A New Class of Yeast Transcriptional Activators

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Summary

We describe yeast transcriptional activators encoded by E. coli genomic DNA fragments fused to the coding sequence of the DNA-binding portion of GAL4. All of the new activating sequences that we have analyzed, like those of GAL4 and GCN4, are acidic; most of these sequences show no obvious sequence homology when compared with the identified activating regions of GAL4 and GCN4 or among themselves. We also describe a fusion protein that contains no yeast protein sequence but activates transcription in yeast.

Introduction

In the yeast Saccharomyces cerevisiae the genes required for galactose catabolism (GAL1, GAL7, and GAL10) are expressed at high levels when cells are grown in medium containing galactose (Hopper et al., 1978; Hopper and Rowe, 1978; St John and Davis, 1981). GAL4, a protein of 981 amino acids (Laughon and Gesteland, 1984; Laughon et al., 1984), is required for the expression of these genes (Douglas and Hawthorne, 1964; Oshima, 1982). The protein binds specifically to an upstream activation sequence (called the UAS0), located midway between the divergently transcribed GAL1 and GAL10 genes, and activates transcription (Guarente et al., 1982; Yncum et al., 1984; West et al., 1984; Johnston and Davis, 1984; Bram and Kornberg, 1985; Giniger et al., 1985; Lohr et al., 1985; Selleck and Majere, 1987a, 1987b). The activity of GAL4 is inhibited by the negative regulator GAL80 when cells are grown in medium lacking galactose (Douglas and Hawthorne, 1964, 1966; Oshima, 1982; Torchia et al., 1984; Nogi et al., 1984; Nogi and Fujisawa, 1984). Glucose represses the expression of the GAL genes whether or not galactose or GAL80 is present, a phenomenon called glucose repression (Adams, 1972; Atsumoto et al., 1981, 1983).

Two lines of evidence show that GAL4 binds DNA using its amino terminus and activates transcription using other parts of the protein. First, amino terminal fragments of GAL4 (residues 1–98 or 1–147) bind to DNA both in vivo and in vitro but fail to activate transcription (Keegan et al., 1986, Ma and Ptashne, 1987a, J. Ma, H. Kakidani, and M. Ptashne, unpublished data). Second, a fusion protein bearing the DNA-binding domain of the bacterial repressor LexA in place of that of GAL4 activates transcription from a lexA operator positioned upstream of a yeast gene (Brent and Ptashne, 1985). More recent experiments show that either of two short regions of the protein (activating region I, residues 148–196 or activating region II, residues 768–881) activates transcription when fused to the DNA-binding portion of the molecule (residues 1–147; Ma and Ptashne, 1987a). The carboxy-terminal 30 amino acids of GAL4, a part of activating region II, are also involved in inhibition by GAL80 (Ma and Ptashne, 1987b; Johnston et al., 1987).

The two activating regions of GAL4, each highly acidic, bear no obvious sequence homology to one another (Ma and Ptashne, 1987a), or to the activating region (also acidic) of another yeast activator, GCN4 (Hope and Struhl, 1986; Etrulli, 1987). Protein sequences that bear little sequence homology but nevertheless manifest identical biological functions have been observed in other cases. For example, signal sequences that direct the secretion of proteins bear little, if any, sequence homology (reviewed by Briggs and Giersasch, 1986; also see von Heijne, 1985); the same is true for signal sequences of mitochondrial proteins (von Heijne, 1986; Chatz, 1987). Recently, Kaiser et al. (1987) and Baker and Schatz (1987) replaced the signal sequences of secreted and of mitochondrial proteins, respectively, with random peptide sequences and found that a large fraction of these random sequences can function as signal sequences.

In this paper we describe a new class of yeast activators; these activators were encoded by genes bearing E. coli genomic DNA fragments fused to the coding sequence of the DNA-binding portion of GAL4. We detected these activating sequences at a high frequency, and most of the new activating sequences show no obvious sequence homology when compared with one another or with the activating regions of GAL4 and GCN4. All of the new activating sequences that we have analyzed are acidic. We also show that a fusion protein bearing one of our new activating sequences attached to the DNA-binding domain of LexA—a protein therefore containing no yeast protein sequence–activates transcription in yeast.

Results

Experimental Design

We fused E. coli genomic DNA fragments (generated with the restriction enzyme Sau3A) to the coding sequence of the DNA-binding portion of GAL4 (amino acids 1–147). Expression of these genes, carried on plasmids (Figure 1), was directed by the ADH1 promoter. We introduced these plasmids into a yeast "tester" strain lacking functional GAL4 but bearing an integrated GAL1-lacZ fusion gene with the UAS0 at its normal location. The product of the GAL1-lacZ fusion gene (β-galactosidase) conferred a distinctive blue color to the yeast colonies on the appropriate indicator plates (X-gal plates); in the absence of a transcriptional activator that recognizes UAS0 the colonies were white. We detected 154 blue colonies among about 15,000 transformants. We isolated plasmids from 35 individual transformants expressing various levels of β-galactosidase. We retransformed 26 plasmids into the tester strain and found that the β-galactosidase-positive pheno-
Figure 1. Structure of pMA424

pMA424 expresses the DNA-binding portion of GAL4 (residues 1-147) from the ADH1 promoter; a polylinker from pEMBL9(+) (Dente et al., 1983) was placed adjacent to the coding sequence of GAL4. The BamHI restriction site (marked with an arrow) is unique on this plasmid, and Sau3A fragments of E. coli genomic DNA were inserted at this BamHI site to generate the GAL4 fusion genes used in this study. The boxed sequences are translational stop codons of each of the three reading frames. The DNA sequence after the underlined G is from the ADH7 gene, and this G is at position 1048 according to Bennetzen and Hall (1982). pMA424 also contains the following sequences: replication origin of pBR322 (ori), ampicillin drug-resistance gene (AmpR), yeast HIS3 selection marker, and yeast 2μ replication origin. This figure is not drawn to scale.

Acidic Activating Sequences Encoded by E. coli DNA Fragments

All of the transcriptional activating sequences encoded by the E. coli DNA fragments have a net negative charge of at least one, and one of them (B42) has a net negative charge of ten (Table 1). The least active members generally have a lower net negative charge (e.g., B9, B34, B36, and B41), but there is no strict correlation between the net negative charge and the activity (Tables 1 and 2). The lengths of the new activating sequences vary from 12 to 81 amino acids (Table 1; also see legend to the table).

Two of our new activating sequences (B9 and B38) are identical, and the sequences of B17 and B15 are the same as the amino terminal halves of B1 and B6, respectively (Table 1). For B1 and B17, the shorter sequence (B17) gave more activity than its longer counterpart (B1); for B6 and B15, the longer sequence (B6) gave more activity (Table 2). We believe that these clones were recovered independently (see legend to Table 1), but we have no good explanation for why certain fragments of these clones were recovered more than once.

All of the new activators activated transcription nearly identically in the presence and absence of galactose (Table 2; also see legend to the Table). This result is expected because none of our new activators bore the carboxy-terminal 30 amino acids of GAL4, the region required for inhibition by GAL80 in the absence of galactose (Ma and Ptashne, 1987b; Johnston et al., 1987). All of our new activators were almost inactive when yeast cells were grown in medium containing glucose (data not shown).

Further Tests

The following experiments suggest that our new activators behave similarly to native yeast activators. First, the transcriptional start sites of the GAL1-lacZ fusion gene activated by various new activators and by wild-type GAL4 were identical, and the levels of mRNA were consistent with the β-galactosidase measurements (Figure 2). Second, none of our new activators activated transcription if no GAL4-binding site was present upstream of a yeast gene (data not shown), nor, where tested, when the TATA sequence was deleted from the GAL1-lacZ fusion gene (J. Ma, M. Lamphier, and M. Ptashne, unpublished). Third, several of our new activators (e.g., B1, B6, and B35) enabled a yeast strain lacking functional GAL1 to grow in medium containing galactose as the only carbon source (Figure 3), indicating that they activated all the chromosomal GAL genes required for galactose as the only carbon source (Figure 3), indicating that they activated all the chromosomal GAL genes required for galactose catabolism. The least active derivatives (B9, B34, B36, and B41) failed this test (Figure 3), as did (to varying extents) B7, B16, and B42. For reasons we do not understand, cells bearing plasmids directing synthesis of the latter three activators grow very poorly...
Yeast Transcriptional Activators

Table 1. New Activating Sequences

<table>
<thead>
<tr>
<th>Name</th>
<th>Lengths</th>
<th>Charge</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>B17</td>
<td>220</td>
<td>-5</td>
<td>ILDLOLACEDG NQGLPEEQGF QTWNAVI</td>
</tr>
<tr>
<td>B42</td>
<td>239</td>
<td>-10</td>
<td>INKDIECENA IEQFDYLG TGGEMPMEMA DQAINVPGM TPKTILHAG PIQPDWLAYN QHEIEADEV YTSLL300</td>
</tr>
<tr>
<td>B6</td>
<td>230</td>
<td>-8</td>
<td>IP LDVMYDV NTAAGNSWMF ENRMRHVVDIG DTYPHSADVI FVKNLESHGE FETTSALHTY FNVGLAAX YVSQGD</td>
</tr>
<tr>
<td>B7</td>
<td>128</td>
<td>-8</td>
<td>ISPEDYGALL DDMFFHDGSD IPTDIEEOD AQVHFHAFME NDL</td>
</tr>
<tr>
<td>S1</td>
<td>245</td>
<td>-8</td>
<td>ILDLOLACEDG NQGLPEEQGF QTWNAVIPQE FQD&amp;VITI~ VVDTAEHSNL FNYGSKSD</td>
</tr>
<tr>
<td>S32</td>
<td>137</td>
<td>-5</td>
<td>FQWAGSGQLE KMVSEALVFD DENFTHTPOQ NIVSAEFQTL LWQSD</td>
</tr>
<tr>
<td>B3</td>
<td>88</td>
<td>-4</td>
<td>IAALENDNDL FHGGLFLV</td>
</tr>
<tr>
<td>B35</td>
<td>158</td>
<td>-7</td>
<td>IPQEYADYGQ EUDIOKALAD NSEITALMIL ASDAFMQANY AQAILWQKMD</td>
</tr>
<tr>
<td>B16</td>
<td>158</td>
<td>-6</td>
<td>IFLDNDDEYQ VSQSGYDGYD TPQDLEAFQ SLVORSNAQ PAOKAVINAP AQO</td>
</tr>
<tr>
<td>B14</td>
<td>85</td>
<td>-4</td>
<td>IDIPPOYRIL ELNALLLOEQE V</td>
</tr>
<tr>
<td>S15</td>
<td>134</td>
<td>-4</td>
<td>IP LDVMYDV NTAAGNSWMF ENRMRHVVDIG DTYPHSADVI FVADG</td>
</tr>
<tr>
<td>B41</td>
<td>79</td>
<td>-1</td>
<td>IPPSELAVWF STPGW</td>
</tr>
<tr>
<td>B34</td>
<td>151</td>
<td>-2</td>
<td>IFDAQGFTLVP ANF</td>
</tr>
<tr>
<td>B38</td>
<td>109</td>
<td>-1</td>
<td>IVPNGLDHYL SW</td>
</tr>
<tr>
<td>B39</td>
<td>189</td>
<td>-1</td>
<td>IVPNGLDHYL SW</td>
</tr>
</tbody>
</table>

* = PSTCQANSGRISYDL

Shown are predicted new activating sequences represented by the standard one-letter code. The residues bearing a positive charge (R and K) are marked with a +, while those bearing a negative charge (D and E) are marked with a −. The lengths of DNA inserts and amino acid sequences encoded by open reading frames are indicated by bp and aa, respectively. In the cases where translation terminates outside the E. coli DNA inserts, the additional sequences encoded by the plasmid DNA, as indicated by *, are shown at the bottom of the table. The column of charge shows the net negative (−) charge of each new activating sequence. The amino acids that link the DNA-binding portion of GAL4 (residues 1–147) and the new activating sequences are PEFPG (see Figure 1 for details); the GAL4 derivative bearing residues 1–147 (pMA241) in Table 2 contains PEF at its carboxyl-terminal (Ma and Ptashne, 1987a). Although B9 and B38 have identical open reading frames, they were recovered independently: B9 has an extra Sau3A DNA fragment in addition to the one identical to B38. The origins of two of our new activating sequences were kindly identified by Dr. R. Doolittle as follows: residues 1–13 of B35 are identical to residues 131–143 of the RsA gene product (Robinson et al., 1984), and residues 1–31 of B42 are identical to residues 215–245 of the envZ gene product (Mizuno et al., 1982). The fisA gene is involved in regulation of E. coli cell cycle (reviewed by Holland, 1987), and the envZ is involved in transcriptional regulation of genes for outer membrane proteins (Garrett and Silhavy, 1987).

in liquid or on plates regardless of the carbon source, and the failure to grow on galactose medium may be a consequence of this inhibiting effect.

A Yeast Activator Bearing No Yeast Protein Sequence

We fused one of our new activating sequences (B42) to the DNA-binding domain of LexA (i.e., the amino-terminal domain) and generated a fusion protein that activated transcription from a lexA operator upstream of a yeast gene (Table 3). Two other LexA fusion molecules bearing the E. coli sequences (B7 and B16) did not activate transcription (data not shown). We suggest the following explanation for these differences. Although the amino terminus of LexA can bind to the lexA operator, efficient binding requires formation of dimers, a function provided primarily by the carboxy-terminal domain in the intact LexA molecule (reviewed by Little and Mount, 1982; Walker, 1984; Brent, 1982; Brent and Ptashne, 1985). We imagine that the B42 sequence provides, in addition to an activation function, a dimerization function, whereas B7 and B16 sequences do not.

Discussion

We have generated a new class of yeast activators encoded by E. coli genomic DNA fragments fused to the coding sequence of the DNA-binding domain of GAL4. In addition, we have constructed a LexA fusion protein bearing one of our new activating sequences fused to the DNA-binding domain of LexA; this fusion protein contains no yeast protein sequence but activates transcription in yeast. Gene activation by GAL4 fusions require a GAL4 binding site (the UASg) in front of the gene, whereas activation by the LexA fusion requires a suitably positioned lexA operator (data not shown). Most of our new activating sequences show no obvious sequence homology when compared with the activating regions of GNC4 (Hope and Struhl, 1986; Struhl, 1987) and GAL4 (Ma and Ptashne, 1987a), or when compared among themselves. About 1% of our fu-
Table 2. Activities of the New Activators

<table>
<thead>
<tr>
<th>Activator</th>
<th>- galactose (gly eth)</th>
<th>+ galactose (gal gly eth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B17</td>
<td>415</td>
<td>794</td>
</tr>
<tr>
<td>B4D</td>
<td>542</td>
<td>756</td>
</tr>
<tr>
<td>B6</td>
<td>429</td>
<td>688</td>
</tr>
<tr>
<td>B7</td>
<td>327</td>
<td>678</td>
</tr>
<tr>
<td>B1</td>
<td>251</td>
<td>468</td>
</tr>
<tr>
<td>B32</td>
<td>223</td>
<td>388</td>
</tr>
<tr>
<td>B3</td>
<td>217</td>
<td>359</td>
</tr>
<tr>
<td>B35</td>
<td>332</td>
<td>347</td>
</tr>
<tr>
<td>B16</td>
<td>221</td>
<td>255</td>
</tr>
<tr>
<td>B4</td>
<td>111</td>
<td>83</td>
</tr>
<tr>
<td>B15</td>
<td>90</td>
<td>73</td>
</tr>
<tr>
<td>B41</td>
<td>61</td>
<td>30</td>
</tr>
<tr>
<td>B34</td>
<td>36</td>
<td>17</td>
</tr>
<tr>
<td>B38</td>
<td>21</td>
<td>9.3</td>
</tr>
<tr>
<td>B9</td>
<td>21</td>
<td>9.2</td>
</tr>
<tr>
<td>no GAL4</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>pMA200</td>
<td>111</td>
<td>1895</td>
</tr>
<tr>
<td>wt GAL4</td>
<td>221</td>
<td>255</td>
</tr>
<tr>
<td>pMA210</td>
<td>111</td>
<td>1895</td>
</tr>
<tr>
<td>GAL4 (1-147) (pMA241)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Plasmids expressing either our new activators, or wild-type GAL4, or a truncated GAL4 derivative bearing only its DNA-binding portion, were introduced into a yeast strain lacking a functional GAL4 gene but bearing a GAL80 gene and an integrated GAL1-lacZ fusion gene (YM335::RY171). β-Galactosidase activities were measured from cells grown in conditions as indicated (gal = galactose; gly = glycerol; eth = ethanol). Wild-type GAL4 was partially active in this experiment in the absence of galactose because it was expressed from the ADH1 promoter and "titrated" GAL80 (Johnston and Hopper, 1982; Hashimoto et al., 1983; Johnston et al., 1986; Ma and Ptashne, 1987b). The new activators are listed according to their activities in the presence of galactose.

Table 3. A Yeast Activator Bearing No Yeast Protein Sequence

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<th>β-Galactosidase Activity</th>
</tr>
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<td>lexAop-GAL1-lacZ</td>
<td>119 (gly eth) 94 (gal gly eth)</td>
</tr>
<tr>
<td>lexAop-CYC1-lacZ</td>
<td>118 (gly eth) 117 (gal gly eth)</td>
</tr>
</tbody>
</table>

Yeast cells (YM335) containing either a plasmid bearing a lexA operator-GAL1-lacZ fusion gene (pRB1145) or a plasmid bearing a lexA operator-CYC1-lacZ fusion gene (pRB1155; Brent and Ptashne, 1985) were transformed with a second plasmid expressing the LexA fusion protein (pMA457). β-Galactosidase activities were measured from cells grown in conditions as indicated. (In the absence of pMA457, less than one unit of β-galactosidase was detected for each case; data not shown.) The β-galactosidase activities were not significantly lowered when the yeast cells were grown in medium containing glucose (data not shown).

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We imagine that GAL4, GCN4, and our new activators activate transcription by touching another component of the transcriptional machinery, e.g., a TATA-binding factor or the RNA polymerase (Brent and Ptashne, 1985; Keegan et al., 1986; Hope and Struhl, 1986; Ptashne, 1986; Struhl, 1987). It is possible that the activating sequences form similar structures in transcriptional complexes. The Chou
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and Fasman (1978) program, which relates the secondary structure to the primary sequence of a protein, predicts that most of our new activating sequences contain \( \beta \)-turns having an acidic residue(s) at the turns and, adjacent to these turns, \( \alpha \)-helices composed of hydrophobic residues in many cases. Most of our activating sequences also have short segments that could form amphiphilic helices, that is, \( \alpha \)-helices with charged residues on one side and hydrophobic residues on the other. Whether any of these features is important remains to be tested.

**Experimental Procedures**

**Strains and Media**

*Yeast strains YM335 and YM335::RY171* were described previously (Johnston and Davis, 1984; Ma and Ptashne, 1987a). Yeast were grown in either YEPI-D-rich medium or minimal media (Sherman et al., 1983), and the carbon sources of minimal media were with (+) galactose (2% [w/v] galactose, 2% [v/v] glycerol, and 2% [v/v] ethanol), or without (−) galactose (2% [w/v] glycerol and 2% [v/v] ethanol). All plasmid constructions were performed using the E. coli strain MM294 and bacteria were grown in LB medium (Miller, 1972).

**Construction of GAL4 Fusion Genes and Screening for New Activators**

pMA424 (Figure 1), which was used to construct the GAL4 fusions, was constructed as follows. The XbaI-EcoRI fragment of CD18 (Ma and Ptashne, 1987a) was inserted at the HindIII site of pEMBLS(+) (+), which had been filled in with Klenow to generate pMA420; the orientation of the insert in pMA420 was confirmed by sequencing the plasmid. pMA420 was ligated to the BamHI site within the polylinker of pMA235 (Ma and Ptashne, 1987a) with that of pMA420. pMA424 was constructed as follows. The XbaI-EcoRI fragment of CD18 (Ma and Ptashne, 1987a) was inserted at the HindIII site of pEMBLS(+) (+), which had been filled in with Klenow to generate pMA420; the orientation of the insert in pMA420 was confirmed by sequencing the plasmid. pMA420 was ligated to the BamHI site within the polylinker of pMA235 (Ma and Ptashne, 1987a) with that of pMA420. pMA424 was constructed by replacing the XbaI-EcoRI fragment of CD18 (Ma and Ptashne, 1987a) with the BamHI-EcoRI fragment of pMA453; the BamHI end was filled in before ligation. The amino acids that link the DNA-binding domain of LexA, was ligated into such treated pMA450 to generate pMA451. pMA452 was constructed by destroying the EcoRI site of pMA451, which is located between the ADH1 promoter and the coding sequence of LexA. A 12-mer EcoRI linker (obtained from New England BioLabs) was inserted at the XhoI site of pMA452, which had been filled in with Klenow to generate pMA453. pMA457 was constructed by replacing the PvuII-EcoRI fragment of pMA457 with the BamHI-EcoRI fragment of pMA453; the BamHI had been filled in before ligation. The amino acids that link the DNA-binding domain of LexA (residues 1–89) and the activating sequence of S424 are REPFO糕.

**Isolation of Plasmids from Yeast and DNA Sequencing**

The procedure of isolation of plasmids from yeast was as described (Filetici, 1985; Ma and Ptashne, 1987a). The Maxam and Gilbert (1980) sequencing technique was used; both strands of each insert were sequenced from EcoRI and Sall sites, respectively (Figure 1).

**Yeast RNA Preparation and Primer Extension**

Procedures of yeast RNA preparation and primer extension are as previously described (Krainer et al., 1984; Ma and Ptashne, 1987a).

**Yeast Transformation and Assay of \( \beta \)-Galactosidase**

Yeast cells were made competent for transformation by treatment with Lithium Acetate (Ito et al., 1983). Liquid \( \beta \)-galactosidase assays were performed as previously reported (Yocum et al., 1984; West et al., 1984), and the standard error was less than 20%.

**Acknowledgments**

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