GAL1–GAL10 divergent promoter region of *Saccharomyces cerevisiae* contains negative control elements in addition to functionally separate and possibly overlapping upstream activating sequences

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The upstream activating sequence (UASc) of the adjacent and divergently transcribed *GAL1* and *GAL10* promoters of *Saccharomyces cerevisiae* regulates the induction of the corresponding genes in response to the presence of galactose. We constructed chimeric yeast promoters in which a different UAS, UASc from the iso-1-cytochrome *c* (CYC1) gene of *S. cerevisiae*, was fused at different locations upstream of *GAL1* (UASc—*GAL1*) promoters or *GAL10* (UASc—*GAL10*) promoters and used to monitor the activity of UASc in cells grown in the presence or absence of galactose. Though the CYC1 promoter is fully induced in yeast grown in glycerol medium, UASc—*GAL* chimeric promoters containing UASc were repressed as much as 400-fold (UASc—*GAL1*) or 1350-fold (UASc—*GAL10*) in this growth medium. Several distinct portions of the GAL1—*GAL10* divergent promoter region blocked the UASc-induced expression of the *GAL1* and *GAL10* promoters, whereas others did not, suggesting that several distinct negative control elements are present that may repress transcription of *GAL1* and *GAL10* in the absence of galactose. The approximate locations of these negative control elements were delimited to sites adjacent to or possibly overlapping the sites at which the positive control protein GAL4 binds in UASG. Deletion derivatives of GAL4 that fail to induce transcription from the wild-type GAL promoters but retain the DNA binding domain significantly derepressed the expression of the UASc—*GAL* chimeric promoters. These results, combined with those of earlier studies, suggest the possibility that GAL4 normally induces transcription of *GAL1* and *GAL10* by blocking the activity of these negative control elements, in addition to stimulating transcription by a mechanism of positive control.

[Key Words: Upstream activating sequence; negative control; GAL1—*GAL10* divergent promoter; *Saccharomyces cerevisiae*; CYC1 gene]

Received June 9, 1987; revised version accepted October 6, 1987.

Transcriptional control of eukaryotic protein-coding genes requires specific regulatory sequences located adjacent to each gene. Proximal regulatory sequences include the TATA box, which in yeast is known to be involved in determining the precise start sites for transcription initiation [Chen and Struhl 1985; Hahn et al. 1985; Nagawa and Fink 1985; McNeil and Smith 1986]. Distal regulatory sequences (upstream promoter elements) respond to specific physiological stimuli to control the amount of transcription initiating downstream [Guarente et al. 1984; Giniger et al. 1985; Hope and Struhl 1985; Arndt and Fink 1986; McKnight and Tjian 1986; Pfeifer et al. 1987]. In *Saccharomyces cerevisiae* the distal regulatory elements have been termed upstream activating sequences, or UASs [Guarente 1984].

One well-characterized yeast UAS is UASc, which controls the adjacent and divergently transcribed *GAL1* and *GAL10* genes [Guarente et al. 1982; Johnston and Davis 1984; West et al. 1984; Yocum et al. 1984]. UASc is about 120 bp in size [nomenclature of Giniger et al. 1985], resides about midway between the translation start sites of *GAL1* and *GAL10*, and is required for galactose-mediated induction of both genes. In galactose (Gal) medium, the positive control protein GAL4 binds to four related, dyad-symmetric sequences in UASc and induces transcription of *GAL1* and *GAL10* [Bram and Kornberg 1985, Giniger et al. 1985]. In glycerol (Gly) medium, though GAL4 is produced constitutively [Johnston and Hopper 1982; Laughon and Gesteland 1982], its
activity is inhibited by the negative regulatory protein GAL80 and transcription of GAL1 and GAL10 is prevented (Lue et al. 1987). GAL80 probably inhibits GAL4 by binding to a distinct region at its carboxyl terminus and blocking a specific domain that is rich in acidic amino acids [see Struhl 1987] and is involved in GAL4’s positive control [transcription-activating] function [Johnston et al. 1987; Ma and Ptashne 1987b].

The DNA-binding domain of GAL4 is located within the amino-terminal 75 amino acids of the 881-amino-acid protein [Brent and Ptashne 1985; Keegan et al. 1986; Johnston 1987; Johnston and Dover 1987]. Data derived from in vivo DMS protection [Giniger et al. 1985], DNase I footprinting [Lohr and Hopper 1985], and photofootprinting studies [Selleck and Majors 1987a, b] indicate that GAL4 may be bound at UASc in Glycer medium as well as Gal medium. This result, and the fact that GAL80 represses transcription of GAL1 and GAL10 by blocking GAL4’s transcription-activating domain, suggests a model where galactose induces transcription by causing GAL80 to dissociate from GAL4 molecules bound at UASc, thus exposing GAL4’s transcription-activating domain to the cellular transcription apparatus [Johnston et al. 1987; Ma and Ptashne 1987b; Selleck and Majors 1987b].

Four other S. cerevisiae genes, GAL7, GAL2, GAL80, and MEL1, are also induced by GAL4 and have GAL4 binding sites in their 5' control regions [Bram et al. 1986]. The MEL1 and GAL80 genes are transcribed at a detectable level in uninduced cells [Post-Beittenmiller et al. 1984; Shimada and Fukasawa 1985], whereas GAL1, GAL10, GAL7, and GAL2 are not [St. John and Davis 1981; West et al. 1984; Yocum et al. 1984; Tajima et al. 1986; Tschopp et al. 1986]. Previous deletion-mapping analysis of the 600-bp GAL1–GAL10 divergent promoter region revealed that a certain portion of UASc (located proximal to the GAL1 promoter), when deleted, increased the uninduced level of GAL1 transcription from an undetectable level to about 5% of the fully induced level [West et al. 1984]. This raised the possibility that a negative control element[s] is also present in UASc that normally represses transcription of GAL1 and GAL10 in uninduced cells.

Here we show evidence suggesting that multiple negative control elements are present in the GAL1–GAL10 divergent promoter region, which may account, in part, for the lack of detectable expression of the respective genes in Glycer medium. The negative control elements lie adjacent or possibly overlap the GAL4 binding sites in UASc, and neither GAL4 nor GAL80 is required for their function. Deletion derivatives of GAL4 that apparently bind to UASc but fail to activate transcription of the wild-type GAL genes, significantly block the activity of the negative control elements, suggesting that normally GAL4 regulates the activity of this repression mechanism.

Results
Experimental design
Our goal was to test for the presence of negative control elements in the GAL1–GAL10 divergent promoter region. To pursue this, we fused a 150-bp fragment containing UASc from the iso-1-cytochrome c [CYC1] gene of S. cerevisiae [Guarente et al. 1984; Pfeifer et al. 1987], at the end points of 5' deletions of GAL1 or GAL10, as shown in Figure 1. Our rationale was that UASc would provide the GAL promoters a basal level of expression independent of galactose and GAL4, allowing us to monitor the capacity of portions of UASc and flanking sequences to inhibit UASc-induced expression of GAL1 or GAL10. Activation of CYC1 transcription by UASc, though inhibited about fivefold by glucose, is essentially constitutive under the growth conditions we normally use to induce or repress the GAL promoters [galactose vs. glycerol and lactate medium, respectively].

To measure the amount of expression from the hybrid promoters, the GAL1 and GAL10 genes were fused to the Escherichia coli lacZ gene, and levels of transcription were determined by assaying for β-galactosidase produced in yeast. The UASc–GAL–lacZ gene fusions were maintained on multicopy plasmids derived from YEp24, as described previously [West et al. 1984; Yocum et al. 1984]. Since UASc and UASs are differentially regulated, expression of the hybrid UASc–GAL promoters should reflect the physiological and genetic conditions normally controlling CYC1 or GAL gene transcription. For this purpose, the plasmids were transformed into the yeast strains YM256 [GAL4+] or YM335 [Δgal4] and the transformants grown in synthetic medium containing either glycerol and lactate (Gly) or galactose plus glycerol and lactate (Gal), prior to assaying for β-galactosidase production.

Expression in Gal medium
The activities of the hybrid promoters shown in Figure 1, when transformed into YM256 [GAL4+] and grown in Gal medium, are presented in Table 1. Only hybrid promoters containing a single copy of UASc, inserted in the normal orientation with respect to a TATA box, are included in Table 1 [see Materials and methods]. The results indicated that if UASc was present in a particular UASc–GAL1 or UASc–GAL10 hybrid promoter, expression of the UASc–GAL1– or UASc–GAL10–lacZ fusions was normally induced, and β-galactosidase levels were often as much as twofold higher than in cells containing a respective GAL1– or GAL10–lacZ fusion lacking UASc. If UASc was absent in a given chimeric promoter, as with plasmids UASs–GAL1–8 and UASs–GAL1–9 in Table 1 for example, expression derived solely from the activity of UASs. These results show that UASc does not affect the activity of UASs other than to increase the total amount of expression from the hybrid promoters and that the GAL1 and GAL10 promoters can be induced by UASs alone when UASc is absent.

Repression in Gly medium
In contrast to the results above, Table 1 also shows that when the same plasmid-containing cells were grown in
Gly medium, β-galactosidase levels decreased substantially as large portions of UASc were present, separating UASc from the respective promoter. Figure 2 shows that for both the GAL1 promoter [Fig. 2A] and the GAL10 promoter [Fig. 2B], β-galactosidase levels dropped drastically (about 400-fold altogether for GAL1 and over 1350-fold for GAL10) as a function of the linear distance separating UASc from the promoter. The results of other experiments suggested that this repression was not due to altered spacing between regulatory elements of the chimeric promoters [Guarente and Hoar 1984; R.W. West, unpubl.], implying instead that inhibition of UASc-induced expression of GAL1 and GAL10 was due to the presence of negative control elements in the GAL1–GAL10 divergent promoter region. Figure 2 also shows that the same portions of UASc that repress the UASc–GAL1 or UASc–GAL10 promoters in cells grown in Gly medium induce their expression in cells grown in Gal medium. This suggests that the putative negative control elements reside in the GAL1–GAL10 divergent promoter region in the same proximity as the GAL4 binding sites of UASc.

**GAL80 and GAL4 are not required for repression**

Since GAL80 negatively regulates transcription of the GAL structural genes, it was possible that repression in Gly medium was due to GAL80 binding at sequences in or about UASc. Alternatively, GAL4 may act as a repressor in the absence of galactose, either alone or as part of a GAL4–GAL80 complex [see introductory section]. Thus, we tested to see if GAL80 or GAL4 (or a GAL4–GAL80 complex) was required for this repression. We transformed each of the UASc–GAL1–lacZ fusions shown in Table 1 into yeast strains YM335 [Agal4], YJ1 [Agal4], and YM709 [Agal4 Agal80] and analyzed their expression. The relative level of β-galactosidase synthesized by each fusion in YM335 [Table 2], YJ1, and YM709 [data not shown] was approximately the same as that in wild-type strain YM256 [Table 1], suggesting that neither GAL80 nor GAL4 is required for this repression. S1 mapping studies confirmed that β-galactosidase levels in YM335 cells accurately reflected the amount of specific mRNA made from each of the hybrid promoters [Fig. 3] and that no transcripts were initiated at positions upstream of the normal start sites [data not shown]. This indicates that inhibition of UASc activity by portions of UASc was not a consequence of transcription [and translation] starting at aberrant upstream locations.

**Table 2** also shows that YM335 [Agal4] transformants grown in Gal (galactose plus glycerol and lactate) medium expressed 2- to 20-fold more β-galactosidase than transformants grown in Gly (glycerol plus lactate) medium, indicating that galactose partially derepresses the expression of UASc–GAL1– and UASc–GAL10–lacZ fusions in the absence of GAL4. The basis of this partial
derepression by galactose is unclear. Nevertheless, factors other than galactose are plainly required to overcome the repression mechanism.

**Multiple negative control elements reside in or about UASG**

The data of Tables 1 and 2 suggested the presence and possible locations of several distinct negative control elements residing in the GAL1-GAL10 divergent promoter region. To analyze this possibility further, restriction fragments containing various parts of the GAL1-GAL10 divergent promoter region were inserted between UASc and the CYC1 TATA box in the wild-type CYC1-lacZ fusion plasmid pLG669Δ-312 (Guarente et al. 1984), as depicted in the schematic diagram of Figure 4. The ability of each fragment to repress the expression of the CYC1 promoter in cells (YM335; Δgal4) grown in Gly medium was then examined. A 365-bp fragment containing UASG and flanking sequences (UASG-365) reduced expression of the CYC1 promoter 1200-fold (Fig. 4). However, smaller portions of the GAL1-GAL10 divergent promoter region repressed the CYC1 promoter much less, if at all. Table 3 shows that a 55-bp fragment (UASG-55; formerly designated UASG', West et al. 1984) containing the GAL4 binding sites 2 and 3 (nomenclature of Giniger et al. 1984) did not repress the CYC1 promoter, suggesting that a GAL4 binding site per se is insufficient for repression. A 75-bp fragment (UASG-75) containing GAL4 binding sites 1, 2, and 3 repressed CYC1 promoter activity twofold, whereas a 110-bp fragment (UASG-110) containing GAL4 binding site 4 and a 120-bp fragment (UASG-120) containing GAL4 bindings sites 2, 3, and 4 repressed the CYC1 promoter fivefold and sevenfold, respectively. A 145-bp fragment (UASG-145) containing all four GAL4 binding sites (Bram and Kornberg 1985; Giniger et al. 1985) reduced expression of the CYC1 promoter 100-fold. The orientation in which these fragments were inserted did not significantly affect their ability to repress the CYC1 promoter (data not shown).

Combined, the data of Tables 1–3 suggest the presence of approximately three negative control elements in the GAL1-GAL10 divergent promoter region, residing adjacent to and possibly overlapping (but separate from) the GAL4 binding sites in UASc. Their apparent locations are defined by small portions of the GAL1-GAL10 divergent promoter region having the most notable ef-

### Table 1. Activities of UASc-GAL1- and UASc-GAL10-lacZ fusions in a GAL4+ strain

| Promoter | 5' Deletion end point* | UASc—UASG Distanceb | Presence of UASc^c | β-Galactosidase activity in | Gly | Gal^d |
|----------|------------------------|----------------------|-------------------|---------------------------|-----|-------|
| CYC1     |                        |                      |                   |                           |     |       |
| wild type|                        |                      |                   |                           |     |       |
| GAL1     |                        |                      |                   |                           |     |       |
| wild type|                        |                      |                   |                           |     |       |
| UASc—GAL1 |                      |                      |                   |                           |     |       |
| 1        | 274                    | 220                  | +                 | 5                         | 4050 (2210) |
| 2        | 301                    | 193                  | +                 | 6                         | 4195 (2601) |
| 3        | 330                    | 164                  | +                 | 14                        | 4965 (2136) |
| 4        | 365                    | 129                  | +                 | 98                        | 4013 (1464) |
| 5        | 376                    | 118*                 | ±                 | 79                        | 3046 (1593) |
| 6        | 390                    | 104*                 | ±                 | 173                       | 3365 (734) |
| 7        | 423                    |                      |                   | 547                       | 529 (0) |
| 8        | 578                    |                      |                   | 2014                      | 790 (0) |
| 9        | 632                    |                      |                   | 1387                      | 862 (0) |
| GAL10    |                        |                      |                   |                           |     |       |
| wild type|                        |                      |                   |                           |     |       |
| UASc—GAL10 |                     |                      |                   |                           |     |       |
| 1        | 592                    | 240                  | +                 | <0.1                      | 882 (576) |
| 2        | 552                    | 200                  | +                 | <0.1                      | 914 (363) |
| 3        | 473                    | 121*                 | ±                 | 1.0                       | 850 (239) |
| 4        | 428                    | 76*                  | ±                 | 0.5                       | 330 (90) |
| 5        | 421                    | 60*                  | ±                 | 1.0                       | 486 (5) |
| 6        | 394                    | 42*                  | ±                 | 1.0                       | 194 (0) |
| 7        | 390                    | 38*                  | ±                 | —                         | 131 (0) |
| 8        | 326                    |                      |                   | 135                       | 158 (0) |
| 9        | 261                    |                      |                   | 92                        | 69 (0) |

β-Galactosidase activities were from YM256 cells (GAL4+) containing the indicated plasmids, grown in Gly or Gal medium.
* Number refers to the 5' deletion end point position in the GAL1–GAL10 divergent promoter region for GAL1 or GAL10, according to the nomenclature of Yocum et al. [1984]. See also Fig. 1.

b Distances, denoted in base pairs, are taken from the center of UASc (Guarente et al. 1984) to the center of UASc (Giniger et al. 1985). Asterisks indicate that one or more of the four GAL4 binding sites of UASc have been removed by the deletion.

^c (±) Contains all four GAL4 binding sites; (±) contains one to three GAL4 binding sites; (—) lacks all four GAL4 binding sites.
^d Activities for GAL1–lacZ fusions that lack UASc are denoted by parentheses and are provided for comparison.
Efects on the expression of both UASG–GAL1 and UASG–GAL10 promoters (Tables 1 and 2) and on the CYC1 promoter in the plasmid pLG669Δ-312 (Table 3). Figure 5 shows that one negative control element, arbitrarily designated GAL operator 1 [GAL O₁], appears to lie in the divergent promoter region between positions 330 and 365 (nomenclature of Yocum et al. 1984), proximal to GAL10 and adjacent to or possibly overlapping GAL1 binding site 1. The position of GAL O₁ is defined by differences in the expression (in Gly medium) of the two sets of promoters UASG–GAL1-3 and UASG–GAL1-4 (sixfold) and UASG–GAL10-7 and UASG–GAL10-8 (twofold). Additional support for this assignment was obtained from UASG–GAL1-3 and UASG–GAL1-4 promoters that were integrated into the yeast genome, where a 15-fold difference in their expression was observed (see Table 4, described below). A second negative control element appears to be located between positions 365 and 394 and is arbitrarily designated GAL O₂. GAL O₂ is defined by differences in repression of the CYC1 promoter by DNA fragments UASG-55 and UASG-75 (twofold), as well as differences in the expression of the G sets of promoters UASG–GAL1-4 and UASG–GAL1-7 (15-fold) and UASG–GAL10-6 and UASG–GAL10-7 (20-fold; see Discussion). GAL O₂ probably overlaps GAL4 binding site 1 and may overlap GAL4 binding site 2 as well. A third negative control element, arbitrarily designated GAL O₃, appears to be located in UASG proximal to GAL1, between positions 473 and 510. GAL O₃ lies adjacent to or overlaps GAL4 binding site 4 and is defined by differences in repression of the CYC1 promoter by DNA fragments UASG-55 and UASG-120 (fivefold), as well as by differences in expression of the two sets of promoters UASG–GAL1-7 and UASG–GAL1-8 (twofold) and UASG–GAL10-2 and UASG–GAL10-3 (twofold). A search for similarities in the DNA sequences at the sites corresponding to GAL O₁, O₂, and O₃ revealed no striking homologies; further refinement of the sizes and locations of the negative control elements may require alternative experimental approaches such as DNase I footprinting.

Though GAL1 and GAL10 transcription is regulated by glucose repression as well as by GAL4 and GAL80 (West et al. 1984; Yocum et al. 1984), partly mediated by cis-acting glucose repression elements present in the GAL promoter region (West et al. 1984; S. Chen and R. West, unpubl.), GAL O₁, O₂, and O₃ acted independently of the glucose repression pathway. GAL4 deletion derivatives significantly derepress UASG–GAL1 promoters

The fact that the GAL operators lie in close proximity to the GAL4 binding sites of UASG (Fig. 5) and that galactose alone is insufficient to derepress significantly the UASG–GAL1 and UASG–GAL10 promoters in a Δgal4 strain (Table 2) suggested the possibility that GAL4 itself regulates the activity of the GAL operators when bound at UASG. To examine this possibility, we devised a specific genetic selection procedure to obtain GAL4 mutants that, although unable to activate transcription of the wild-type GAL promoters, might block the activity of the GAL operators and allow expression of UASG–GAL promoters (for details, see Materials and methods). Three mutant gal4 genes whose products activated an integrated chimeric promoter, UASG–GAL1-
Negative elements in a yeast GAL promoter

1, but not the endogenous wild-type GAL promoters, were obtained by this procedure. Each contained a single base transition that created a translation termination codon between the DNA-binding domain at the amino terminus and the transcription-activating domain at the carboxyl terminus of GAL4. Two of these mutations placed a stop codon at amino acid position 174 [GAL4-174], whereas the third placed a stop codon at amino acid position 404 [GAL4-404] of the 881-amino-acid sequence, yielding derivatives of GAL4 with carboxy-terminal truncations. Figure 6 shows that both of these GAL4 deletion mutants contain the DNA-binding domain [Fig. 6, box 1], but lack a region required for transcription-activation [Fig. 6, box 2]. Both the gal4-174 and the gal4-404 genes, when located on a multicopy plasmid and overexpressed by a yeast constitutive promoter, gave rise to protein products that failed to cause significant expression of a wild-type GAL1–lacZ fusion [lacking UASc] that had been integrated at the URA3 locus of chromosome 5 [Fig. 6 and Table 4].

To determine how efficiently GAL4-174 and GAL4-404 activated the expression of the UASc–GAL1–lacZ promoters, seven of the nine different UASc–GAL1–lacZ fusion plasmids of Table 1 were integrated into the URA3 gene of strain YJ1 [Δgal4] [see Materials and methods]. Seven independent strains resulted, designated 274.3, 301.1, 330.3, 365.1, 390.1, and 578.1 [Table 4]. Table 4 shows that the amount of β-galactosidase produced by each integrated promoter was roughly an order of magnitude less than that produced by the respective promoter located on a multicopy plasmid, and the activity of each was inversely proportional to the number of GAL operators it contained. Multicopy plasmids containing the gal4-174 or gal4-404 genes were then transformed into each of the strains of Table 4, and the amount of β-galactosidase in cells grown in Gly or Gal medium was measured. Table 4 shows that GAL4-174 significantly increased the expression of each UASc–GAL1 promoter in both media. For example, the chimeric promoter UASc–GAL1-2 was expressed at a level 63-fold higher in Gly medium and 133-fold higher in Gal medium in the presence of GAL4-174. GAL4-404 significantly derepressed the UASc–GAL1 promoters in Gal medium but not Gly medium [Table 4]. Figure 7 shows that in Gal medium, in the presence of GAL4-404, integrated chimeric promoters containing one or more GAL operators produced only slightly less [about two- to threefold] β-galactosidase than UASc–GAL1-8 which lacks the GAL operators. Furthermore, each chimeric promoter was expressed at roughly the same level, regardless of the total number of GAL operators present. These results suggest that GAL4 is a major factor responsible for blocking the activity of the GAL operators and that its derepressing function normally may be physiologically regulated in response to the presence or absence of galactose.

Even in the presence of GAL4-404 or GAL4-174, UASc–GAL1 promoters containing one or more GAL...
Table 2. Activities of UASc−GAL1 and UASc−GAL10−lacZ fusions in a Δgal4 strain

| Promoter          | β-Galactosidase activity in Gly | Fold derepression by galactose |
|-------------------|--------------------------------|-------------------------------|
| CYC1 wild type    | 842                            | 777                           |
| GAL1 wild type    | <0.1                           | <0.1                          |
| UASc−GAL1         |                                |                               |
| 1                 | 11                             | 4                             |
| 2                 | 17                             | 9                             |
| 3                 | 40                             | 6                             |
| 4                 | 118                            | 3                             |
| 5                 | 210                            | 3                             |
| 6                 | 257                            | 2                             |
| 7                 | 1194                           | 2                             |
| 8                 | 1372                           | 1                             |
| 9                 | 1509                           | 1                             |
| GAL10 wild type   | <0.1                           | <0.1                          |
| UASc−GAL10        | |                               |
| 1                 | <0.1                           | —                             |
| 2                 | <0.1                           | —                             |
| 3                 | 22                             | 22                            |
| 4                 | 9                              | 9                             |
| 5                 | 8                              | 8                             |
| 6                 | 18                             | 18                            |
| 7                 | 213                            | 3                             |
| 8                 | 319                            | 2                             |
| 9                 | 184                            | 1                             |

The Δgal4 strain used was YM335. Activities in brackets were from an isogenic GAL4+ strain (YM256). For additional information see Table 1.

operators were normally repressed two- to ninefold in Gly or Gal medium [Table 4; Fig. 7]. Apparently, either the loss of a portion of the GAL4 protein diminishes the capacity of GAL4-174 or GAL4-404 to inhibit the activity of the GAL operators or the GAL4-404 and GAL4-174 proteins themselves partially inhibit the function of these promoters [see, e.g., Keegan et al. 1986].

Activation of the UASc−GAL1 chimeric promoters by GAL4-174 or GAL4-404 was dependent on the presence of UASc as well as the presence of a wild-type allele of the HAPI gene, which encodes a positive control protein that binds to UASc [Guarente et al. 1984; Pfeifer et al. 1987], showing that the GAL4 deletion derivatives activated expression by removing a block on UASc-induced transcription of the GAL1 promoter [data not shown].

Discussion

We have shown evidence suggesting that transcriptional regulation of the S. cerevisiae GAL1 and GAL10 genes involves negative control elements as well as inducing sequences in the GAL promoter region. Since transcription of the GAL structural genes is also inhibited by GAL80, we concur that at least two distinct pathways are involved in repressing GAL1 and GAL10 in Gly medium. The pathway we characterized is independent of GAL4 and GAL80, but may impose an equal amount of control on the expression of the GAL structural genes. Combined, the two control mechanisms moderate transcriptional levels of GAL1 and GAL10 by over four orders of magnitude [West et al. 1984; Yocum et al. 1984].

To a first approximation, the three putative negative control elements we defined, tentatively designated GAL O1, O2, and O3, map to positions either adjacent to or overlapping GAL4's binding sites in UASc. This fact may have precluded their identification during the course of biochemical procedures used to characterize the sites of GAL4 binding in UASc. To confirm the number of negative control elements and their precise sizes and locations will require considerable more study. Nevertheless, our results suggest that in the case of UASc, the term UAS may be a misnomer and that the structure and function of UASc are more complicated than previously imagined.

The mechanism by which the GAL operators inhibit transcriptional activation is unclear. In conjunction with previous data showing that a 365-bp fragment containing UASc [see Fig. 4] did not repress expression of the CYC1 promoter when positioned upstream of UASc in the plasmid pLG669A-312 [Guarente and Hoar 1984], our data suggest that the GAL operators repress transcription if located downstream of a UAS but not upstream of it. We recently confirmed this notion by inserting a single copy of UASc at two independent positions between UASc and the GAL1 TATA box; expression of these "reverse" chimeric promoters was roughly equivalent to that of UASc−GAL1-8, which was nonrepressed [Table 1; R.W. West, unpubl.]. Thus, the GAL negative control elements may be functionally

Table 3. Repression of the CYC1 promoter by parts of the GAL promoter region

| Promoter          | β-Galactosidase activity in Gly | Fold repression |
|-------------------|--------------------------------|-----------------|
| CYC1 wild type    | 1050                           | 1240            |
| UASc-365          | 2713                           | 1               |
| UASc-145          | 2617                           | 12              |
| UASc-55           | 1980                           | 1188            |
| UASc-75           | 2516                           | 650             |
| UASc-110          | 1421                           | 171             |
| UASc-120          | 2031                           | 178             |

The plasmid pLG669A-312 [Guarente et al. 1984], containing the wild-type CYC1 promoter fused to lacZ, was used as a control. The strain used was YM335 (Δgal4). Fold repression values indicate the ratio of the β-galactosidase activity produced from the wild-type CYC1 promoter relative to that of the given hybrid promoter, in Gly medium. For additional information, see Figs. 4 and 5 and the text.
Figure 4. Repression of the CYC1 promoter by UAS(G. A 365-bp SalI fragment containing UAS(G [UASG-365], which spans the GAL1-GAL10 divergent promoter region from a unique DdeI site at position 300 to a unique Sau3a site at position 660, was inserted into the unique XhoI site of the wild-type CYC1-lacZ fusion plasmid pLG669A-312. At right are shown β-galactosidase activities for the wild-type CYC1-lacZ fusion plasmid pLG669A-312 (A), the chimeric UASc-UASc-CYC1-lacZ fusion plasmid (B), and the wild-type GAL1-lacZ fusion plasmid pRY131 (C) in YM335 [Δgal4] cells grown in Gly or Gal medium.
Figure 5. GAL negative control elements map to three locations. At center is shown the GAL1–GAL10 divergent promoter region, drawn approximately to scale, from the TATA box (T) of GAL10 (left) to GAL1 (right). Positions are denoted by the scale at the top of the figure. The locations of UASG and GAL4 binding sites 1–4 (Bram and Kornberg 1985; Giniger et al. 1985) are noted. The approximate positions of three negative control elements, tentatively designated GAL01, 02, and 03, are indicated by double lines above the sequence. [A] Arrows descending from above the sequence indicate deletion end point positions of 5' deletions of GAL1 used in constructing a respective UASG–GAL1-lacZ fusion. The number adjacent to the arrow refers to the respective promoter of Fig. 1 and Table 1. The number in parentheses above the arrow indicates the fold-repression of UASG at that position. [B] Arrows descending from below the sequence denote deletion end point positions of 5' deletions of GAL10 used to construct a respective UASG–GAL10-lacZ fusion, and numbers adjacent to the arrows denote the corresponding hybrid promoters of Fig. 1 and Table 1. Fold-repression values for A and B were calculated from the average activity obtained for a given plasmid from Tables 1 and 2 (in Gly medium in YM256 and in Gly or Gal medium in YM335), relative to the activity of UASG–GAL1-8 for UASG–GAL1-lacZ fusions or of UASG–GAL10-8 for UASG–GAL10-lacZ fusions. (C) Bars designate the position and extent (bp) of sequences of UASG that were cloned into the wild-type CYC1 promoter, whereas the number in parentheses adjacent to each bar denotes the fold-repression occurring in Gly medium (see Table 3).

Materials and methods

Strains and plasmids

*S. cerevisiae* YM335 (Δgal4-537 ura3-52 ade2-101 lys2-801 his3-200 met –) and the isogenic GAL4+ strain YM256, as well as YM709 (Δgal4-542 Δgal80-538 ura3-52 his3-200 ade2-101 lys2-801 trp1-901 tyr1-501 met – CAN4) were kindly provided by M. Johnston. *S. cerevisiae* Y11 (Δgal4 leu2-3 leu2-112 ura3-52 his – MEL1) was a gift of S. Johnston. JG115 (Δgal7 ade8 HIS+) was provided by Jim Yarger. The strain
Negative elements in a yeast GAL promoter

**Figure 6.** Structure and activity of GAL4 and its deletion derivatives. Linear representations of wild-type GAL4 protein (881 codons) and the GAL4 deletion derivatives, GAL4-174 (173 codons) and GAL4-404 (403 codons), are drawn approximately to scale. Positions of the in-frame translation stop codons of the respective GAL4 deletion derivatives are indicated. The major functional domains of GAL4 are denoted by boxes above the GAL4 + map, designating the position of the DNA-binding domain at the amino terminus (box 1) and the transcription-activating domain at the carboxyl terminus (box 2). Activities of GAL4 and its deletion derivatives are shown at right. The GAL4 + gene and the gal4-174 and gal4-404 genes were present on the multicopy plasmid AAH5 and transcribed from the constitutive ADC1 promoter. Values indicate the amount of ß-galactosidase synthesized in strain 131.1 (AGal4 GALl LacZ::URA3) following growth in Gly or Gal medium. Overproduction of wild-type GAL4 protein causes the GAL1-lacZ fusion to be expressed in noninduced cells (Gly medium; see Johnston and Hopper 1982). For further information, see Tables 4 and 5 and the text.

Table 4. GAL4 deletion derivatives significantly derepress integrated UASc-GAL1 promoters

| Strain | Integrated promoter | Control β-Galactosidase activity | Fold derepression |
|--------|---------------------|---------------------------------|------------------|
|        |                     | Gly                     | gal4-174 Gly | gal4-404 Gly | gal4-174 Gly | gal4-404 Gly | gal4-174 Gly | gal4-404 Gly |
| 131.1  | GAL1 (wild type)    | 0.1                      | 0.1          | 0.1          | 0.1          | 0.1          | 0.1          | 0.1          |
| 274.3  | UASc-GAL1-1         | 0.3                      | 0.3          | 0.3          | 0.3          | 0.3          | 0.3          | 0.3          |
| 301.1  | UASc-GAL1-2         | 0.2                      | 0.2          | 0.2          | 0.2          | 0.2          | 0.2          | 0.2          |
| 330.3  | UASc-GAL1-3         | 0.1                      | 0.1          | 0.1          | 0.1          | 0.1          | 0.1          | 0.1          |
| 365.1  | UASc-GAL1-4         | 0.4                      | 0.4          | 0.4          | 0.4          | 0.4          | 0.4          | 0.4          |
| 376.1  | UASc-GAL1-5         | 0.5                      | 0.5          | 0.5          | 0.5          | 0.5          | 0.5          | 0.5          |
| 390.1  | UASc-GAL1-6         | 0.6                      | 0.6          | 0.6          | 0.6          | 0.6          | 0.6          | 0.6          |
| 578.1  | UASc-GAL1-8         | 0.7                      | 0.7          | 0.7          | 0.7          | 0.7          | 0.7          | 0.7          |

Strains were transformed with either the expression vector AAH5 (control plasmid, lacking GAL4 coding sequences) or AAH5 containing gal4-174 or gal4-404.

a Strains were derived from Y111 (Δgal4) by integrating a plasmid containing the respective GAL1 or UASc-GAL1 promoter, fused to lacZ, into the URA3 gene (see Materials and methods). Strain designations for UASc-GAL1 promoters also indicate the position in the GAL promoter region at which UASc was inserted.

b See Fig. 1 and Table 1.

c Values indicate units of ß-galactosidase activity for strains (containing the respective plasmids) grown in either Gly or Gal medium.

d Values indicate ratios of the amount of expression in strains containing AAH5 plus gal4-174 or gal4-404 relative to strains containing AAH5 alone, for a given medium.
Figure 7. Galactose-induced derepression of integrated UAS_c–GAL1 promoters by GAL4-404. Shown at top is a map of the GAL1 promoter, drawn approximately to scale, with the positions of UAS_c, GAL4 binding sites 1–4, GAL operators O₁, O₂, and O₃, GAL1 TATA box (filled box), GAL1 gene, and 5′ mRNA cap site (star) and transcriptional orientation (arrow) denoted. Vertical arrows beneath the map indicate the positions at which UAS_c was inserted in the GAL1 promoter region, corresponding to the respective chimeric promoters UAS_c–GAL1-1 (arrow 1) to UAS_c–GAL1-8 (arrow 8). Below the map is a semilogarithmic plot of units of β-galactosidase (from 0.1 to 100 units) produced by each corresponding integrated UAS_c–GAL1 promoter, in cells grown in Gal (▲) or Gly (△) medium. For additional information, see Table 4.

Yeasts were transformed using either the spheroplast (Sherman et al. 1986) or the lithium acetate (Ito et al. 1983) techniques. Transformants were selected on SD medium (lacking the appropriate amino acid) containing 2% glucose. β-Galactosidase assays were performed as described previously (West et al. 1984). Samples were analyzed in triplicate cultures and the results averaged.

S1 mapping

Total S. cerevisiae RNA was isolated as described by Sherman et al. (1986), and the 5′ ends of GAL1-encoded transcripts were mapped by S1 nuclease analysis (Weaver and Weissman 1979). Reaction mixtures contained 10 μg of RNA, 500 U/ml of S1 nuclease (Sigma Co.), and an excess of single-stranded 32P-labeled DNA probe. The downstream probe extended from position 300 to 660, which extended from position 688 to 930 (Figs. 1 and 3) and was isolated and 32P-labeled as described previously (West et al. 1984). The upstream probe, which extended from position 300 to 660, was isolated as a 365-bp BglII fragment from the plasmid pRY24 (a gift of R. Yocum) and 32P-labeled and strand separated as described previously (West et al. 1984).

Construction of UAS_c–GAL1 and UAS_c–GAL10 hybrid promoters

The 5′ deletion mutants of GAL1 and GAL10 were previously described by West et al. (1984), and the precise end points of each deletion in the GAL1–GAL10 divergent promoter region are provided in Table 1. A 134-bp SmaI–XhoI fragment harboring UAS_c was obtained from the plasmid pLG669-Z (Garrente and Ptashne 1981). An 8-bp linker was placed at the SmaI end, giving a nominal 150-bp XhoI fragment. The XhoI fragment containing UAS_c was then inserted into the unique XhoI sites located at the end points of the GAL1 and GAL10 5′ deletion mutants (Fig. 1). The orientation and number of copies of UAS_c inserted at each 5′ deletion end point of GAL1 and GAL10 were determined by performing restriction mapping experiments using the enzymes MluI and EcoRI. The former enzyme cuts at an asymmetric location within the 150-bp XhoI fragment, whereas the latter cleaves the plasmid vectors at a site corresponding to the fusion junction of GAL1 or GAL10 with the lacZ gene (see, e.g., Fig. 1 of West et al. 1984). The presence of two or more copies of UAS_c in a particular hybrid promoter was detected by the presence of an additional 150-bp MluI fragment (derived from tandem 150-bp XhoI fragments containing UAS_c) on polyacrylamide gels.

The original mutant GAL1 promoters and the corresponding hybrid UAS_c–GAL1 promoters are as follows: 121–274, UAS_c–GAL1-1; 121–301, UAS_c–GAL1-2; 121–330, UAS_c–GAL1-3; 121–365, UAS_c–GAL1-4; 121–376, UAS_c–GAL1-5; 121–390, UAS_c–GAL1-6; 121–423, UAS_c–GAL1-7; 121–578, UAS_c–GAL1-8; 121–632, UAS_c–GAL1-9. The original mutant GAL10 promoters and the corresponding hybrid UAS_c–GAL10 promoters are: 123–592, UAS_c–GAL10-1; 123–552, UAS_c–GAL10-2; 123–473, UAS_c–GAL10-3; 123–428, UAS_c–GAL10-4; 123–412, UAS_c–GAL10-5; 123–394, UAS_c–GAL10-6; 123–390, UAS_c–GAL10-7; 123–326, UAS_c–GAL10-8; 123–261, UAS_c–GAL10-9. Wild-type CYC1, GAL1,
and GAL10 promoters, as well as the UASc–GAL1 and UASc–GAL10 hybrid promoters, were fused to the lacZ gene (described previously; Yocum et al. 1984; West et al. 1984) and promoter activities measured as a function of β-galactosidase and Xgal synthesis.

Subcloning parts of UASc in pLG669A-312

A 365-bp SalI fragment (UASc-365) containing the sequence from position 300 to position 660 of the GAL1–GAL10 divergent promoter region (Guarente et al. 1982; Yocum et al. 1984) was obtained from the plasmid pRY26, a gift of R. Yocum. The 145-bp fragment containing UASc (UASc-145) was obtained by digesting the GAL1–lacZ fusion plasmid 121–365 (West et al. 1984) with Xhol and Alul, which cleave at positions 365 and 510 of the divergent promoter region (respectively), and placing an 8-bp Xhol linker at the Alul end. The 75-bp fragment containing UASc (UASc-75) was obtained by digesting 121–365 with Xhol and Hpal, the latter enzyme cutting within UASc at position 440. The Hpal end was filled in using Klenow fragment and dNTPs prior to ligation to a Xhol linker. The 120-bp fragment (UASc-120) was obtained by cutting the GAL1–lacZ fusion plasmid 121–390 with Xhol and Alul. The former enzyme cuts at position 390 in UASc. The 55-bp (UASc-55) and 110-bp (UASc-110) fragments were obtained by digesting 121–390 with Xhol and Hpal, as well as BstNI (position 550), gel purifying the 55-bp and 110-bp fragments, filling in the ends using Klenow fragment and dNTPs, and ligating to Xhol linkers. The 55-bp fragment, formerly designated UASc′, was described previously (West et al. 1984). The SalI or Xhol fragments derived from these procedures were then cloned into the unique Xhol site of the plasmid pLG669Δ-312.

![Figure 8](https://example.com/fig8.png)

**Figure 8.** Hypothetical scheme depicting GAL1 and GAL10 regulation in the presence (+) or absence (−) of galactose. In the absence of galactose (Gly medium; top), an inactive GAL4–GAL80 complex binds to UAS G (for clarity, only a monomer of the GAL4–GAL80 complex is shown), and a hypothetical repressor protein (R) binds to GAL O, O2, and O3. In the presence of galactose (Gal medium; bottom), galactose itself or a metabolic derivative (Δ) binds to GAL80, causing it to dissociate from GAL4. Subsequently, GAL4 undergoes a structural transition (○), allowing it to block the activity of the hypothetical repressor (possibly causing the repressor to dissociate from the GAL operators; “a” arrows) and to stimulate RNA polymerase II to transcribe GAL1 and GAL10 (“b” arrows). The effects of glucose repression (Yocum et al. 1984; Selleck and Majors 1987b) are not considered in this model.

Integrating UASc–GAL1–lacZ fusion strains

Plasmids containing UASc–GAL1–lacZ fusions were integrated by gene transplacement (Rothstein 1983) into the URA3 locus of S. cerevisiae strain Y11, using the procedure described by Brent and Ptashne [1984]. Yip derivatives [lacking 2μ replicon sequences] of multicopy plasmids containing UASc–GAL1–lacZ fusions were constructed by partial digestion with EcoRI and reclosure with T4 DNA ligase. The plasmids were then linearized in the URA3 coding sequence by cutting with either ApoI or StuI, and the linearized plasmids were transformed into strain Y11. Ura* transformants were selected, and stable integrants were identified by plasmid segregation analysis and Southern blotting procedures. The strains derived from Y11 in this manner were designated as follows: 131.1 (wild-type GAL1–lacZ::URA3), 274.3 (UASc–GAL1-1::URA3), 301.1 (UASc–GAL1-2::URA3), 330.1 (UASc–GAL1-3::URA3), 365.1 (UASc–GAL1-4::URA3), 376.1 (UASc–GAL1-5::URA3), 390.1 (UASc–GAL1-6::URA3), 578.1 (UASc–GAL1-8::URA3).

Genetic selection of gal4 mutants

We first constructed a Δgal7 derivative of the strain Y11 (Δgal4) designated Y11-7 (Δgal4 Δagal7) and then integrated a Yip derivative of a plasmid that contained the chimeric promoter UASc–GAL1-1 fused to lacZ (Fig. 1; Table 1) into the URA3 gene of Y11-7 to yield the strain PCI-3 (Δgal4 Δgal7 UASc–GAL1-1::URA3). We then transformed PCI-3 with a multicopy plasmid (pLPK–C15) containing the wild-type GAL4 gene, transcribed by the constitutive ADC1 promoter (Ammerer 1983). When the resulting strain is grown in the presence of galactose, the Δgal7 mutation causes the toxic substrate galactose-1-
phosphate to accumulate, providing a positive selection for mutations (including gal4 mutants) in the GAL pathway [Matsumoto et al. 1980]. We then mutagenized this strain with EMS, plated the cells onto selective medium containing galactose plus glycerol and lactate, as well as the indicator dye Xgal, and screened for survivors of gal7 killing (gal1- or gal4-) that were blue. GAL4 mutants that were incapable of binding to UAS_G produced white colonies, whereas GAL4 mutants that bound to UAS_G and blocked the activity of the GAL operators (but failed to activate transcription of the endogenous GAL1 gene on chromosome 2) produced blue colonies. Plasmid DNA from 16 blue PC1-3 colonies was then isolated, using the method of Sherman et al. (1986). By recombining complementary pairs of restriction fragments containing either the GAL4 gene or vector sequences obtained from wild-type or mutagenized plasmid DNA, we determined that 15 of the 16 plasmids contained mutant gal4 genes whose products failed to activate transcription of the wild-type GAL1-lacZ fusion in strain 131.1. For four of the 15, the approximate positions of mutations in the GAL6 coding sequence [Laughon and Gesteland 1984] were mapped by recombining complementary pairs of restriction fragments obtained from mutagenized and wild-type GAL4 DNA. After localizing a given mutation to a specific restriction fragment, the precise base alteration was found by DNA sequencing (both strands) using either the Sanger or Maxam and Gilbert techniques.

Expression of wild-type and mutant gal4 genes in yeast

The yeast expression vector AAHS (Ammerer 1983) was used to express the wild-type and mutant gal4 genes in S. cerevisiae. AAHS contains the ADC1 promoter, a 2 µm replicon sequence for maintenance in multiple copies, a LEU2 gene for selection in yeast, and sequences required for selection and maintenance in E. coli. HindIII fragments 2.9 kb in size, containing the wild-type or mutant gal4 genes, were cloned into a unique HindIII site located immediately downstream of the ADC1 promoter.

Acknowledgments

We especially thank Lenny Guarente and Rog Yocum for many helpful discussions during the early phases of this work and for providing many of the necessary strains and plasmids. R.W.W. wishes to thank Mark Prashe, in whose laboratory parts of this project were initiated. We thank Mark Johnston, Stephen John- ston, and Jim Yarger for yeast strains and Ray Judware for helping to construct a YIp derivative of the plasmid containing UAS_C—GAL1-8. We also thank Russ Finley, Peter Hahn, Joseph L. Messina, Dave Mitchell, and Michael Schechman for a critical reading of the manuscript.

This work was supported by grants to R.W.W. from the American Cancer Society [MV-269], the Alexandra and Alexander Sinsheimer Fund [PN72675], and the New York State Health Research Council [15-068].

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*Genes Dev.* 1987, 1:
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