DNA-Bound Fos Proteins Activate Transcription in Yeast

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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; Ruijter, 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). Moreover, c-fos transcription is induced by a number of treatments that cause cell differentiation or potentiate nerve cell activity (Kruger 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," positively or negatively 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 αP2 gene (Distel et al., 1987). Second, another nuclear localized oncogene product, γ-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 γ-jun (Vogt et al., 1987).

Here we present an analysis of transcription activation by Fos and Myc proteins. Thesc experimentre 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 (Ginger 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., 1986). We recently expressed new transcription activators in yeast in which the DNA binding portion of the E. coli LexA repressor protein was fused to GAL1 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) GAL7-IacZ fusion gene with UASo. The major GAL7 transcription startpoint is 340 nucleotides from UASo. (b) Deletion of UASa abolishes transcription. (c) Substitution of a LexA operator for UASo 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 protein represses GAL1 transcription if it is bound to LexA operators positioned at any of a number of locations downstream of UASo 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 CYC7 promoter derivatives (Brent and Ptashne, 1985; unpublished data).

Figure 2 shows activation of transcription by LexA-Fos fusion proteins. Both LexA-vFos and LexA-cFos stimulated transcription of GAL1-IacZ and CYC7-IacZ genes whose upstream activation sites had been replaced with a single LexA operator. Estimated very conservatively, LexA-cFos and LexA-vFos activated CYC7-IacZ and CYC7-IacZ genes by factors of 160x and 210x, respectively. For the CYC7-IacZ 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-IacZ gene were about 98% and 280% (see Figure 3). In a different set of experi-
LexA-Fos Proteins Activate Transcription in Yeast

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

<table>
<thead>
<tr>
<th>Activator</th>
<th>Target Gene</th>
<th>β-Galactosidase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LexA-vFos</td>
<td>lexAop-CYC1-lacZ</td>
<td>295</td>
</tr>
<tr>
<td>LexA-vMyc</td>
<td>lexAop-CYC1-lacZ</td>
<td>55</td>
</tr>
<tr>
<td>GAL4</td>
<td>GAL4 17-mer-CYC1-lacZ</td>
<td>160</td>
</tr>
<tr>
<td>B17</td>
<td>GAL4 17-mer-CYC1-lacZ</td>
<td>120</td>
</tr>
<tr>
<td>B42</td>
<td>GAL4 17-mer-CYC1-lacZ</td>
<td>40</td>
</tr>
<tr>
<td>B3</td>
<td>GAL4 17-mer-CYC1-lacZ</td>
<td>60</td>
</tr>
<tr>
<td>LexA-vFos</td>
<td>lexAop-GAL1-lacZ</td>
<td>450</td>
</tr>
<tr>
<td>LexA-vMyc</td>
<td>lexAop-GAL1-lacZ</td>
<td>40</td>
</tr>
<tr>
<td>GAL4</td>
<td>GAL4 17-mer-GAL1-lacZ</td>
<td>450</td>
</tr>
<tr>
<td>B17</td>
<td>GAL4 17-mer-GAL1-lacZ</td>
<td>340</td>
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<tr>
<td>B42</td>
<td>GAL4 17-mer-GAL1-lacZ</td>
<td>150</td>
</tr>
<tr>
<td>B3</td>
<td>GAL4 17-mer-GAL1-lacZ</td>
<td>70</td>
</tr>
</tbody>
</table>

Shown are units of β-galactosidase activity in cultures of RBY52. 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, 1967b), 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).

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 11 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 assessed 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 transcribed 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 stimulation by other DNA binding eukaryotic phosphoproteins might similarly be due to negative charges from their phosphate groups (see, for example, Sörger 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 (Brent 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-a2, LexA-Myc, and LexA-Fos bind LexA operators. This fact is most easily explained by postulating that, like 434 repressor and MATa2 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 (a ura3 leu2) was used as host in most experiments. For those experiments requiring a gal+ 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% galactose, 2% ethanol, and 2% glycerol as a carbon source (Sherman et al., 1983). ß-galactosidase assays of cultures of plasmid-bearing yeast strains were performed as in Tocum et al. (1984). RBS25 hadR thA avdA and JM701 sup3 thA 5 (lac-proAB/F' traD36 proA+ proB') lacZ/M15 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. LRI3.1 and pLC670Z have been described (West et al., 1985; Brent and Ptashne, 1983). 1940 and 1107 are respectively identical to 1145 and 1155, which have been described (Figure 2; Brent and Ptashne, 1983). SV15 and SV14 are identical to 1140 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-end fused gene were inserted into the HindIII site of pAAH8. pAAH8 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). pCB195, 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). PR19001 and PR19004, 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-Fos and LexA-Myc expression plasmids. The expression plasmids, pKA1190, pKA1198, and pKL222, carry the LexA-Fos, LexA-Myc, and LexA-a2 fusion genes, respectively. Construction of these fusion genes is described below.
LexA-Fos Proteins Activate Transcription in Yeast

LexA-Fos Proteins Activate Transcription in Yeast (Reddy et al., 1983). We cut this plasmid at an Sspl 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, cloned the PstI-HindIII piece into pUC18 to generate pKAC8. In a separate series of constructions, we ligated a BamHI-Xmnl piece from pRB480 (Brent and Plaschke, 1984), which encoded the amino-terminal portion of LexA, to a double-stranded adapter of the sequence CGGGGAGCTGCA.

GCCTCG

We inserted the resulting fragment into BamHI-PstI cut pUC18 to yield the plasmid pKA144. BamHI cuts in the tetracycline gene of pBR480 and pBR322 (Sutcliffe, 1978). We ligated the BamHI-PstI LexA piece from pKA144 and the PstI-HindIII v-fos piece from pKAC8 with the HindIII-BamHI piece from pBR322. We screened plasmid DNA from bacterial colonies on tetracycline-containing LB plates. One such plasmid, pKA20, contains the first 87 codons of lexA, three codons contributed by the adapter fragment, and the last 373 codons of v-fos. This fusion gene encodes a 465 amino acid protein whose sequence across the fusion junction is pro gly leu glu gin pro, where pro is amino acid 87 of LexA and gin is amino acid 51 of native v-myc protein (see Figure 2).

LexA-vFos

Plasmid pFB2 contains an FBJ-MuSV provirus in a 5800 bp HindIII fragment inserted into the HindIII site of pBR322 (Van Beveren 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 3300 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-Xmnl 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 v-fos and the first ala is amino acid 23 of the v-fos product.

LexA-Myc

pKA144 contains two Smal sites, one in the adapter at the carboxyl terminus of the lexA fragment, the other in the pUC18 polylinker. We eliminated the Smal site in the polylinker by T4 DNA polymerase treatment of an overlapping KpnI site. pKA1035, a typical resulting plasmid, contains a unique Smal site after the 87th codon of lexA. We inserted into this Smal site a 1600 bp Dral-Dral fragment from HMLa (Astell et al., 1981). This fragment encoded the MATa carboxyl terminus. 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 MATa2.

Acknowledgments

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References


