Cell, Vol. 52, 179–184, January 29, 1988, Copyright © 1988 by Cell Press

DNA-Bound Fos Proteins Activate Transcription in Yeast

Karen Lech, Kate Anderson, and Roger Brent
Department of Molecular Biology
Massachusetts General Hospital
Boston, Massachusetts 02114
and Department of Genetics
Harvard Medical School
Boston, Massachusetts 02115

Summary

We constructed genes encoding the DNA binding region of the bacterial LexA repressor fused to the v-*fos* and c-*fos* oncogene products. The resulting LexA-Fos fusion proteins activated transcription in yeast. Transcription activation by these proteins was as strong as transcription activation by proteins native to yeast. LexA-Fos fusion proteins only activated transcription of genes when they were bound to LexA binding sites inserted upstream of those genes. Transcription was activated less strongly by similar proteins in which the DNA binding region of LexA was fused to vMyc and cMyc. Transcription was not activated by native LexA or by proteins containing the DNA binding domain of LexA fused to bacteriophage 434 repressor or yeast MATα2 protein. These results demonstrate that Fos proteins activate eukaryotic gene expression when they are bound to promoter DNA, and thus suggest that Fos proteins exert some of their effects because they stimulate transcription of cellular genes. Regulation of transcription by Fos and Myc proteins in yeast provides a phenotype that may facilitate genetic analysis of the function of these proteins in higher organisms.

Introduction

The viral and cellular *fos* and *myc* oncogenes encode interesting proteins. Perhaps the most striking property of these gene products (here called Fos and Myc proteins) is that overexpression of either of them in normal rat fibroblasts, together with expression of the activated *ras* oncogene product, transforms the cells and endows them with the ability to form tumors in living animals (Land et al., 1983; Ruley, 1983). In addition to this tumorigenic effect, expression of large amounts of either Fos or Myc proteins in a variety of cell types allows the cells to grow indefinitely in cell culture (reviewed in Bishop, 1985, and Weinberg, 1985). In contrast to the effects the proteins have when expressed inappropriately, little is known about the effects of their normal expression. Transcription of cellular *fos* and *myc* genes is often induced in response to treatments that stimulate cell growth (Kelly et al., 1983; Greenberg and Ziff, 1984). Moreover, c-*fos* transcription is induced by a number of treatments that cause cell differentiation or potentiate nerve cell activity (Kruijer et al., 1985; Greenberg et al., 1985, 1986).

Still less is known about the mechanisms by which Fos and Myc proteins exert their effects. It is known that the proteins are phosphorylated, localized to the cell nucleus, and possess an affinity for DNA (Donner et al., 1982; Watt et al., 1985; Renz et al., 1987). One plausible idea for the Fos proteins is that they exert their effects by altering gene expression (see, for example, Varmus, 1987). Similarly, it is possible that Myc proteins might exert some of their effects on cell growth because they alter gene expression (see, for example, Kingston et al., 1985; Bishop, 1985; Weinberg, 1985), although alternative roles for Myc in RNA processing and DNA replication have also been proposed (see, for example, Sullivan et al., 1986; Studzinski et al., 1986).

A strong formulation of the transcriptional regulatory hypothesis for Fos and Myc proteins is that these proteins might immortalize cells and cause cancer because they bind to the promoter regions of specific genes and activate or repress their transcription. There is some evidence that supports this idea. Both Fos and Myc proteins reportedly cause "transactivation," respectively stimulating transcription of transiently expressed transfected mouse α1 collagen and human *hsp70* genes (Setoyama et al., 1986; Kingston et al.,1984; Kaddurah-Daouk et al., 1987). Interpretation of these results has been clouded by the fact that transactivation has not been shown to depend on a direct interaction between the oncogene products and the promoters of the genes whose transcription they reportedly stimulate. However, three recent findings make such interactions plausible. First, Fos or an antigenically similar protein has been found to be associated with the promoter of at least one gene, the adipocyte aP2 gene (Distel et al., 1987). Second, another nuclear localized oncogene product, v-*jun*, binds to specific sites on DNA (Struhl, 1987) and is homologous to the c-*jun* product, which also binds specific sites on DNA and which presumably is the transcription factor AP-1 (Bohmann et al., 1987). Finally, both Fos and Myc proteins contain sequences that might be involved in site-specific DNA binding, homologous to the DNA binding portions of GCN4 and v-*jun* (Vogt et al., 1987).

Here we present an analysis of transcription activation by Fos and Myc proteins. These experiments relied on recent research on control of transcription in yeast. Upstream of most yeast genes (for example, the *GAL1* gene shown or the CYC1 gene), there is a stretch of nucleotides called a UAS, or upstream activation site. UAS's contain binding sites for transcription activator proteins such as GAL4, HAP1, and GCN4 (Giniger et al., 1985; Pfeifer et al., 1987; Hill et al., 1986) (Figure 1a). Deletion of the UAS abolishes transcription (Figure 1b) (Lalonde et al., 1986; West et al., 1984). We recently expressed new transcription activators in yeast in which the DNA binding portion of the E. coli LexA repressor protein was fused to GAL4 and GCN4. These new proteins, LexA-GAL4 and LexA-GCN4, stimulate transcription of genes if and only if a LexA operator is inserted into nearby upstream DNA (Fig-

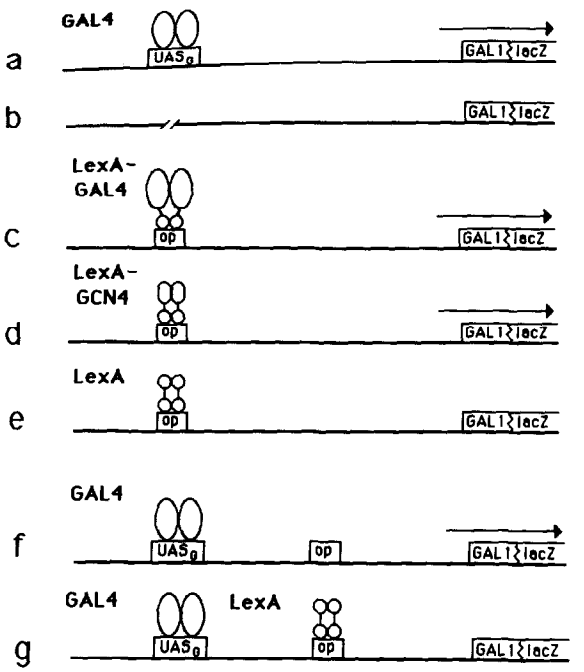

Figure 1. Activating and Repressing Yeast Genes

(a) *GAL1-lacZ* fusion gene with UAS_G. The major *GAL1* transcription startpoint is 340 nucleotides from UAS_G. (b) Deletion of UAS_G abolishes transcription. (c) Substitution of a LexA operator for UAS_G allows DNA binding and transcription activation by LexA-GAL4 and (d) LexA-GCN4 hybrid proteins but not by native LexA (e) (Brent and Ptashne, 1985). However, native LexA represses *GAL1* transcription if it is bound to LexA operators positioned at any of a number of locations downstream of UAS_G but upstream of the transcription start (f) and (g) (Brent and Ptashne, 1984). All results depicted here for LexA and LexA fusion proteins are valid in *CYC1* promoter derivatives (Brent and Ptashne, 1985; unpublished data).

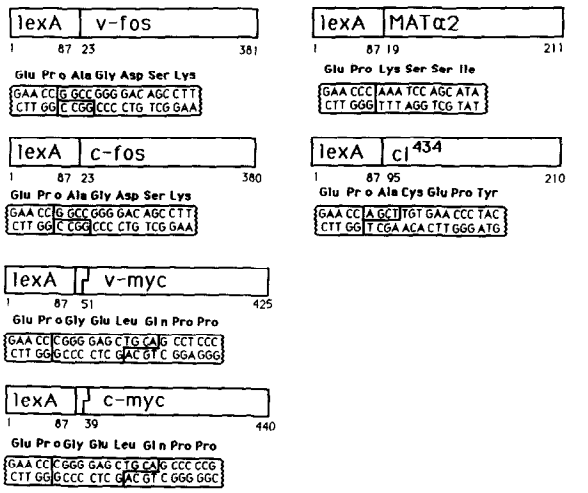

Figure 2. Fusion Proteins Used in These Experiments

Each LexA fusion protein is shown as a bar, and the predicted sequence in the vicinity of the junction with the LexA coding sequence is shown below it.

ures 1c and 1d). Native LexA does not stimulate transcription of the same genes (Figure 1e) (Brent and Ptashne, 1985; see also, Hope and Struhl, 1986). Portions of GCN4 and GAL4 necessary to activate transcription are distinguished by the fact that they contain stretches of acidic amino acids (Hope and Struhl, 1986; Ma and Ptashne, 1987a). Recently, Ma and Ptashne have shown that proteins that contain the DNA-binding region of the GAL4 protein fused to acidic amino acids also activate transcription when bound to DNA (1987b).

We show in this paper that mouse cellular Fos and avian viral Fos stimulate gene expression in yeast. Transcription activation occurs if and only if the proteins are bound to DNA upstream of target genes. In these experiments, we expressed in yeast proteins that contained the DNA binding region of E. coli LexA protein joined to cFos and vFos. These proteins activated transcription of target genes that had LexA operators positioned upstream of their transcription starts. Both LexA-Fos proteins activated transcription strongly, as strongly as powerful activator proteins native to yeast. The strength of transcription activation by Fos proteins in yeast suggests that this phenotype reflects one of their functions in higher cells.

Results

We made use of fusion genes containing the LexA amino terminus joined to avian vFos, mouse cFos, avian vMyc, human cMyc, bacteriophage 434 repressor, and yeast MATα2 gene product. These genes, which encoded proteins here called LexA-vFos, LexA-cFos, LexA-vMyc, LexA-cMyc, LexA-434, and LexA-α2 were carried on *LEU2*$^+$ 2μ-containing yeast expression plasmids. Structures of the fusion genes are shown in Figure 2. The yeast expression plasmids, and a previously constructed vector that directed the synthesis of native LexA protein, were each transformed into *leu2*$^-$ *ura3*$^-$ yeast. These cells had already been transformed with a plasmid that contained a target gene: either a *CYC1-lacZ* or a *GAL1-lacZ* fusion gene that either carried or did not carry an upstream LexA operator (Figure 3; Brent and Ptashne, 1985). In addition, these plasmids carried a 2μm replicator and a *URA3*$^+$ selectable marker. Maintenance of both plasmids in most of the cells in the culture was ensured by growing the cells in medium that lacked leucine and uracil. Transcription of the target *GAL1-lacZ* and *CYC1-lacZ* genes was measured by assaying the amount of β-galactosidase activity produced by cultures of cells containing these plasmids.

Figure 3 shows activation of transcription by LexA-Fos fusion proteins. Both LexA-vFos and LexA-cFos stimulated transcription of *GAL1-lacZ* and *CYC1-lacZ* genes whose upstream activation sites had been replaced with a single LexA operator. Estimated very conservatively, LexA-cFos and LexA-vFos activated CYC1 transcription by factors of 180× and 210×, and GAL1 transcription by factors of 470× and 160×. For the *CYC1-lacZ* gene, transcription activation by LexA-vFos was about 97% as strong, and by LexA-cFos about 83% as strong, as by LexA-GAL4 (see Figure 3). The corresponding levels of LexA-vFos and LexA-cFos activation of the *GAL1-lacZ* gene were about 98% and 280% (see Figure 3). In a different set of experi-

	Promoter Derivatives	LexA-v-Fos	LexA-c-Fos	LexA-v-Myc	LexA-c-Myc	LexA-GAL4	LexA	LexA-MATα2	LexA-434
a	OP — GAL1 ⟩ lacZ	1480	3300	120	30	1680	1	5	2
b	GAL1 ⟩ lacZ	9	7	11	10	10	4	5	2
c	OP — CYC1 ⟩ lacZ	1480	1800	230	25	2620	5	14	3
d	CYC1 ⟩ lacZ	7	10	18	9	12	5	20	3

Figure 3. Activation of Transcription by Fos and Myc

The numbers denote units of β-galactosidase activity measured in cultures of the yeast strain DBY745. Cells contain two plasmids: a "target plasmid," which carries one of the promoter derivatives diagrammed on the left, and an expression plasmid, which directs the synthesis of one of the proteins listed on the top. Line 1 shows 1840, identical to plasmid 1145 (Brent and Ptashne, 1985). The LexA operator in this plasmid is 167 nucleotides from the major *GAL1* transcription start. Line 2 shows LR1Δ1, which is identical to 1840 except that it lacks a LexA operator. Line 3 shows 1107, identical to plasmid 1155 (Brent and Ptashne, 1985), which carries a LexA operator 178 nucleotides upstream of the most upstream *CYC1* transcription start. Line 4 shows pLG670Z, which is otherwise identical except that it lacks a LexA operator. Plasmids that direct the synthesis of LexA-containing proteins are described in Experimental Procedures.

Table 1. Transcription Activation by LexA-vFos and LexA-vMyc

Activator	Target Gene	β-Galactosidase Activity
LexA-vFos	*lexAop-CYC1-lacZ*	295
LexA-vMyc	*lexAop-CYC1-lacZ*	55
GAL4	*GAL4 17-mer-CYC1-lacZ*	160
B17	*GAL4 17-mer-CYC1-lacZ*	120
B42	*GAL4 17-mer-CYC1-lacZ*	40
B3	*GAL4 17-mer-CYC1-lacZ*	60
LexA-vFos	*lexAop-GAL1-lacZ*	450
LexA-vMyc	*lexAop-GAL1-lacZ*	40
GAL4	*GAL4 17-mer-GAL1-lacZ*	450
B17	*GAL4 17-mer-GAL1-lacZ*	340
B42	*GAL4 17-mer-GAL1-lacZ*	150
B3	*GAL4 17-mer-GAL1-lacZ*	70

Shown are units of β-galactosidase activity in cultures of RBY52 cells. These cells contain two plasmids, a transcription activator plasmid, which directs the synthesis of either LexA fusion proteins, GAL4-acidic amino acid fusion proteins, or native GAL4, and a target plasmid, which contains either a single LexA binding site (LexAop) or a single GAL4 binding site (GAL4 17-mer) inserted an identical distance upstream of either a *GAL1-lacZ* or a *CYC1-lacZ* fusion gene. Cells were grown in galactose-glycerol-ethanol medium as described (Ma and Ptashne, 1987b), under which conditions the GAL4-acidic amino acid fusion proteins showed maximum transcription stimulation. Under these conditions, GAL4-acidic amino acid fusion proteins activated transcription of *GAL1-lacZ* and *CYC1-lacZ* fusion genes less strongly than LexA-vFos and GAL4, but with intensities comparable to or greater than those of LexA-vMyc. In control experiments, we showed that LexA fusion proteins did not stimulate transcription of target genes with upstream GAL4 binding sites, and GAL4 fusion proteins did not stimulate transcription of target genes with upstream LexA binding sites (not shown). β-galactosidase activity measured in the absence of an activator protein was shown to be <1 unit for all target genes (not shown).

ments (see Table 1), we showed that transcription activation by the LexA-vFos fusion protein bound to a single site upstream of *GAL1-lacZ* or *CYC1-lacZ* fusion genes was equal to or stronger than that caused by native GAL4 protein bound to a single GAL4 binding site positioned upstream of the same genes (see Figure 1). Depending on the target used, transcription stimulation by LexA-Fos proteins was 1.5–7.5 times stronger than that caused by the most powerful GAL4-acidic amino acid fusion proteins of Ma and Ptashne, kindly provided by Jun Ma (see Table 1).

Transcription activation by Fos fusion proteins in this system only occurred when the proteins were bound to DNA near startpoints of transcription. This fact is shown in Figure 3. LexA-vFos and LexA-cFos did not activate transcription of otherwise identical target genes that did not bear upstream LexA operators (see Figure 3).

LexA-cMyc and LexA-vMyc fusion proteins also stimulated transcription. Transcription activation by LexA-Myc fusion proteins was much weaker than that by LexA-Fos fusion proteins, but showed the same absolute dependence on DNA binding upstream of target genes (see Figure 3).

Native LexA, LexA-α2, and LexA-434 did not stimulate transcription of LexA operator containing constructions (see Figure 3). Because they did not stimulate transcription, it was necessary to demonstrate that these proteins actually bound LexA operators in yeast. We demonstrated operator binding by showing that these proteins repressed transcription of other promoter constructions (see Figures 1f and 1g; C. Besmond, unpublished data) that carried LexA operators between the *GAL1* UAS and the startpoint of transcription (data not shown).

Discussion

We expressed cFos and vFos in yeast as fusion proteins that contained the DNA binding region of the bacterial LexA repressor protein at their amino termini. We assayed the ability of the fusion proteins to stimulate transcription when bound to LexA operators positioned upstream of target genes. LexA-cFos and LexA-vFos strongly activated transcription. Similar LexA-vMyc and LexA-cMyc proteins also activated transcription in yeast, but less strongly. Other proteins, LexA, LexA-434, and LexA-α2, bound to LexA operators but did not activate transcription.

Fos proteins are powerful activators of transcription in yeast, as powerful as the native GAL4 activator protein (see Figure 3 and Table 1). It has recently been shown that the transcription apparatus is sufficiently conserved between yeast and mammalian cells to permit transcription activators from yeast to function in mammalian cells (Kakidani and Ptashne, 1988; Webster et al., 1988). Because of the strength of transcription activation by Fos proteins in yeast, we believe that Fos proteins are likely to be potent transcription factors when bound to DNA upstream of genes in higher cells. Transcription activation by

Myc proteins in yeast is less powerful, and we are not sure whether it reflects transcription activation by the proteins in higher cells. We do not know whether transcription activation by either Fos or Myc is relevant to their oncogenic effects. Analysis in mammalian cells of Fos and Myc proteins mutant in the yeast transcription function (K. Lech, unpublished data) may eventually allow us to settle this point.

In these experiments, transcription activation by Fos requires a direct interaction between the Fos protein and the promoter of a gene, that is, it is only observed when LexA-cFos or LexA-vFos are bound to LexA operators positioned upstream of target genes. The weaker transcription activation by LexA-vMyc and LexA-cMyc also depends on a direct interaction of the fusion protein with the promoter. Based on these experiments, we think it likely that "transactivation" of transfected gene expression by Fos (and perhaps by Myc) requires a direct interaction between the protein and the promoters of the activated genes. However, it is possible that some or all of these proteins' promoter binding specificity might normally be caused by an association with other proteins, and that bringing the proteins to DNA with LexA in our experiments has circumvented this requirement.

It is tempting to speculate that, as for other yeast transcription activators, cFos and vFos might stimulate transcription because they contain stretches of acidic amino acids (Hope and Struhl, 1986; Ma and Ptashne, 1987a, 1987b). In fact, Fos proteins contain a modestly acidic stretch (albeit flanked by basic amino acids), and this stretch lies within a region of the protein that has been shown to be important for its transforming and immortalizing function (Jenuwein and Mueller, 1987). However, Fos proteins are much better transcription activators than the more acidic Myc proteins and GAL4-acidic amino acid proteins, and this fact makes us consider alternative explanations for its transcription stimulation. One possible explanation is that Fos displays its modestly acidic stretch in a way that is particularly attractive to some component of the transcription apparatus. Another idea, which we favor, is that transcription activation by Fos is due to negative charges on the surface of the protein, but that most of those negative charges are contributed by phosphate groups rather than by acidic amino acids. We speculate that transcription stimulatory activity of other DNA binding eukaryotic phosphoproteins might similarly be due to negative charges from their phosphate groups (see, for example, Sorger et al., 1988).

The amino-terminal portion of LexA present in the Fos and Myc fusion proteins does not itself bind LexA operators efficiently (Schnarr et al., 1985; Hurstel et al., 1986). This fact can be explained by positing that LexA's amino terminus does not dimerize efficiently, but that dimerization must occur before the amino terminus can bind to DNA (Brent and Ptashne, 1985). Consistent with this idea, we have been unable to demonstrate operator binding by the free amino terminus of LexA when that protein is synthesized in yeast (Besmond and Brent, unpublished data), but we and others have observed efficient operator binding when LexA's amino terminus is fused to proteins known to form dimers or tetramers (Brent and Ptashne, 1985; Anderson and Brent, unpublished data; Hope and Struhl, 1986). In these experiments, we have observed that LexA-434, LexA-α2, LexA-Myc, and LexA-Fos bind LexA operators. This fact is most easily explained by postulating that, like 434 repressor and MATα2 protein, native Myc and Fos proteins are dimers or tetramers.

Finally, we note that we have described a very easily scored effect of Fos and Myc gene expression. Yeast containing Fos and Myc fusion proteins and the appropriate target genes form blue colonies on standard Xgal indicator medium. We believe that exploitation of this phenotype will facilitate sophisticated genetic analysis of Fos and Myc. Moreover, we hope that application of these sorts of yeast genetic techniques will aid in the analysis of other putative transcriptional regulatory proteins: other nuclear localized oncogene products and proteins important for proper development of higher organisms.

Experimental Procedures

Microbiological Work

DBY745 (*α ura3 leu2*) was used as host in most experiments. For those experiments requiring a *gal4* host RBY52 (*a Δgal4 ura3-52 leu2 his3*) was used. Yeast transformation was performed by a minor modification of the LiCl technique of Ito et al. (1983). Yeast were grown in appropriately supplemented medium which contained either 2% glucose or 2% glycerol, 2% ethanol, and 2% galactose as a carbon source (Sherman et al., 1983). β-galactosidase assays of cultures of plasmid-bearing yeast strains were performed as in Yocum et al. (1984). RB926 *hsdR thiA endA* and JM101 *supE thiA Δ (lac-proAB)/F' traD36 proA$^+$ proB$^+$ lacI^q lacZΔM15* were used as hosts for plasmid DNA construction. Bacteria were grown using standard techniques (Miller, 1972; Ausubel et al., 1987).

DNAs

Plasmids were constructed by standard techniques (Maniatis et al., 1982; Ausubel et al., 1987).

Target Plasmids

All carried the *URA3*$^+$ gene, a 2 μm replicator, and promoter elements as shown in Figure 3. LR1Δ1 and pLG670Z have been described (West et al., 1985; Brent and Ptashne, 1985). 1840 and 1107 are respectively identical to 1145 and 1155, which have been described (Figure 2; Brent and Ptashne, 1985). SV15 and SV14 are identical to 1840 and 1107 respectively except that the LexA operator has been replaced by a synthetic 17 bp consensus GAL4 binding site (Giniger et al., 1985).

LexA-Fusion Gene Plasmids

HindIII-ended fusion genes were inserted into the HindIII site of pAAH5. pAAH5 carries the *LEU2*$^+$ gene, a portion of the 2μ plasmid to allow replication in yeast, and a DNA fragment containing the ADH1 promoter and transcription terminator flanking the HindIII site (Ammerer, 1983). pRB500, the prototype plasmid that directs the synthesis of native LexA in yeast, has been described (Brent and Ptashne, 1984). pCB109, which directs the synthesis of LexA-434 in yeast, was a gift from Claude Besmond. It was constructed by similar techniques and encodes a 203 amino acid protein whose sequence across the fusion junction is pro ala cys glu, where pro is amino acid 87 of LexA and ala is amino acid 95 of 434 repressor (see Figure 2). pVR1001 and pVR1004, which direct the synthesis in yeast of LexA-cFos and LexA-cMyc (see Figure 2), were gifts from Vic Rivera, who constructed them from cDNAs encoding mouse cFos and human cMyc by methods analogous to those used to construct the LexA-vFos and LexA-vMyc expression plasmids. The expression plasmids, pKA1190, pKA389, and pKL222, carry the LexA-vFos, LexA-vMyc, and LexA-α2 fusion genes, respectively. Construction of these fusion genes is described below.

LexA-vMyc

Plasmid MC38 contains the entire avian MC29 avian myelocytomatosis virus (Reddy et al., 1983). We cut this plasmid at an SspI site downstream of the viral *gag-myc* gene, treated the mixture with T4 DNA ligase in the presence of HindIII linkers, treated the ligation mixture with PstI and HindIII, isolated the PstI–HindIII piece that contained most of the *myc* gene, and inserted it into PstI–HindIII cut pUC18 to generate pKA58. In a separate series of constructions, we ligated a BamHI–XmnI piece from pRB480 (Brent and Ptashne, 1984), which encoded the amino terminal portion of LexA, to a double-stranded adaptor of the sequence CGGGGAGCTGCA.
GCCCCTCG We inserted the resulting fragment into BamHI–PstI cut pUC18 to yield the plasmid pKA144. BamHI cuts in the tetracycline gene of pRB480 and pBR322 (Sutcliffe, 1978). We ligated the BamHI–PstI LexA piece from pKA144 and the PstI–HindIII v-*myc* piece from pKA58 with the HindIII–BamHI piece from pBR322. We screened plasmid DNA from bacterial colonies on tetracycline-containing LB plates. One such plasmid, pKA210, contains the first 87 codons of *lexA*, three codons contributed by the adaptor fragment, and the last 373 codons of v-*myc*. This fusion gene encodes a 465 amino acid protein whose sequence across the fusion junction is pro gly glu leu gln pro, where pro is amino acid 87 of LexA and gln is amino acid 51 of native v-myc protein (see Figure 2).

LexA-vFos

Plasmid pFBJ-2 contains an FBJ-MuSV provirus in a 5800 bp HindIII fragment inserted into the HindIII site of pBR322 (Van Beverens et al., 1983). We cut this plasmid with EagI and filled in the 5′ overhang with Klenow. We then cut the plasmid with HindIII and isolated the 3600 bp fragment that contained the carboxy-terminal portion of v-fos protein. We ligated this v-*fos* EagI (filled)–HindIII piece to the *lexA* BamHI–XmnI piece from pRB480 and to the HindIII–BamHI backbone fragment of pBR322. E. coli containing this construction were identified by their tetracycline resistance. A typical resulting plasmid, pKA195, contained the first 87 codons of *lexA* fused directly to codons 23–381 of v-*fos*. This fusion gene encodes a 446 amino acid protein whose sequence across the fusion junction is pro ala gly, where pro is amino acid 87 of LexA and the first ala is amino acid 23 of the v-*fos* product.

LexA-α2

pKA144 contains two SmaI sites, one in the adaptor at the carboxyl terminus of the *lexA* fragment, the other in the pUC18 polylinker. We eliminated the SmaI site in the polylinker by T4 DNA polymerase treatment of an overlapping KpnI site. pKA1035, a typical resulting plasmid, contains a unique SmaI site after the 87th codon of LexA. We inserted into this SmaI site a 1600 bp DraI–DraI fragment from HMLα (Astell et al., 1981). This fragment encoded the MATα2 carboxyl terminus. The resulting plasmid, pKL111, contains a HindIII–HindIII fragment that contains a gene composed of codons 1–87 of *lexA* and codons 19 to 211 of MATα2. The gene encodes a 280 amino acid protein whose sequence across the fusion junction is pro lys ser ser, where pro is amino acid 87 of native LexA and lys is amino acid 19 of MATα2.

Acknowledgments

We are extremely grateful to Vic Rivera for his gift of LexA-cFos and LexA-cMyc fusion expression plasmids, which he constructed and showed activated yeast transcription during a rotation in this laboratory; to Claude Besmond for plasmids and for communicating unpublished data; to Michael Greenberg and Joel Belasco for a plasmid containing cFos cDNA; to Adrian Hayday for a plasmid containing cMyc cDNA; and to Jun Ma for plasmids that synthesize GAL4-acidic amino acid fusion proteins. We thank David Eisenmann, Anne Ephrussi, Robert E. S. D. Kingston, Gary B. Ruvkun, Jack Szostak, Cliff Tabin, Richard Treisman, and Lou Zumstein for helpful discussions and/or comments on the manuscript. This work was supported by a grant from Hoechst AG and an award to R. B. from the Pew Scholars Program.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 23, 1987; revised December 21, 1987.

References

Ammerer, G. (1983). Expression of genes in yeast using the ADCI promoter. Meth. Enzymol. *101*, 192–210.

Astell, C. R., Ahlstrom-Jonasson, L., Smith, M., Tatchell, K., Nasmyth, K. A., and Hall, B. D. (1981). The sequence of the DNAs coding for the mating-type loci of Saccharomyces cerevisiae. Cell *27*, 15–23.

Ausubel, F. M., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., and Struhl, K. (1987). Current Protocols in Molecular Biology (New York: Greene Publishing Associates).

Bishop, J. M. (1985). Viral oncogenes. Cell *42*, 23–38.

Brent, R. (1985). Repression of transcription in yeast. Cell *42*, 3–4.

Brent, R., and Ptashne, M. (1984). A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene. Nature *312*, 612–615.

Brent, R., and Ptashne, M. (1985). A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell *43*, 729–736.

Bohmann, D., Bos, T. J., Admon, A., Nishimura, T., Vogt, P. K., and Tijan, R. (1987). Human oncogene *c-jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science *238*, 1386–1392.

Distel, R. J., Ro, H.-S., Rosen, B. S., Groves, D. L., and Spiegelman, B. M. (1987). Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-*fos*. Cell *49*, 835–844.

Donner, P., Greiser-Wilke, I., and Moelling, K. (1982). Nuclear localization and DNA binding of the transforming gene product of the avian myelocytomatosis virus. Nature *296*, 262–266.

Giniger, E., Varnum, S. M., and Ptashne, M. (1985). Specific DNA binding of GAL4, a positive regulatory protein of yeast. Cell *40*, 767–774.

Greenberg, M. E., and Ziff, E. B. (1984). Stimulation of 3T3 cells induces transcription of the *c-fos* proto-oncogene. Nature *311*, 433–438.

Greenberg, M. E., Greene, L. A., and Ziff, E. B. (1985). Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. J. Biol. Chem. *260*, 14, 101–14,110.

Greenberg, M. E., Ziff, E. B., and Greene, L. A. (1986). Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. Science *234*, 80–83.

Hill, D. E., Hope, I. A., Macke, J. P., and Struhl, K. (1986). Saturation mutagenesis of the yeast *HIS3* regulatory site: requirements for transcriptional induction and for binding by GCN4 activator protein. Science *43*, 177–188.

Hope, I. A., and Struhl, K. (1986). Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell *46*, 885–894.

Hurstel, S., Granger-Schnarr, M., Daune, M., and Schnarr, M. (1986). *In vitro* binding of LexA repressor to DNA: evidence for the involvement of the amino-terminal domain. EMBO J. *5*, 793–798.

Ito, H., Fukada, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *53*, 163–168.

Jenuwein, T., and Müller, R. (1987). Structure–function analysis of *fos* protein: a single amino acid change activates the immortalizing potential of v-*fos*. Cell *48*, 647–657.

Johnson, A. D., and Herskowitz, I. (1985). A repressor (*MAT*α2 product) and its operator control expression of a set of cell type specific genes in yeast. Cell *42*, 237–247.

Kaddurah-Daouk, R., Greene, J. M., Baldwin, A., Jr., and Kingston, R. (1987). Activation and repression of mammalian gene expression by the c-myc protein. Genes and Development *1*, 347–357.

Kakidani, H., and Ptashne, M. (1988). GAL4 activates gene expression in mammalian cells. Cell *52*, 000–000.

Keegan, L., Gill, G., and Ptashne, M. (1986). Separation of DNA binding from the transcription-activation function of a eukaryotic regulatory protein. Science *231*, 699–704.

Kelly, K., Cochran, B. H., Stiles, C. D., and Leder, P. (1983). Cell-specific regulation of the *c-myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell *35*, 603–610.

Kingston, R. E., Baldwin, A. S., Jr., and Sharp, P. A. (1984). Regulation of heat shock protein 70 gene expression by c-myc. Nature *312*, 280–282.

Kingston, R. E., Baldwin, A. S., and Sharp, P. A. (1985). Transcription control by oncogenes. Cell *41*, 3–5.

Koenen, M., Ruether, U., and Mueller-Hill, B. (1982). Immunoenzymatic detection of expressed gene fragments cloned in the *lacZ* gene of *E. coli*. EMBO J. *1*, 509–512.

Kruijer, W., Cooper, J. A., Hunter, T., and Verma, I. M. (1985). Induction of the proto-oncogene *fos* by nerve growth factor. Proc. Natl. Acad. Sci. USA *82*, 7330–7335.

LaLonde, B., Arcangioli, B., and Guarente, L. (1986). A single yeast upstream activation site UAS1 has two distinct regions necessary for its activity. Mol. Cell. Biol. *6*, 4640–4696.

Land, H., Parada, L. F., and Weinberg, R. A. (1983). Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature *304*, 596–601.

Ma, J., and Ptashne, M. (1987a). Deletion analysis of GAL4 defines two transcriptional activating segments. Cell *48*, 847–853.

Ma, J., and Ptashne, M. (1987b). A new class of yeast transcriptional activators. Cell *51*, 113–119.

Maniatis, T., Fritsch, E., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Miller, J. (1972). Experiments in Molecular Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Pfeifer, K., Prezant, T., and Guarente, L. (1987). Yeast HAP1 activator binds to two upstream activation sites of different sequence. Cell *49*, 19–27.

Reddy, E. P., Reynolds, R. K., Watson, D. K., Schultz, R. A., Lautenberger, J., and Papas, T. S. (1983). Nucleotide sequence analysis of the proviral genome of avian myelocytomatosis virus (MC29). Proc. Natl. Acad. Sci. USA *80*, 2500–2504.

Renz, M., Verrier, B., Kurz, C., and Mueller, R. (1987). Chromatin association and DNA binding properties of the c-fos proto-oncogene product. Nucl. Acids Res. *15*, 277–292.

Ruley, H. E. (1983). Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature *304*, 602–606.

Schnarr, M., Pouyet, J., Granger-Schnarr, M., and Daune, M. (1985). Large-scale purification, oligomerization, equilibria, and specific interaction of the LexA repressor of *Escherichia coli*. Biochemistry *24*, 2812–2818.

Setoyama, C., Frunzio, R., Liau, G., Mudryj, M., and de Crombrugghe, B. (1986). Transcriptional activation encoded by the v-fos gene. Proc. Natl. Acad. Sci. USA *83*, 3213–3217.

Sherman, F., Fink, G. R., and Lawrence, C. W. (1983). Laboratory Manual for a Course in Yeast Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Sorger, P. K., Lewis, M. J., and Pelham, H. R. B. (1987). Heat shock factor is regulated differently in yeast and HeLa cells. Nature *329*, 81–84.

Struhl, K. (1987). The DNA-binding domains of the jun oncoprotein and the yeast GCN4 transcriptional activator proteins are functionally homologous. Cell *50*, 841–846.

Studzinski, G. P., Brelvi, Z. S., Feldman, S. S., and Watt, R. A. (1986). Participation of c-myc protein in DNA synthesis of human cells. Science *234*, 467–470.

Sullivan, N. F., Watt, R. A., Dellannoy, M. R., Green, C. L., and Spector, D. L. (1986). Co-localization of the myc oncogene protein and small nuclear ribonucleoprotein particles. Cold Spring Harbor Symp. Quant. Biol. *51*, 943–947.

Sutcliffe, J. G. (1978). Sequence of the plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. *43*, 77–90.

Van Beveren, C., van Straaten, F., Curran, T., Müller, R., and Verma, I. M. (1983). Analysis of FBJ-MuSV provirus and *c-fos* (mouse) gene reveals that viral and cellular *fos* gene products have different carboxy termini. Cell *32*, 1241–1255.

Varmus, H. E. (1987). Oncogenes and transcriptional control. Science *238*, 1337–1339.

Vogt, P. K., Bos, T. J., and Doolittle, R. F. (1987). Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxyl-terminal region of a protein coded for by the oncogene *jun*. Proc. Natl. Acad. Sci. USA *84*, 3316–3319.

Yocum, R. R., Hanley, S., West, R., Jr., and Ptashne, M. (1984). Use of *lacZ* fusions to delimit regulatory elements of the inducible GAL1–GAL10 promoter in Saccharomyces cerevisiae. Mol. Cell. Biol. *4*, 1985–1988.

Watt, R. A., Schatzman, A. M., and Rosenberg, M. (1985). Expression and characterization of the human c-myc DNA-binding protein. Mol. Cell. Biol. *5*, 448–456.

Webster, N., Jin, J. R., Green, S., Hollis, M., and Chambon, P. (1988). The yeast UAS_G is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 *trans*-activator. Cell *52*, 169–178.

Weinberg, R. A. (1985). The action of oncogenes in the cytoplasm and nucleus. Science *230*, 770–776.

West, R. W., Jr., Yocum, R. R., and Ptashne, M. (1984). *Saccharomyces cereviseae* GAL1–GAL10 divergent promoter region: location and function of the upstream activator sequence UAS_G. Mol. Cell. Biol. *4*, 2467–2478.

Wharton, R. P., Brown, E. L., and Ptashne, M. (1984). Substituting an α-helix switches the sequence-specific DNA interactions of a repressor. Cell *38*, 361–369.