GAL4 Mutations That Separate the Transcriptional Activation and GAL80-Interactive Functions of the Yeast GAL4 Protein

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ABSTRACT

The carboxy-terminal 28 amino acids of the *Saccharomyces cerevisiae* transcriptional activator protein GAL4 execute two functions—transcriptional activation and interaction with the negative regulatory protein, GAL80. Here we demonstrate that these two functions are separable by single amino acid changes within this region. We determined the sequences of four $GAL4^C$-mutations, and characterized the abilities of the encoded $GAL4^C$ proteins to activate transcription of the galactose/melibiose regulon in the presence of *GAL80* and superrepressible $GAL80^S$ alleles. One of the $GAL4^C$ mutations can be compensated by a specific $GAL80^S$ mutation, resulting in a wild-type phenotype. These results support the idea that while the GAL4 activation function tolerates at least minor alterations in the GAL4 carboxyl terminus, the GAL80-interactive function is highly sequence-specific and sensitive even to single amino acid alterations. They also argue that the $GAL80^S$ mutations affect the affinity of GAL80 for GAL4, and not the ability of GAL80 to bind inducer.

THE *GAL4* gene of *Saccharomyces cerevisiae* encodes a protein that activates transcription of galactose and melibiose catabolic enzyme genes (DOUGLAS and HAWTHORNE 1966; JOHNSTON and HOPPER 1982; LAUGHON and GESTELAND 1982; HASHIMOTO *et al.* 1983). Activation is achieved primarily through two functions of the GAL4 protein; an amino-terminal DNA binding function which positions GAL4 upstream of regulated structural genes (GUARENTE, YOCUM and GIFFORD 1982; JOHNSTON and DAVIS 1984; WEST, YOCUM and PTASHNE 1984; YOCUM *et al.* 1984; BRAM and KORNBERG 1985; GINIGER, VARNUM and PTASHNE 1985; BRAM, LUE and KORNBERG 1986; KEEGAN, GILL and PTASHNE 1986), and a carboxy-terminal "activation" function which interacts with cellular transcription factors (BRENT and PTASHNE 1985; JOHNSTON *et al.* 1986; JOHNSTON, SALMERON and DINCHER 1987; MA and PTASHNE 1987a). Expression of the wild-type galactose/melibiose regulon is not constitutive, due to the activity of the negative regulatory gene *GAL80* (DOUGLAS and HAWTHORNE 1966; NOGI *et al.* 1984; YOCUM and JOHNSTON 1984). In the absence of the regulon inducer (galactose or a metabolite thereof), the GAL4 protein binds target DNA sequences but does not activate transcription, due to the binding of the *GAL80*-encoded protein to GAL4 (JOHNSTON and HOPPER 1982; GINIGER, VARNUM and PTASHNE 1985; SELLECK and MAJORS 1987; LUE *et al.* 1987; MA and PTASHNE (1988). Inducer frees GAL4 of GAL80-mediated negative regulation (DOUGLAS and HAWTHORNE 1966). Mutations in the *GAL80* gene, termed $GAL80^S$, result in proteins that block activation by GAL4 under both noninducing and inducing conditions (DOUGLAS and HAWTHORNE 1972; NOGI and FUKASAWA 1984). $GAL80^S$ proteins are hypothesized either to have lost inducer binding function (DOUGLAS and HAWTHORNE 1972; NOGI and FUKASAWA 1984), or to possess a high affinity for GAL4 (SALMERON, LANGDON and JOHNSTON, 1989).

The protein encoded by the *gal4*-62 nonsense allele is defective because it lacks the carboxy-terminal 28 amino acids of wild-type GAL4, which contain the activation function (JOHNSTON, SALMERON and DINCHER 1987; MA and PTASHNE 1987a; Figure 1). We previously characterized a Gal^+ revertant of *gal4*-62, $GAL4^C$-62. $GAL4^C$ alleles encode proteins that escape regulation by GAL80 (DOUGLAS and HAWTHORNE 1966). The $GAL4^C$-62 mutation causes a frameshift that replaces the last four amino acids of the gal4-62 protein with three new amino acids, but does not restore the wild-type GAL4 carboxyl terminus (JOHNSTON, SALMERON and DINCHER 1987; also see Figure 1). Its constitutive phenotype indicates that the carboxy-terminal 28 amino acids of GAL4 contain a GAL80-interactive function as well as an activation function.

Similar results are obtained by MA and PTASHNE (1987a, b), who analyzed the effects of *in vitro* generated deletions of the *GAL4* gene on GAL4 protein activity. Together these findings prompted a model whereby the transcriptional activity or inactivity of the GAL/MEL regulon is determined by the binding

of either GAL80 or transcription factors to the carboxyl terminus of GAL4 (JOHNSTON, SALMERON and DINCHER 1987; MA and PTASHNE 1987b).

Although these results provide information critical to the understanding of GAL4-GAL80 interaction, many questions remain. Three of the most important are: (1) are the transcriptional activation and GAL80-interactive functions of the GAL4 carboxyl terminus separable?, (2) what are the specific amino acid interactions that occur between GAL4 and GAL80? and (3) what is the nature of *GAL80^S* mutations?

To address these questions, we characterized and sequenced four additional *GAL4^C* mutations. As concerns the first question it appears there are minimal primary sequence requirements for the GAL4 transcriptional activation. JOHNSTON and DOVER (1988) characterized 41 *gal4* missense mutations and found none that affected the GAL4 carboxyl terminus, indicating that most single amino acid changes do not eliminate the GAL4 activation function. GINIGER and PTASHNE (1987) described a synthetic 15-amino-acid peptide that bore little similarity to the GAL4 carboxyl terminus yet activated transcription when fused to the GAL4 DNA binding region. Most strikingly, MA and PTASHNE (1987c) identified multiple peptides, encoded by random *Escherichia coli* DNA fragments, that activate transcription in yeast when fused to the GAL4 DNA binding region. Thus, the GAL4 activation region appears to have minimal primary sequence requirements, at least when the gene is highly overexpressed.

In contrast, the primary sequence requirements for GAL4-GAL80 protein-protein interaction appear more stringent. Specific allelic relationships can exist between *GAL4^C* and *GAL80^S* alleles (NOGI *et al.* 1977), suggesting at least that the interactions between GAL4^C and GAL80^S proteins are sequence-specific. In addition, all the *E. coli* peptides that function as activating regions in GAL4 are not negatively regulated by the GAL80 protein (MA and PTASHNE 1987c). Together, these results predict that some *GAL4^C* mutations should be missense mutations that selectively eliminate the GAL80-interactive function of the GAL4 carboxyl terminus. On the other hand, if the two functions of the GAL4 carboxyl terminus are not separable, all *GAL4^C* alleles should contain nonsense mutations and encode carboxy-terminally truncated proteins such as GAL4^C-62.

Analysis of *GAL4^C* mutations in combination with specific *GAL80^S* alleles could yield insight into the other two questions listed above. NOGI and FUKASAWA (1984) determined the amino acid sequences of three *GAL80^S* mutations. The three alterations span a small (47 amino acid) region of the GAL80^S protein. One mutation, *GAL80^{s-1}*, was found by NOGI *et al.* (1977) to interact specifically with a *GAL4^C* mutation, termed *GAL81-1*, to produce an inducible phenotype. Such interactions between *GAL4^C* and *GAL80^S* alleles may define specific amino acid interactions between *GAL4* and *GAL80*. Finally, analysis of the *GAL4^C/GAL80^S* combinations could yield insight into the nature of the *GAL80^S* mutations themselves.

MATERIALS AND METHODS

Yeast strains: *GAL4^C* alleles were analyzed from the following strains: Sc99 (*a GAL4^c*-Cl *GAL80 trp1 his$^-$ ade$^-$ MEL1*), Sc252 (a *GAL4^c GAL80 ura3-52 leu2-3, 112 MEL1*), Sc258R10 (*a GAL4^c GAL80 ura3-52 leu2-3, 112 MEL1*), and Sc272 (*a GAL4^c*-12 *GAL80 gal7-2 gal82 leu1*). Sc99 was a gift of ROGERS YOCUM, Sc258R10 was a gift of JACQUELINE BROMBERG, and Sc272 was a gift of KUNIHIRO MATSUMOTO. Sc99 was isolated as a Gal$^+$ revertant of a ρ^- *GAL4 GAL80 gal3* strain (for details, see DOUGLAS and HAWTHORNE 1966). Sc252 was isolated as a Gal$^+$ revertant of a *GAL4 GAL80^{S-100}* strain (JOHNSTON and HOPPER 1982). Sc272 was isolated as a Gal$^+$ revertant of a *GAL4/GAL4 GAL80^{S-2}/GAL80^{S-2}* diploid (for details, see MATSUMOTO *et al.* 1980). Sc258R10 was isolated from a *gal4-62* strain (JOHNSTON, SALMERON and DINCHER 1987). For dominance tests, *GAL4^c* strains were crossed to strain Sc128 (*α GAL4 GAL80 ura1 trp1 MEL1*). The *GAL4 GAL80* control strain was SJ21R (JOHNSTON and HOPPER 1982). For assaying *GAL4^c* alleles in yeast, *gal4 gal80* strain YJ0 (SALMERON, LANGDON and JOHNSTON 1989) was used.

Sequencing of *GAL4^c* mutations: Genomic DNA was isolated from the *GAL4^c* strains by the detergent/alkaline lysis method (HIRT 1967). Samples of 0.5 μg each of the oligonucleotide primers 5′ TCCAGATTGTGTCTACGTAATGCAC$^{3'}$ and $^{5'}$AACGTTCTACTTTACACTGGTGGTAG$^{3'}$, corresponding to nucleotides 375–399 of the coding strand and nucleotides 3436–3411 of the noncoding strand of the *GAL4* gene (coordinates according to LAUGHON and GESTELAND 1984) were synthesized on an Applied Biosystems 380B DNA Synthesizer and used for direct amplification (SAIKI *et al.* 1988) of the *GAL4* coding sequence from 0.1 μg genomic DNA using a Perkin-Elmer DNA Thermal Cycler. The region amplified included the entire *GAL4* coding sequence and 67 and 326 nucleotides of its 5′ and 3′ flanking sequences, respectively. The first amplification reaction cycle consisted of a 94° incubation for 1 min, a 30° incubation for 3 min, a 4-min incubation during which the temperature was increased linearly to 72°, and an incubation at 72°, for 3 min. For each successive cycle (up to a total of 25), the 72° incubation time was increased 15 sec. Reaction products were electrophoresed through an 0.8% agarose (International Biotechnologies, Inc.) gel, and the major product of 3.0 kb was eluted. The eluted DNA fragments were cleaved with *Sph*I and *Hin*dIII restriction endonucleases (Fisher/Promega) and cloned into the multiple cloning site of pUC119 (VIEIRA and MESSING 1987) to form pUC119-99, pUC119-252, pUC119-258R10 and pUC119-272. Single-stranded DNAs were prepared from these constructions (VIEIRA and MESSING 1987) and subjected to DNA sequence analysis (SANGER, NICKLEN and COULSON 1977) using oligonucleotide primers spaced uniformly throughout the *GAL4* gene. In the case of each pUC119 derivative, sequencing was performed on a pool of eight isolates to avoid detection of mutations arising randomly during the amplification. The sequence from *GAL4* nucleotides 1679 (the location of a *Sal*I restriction site) to 3332 (a point 247 nucleotides downstream of the GAL4 termination codon) was determined in each case.

Yeast centromeric plasmids: Sequencing of the GAL4 *Sal*I-*Hin*dIII fragment of pUC119-99, pUC119-258R10 and SJ3XHOGAL4^{C}-62 (JOHNSTON and HOPPER 1982) revealed that the mutation in the extreme 3′ end of the *GAL4* gene, as reported in the Results section, is the only one found in the cloned fragment. To construct yeast centromeric plasmids, the *Pvu*II-*Hin*dIII fragment encoding the carboxyl-terminal amino acids of GAL4 was excised from the *LEU2 CEN4* plasmid pSB32 containing the *Bam*HI-*Hin*dIII fragment of *GAL4* (SALMERON, LANGDON and JOHNSTON 1989), and replaced with the corresponding *Pvu*II-*Hin*dIII fragment from either pUC119-99, pUC119-258R10 or SJ3XHOGAL4^{C}-62, to form pJCGAL4I859, pJCGAL4M851 and pJCGAL4^{C}-62, respectively. DNA sequence analysis of pUC119-272 and pUC119-252 revealed sequence polymorphisms within the *GAL4* coding region. To confirm that the single nucleotide change in the extreme 3′ end of the *GAL4* coding region is responsible for the constitutive phenotype conferred by these *GAL4*^{C} alleles, as reported in the Results section, single stranded DNA was prepared from pUC119-GAL4 (SALMERON, LANGDON and JOHNSTON 1989) and subjected to site-directed mutagenesis using an Amersham *in vitro* mutagenesis kit. The oligonucleotide primer 5′ ATGGATGATG*AA*TATAACTAT 3′ was used to convert the GTA (Val) codon at *GAL4* position 864 to GAA (Glu); the oligonucleotide primer 5′ TATAACTAT*CC*ATTCGATGATG 3′ was used to convert the CTA (Leu) codon at *GAL4* position 868 to CCA (Pro). Introduction of the single nucleotide changes was confirmed by sequencing. The *Sal*I-*Hin*dIII fragments from the resulting plasmids were used to replace the corresponding wild-type *GAL4* fragment of pSB32-GAL4 as described above, to form pJCGAL4E864 and pJCGAL4Pro868, respectively. Centromeric plasmids were introduced into yeast by the lithium chloride method (ITO *et al.* 1983).

Assays: α-Galactosidase assays were performed with intact cells (KEW and DOUGLAS 1976). Carbon sources were 3% glycerol, 2% lactic acid for uninduced cultures, and 3% glycerol, 2% lactic acid, 2% galactose for induced cultures. To assay expression of the *GAL1* gene, yeast strains were plated to selective media containing either 3% glycerol, 2% lactic acid, and 0.5% 2-deoxygalactose (Sigma Chemical Co.), or 3% glycerol. 2% lactic acid, 2% galactose.

RESULTS

Genetic characterization of the *GAL4*^{c}-alleles: Given the diversity in the genetic backgrounds of the strains employed in this study (see MATERIALS AND METHODS), it was desirable to confirm that the *GAL4* alleles they possessed were in fact *GAL4*^{c} alleles. By definition, *GAL4*^{c} alleles are either dominant or semidominant over the wild-type *GAL4* allele (DOUGLAS and HAWTHORNE 1966; NOGI *et al.* 1977; MATSUMOTO *et al.* 1980), in contrast to a *gal80* allele which produces a constitutive phenotype in yeast but is recessive to the wild-type *GAL80* allele. To test the dominance of the four mutations analyzed in this study, strains bearing these mutations were crossed to a *GAL4 GAL80* strain. Diploids were purified on selective media, and patched to test plates containing the noninducing carbon sources glycerol and lactic acid, and 2-deoxygalactose, a chemical toxic to cells expressing the *GAL1* gene (PLATT 1984). On these plates the haploid strains die due to constitutive expression of the *GAL1* gene (Table 1, column 1, lines 1–4). In contrast, the *GAL4 GAL80* control strain SJ21R grows (line 5). For each strain of interest, the diploid produced from a cross to the *GAL4 GAL80* strain Sc128 fails to grow on the test medium (column 2, lines 1–5). The diploid produced from the control *GAL4 GAL80* × *GAL4 GAL80* cross grows (line 5). We conclude that all four mutations are either dominant or semidominant, and that all four strains harbor *GAL4*^{c} alleles.

TABLE 1

Dominance of GAL4^{c} mutations

Line	Yeast strain	Growth on glycerol/lactic acid + 2-deoxygalactose medium	
		1x[a]	*2x*[b]
1	Sc99	−	−
2	Sc258R10	−	−
3	Sc260	−	−
4	Sc272	−	−
5	SJ21R	+	+
6	Sc128	+	NA

[a] Haploid strain.
[b] Diploid produced from cross of haploid strain with Sc128.

Localization of *GAL4*^{c} mutations: The *GAL4* locus strain was subjected to DNA sequence analysis. Each *GAL4*^{c} lesion was identified as a point mutation in the extreme 3′ end of the *GAL4* coding sequence (Figure 1). Three of the alleles derived from wild-type *GAL4* alleles, encode single amino acid alterations within the carboxy-terminal GAL80-interactive region of GAL4. The amino acid alterations are Thr859 > Ile, Val864 > Glu and Leu868 > Pro. The fourth allele, derived from the *gal4-62* allele (JOHNSTON, SALMERON and DINCHER 1987), encodes a Ile851 > Met alteration but does not restore the wild-type GAL4 carboxyl terminus lacking in the gal4-62 protein, and thus is similar to the *GAL4*^{c}-62 allele. The *GAL4*^{c} alleles were designated *GAL4*-Ile859, *GAL4*-Glu864, *GAL4*-Pro868, and *GAL4*-62Met851 based on their encoded amino acid alterations.

Activation of the *MEL1* gene: The *GAL4*^{c} alleles were cloned into yeast centromeric plasmids (for details see MATERIALS AND METHODS) and introduced into the *gal4 gal80* strain YJ0 in combination with either *GAL80* or *GAL80*^{s} alleles borne on the URA3-, CEN3- containing plasmid YCp50 (SALMERON, LANGDON and JOHNSTON 1989). We assayed the level of α-galactosidase (the product of the regulon structural gene MEL1; KEW and DOUGLAS 1976) activated in each of the transformants under inducing and noninducing conditions. The wild-type *GAL4* allele activates constitutive expression of the *MEL1* gene in the presence of a *gal80* null allele (Table 2, line 1a). In the

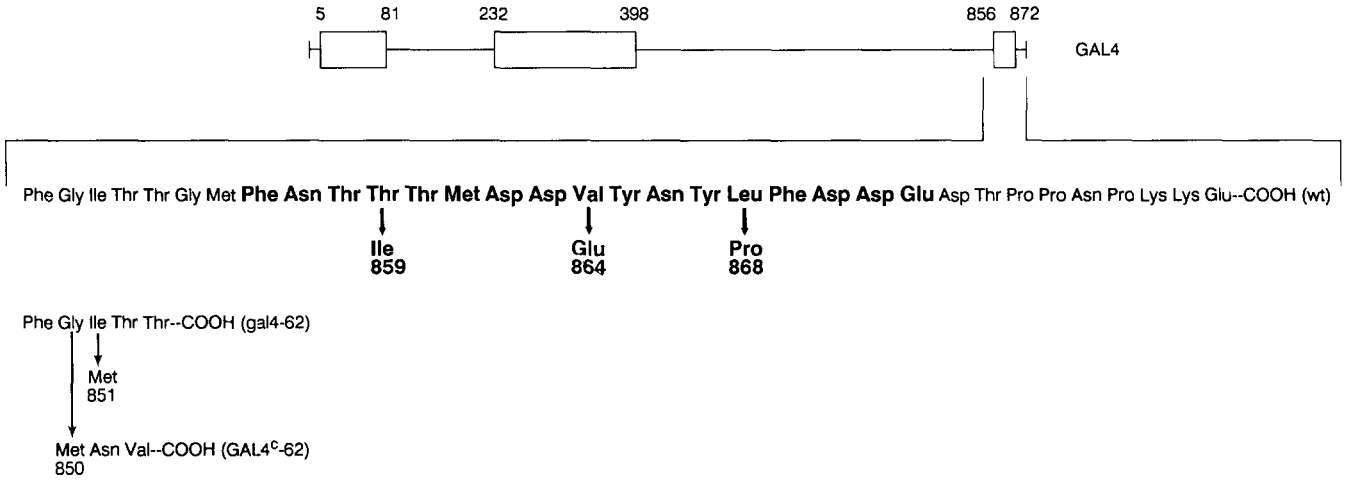

FIGURE 1.—Amino acid sequence of the carboxyl termini of the *GAL4*- and *gal4*-62-encoded proteins, and the amino acid alterations caused by *GAL4*^{c} mutations derived from these genes. Numbers indicate the position of each alteration in the GAL4 amino acid sequence. The region in bold type are 88% amino acid similarity to the carboxyl terminus of the GAL4 homolog from *K. lactis*, LAC9 (SALMERON and JOHNSTON 1986). A diagram of the GAL4 protein (top, with boxes representing regions of similarity with the LAC9-encoded protein) is included to orient the positions of the GAL4^c alterations in the GAL4 protein.

presence of the wild-type *GAL80* allele, *MEL1* expression is inducible (line 1b). In the presence of any of the *GAL80*^{s} alleles, expression is uninducible (lines 1c–e). By contrast, the *GAL4*-Glu864 allele (lines 2a–e), under both inducing and noninducing conditions, and in combination with either the *GAL80* or *GAL80*^{s} alleles, activates *MEL1* gene expression at levels nearly equal that activated in a *GAL4 GAL80* strain under inducing conditions (line 1b). The *GAL4*-Pro868 allele also activates *MEL1* constitutively, at *GAL4*-like levels in combination with the wild-type *GAL80* allele (line 3b), and, interestingly, at levels ~3-fold lower in combination with any of the *GAL80*^{s} alleles (lines 3c–e). In contrast, the *GAL4*-62Met851 and *GAL4*^{c}-62 alleles activate *MEL1* expression constitutively (lines 4a-b and 5a-b), to levels approximately 25% and 33%, respectively, of the level activated in the *GAL4 GAL80* strain under inducing conditions, and are not affected by the presence of the *GAL80*^{s} alleles (lines 4c–e and 5c–e). The *GAL4*-Ile859 allele activates constitutive *MEL1* expression at high levels in combination with the *GAL80* or *GAL80*$^{s-0}$ alleles (lines 6b, c). Expression is somewhat lower in combination with *GAL80*$^{s-0}$. In combination with the *GAL80*$^{s-2}$ allele (line 6e), activation is constitutive, but at only half the level observed in the *GAL4*-Ile859 *GAL80*$^{s-0}$ strain. Strikingly, in combination with the *GAL80*$^{s-1}$ allele (line 6d), the *GAL4*-Ile859 allele exhibits an inducible phenotype identical to that exhibited by the wild-type *GAL4* and *GAL80* alleles (line 1b).

Activation of GAL genes: We also assayed the *GAL4*^{c}-*GAL80*^{s} strains for expression of the GAL structural genes under both inducing and noninducing conditions, by testing for growth on either galactose medium (Table 3A) or glycerol/lactic acid medium containing 2-deoxygalactose (Table 3B). All transformants grew on a control medium containing glycerol/lactic acid without 2-deoxygalactose (data not shown). In general, the results parallel those of Table 2. In the presence of the *GAL80*^{s} alleles, the *GAL4* allele fails to activate expression of the *GAL* genes at a level sufficient for galactose growth (Table 3A, line 1), indicating that the GAL80^s proteins repress the activity of the GAL4 protein under inducing conditions. By contrast, the *GAL4*-Ile859, *GAL4*-Glu864, *GAL4*-Pro868, *GAL4*-62Met851 and *GAL4*^{c}-62 alleles all support galactose growth in the presence of all three *GAL80*^{s} alleles (lines 2–6), although growth of the *GAL4*-Ile859 *GAL80*$^{s-2}$ and *GAL4*-62Met851 strains is slow (line 2). Growth of all the *GAL4* GAL80 and GAL4 *GAL80*^{s} strains on glycerol/2-deoxygalactose (Table 3B) indicates that repression of *GAL1* expression occurs in these strains under noninducing conditions (line 1). The *GAL4*-Glu864, *GAL4*-Pro868, and *GAL4*^{c}-62 strains fail to grow on glycerol/2-deoxygalactose medium, regardless of whether *GAL80* or *GAL80*^{s} alleles are present (lines 3, 4 and 6), indicating that *GAL1* expression in all these strains is constitutive. The *GAL4*-62Met851 strain grows weakly on the 2-deoxygalactose-containing medium (line 5), indicating a low level of constitutive *GAL1* expression. The *GAL4*-Ile859 *GAL80*$^{s-0}$ strain (line 3) does not grow, indicating that *GAL1* expression is constitutive. However, the *GAL4*-Ile859 *GAL80*$^{s-1}$ and *GAL4*-Ile859 *GAL80*$^{s-2}$ strains grow, indicating that the *GAL80*$^{s-1}$ and *GAL80*$^{s-2}$ proteins severely repress the ability of the GAL4-Ile859 protein to activate GAL1 expression under noninducing conditions.

DISCUSSION

We studied four *GAL4*^{c} mutations in order to address questions concerning the interaction between

TABLE 2

MEL1 expression activated by combinations of *GAL4* and *GAL80* alleles

Line	*GAL4* allele[a]	*GAL80* allele[b]	α-Galactosidase level		R^c
			Gly	GlyGal	
1a	*GAL4*	*gal80*	97.5	114.9	1.2
b		*GAL80*	16.5[d]	100.0[e]	6.1
c		*GAL80*$^{s-0}$	18.6	26.0	1.4
d		*GAL80*$^{s-1}$	12.8	24.0	1.9
e		*GAL80*$^{s-2}$	14.1	16.5	1.2
2a	*GAL4*-Glu864	*gal80*	55.0	82.6	1.5
b		*GAL80*	87.2	95.0	1.1
c		*GAL80*$^{s-0}$	78.9	82.2	1.0
d		*GAL80*$^{s-1}$	46.7	74.0	1.6
e		*GAL80*$^{s-2}$	53.3	74.0	1.4
3a	*GAL4*-Pro868	*gal80*	105.8	95.0	0.9
b		*GAL80*	98.8	104.5	1.1
c		*GAL80*$^{s-0}$	27.3	34.3	1.3
d		*GAL80*$^{s-1}$	26.9	36.4	1.4
e		*GAL80*$^{s-2}$	31.8	31.8	1.0
4a	*GAL4*-62Met851	*gal80*	14.9	24.8	1.7
b		*GAL80*	23.1	28.9	1.3
c		*GAL80*$^{s-0}$	15.7	20.2	1.3
d		*GAL80*$^{s-1}$	23.1	33.9	1.5
e		*GAL80*$^{s-2}$	21.1	27.3	1.3
5a	*GAL4*^{c}-62	*gal80*	30.2	40.9	1.4
b		*GAL80*	22.3	31.4	1.4
c		*GAL80*$^{s-0}$	26.4	43.8	1.7
d		*GAL80*$^{s-1}$	27.3	31.4	1.2
e		*GAL80*$^{s-2}$	23.1	33.1	1.4
6a	*GAL4*-Ile859	*gal80*	113.2	123.6	1.1
b		*GAL80*	96.3	132.2	1.4
c		*GAL80*$^{s-0}$	52.9	78.9	1.5
d		*GAL80*$^{s-1}$	19.0	104.5	5.5
e		*GAL80*$^{s-2}$	28.5	47.1	1.7

[a] Activator allele borne on pSB32 in strain YJ0.

[b] *GAL80* allele borne on YCp50 in strain YJ0.

[c] Induction ratio (induced level/uninduced level of α-galactosidase).

[d] The relatively high level of α-galactosidase (*cf.* JOHNSTON and HOPPER 1982) activated under noninducing conditions is characteristic of strains in which the *GAL80* gene is plasmid-borne.

[e] The reference value 100.0 represents an increase in optical density at 400 nm of 0.242 per min per 10^7 cells.

the GAL4 and GAL80 proteins in *S. cerevisiae*. First, are the transcriptional activation and GAL80 interactive functions of the GAL4 carboxyl terminus separable? We identified three *GAL4*^{c} mutations that cause single amino acid substitutions within the carboxy-terminal activation region of the GAL4 protein. The mutations have at most minor effects on the GAL4 function (Table 2, lines 1b, 2b, 3b and 6b, induced), while eliminating the GAL80-interactive function (uninduced). Thus, the two functions executed by the carboxy-terminal 28 amino acids of the GAL4 protein are separable, and the GAL80-interactive function appears to be a higher degree of sequence specificity than the activation function.

Although some amino acid alterations of the GAL4 carboxyl terminus eliminate the GAL80-interactive function, others do not. The GAL4 carboxyl terminus is very similar to that of its homolog from *Kluyveromyces lactis*, LAC9, but contains three regions of sequence dissimilarity (SALMERON and JOHNSTON 1986; WRAY *et al.* 1987). First, GAL4 contains a Leu residue at position 868 (Figure 1), whereas LAC9 contains Ile at the analogous position. Second, three residues downstream of the isoleucine residue in LAC9 there is an Asn residue not present in GAL4. Third, LAC9 terminates following the final acidic amino acid in the region of similarity with GAL4, Glu, while GAL4 continues for another nine amino acids. Despite these differences, the carboxyl terminus of LAC9 can functionally substitute for the GAL4 carboxyl terminus in both transcriptional activation and GAL80-interaction (SALMERON, LANGDON and JOHNSTON 1989).

Second, we attempted to identify combinations of *GAL4*^{c} and *GAL80*^{s} alleles that produce an inducible phenotype, in hopes of identifying possible sites of amino acid-amino acid interaction between GAL4 and GAL80. We found that the combination of the *GAL4*-Ile859 and *GAL80*$^{s-1}$ alleles elicits an inducible phenotype (Table 2, line 6d). This result was described initially by NOGI et al. (1977), who later determined that the *GAL80*$^{s-1}$ mutation leads to a Gly > Arg change at amino acid 323 of GAL80 (NOGI and FUKASAWA 1984). The inducible nature of this allelic combination suggests that the wild-type amino acids of GAL4 and GAL80, Thr859 and Gly323, respectively, may interact *in vivo*.

Third, we wished to gain information about the nature of *GAL80*^{s} mutations in general. The inducible nature of the *GAL4*-Ile859/*GAL80*$^{s-1}$ combination argues strongly against the idea that the *GAL80*$^{s-1}$ mutation simply affects the ability of the GAL80^{s-1} protein to bind inducer (a possibility suggested by NOGI and FUKASAWA 1984 based on the nature of *laci*^{s} mutations in *E. coli*), and instead supports the idea that the mutation increases the affinity of GAL80 for GAL4. Further support for this idea comes from the fact that although the proteins encoded by the *GAL4*^{c} missense mutations (GAL4-Ile859, GAL4-Glu864 and GAL4-Pro868) activate transcription constitutively in combination with the GAL80^{s} proteins (except for the *GAL4*-Ile859/*GAL80*$^{s-1}$ combination described above), activation is lower in the presence of a GAL80^{s} protein than with the GAL80 protein (Table 2A, lines 2b–e, 3b–e, 6b, c, e). This implies a stronger interaction of these GAL4^{c} proteins with the GAL80^{s} proteins. By contrast, the GAL4^{c} proteins truncated at the carboxyl terminus (GAL4^{c}-62, GAL4-62Met851) are unaffected by the GAL80^{s} proteins (lines 4b–e, 5b–e). Finally, although normally the GAL4 homolog from *K. lactis*, LAC9, activates transcription constitutively in *S. cerevisiae* (SALMERON

TABLE 3

GAL gene expression by combinations of *GAL4*^{c} and *GAL80*^{s} alleles

Line	*GAL4* allele[a]	*gal80* allele[b]				
		gal80	*GAL80*	*GAL80*$^{s-0}$	*GAL80*$^{s-1}$	*GAL80*$^{s-2}$
A. Growth on galactose medium						
1	*GAL4*	+++[c]	++	−	−	−
2	*GAL4*-Ile859	+++	+++	++	++	+
3	*GAL4*-Glu864	+++	+++	++	++	++
4	*GAL4*-Pro868	+++	+++	++	++	++
5	*GAL4*-62Met851	+	+	+	+	+
6	*GAL4*^{c}-62	++	++	++	++	++
B. Growth on glycerol/lactic acid + 2-deoxygalactose medium						
1	*GAL4*	−	+++	+++	+++	+++
2	*GAL4*-Ile859	−	−	−	+++	++
3	*GAL4*-Glu864	−	−	−	−	−
4	*GAL4*-Pro868	−	−	−	−	−
5	*GAL4*-62Met851	+	+	+	+	+
6	*GAL4*^{c}-62	−	−	−	−	−

[a] *GAL4* allele borne on pSB32 in strain YJ0.
[b] *GAL80* allele borne on YCp50 in strain YJ0.
[c] +++, robust growth detectable after 24 h; ++, robust growth detectable after 48 h; +, weak growth detectable after 72 h; −, no growth.

and JOHNSTON 1986; WRAY *et al.* 1987), the combination of *LAC9* and *GAL80*$^{s-1}$ genes is inducible, and the *LAC9*/*GAL80*$^{s-2}$ combination is uninducible (SALMERON, LANGDON and JOHNSTON 1989). Together, these results support the idea that the *GAL80*^{s} mutations increase the affinity of GAL80 for GAL4, and not that they simply eliminate the ability of GAL80 to bind inducer. The forces involved in forming the GAL4-GAL80 protein complex might be primarily electrostatic. Increased stability may then result in part from the fact that *GAL80*^{s} mutations increase the net positive charge of GAL80 (NOGI and FUKASAWA 1984), while the GAL80-interactive region of GAL4 is acidic (JOHNSTON, SALMERON and DINCHER 1987; MA and PTASHNE 1987b).

The stringent sequence requirements for interaction between positive and negative regulatory proteins may explain the extreme similarity between the carboxyl termini of GAL4 and LAC9 (SALMERON and JOHNSTON 1986; WRAY *et al.* 1987). Since a functional homolog to GAL80, LAC10, exists in *K. lactis* (DICKSON, SHEETZ and LACY 1981), and since the GAL80 protein is able to repress the LAC9 protein (SALMERON, LANGDON and JOHNSTON 1989), we believe that negative regulation in both systems takes place in a similar manner, by interaction of a negative regulatory protein with the carboxyl terminus of either GAL4 or LAC9.

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