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 UASc. This region contains four 17 bp sites of relation 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 UASc 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 UASc inefficiently under these conditions (Giniger et al., 1985).

Upstream activation sites (UASs) have been found upstream of all RNA polymerase II-dependent yeast genes in the regulatory regions of which have been carefully studied. For example, upstream of CYC1 are two sites called UASc1 and UASc2. Cellular gene products, probably encoded by the HAP1, HAP2, and HAP3 genes (Guarente et al., 1994), presumably interact with these sites. If UASc is inserted upstream of CYC1 in place of UASc1 and UASc2, 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, UASc and the two UASc2s 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 (e.g., 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., 1995). 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.
Cell 730

Table 1. \( \text{LexA-GAL4 Represses} \ \text{/exA Transcription} \)

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Regulatory Protein</th>
<th>Units of ( \beta )-galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBR322</td>
<td>none</td>
<td>2000</td>
</tr>
<tr>
<td>pKB280</td>
<td>Lambda repressor</td>
<td>1800</td>
</tr>
<tr>
<td>PRBA451</td>
<td>LexA</td>
<td>120</td>
</tr>
<tr>
<td>1109</td>
<td>LexA-GAL4</td>
<td>140</td>
</tr>
</tbody>
</table>

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 \( \beta \)-galactosidase 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 \( 1 \times 10^9 \text{cells/mL} \) in LB medium that contained 80 \( \mu \text{g/mL} \) kanamycin. At this point IPTG was added to the culture to a concentration of \( 5 \times 10^{-4} \text{M} \). Growth was resumed for 4 hr, and \( \beta \)-galactosidase was assayed, and numbers refer to units of \( \beta \)-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 tat promoter, and in the other case in yeast under the control of the ADH7 promoter (see Experimental Procedures and Figure 1).

LexA-GAL4 Recognizes /exA Operators in E. coli

Two experiments show that, in E. coli, LexA-GAL4 recognizes /exA 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 /exA promoter, so that the amount of \( \beta \)-galactosidase in the strain was a measure of transcription from that promoter. The strain also carried a mutation that inactivated the chromosomal lacZ 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). Protein analysis 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).
Transcriptional Activation by LexA-GAL4 Hybrid

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).

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: UASo, the 17-mer, UASc, and UASc*, 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 GALA in cells grown in glucose medium was diminished by about a factor of three. 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 UASo but no lexA operator, nor from an integrated Gall-lacZ fusion gene, nor did it complement the
Table 2. LexA-GAL4 Activates Transcription of a CYC1-lacZ Fusion Gene

<table>
<thead>
<tr>
<th>Growth Medium</th>
<th>Upstream Element</th>
<th>LexA</th>
<th>LexA-GAL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>no UAS (f)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>lexA op at -178 (g)</td>
<td>&lt;1</td>
<td>590</td>
</tr>
<tr>
<td></td>
<td>lexA op at -577 (h)</td>
<td>&lt;1</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>UAS(<em>{\alpha}) and UAS(</em>{\beta}) (a)</td>
<td>550</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>UAS(_{\alpha}) (l)</td>
<td>1950</td>
<td>1950</td>
</tr>
<tr>
<td></td>
<td>17-mer (j)</td>
<td>600</td>
<td>620</td>
</tr>
<tr>
<td>Glucose</td>
<td>no UAS (f)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>lexA op at -178 (g)</td>
<td>&lt;1</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>lexA op at -577 (h)</td>
<td>&lt;1</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>UAS(<em>{\alpha}) and UAS(</em>{\beta}) (a)</td>
<td>180</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>UAS(_{\alpha}) (l)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>17-mer (j)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Growth Medium</th>
<th>Upstream Sequence</th>
<th>LexA</th>
<th>LexA-GAL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>no UAS (b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>lexA op at -167 (c)</td>
<td>&lt;1</td>
<td>520</td>
</tr>
<tr>
<td></td>
<td>17-mer (d)</td>
<td>1050</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>UAS(_{\beta}) (a)</td>
<td>1800</td>
<td>1800</td>
</tr>
<tr>
<td>Glucose</td>
<td>no UAS (a)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>lexA op at -167 (c)</td>
<td>&lt;1</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>17-mer (c)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>UAS(_{\beta}) (a)</td>
<td>160</td>
<td>150</td>
</tr>
</tbody>
</table>

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.

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

Cells were grown in glucose medium, RNA was extracted, 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 LR1A206 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 LR1A206 and PRB500; LR1A206 lacks a UAS, and PRB500 directs the synthesis of LexA protein. Lane 3, LR1A206, which contains UAS\(_{\alpha}\) and PRB500. Lanes 4 and 5, 1145, which contains a lex4 operator but does not contain UAS\(_{\alpha}\), and PRB500. Lane 6, LR1A1 and 1027, which directs the synthesis of LexA-GAL1. Lane 7, LR1A206, which contains UAS\(_{\alpha}\) and TUG2. 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 5'90 nucleotides upstream of the nearest transcription start site than when it was 178 nucleotides upstream (not shown). In experiments using these strains, we 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\(_{\alpha}\). 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\(_{\alpha}\) upstream but no lexA operator. In this experiment, cells were grown on glucose medium.
Table 4. LexA-GAL4 Induces GALI-lacZ Expression

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Regulator Protein</th>
<th>Units of β-galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAAH5</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>pRB500</td>
<td>LexA</td>
<td>0</td>
</tr>
<tr>
<td>C15</td>
<td>GAL4</td>
<td>0</td>
</tr>
<tr>
<td>1027</td>
<td>LexA-GAL4</td>
<td>75</td>
</tr>
</tbody>
</table>

In a GAL4" strain, synthesis of large amounts of LexA-GAL4 induces expression of a GALI-lacZ fusion gene. 745::pRY171, which contains an integrated GALI-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.

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 UASo (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 UASo upstream of the CYC7 coding sequence, which is fused to a fragment containing a portion of RPS1, a gene of which the transcript is spliced. The RPS1 fragment contains part of the first exon, the intron, and part of the second exon. The second exon of RPS1 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 RPS1 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 UASo. β-galactosidase activity in this experiment was about 4% of the level observed in a GAL4" strain when transcription was activated from UASo 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 UASo. 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 UASo 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 UASo, 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.
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 GAL7 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 GAL7 that is present in our construct (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 UAS0, and a C-terminal domain that stimulates transcription, interacts with GAL80 (Loughn and Gootzland, 1984), and directs oligomerization.

We have recently constructed a hybrid protein composed of LexA and a positive regulatory 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 a leu2 ura3. SHC22C, a gal4 ura3 leu2, was a gift of Susan Hanley. SC294, a (gal4-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 GAL4 lacZ fusion gene, with Apa I in the URA3 gene, transforming the strains with the linearized plasmid DNA, and selecting stable URA3 transformants. Bacterial strain JM101 (Messing et al., 1981) was the host for most plasmid constructions.

**Plasmids**

All plasmids carry the URA3 gene, a 2μ replicator, and relevant portions diagrammed in Figure 2. LR120B, which contains UAS0 and the GAL7 gene fused to lacZ, and LR141, which does not contain UAS0, have been described (West et al., 1984). 1155 was made from LR141 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, that contains the "17-mer" (the 17 bp near-consensus GAL4 DNA site of action [Giniger et al., 1985]). pGL669-Z, which carries UAS0 and UAS0 upstream of a CYC7-lacZ fusion gene, and pGL506S, which carries UAS0 upstream of the CYC7-lacZ gene, have been described (Guarente and Ptashne, 1981; Guarente et al., 1983). pGL670Z, a gift of L. Guarente, is a derivative of pGL669-Z from which the Xho I-Xho I fragment that contains the upstream activation sites has been deleted. 1057 and 1155 were constructed from pGL670Z by inserting a single lexA operator at the Xho I site or the Sal I site, 178 and 577

![Diagram](https://via.placeholder.com/150)

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

<table>
<thead>
<tr>
<th>Downstream Element Found in First Plasmid</th>
<th>LexA</th>
<th>LexA-GAL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Operator</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>lexA Operator</td>
<td>&lt;1</td>
<td>30</td>
</tr>
</tbody>
</table>

Constructions are shown in Figure 6 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.
Transcriptional Activation by LexA-GAL4 Hybrid

nucleotides upstream of the most upstream CYC1 transcription start site, respectively. pSVy14 is a derivative of plp807072 in which a fragment of DNA that contains the T7-mer has been inserted at the Xho I site 178 nucleotides upstream of the CYC1-His2 fusion gene. H1916, a gift of John Teem, was described by Teem and Rosbash (1983). It contains a Sal I site in the RPS1 intron about 160 nucleotides downstream from the most upstream CYC1 transcription start site. 1146 was constructed from H1916 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 Pat I-Xmn I piece was isolated from pRB451, a tac promoter derivative of pRB191 (Brent and Plashe, 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 Hpa I. The Xho I ends were then filled in by treatment with the Klenow fragment of DNA polymerase I and then were cut with Hind III to isolate 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 861. These two pieces were inserted into a Pat I-Hind III backbone fragment of pBR322 (Boivier 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, 1981). This fragment contained DNA 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 pEP-8, a gift of R. R. Yocum, that confers resistance to tetracycline if Hind III-inverted 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-inverted lexA-GAL4 fragment was inserted into pAHA5, a plasmid made by Gustav Amman that contains the Ada1 promoter, the Leu2 selectable marker, and the 2 µ replicator, to create plasmid 1184. RNA made in vitro by SP6 polymerase from plasmid 1184 is complementary to RNA made in vivo from GAL4. Since any transcription from the chromosomal GAL4 gene would produce message that could also hybridize with the SP6 probe, we used strain S1054, which contains a deletion of the GAL4 and GAL10 genes, for these experiments. Cells contained the plasmids shown in the legend to Figure 4. Since gatt* strains cannot grow on galactose medium, we grew the cells on glucose medium and used cells transformed with the plasmid LR1202B, 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 developed by K. Durban, very similar to that used by Carlso and Botstein (1989). 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 pBR322 was digested with Hpa II (Sutcliffe, 1978), and the fragments were labeled with 32P (Maniatis et al., 1982).

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, carbencillin at 60 µg/ml, and isopropyl-β-D-thiogalactopyranoside (IPTG) at 5 x 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 ("galactose medium") and 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 pUA1 or another LexA-GAL4 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 activity in liquid cultures of E. coli was measured according to Miller (1979) 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-D-galactopyranoside, as described (Brent and Ptashne, 1984; Yocum et al., 1984). In DBY745, results of 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 lacZ mutation, was transformed separately with four similar plasmids that contain a tac promoter as follows: pph2, which does not direct the synthesis of a regulatory protein; pEA80, which direct the synthesis of bacteriophage lambda repressor (Amman et al., 1990); pID451, 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 x 108 cells/ml. IPTG was added to these cultures to a concentration of 5 x 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 LR114, which contains DNA from the boundary between GAL1 and lecZ and extends upstream to 167 nucleotides 5' of the primary GAL1 transcription start, was inserted into the SP6 vector pSP64 (Metton et al., 1994) 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 GAL4. Since any transcription from the chromosomal GAL4 gene would produce message that could also hybridize with the SP6 probe, we used strain S1054, 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 gatt* strains cannot grow on galactose medium, we grew the cells on glucose medium and used cells transformed with the plasmid LR1202B, 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 developed by K. Durban, very similar to that used by Carlso and Botstein (1989). 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 pBR322 was digested with Hpa II (Sutcliffe, 1978), and the fragments were labeled with 32P (Maniatis et al., 1982).

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