3 also shows that, growth of cells in glucose medium decreased that stimulation by about half. Native LexA did not stimulate β-galactosidase production.

The experiments described in Table 2 and Table 3 were performed in a *GAL4*$^+$ host. We have performed similar experiments in two other strains, one of which carried a *gal4* point mutation, the other a *gal4* deletion. In these strains, LexA-GAL4 stimulated β-galactosidase production from the CYC1-lacZ and GAL1-lacZ plasmids if and only if they contained a *lexA* operator. In particular, LexA-GAL4 did not stimulate β-galactosidase production from plasmids that contained UAS$_G$ but no *lexA* operator, nor from an integrated *Gal1-lacZ* fusion gene, nor did it complement the

Table 2. LexA-GAL4 Activates Transcription of a *CYC1-lacZ* Fusion Gene

Growth Medium	Upstream Element	Regulatory Protein	
		LexA	LexA-GAL4
Galactose	no UAS (f)	<1	<1
	lexA op at −178 (g)	<1	590
	lexA op at −577 (h)	<1	420
	UAS_{C1} and UAS_{C2} (e)	550	500
	UAS_G (i)	950	950
	17-mer (j)	600	620
Glucose	no UAS (f)	<1	<1
	lexA op at −178 (g)	<1	210
	lexA op at −577 (h)	<1	140
	UAS_{C1} and UAS_{C2} (e)	180	160
	UAS_G (i)	<1	<1
	17-mer (j)	<1	<1

The letter in parentheses in each case refers to the construction diagrammed in Figure 3 and described in Experimental Procedures. Numbers denote units of β-galactosidase activity measured in cultures of doubly transformed yeast as described in Experimental Procedures.

Table 3. LexA-GAL1 Activates Transcription of a *GAL-lacZ* Fusion Gene

Growth Medium	Upstream Sequence	Regulatory Protein	
		LexA	LexA-GAL4
Galactose	no UAS (b)	0	0
	lexA op at −167 (c)	<1	520
	17-mer (d)	1050	1000
	UAS_G (a)	1800	1800
Glucose	no UAS (a)	0	0
	lexA op at −167 (c)	<1	190
	17-mer (c)	<1	<1
	UAS_G (a)*	160	150

The letter in parentheses in each case refers to the construction diagrammed in Figure 3 and described in Experimental Procedures. Numbers denote units of β-galactosidase activity measured in cultures of doubly transformed cells.

* This particular plasmid, LR1Δ20B, directs a low level of GAL1 transcription and β-galactosidase production when cells containing it are grown on glucose medium (West et al., 1984).

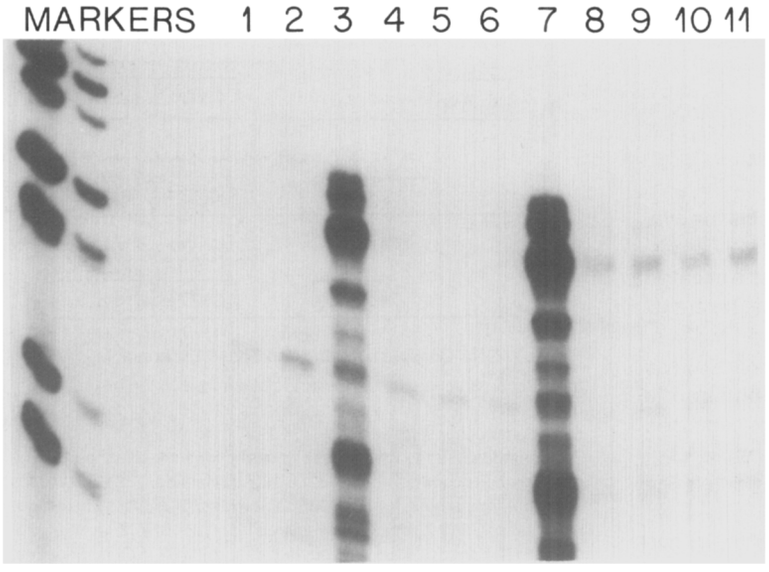

Figure 4. 5′ Ends of RNAs Made from *GAL1* Derivatives in the GAL4⁺ Strain Sc294

Cells were grown in glucose medium, RNA was mapped, and an autoradiogram was generated as described in Experimental Procedures. The lanes on the left contain size markers. In this experiment, Sc294 carried two plasmids, the relevant features of which are given below. The plasmid LR1Δ20B was used as a control because it directs the transcription of correctly 5′-ended GAL1 mRNA when cells harboring it are grown in glucose medium (West et al., 1984). Lane 1 contains the probe alone. Lane 2, RNA extracted from GAL4⁺ strain Sc294 transformed with LR1Δ1 and PRB500; LR1Δ1 lacks a UAS, and PRB500 directs the synthesis of LexA protein. Lane 3, LR1Δ20B, which contains UAS_G, and PRB500. Lanes 4 and 5, 1145, which contains a *lexA* operator but does not contain UAS_G, and pRB500. Lane 6, LR1Δ1 and 1027, which directs the synthesis of LexA-GAL4. Lane 7, LR1Δ20B, which contains UAS_G, and 1027. Lanes 8, 9, 10, and 11, 1145 and 1027.

inability of the *gal4*⁻ strain to grow on galactose medium (not shown). As above, LexA-GAL4 directed synthesis of less β-galactosidase production from the *CYC1-lacZ* fusion gene when the lexA operator was located 590 nucleotides upstream of the nearest transcription start site than when it was 178 nucleotides upstream (not shown). In experiments using these strains, we in most cases estimated β-galactosidase levels from the color of colonies on indicator plates (see Experimental Procedures).

Figure 4 shows that, when LexA-GAL4 stimulated transcription of *GAL1* derivatives, the RNAs made had the same 5′ ends as the RNAs made from a plasmid that contained wild-type UAS_G. LexA-GAL4 directed the synthesis of 5% as much *GAL1* transcript as was expected from the amount of β-galactosidase activity in these cultures. We do not yet know the cause of this apparent discrepancy between these two measures of the amount of transcription.

LexA-GAL4 May Interact with GAL80

Table 4 shows that, in a *GAL4*⁺ strain, LexA-GAL4 induced synthesis of β-galactosidase from a *GAL1-lacZ* fusion gene that carried UAS_G upstream but no *lexA* operator. In this experiment, cells were grown on glucose medium.

Table 4. LexA-GAL4 Induces *GAL1-lacZ* Expression

Plasmid	Regulatory Protein	Units of β-galactosidase
pAAH5	none	0
pRB500	LexA	0
C15	GAL4	80
1027	LexA-GAL4	75

In a *GAL4*⁺ strain, synthesis of large amounts of LexA-GAL4 induces expression of a *GAL1-lacZ* fusion gene. 745::pRY171, which contains an integrated *GAL1-lacZ* fusion gene, was transformed with the plasmids below (described in Experimental Procedures). Cells were grown on glucose medium, and β-galactosidase levels were determined as described in Experimental Procedures.

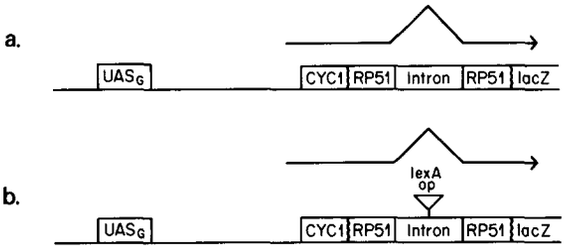

Figure 5. DNA Constructions Used in Downstream Activation Experiment

Constructions are described in more detail in Experimental Procedures. Arrow represents transcript when transcription is activated by UAS_G upstream. The distance from the Sal I site in the *RP51* intron to the start of the most upstream *CYC1* transcript is about 160 nucleotides. (a) the plasmid HZ18 (Teem and Rosbash, 1983). (b) the derivative 1146, which bears a *lexA* operator in the RP51 intron.

This aspect of the behavior of LexA-GAL4 is consistent with a model in which large amounts of the C-terminus of GAL4 titrate the negative regulator GAL80, so that the wild-type GAL4 present in the cell is free to activate transcription from UAS_G (see Discussion) (Laughon and Gesteland, 1982; Johnston and Hopper, 1982).

LexA-GAL4 Activates Transcription from a Downstream Site

To test whether LexA-GAL4 could activate transcription from a site downstream of the normal transcription start, we inserted a *lexA* operator into the intron of a spliced yeast gene, downstream of the normal transcription start site. We adopted an approach first used by Guarente and Hoar (1984), and inserted the *lexA* operator into the plasmid diagrammed in Figure 5 (Teem and Rosbash, 1983). This plasmid contains UAS_G upstream of the *CYC1* coding sequence, which is fused to a fragment containing a portion of *RP51*, a gene of which the transcript is spliced. The *RP51* fragment contains part of the first exon, the intron, and part of the second exon. The second exon of *RP51* is fused to *lacZ*. Insertion of a *lexA* operator into the intron allowed us to test whether LexA-GAL4 activated transcription when bound downstream of the transcription start point, by measuring β-galactosidase produced by the plasmid.

Table 5 shows that LexA-GAL4 stimulated production of β-galactosidase if and only if the *RP51* intron contained a *lexA* operator. No β-galactosidase was produced when LexA was present instead of LexA-GAL4. This experiment was done in a *gal4*⁻ strain to eliminate upstream activation by UAS_G. β-galactosidase activity in this experiment was about 4% of the level observed in a *GAL4*⁺ strain when transcription was activated from UAS_G upstream (not shown).

Discussion

We have described a new protein, LexA-GAL4, that activates transcription in yeast. Our most important conclusion is diagrammed in Figure 6. Although LexA-GAL4 and LexA both bind *lexA* operators in yeast (this paper, and Brent and Ptashne, 1984), LexA-GAL4 activates transcription, while LexA does not. LexA-GAL4 does not interact with UAS_G. Activation of transcription by LexA-GAL4 is less effective when the *lexA* operator is far upstream of the transcription start point than when it is close. In the one case tested, the mRNAs for which synthesis is stimulated by LexA-GAL4 have the same 5′ ends as those generated by the wild-type promoter, but are present at an unexpectedly low level. LexA-GAL4 activates transcription, at further reduced efficiency, when its binding site lies downstream of the normal transcription start. In this case, we have not yet determined the location of the 5′ end of the RNA.

Since LexA-GAL4 stimulates transcription when bound to a *lexA* operator, but native LexA does not, our results argue against any model of GAL4 action that would posit that the sequence-specific contact GAL4 makes with UAS_G changes the structure of DNA and that this change is crucial to gene activation. If gene activation by DNA-bound GAL4 is not effected via a change in the structure of DNA, we infer that gene activation depends on the interaction of GAL4 with other cellular components, most likely proteins. This suggestion has received further support from the work of Keegan, Gill, and Ptashne (unpublished), which shows that a GAL4-β-galactosidase hybrid protein containing only the first 74 amino acids of GAL4 binds UAS_G but cannot stimulate transcription.

Indirect immunofluorescence using anti-lexA antibody shows that, LexA-GAL4, which lacks the portion of GAL4 thought to be necessary for its nuclear localization, is not concentrated to the nucleus, but is dispersed throughout the cell (P. Silver, R. Brent, and M. Ptashne, unpublished). We imagine that LexA-GAL4 (monomer weight 99,000 daltons) enters the nucleus by diffusion through the nuclear pores. This idea is not unprecedented, at least for a smaller protein; native LexA (monomer molecular weight 20,000 daltons) binds *lexA* operators in the nucleus even though it is not localized to the nucleus (Brent and Ptashne, 1984; Silver, Brent, and Ptashne, unpublished). LexA-GAL4 functions in cells that contain deletions of the GAL4 (this paper) and GAL80 genes (C. L. Anderson and Brent, unpublished), suggesting that its entry into the nucleus is not facilitated by an interaction with either of these gene products.

Table 5. LexA-GAL4 Stimulates Synthesis of β-galactosidase when Bound to a Site Downstream of the Normal Transcription Start Site

Downstream Element Found in First Plasmid	Regulatory Protein Produced by Second Plasmid	
	LexA	LexA-GAL4
No Operator	<1	<1
lexA Operator	<1	30

Constructions are shown in Figure 5 and are described in Experimental Procedures. β-galactosidase activity was measured in cultures of the gal4⁻ strain SHC22C grown in glucose medium, which were doubly transformed with the indicated plasmids. Numbers refer to units of β-galactosidase activity measured as described in Experimental Procedures.

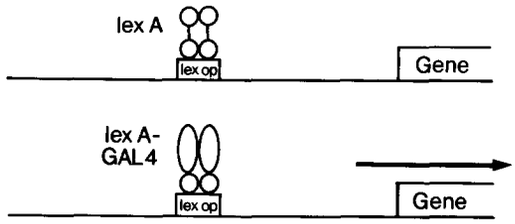

Figure 6. Activation of Transcription by LexA-GAL4

The first line shows that LexA protein is unable to activate transcription when bound to its operator upstream of a yeast gene. The second line shows that LexA-GAL4 activates transcription when bound to the same site.

GAL4 is thought to form oligomers (Oshima, 1983; Giniger et al., 1985). The fact that it recognizes a 17 bp sequence that has approximate 2-fold rotational symmetry is consistent with the idea that the DNA binding form of GAL4 is a dimer or tetramer (Giniger et al., 1985). The DNA binding form of LexA is also likely to be a dimer (R. Brent, Ph.D. thesis, 1982). We think it likely that the part of the LexA hinge region contained in LexA-GAL4 provides sufficient flexibility to allow the amino-terminal LexA moieties to assume a conformation identical with the one they have when they are part of a dimer of native LexA.

In contrast to GAL4 activity in a wild-type cell, the activity of LexA-GAL4 in our experiments did not depend on the presence of galactose in the medium. We can explain the galactose-independence of the activity of LexA-GAL4 by assuming that LexA-GAL4 retains the portion of GAL4 that interacts with GAL80, an assumption supported by the experiment shown in Table 4. Since, in our experiments, the synthesis of LexA-GAL4 was directed by the *ADH1* promoter, we suspect that GAL80 was titrated, and the excess, uncomplexed LexA-GAL4 was free to activate transcription. We interpret the relative insensitivity of LexA-GAL4 activity to the presence of glucose in the medium to mean that, under these conditions, LexA-GAL4 retains normal ability to bind DNA. We think, at least in the case of LexA-GAL4-dependent *GAL1* transcription, that the residual 2-fold glucose repression we observe arises from a different mechanism that depends for its action on a specific sequence upstream of *GAL1* that is present in our construction (West et al., 1984; M. Lamphier and M. Ptashne, unpublished).

Our experiments are thus consistent with a picture in which GAL4 is divided into distinct functional domains; an amino-terminal domain that directs nuclear localization (Silver et al., 1984) and binds UAS_G, and a C-terminal domain that stimulates transcription, interacts with GAL80 (Loughn and Gesteland, 1984), and directs oligomerization.

We have recently constructed a hybrid protein composed of LexA and a positive regulator of amino acid biosynthesis called GCN4 (Lucchini et al., 1984; Driscoll-Penn et al., 1983). LexA-GCN4 activates transcription from the *lexA* operator containing constructions used in this paper (Brent and C. L. Anderson, unpublished). This fact suggests that GCN4 activates transcription, if and only if it is bound to DNA. From this experiment, we cannot exclude the possibility that GCN4 normally interacts with some other protein that brings it to the DNA, and that fusion of LexA to GCN4 has circumvented this mechanism. However, GCN4 has recently been shown to interact with DNA directly (Hope and Struhl, 1985). Construction of analogous hybrid proteins may prove to be a useful tool for identifying and studying transcriptional activation functions in other eukaryotic regulatory proteins.

Experimental Procedures

Strains

DBY745 is *α leu2 ura3*. SHC22C, *α gal4 ura3 leu2*, was a gift of Susan Hanley. Sc294, a (*gal1-gal10*) *ura3 leu2*, was a gift of Jim Hopper. DBY745::pRY171 and SHC22C::pRY171 were made by cutting pRY171 (Yocum et al., 1984), which contains a *GAL1-lacZ* fusion gene, with Apa I in the *URA3* gene, transforming the strains with the linearized plasmid DNA, and selecting stable URA⁺ transformants. Bacterial strain JM101 (Messing et al., 1981) was the host for most plasmid constructions. XA90 (Amman et al., 1983), which contains the *lacI*^{q1} mutation, and thus contains large amounts of lac repressor, was used as the host in the ultraviolet killing experiments. RB1003 F′ (*lacI*^{q1}/*lacZ*::Tn10)/*lexA*(def) Δ (*lac-pro*) *arg ile val thiA str*^{r} was constructed by standard bacterial techniques and lysogenized with RB230, an *imm*^{21} phage that contains the *lexA* promoter fused to an operon that contains *trpA* and *lacZ*, similar to RB200 (Brent and Ptashne, 1980).

Plasmids

Plasmids were constructed by standard techniques (Maniatis et al., 1982).

lacZ Fusion Plasmids

All plasmids carry the *URA3*⁺ gene, a 2μ replicator, and relevant portions diagrammed in Figure 2. LR1Δ20B, which contains UAS_G and the *GAL1* gene fused to *lacZ*, and LR1Δ1, which does not contain UAS_G, have been described (West et al., 1984). 1155 was made from LR1Δ1 by inserting a single synthetic consensus *lexA* operator (Brent and Ptashne, 1984) into the Xho I site of the plasmid, 167 nucleotides from the primary transcription start site. pSV15 is a derivative of LR1Δ20B in which UAS_G has been deleted and replaced with a DNA fragment, 128 nucleotides upstream of the primary *GAL1* transcription start site, that contains the "17-mer" (the 17 bp near-consensus GAL4 site of action [Giniger et al., 1985]). pLG669-Z, which carries UAS_{C1} and UAS_{C2} upstream of a *CYC1-lacZ* fusion gene, and pLGSD5, which carries UAS_G upstream of the *CYC1-lacZ* gene, have been described (Guarente and Ptashne, 1981; Guarente et al., 1982b). pLG670Z, a gift of L. Guarente, is a derivative of pLG669-Z from which the Xho I–Xho I fragment that contains the upstream activation sites has been deleted. 1057 and 1155 were constructed from pLG670Z by inserting a single *lexA* operator at the Xho I site or the Sal I site, 178 and 577

nucleotides upstream of the most upstream *CYC1* transcription start site, respectively. pSV14 is a derivative of pLG670Z in which a fragment of DNA that contains the 17-mer has been inserted at the Xho I site 178 nucleotides upstream of the *CYC1-lacZ* fusion gene. HZ18, a gift of John Teem, was described by Teem and Rosbash (1983). It contains a Sal I site in the RP51 intron about 160 nucleotides downstream from the most upstream *CYC1* transcription start site. 1146 was constructed from HZ18 by inserting a lexA operator into the Sal I site.

LexA-GAL4 Hybrid Plasmids

Plasmid 1109, which directs the synthesis of LexA-GAL4 in E. coli, was constructed from three DNA fragments. In this construction, a Pst I–Xmn I piece was isolated from pRB451, a tac promoter derivative of pRB191 (Brent and Ptashne, 1981). This piece of DNA contained the tac promoter, a hybrid ribosome binding site, and *lexA* DNA from codon 1 to within codon 87. Plasmid C15, a gift of Liam Keegan, which contains the S. cerevisiae *GAL4* gene transcribed by its own promoter, was cut with Xho I. The Xho I ends were rendered flush by treatment with the Klenow fragment of DNA polymerase I and then were cut with Hind III to liberate a fragment of DNA that encoded the C-terminus of GAL4, extending from the filled-in Xho I site within codon 74 (Laughon and Gesteland, 1984) to about 250 nucleotides beyond the termination codon after amino acid 881. These two pieces were inserted into a Pst I–Hind III backbone fragment of pBR322 (Bolivar et al., 1977). Plasmid 1027, which directs the synthesis of LexA-GAL4 in S. cerevisiae, was constructed in two steps. In the first, we isolated a Hind III–Xmn I fragment from pRB500, a plasmid that directs the synthesis of *lexA* in yeast (Brent and Ptashne, 1984). This fragment contained DNA from immediately upstream of the *lexA* coding sequence to within codon 87. We inserted this fragment, and the same *GAL4* fragment used in constructing 1109 above, into plasmid pi^{a}-8, a gift of R. R. Yocum, that confers resistance to tetracycline if Hind III-ended fragments are inserted into it. This ligation created plasmid 1002, which contained the *lexA-GAL4* hybrid gene flanked by Hind III sites. The Hind III-ended *lexA-GAL4* fragment was inserted into pAAH5, a plasmid made by Gustav Ammerer that contains the *ADH1* promoter, the *LEU2* selectable marker, and the 2 μ replicator, to create plasmid 1027. Like all plasmids from this construction that contained the gene encoding LexA-GAL4 in the correct orientation, purified 1027 transformed JM101 at 1% of the frequency expected from the amount of DNA used, and most transformed cells grew slowly. About 10% of the JM101 cells transformed to carbenicillin resistance from a preparation of 1027, grew quickly, and proved to contain plasmids that carried deletions of portions of the gene encoding LexA-GAL4 (not shown).

Cell Growth, Transformation, and Assay of β-galactosidase

Bacterial strains were grown in LB medium that contained, when appropriate, tetracycline at a concentration of 15 μg/ml, carbenecillin at 60 μg/ml, and isopropyl-β-D-thio-galactopyranoside (IPTG) at 5×10^{-4} M. Yeast were grown on YEPD medium, or, when they contained plasmids, on minimal medium containing either glucose 2% weight/volume ("glucose medium") or galactose at the same concentration ("galactose medium") but lacking either leucine or uracil or both (Sherman et al., 1983). Yeast were made competent by treatment with lithium acetate (Ito et al., 1983). For assay of LexA-GAL4 activity, cells were first transformed with 1027 or another *LEU2*$^{+}$ plasmid. As determined by their ability to activate β-galactosidase production from a plasmid that contained a *lexA* operator near a *lacZ* fusion gene, about 90% of the 1027 transformants produced LexA-GAL4. The amount of β-galactosidase in liquid cultures of E. coli was measured according to Miller (1972) and determined for S. cerevisiae, in liquid culture and from the degree of blue color on indicator plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, as described (Brent and Ptashne, 1984; Yocum et al., 1984). In DBY745, results of liquid assays were quite consistent from day to day; in contrast, due to a tendency of cells in cultures of the *gal4*$^{-}$ strain SHC22C to form clumps on some days but not on others, liquid assays had to be repeated many times to generate reliable data. For all strains used, estimation of β-galactosidase activity from color on indicator plates was consistent from day to day.

Measurement of Sensitivity to Ultraviolet Radiation

In the experiment shown in Figure 2, E. coli XA90, which contains large amounts of *lac* repressor because of its *lacI*^{q1} mutation, was transformed separately with four similar plasmids that contain a tac promoter as follows: ptac12, which does not direct the synthesis of a regulatory protein; pEA305, which directs the synthesis of bacteriophage lambda repressor (Amman et al., 1983); pRB451, which directs the synthesis of LexA; or 1109, which directs the synthesis of LexA-GAL4. Since the amount of wild-type LexA that can be disposed of by proteolysis after DNA damage is limited, cells that contain large amounts of wild-type LexA display a sensitivity to killing by ultraviolet radiation equivalent to that observed for cells that contain a mutant LexA that cannot be proteolysed (Brent and Ptashne, 1980; Little and Mount, 1982; Walker, 1984). Cells were grown at 37°C in LB medium that contained 60 μg/ml of carbenicillin to a density of 2×10^{7} cells/ml. IPTG was added to these cultures to a concentration of 5×10^{-4} M to derepress the tac promoters, and growth was resumed for 90 min. Cells were then irradiated with ultraviolet light, and a series of 10-fold dilutions of the cultures was plated immediately on LB plates that contained carbenicillin at 60 μg/ml but that lacked IPTG. The fraction of cells surviving was determined by counting colonies on plates that had been incubated in the dark at 37°C for 2 days.

RNA Mapping

A Bam HI–Xho I fragment from LR1Δ1, which contains DNA from the boundary between *GAL1* and *lacZ* and extends upstream to 167 nucleotides 5′ of the primary *GAL1* transcription start, was inserted into the SP6 vector pSP64 (Melton et al., 1984) that had been cut with Sal I and Bam HI to create plasmid 1164. RNA made in vitro by SP6 polymerase from plasmid 1164 is complementary to RNA made in vivo from *GAL1*. Since any transcription from the chromosomal *GAL1* gene would produce message that could also hybridize with the SP6 probe, we used strain Sc294, which contains a deletion of the *GAL1* and *GAL10* genes, for these experiments. Cells contained the plasmids shown in the legend to Figure 4. Since *gal1*$^{-}$ strains cannot grow on galactose medium, we grew the cells on glucose medium and used cells transformed with the plasmid LR1Δ20B, which constitutively produces correctly 5′-ended RNA under these conditions (West et al., 1984), as a positive control. RNA was extracted from cells according to a procedure provided by K. Durban, very similar to that used by Carlson and Botstein (1982). Precise mapping of the RNAs made from the *GAL1* promoter was done after hybridization with probe and digestion with RNAases A and T1 according to Zinn et al. (1983). To generate size markers, plasmid pRB322 was digested with Hpa II (Sutcliffe, 1978), and the fragments were labeled with ^{32}P (Maniatis et al., 1982).

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