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Genetics

Regulated expression of the *GAL4* activator gene in yeast provides a sensitive genetic switch for glucose repression

(repressors/weak promoters/synergism)

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ABSTRACT **Glucose (catabolite) repression is mediated by multiple mechanisms that combine to regulate transcription of the *GAL* genes over at least a thousandfold range. We have determined that this is due predominantly to modest glucose repression (4- to 7-fold) of expression of *GAL4*, the gene encoding the transcriptional activator of the *GAL* genes. *GAL4* regulation is affected by mutations in several genes previously implicated in the glucose repression pathway; it is not dependent on GAL4 or GAL80 protein function. *GAL4* promoter sequences that mediate glucose repression were found to lie downstream of positively acting elements that may be "TATA boxes." Two nearly identical sequences (10/12 base pairs) in this region that may be binding sites for the MIG1 protein were identified as functional glucose-control elements. A 4-base-pair insertion in one of these sites causes constitutive GAL4 synthesis and leads to substantial relief (50-fold) of glucose repression of *GAL1* expression. Furthermore, promoter deletions that modestly reduce *GAL4* expression, and therefore presumably the amount of GAL4 protein synthesized, cause much greater reductions in *GAL1* expression. These results suggest that GAL4 works synergistically to activate GAL1 expression. Thus, glucose repression of *GAL1* expression is due largely to a relatively small reduction of GAL4 protein levels caused by reduced *GAL4* transcription. This illustrates how modest regulation of a weakly expressed regulatory gene can act as a sensitive genetic switch to produce greatly amplified responses to environmental changes.**

Expression of the *GAL1*, *-7*, and *-10* genes, which are required for galactose catabolism in *Saccharomyces cerevisiae*, is regulated at two levels (1). (*i*) Galactose induces their transcription by preventing GAL80 protein from inhibiting function of the GAL4 transcriptional activator. (*ii*) Glucose causes severe repression of *GAL* gene transcription by a process to which several different mechanisms contribute. Some operate to reduce the amount of inducer available to inactivate GAL80 by reducing expression of *GAL3*, required for inducer synthesis (2), and of *GAL2*, encoding the galactose transporter (3), and by inactivation of preexisting galactose permease in the cell (4). Other mechanisms of repression operate through sites in the *GAL* promoters termed the upstream activation sequence (UAS) and the upstream repression sequence (URS) and, therefore, act more directly to repress transcription.

The UAS and URS regions from the *GAL1* promoter are capable of independently mediating glucose repression (5). The repression that operates through the UAS region, which contains four binding sites for the GAL4 activator, probably reflects reduced levels or reduced function of the GAL4 protein in glucose-grown cells. Repression mediated by the URS, which lies between the UAS and the "TATA element," is presumably due to unidentified repressors that bind to this region.

UAS-mediated repression is characterized by the failure of GAL4 to bind the UAS in cells growing in the presence of glucose (6, 7). This could be due to glucose-induced modifications of GAL4 that affect DNA binding, to glucose-induced proteolysis of the GAL4 protein, or to glucose repression of *GAL4* gene expression. We describe experiments that show that *GAL4* expression is modestly reduced by glucose through the action of specific negatively acting elements in the *GAL4* promoter. The resulting reduction in intracellular GAL4 activator levels leads to a greatly amplified effect on expression of *GAL1* and accounts for a substantial portion of glucose repression of *GAL1* expression.

MATERIALS AND METHODS

Strains and Growth Conditions. All yeast strains used in this study (except YM3322) contain *ura3-52*, *Δhis3-200*, *ade2-101*, *lys2-801*, *LEU2*::pRY181 (*GAL1/lacZ*) (pRY181 is described in ref. 8). All cultures were grown at 30°C in YP medium (9) containing the described carbon sources. The presence of 0.1% glucose in medium with 5% (vol/vol) glycerol stimulated the growth of strains but caused no detectable glucose repression, as has been noted (5).

Plasmids Designed for Construction and Chromosomal Integration of Modified Promoters and Fusions. A detailed description of the construction of these plasmids will be presented elsewhere. Briefly, *GAL4* and 1.5–2.0 kilobases of DNA flanking each end were cloned into a modified pBluescript SK+ (Stratagene) vector. A 1.1-kilobase *Hin*dIII fragment containing the selectable gene *URA3* was then inserted into a *Hin*dIII site adjacent to the 3′ end of the *gal4* coding region. Fusions were constructed by replacing an internal restriction fragment of *GAL4* with fragments carrying the gene for chloramphenicol acetyltransferase (CAT) or *HIS3* such that *GAL4* was fused in-frame at its *Sph* I site at codon 11 to the first codon of the reporter genes. Deletions and linker insertions in the *GAL4* promoter were constructed using PCR methodology, which will be described in detail elsewhere. To integrate the various mutations and fusions, yeast were transformed with the products of a restriction digestion that releases the cloned insert from the vector. Since the recipients usually contained a deletion removing the entire *GAL4* coding region and since the ends of DNA fragments are highly recombinogenic (10), URA^+ transformants arise by recombination between sequences flanking *GAL4*. Southern blot analysis confirmed the proper integration of mutations in all transformants tested.

Enzyme Assays. For CAT assays, cells from 5-ml cultures grown to an A_{600} of 0.8–1.5 in YP medium with the appropriate carbon sources were washed with 0.5 ml of 0.25 M Tris·HCl (pH 7.5) and frozen in liquid nitrogen. To prepare

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Abbreviations: UAS, upstream activation sequence; URS, upstream repression sequence; CAT, chloramphenicol acetyltransferase.

extracts from thawed pellets in 1.5-ml microcentrifuge tubes, 0.2 ml of ice-cold 0.25 M Tris·HCl (pH 7.5) was added to resuspend the cells, acid-washed glass beads were added to a level 1–2 mm below the meniscus, and the tubes were shaken at maximum speed on the 6-inch platform head (1 inch = 2.54 cm) of a Vortex Genie 2 (Scientific Industries, Bohemia, NY) at 4°C for eight 20-s periods, with 20-s pauses between each period of shaking. The tubes were centrifuged in a standard microcentrifuge at 4°C for 5 min, and samples of the supernatants were stored at −70°C. The concentration of protein in each extract was determined by the method of Bradford (11). CAT activities were determined by the phase-extraction method described by Seed and Sheen (12). Typically, 3–10 μg of protein were assayed in 100-μl reaction volumes. Units of CAT activity are defined as cpm measured in the organic phase and expressed as a percentage of total cpm (% conversion) divided by the amount of protein assayed (μg) and the time of incubation (min). Assay of β-galactosidase activity was carried out on permeabilized cells as described by Yocum *et al.* (8).

RESULTS

Expression of *GAL4* Is Regulated by Glucose. *GAL4* is an extremely weakly expressed gene (13). To provide a sensitive assay for measuring *GAL4* expression, we constructed plasmids containing chimeric genes in which several kilobases of DNA upstream of and including codon 11 of *GAL4* are fused to either the CAT gene or *HIS3*. A single copy of these fusions was integrated into the yeast genome without any associated vector sequences by recombination at the *GAL4* locus such that all sequences native to the region upstream of the fusion junctions were retained.

The data in Table 1 show that expression of the *GAL4*–CAT fusion in glucose-grown cells was 5- to 7-fold lower than in cells grown on glycerol. This effect was similar in strains containing wild-type and null alleles of *GAL4* and *GAL80*. These results confirm earlier work showing GAL4 does not regulate its own synthesis (13) and suggest the existence of a mechanism for regulating the synthesis of GAL4 protein in response to glucose.

Regulation of *GAL4* was also apparent from the growth of a strain containing a *GAL4–HIS3* fusion on minimal plates lacking histidine (Fig. 1). When the carbon source was raffinose, which does not cause repression of the galactose metabolizing pathway, *GAL4* promoter activity was sufficient to produce a His^+ phenotype; growth on glucose apparently reduced the expression of the hybrid gene to a level that was inadequate to support colony formation.

Trans-Acting Mutations Affecting *GAL4* Regulation. After extended incubation (4–6 days) of the *GAL4–HIS3*-containing strain in the presence of glucose, His^+ mutants resistant to glucose repression arose at a frequency of 10^{-5}–10^{-6} (Fig. 1). Analysis of several of these mutants revealed that some contained recessive defects that were complemented by *GRR1*, *SSN6*, or *TUP1*, genes that have been described and are required for glucose repression of *GAL1* and other genes (14–16). Furthermore, glucose repression of *GAL4*–CAT activity was relieved in strains with characterized mutations in these genes and in several others implicated in glucose regulation (1, 14, 16–18) (Fig. 2*A*). The *GAL11* gene, which is required for full expression of *GAL1* but appears not to be involved in glucose repression (19), had no effect on either the level of *GAL4* expression or its regulation. Function of SNF1, a protein kinase essential for release from repression of all glucose-regulated genes analyzed to date (20), was also required for derepression of *GAL4*–CAT activity (Fig. 2*B*). A mutation in *SSN6*, which is a suppressor of *snf1* mutations (15), resulted in constitutive *GAL4* expression (Fig. 2*B*). Thus, all of these genes affect *GAL4* in the same manner in which they affect other glucose-repressed genes.

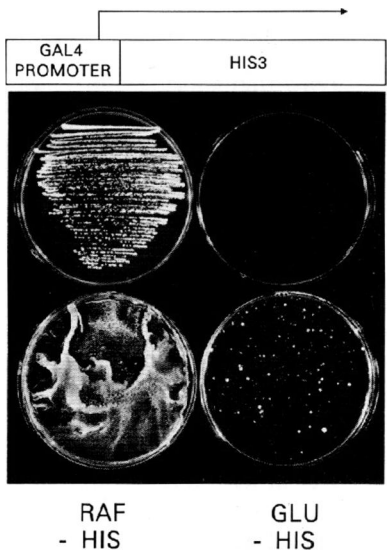

FIG. 1. Carbon-source-dependent growth of a strain containing a *GAL4–HIS3* fusion. Strain YM3182 containing the *GAL4–HIS3* fusion integrated at *GAL4* was streaked (upper plates) and spread (lower plates at 1×10^7 cells) on SD plates lacking histidine and incubated at 30°C for 3–5 days. RAF, raffinose; Glu, glucose.

Table 1. Regulation of *GAL4*–CAT expression by glucose

Strain	Genotype	CAT activity		
		Glycerol	Glucose	Fold decrease
YM2632	*gal4⁻ gal80⁻*	4.4	0.9	4.7 ± 1.1
YM2631	*gal4⁻ GAL80⁺*	2.6	0.4	6.8 ± 1.2
YM3544	*GAL4⁺ gal80⁻*	2.2	0.3	7.5 ± 1.5
YM3543	*GAL4⁺ GAL80⁺*	2.5	0.4	5.9 ± 2.5

All strains contain the CAT gene fused to *GAL4* at the *GAL4* locus. *GAL4⁺* strains contain a single copy of the functional gene integrated at *LYS2*. CAT assays were performed on exponentially growing cells in YP medium containing either 5% glycerol and 0.1% glucose or 2% glucose as indicated by glycerol or glucose, respectively. Activities represent the average calculated from at least three experiments. Data for fold decrease are expressed as mean ± SEM.

Identification of a *GAL4* Promoter Element Controlling Glucose Repression. We tested for the existence of promoter sequences necessary for glucose regulation of *GAL4* by examining the effects of internal deletions constructed upstream of the *GAL4*–CAT gene. As shown in Fig. 3, a 50-base-pair (bp) region (positions −77 to −25) required for glucose repression was identified (line A). This region lies ≈40 bp upstream from the most promoter-proximal site for transcription initiation (21) and lies downstream from positively acting elements that we have identified from a more extensive analysis of the *GAL4* promoter. These positively acting elements include two that we believe may be TATA boxes because (*i*) at least one of them is required for any *GAL4* expression, (*ii*) their sequences are A+T-rich, and (*iii*) they are in a location characteristic of TATA elements (our complete analysis of the *GAL4* promoter will be presented elsewhere).

Within the glucose control region, we recognized a directly repeated sequence (10/12-bp identity), each copy of which should lie on the same face of the DNA helix (i.e., separated by 21 bp; Fig. 3). All deletions or linker insertions that disrupted the upstream copy resulted in completely constitutive promoter activity (constructs A, B, E, F, G, and H); a deletion removing the downstream copy (construct C) eliminated most, though possibly not all, repression. Muta-

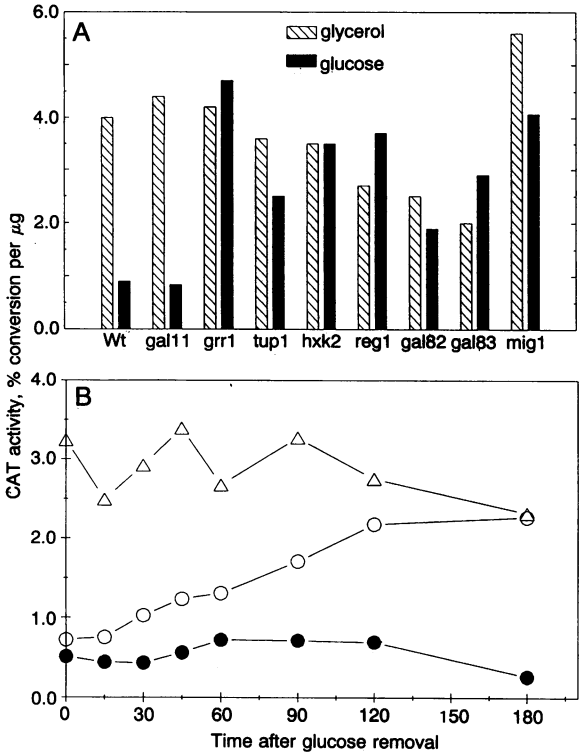

FIG. 2. *GAL4* expression in various glucose repression mutants. (*A*) *GAL4*–CAT activity measured in cells growing exponentially in YP medium containing 5% glycerol and 0.1% glucose or containing 2% glucose. Strains: Wt (wild type, YM3216), *gal11* (YM3220), *grr1* (YM3317), *tup1* (YM3390), *hxk2* (YM3313), *reg1* (YM3316), *gal82* (YM3314), *gal83* (YM3315), and *mig1* (*YM3733*). All alleles except *gal82* and *gal83* are gene disruptions. (*B*) Time course of derepression of *GAL4*–CAT activity in wild-type (YM3216) (○), Δ*snf1* (YM3322) (●), and Δ*ssn6* (YM3319) (△) backgrounds. Cells grown to early logarithmic phase in YP containing 2% glucose were centrifuged and resuspended in YP containing 5% glycerol and 0.1% glucose. Incubation was continued and samples were removed at various times (min) for assay.

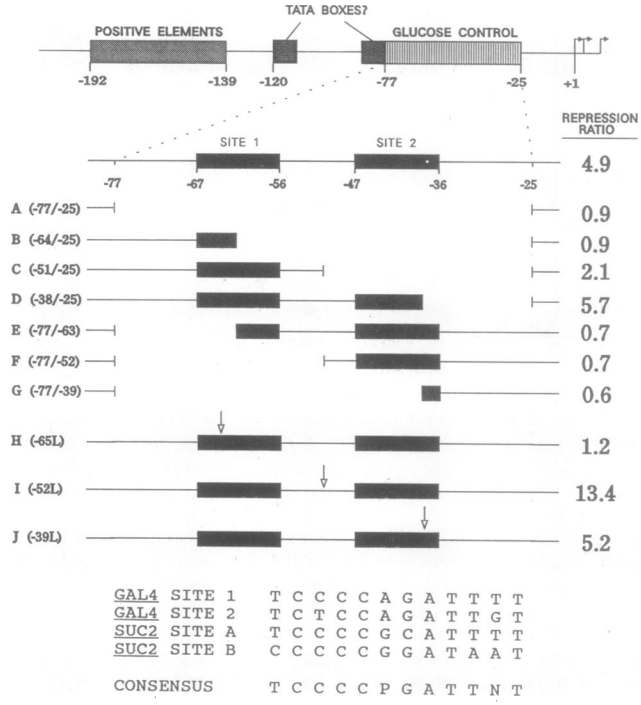

FIG. 3. Delineation of sites in the *GAL4* promoter required for glucose repression. The first of three previously identified (21) positions for transcription initiation is designated +1. A 6-bp insertion containing a *Bam*HI site lies between the indicated end points of all deletions. Vertical arrows designate insertion mutations that form a new *Bam*HI site beginning 1 bp downstream of the nucleotide indicated at the left end of each line. Each modified promoter was fused to the CAT gene and integrated at the *GAL4* locus in YM2632. CAT activity was assayed as described in Table 1, and the repression ratio was calculated as the activity on glycerol divided by activity on glucose. The sequences of sites 1 and 2 (solid boxes) are shown at the bottom and are compared to similar sites from the *SUC2* promoter.

tions in the region that left both copies intact (constructs D and I), or nearly intact (construct J), preserved normal glucose regulation.

The directly repeated element in *GAL4* resembles a repeated sequence present in an inverted orientation in the promoter of the glucose-repressed *SUC2* gene (Fig. 3). In *SUC2*, these sequences are binding sites for a protein known as MIG1 (18). The presence of *MIG1* on a high copy number plasmid causes reduced expression of *SUC2* and inhibits growth on galactose, raffinose, and other nonrepressing sugars; disruptions of *MIG1* relieve glucose repression of *SUC2* (18). Thus, MIG1 exhibits the properties of a glucose-sensitive repressor. *MIG1* function is also required for regulation of *GAL4*, since a *mig1* null mutation relieves glucose repression of *GAL4* expression (Fig. 2*A*). Recent *in vitro* footprinting experiments have confirmed MIG1 binding to the upstream motif (site 1) in the *GAL4* promoter (30). Although no MIG1 binding was detected at the downstream motif (site 2), our results show that its deletion does affect regulation *in vivo* (Fig. 3, construct C). Nevertheless, the residual repression observed in the absence of the downstream site (Fig. 3, construct C), the complete loss of repression caused by mutation of the upstream site (constructs E and H), the footprinting experiments, and the greater sequence similarity of the upstream site to the MIG1 binding sites in *SUC2* implicate the upstream motif (site 1) in *GAL4* as the primary binding site for MIG1.

Significance of *GAL4* Regulation in the Galactose Catabolite Repression System. In *gal80*⁻ cells, where mechanisms of repression that operate to reduce inducer levels are irrelevant, transcription of the *GAL1*, *-7*, and *-10* structural genes is reduced about a hundredfold by glucose (22). Since the observed effect of glucose is to reduce *GAL4* expression only ≈5-fold, it was conceivable that this regulation would have only a minor role in the overall process of repression of the genes that GAL4 activates.

To correlate changes in *GAL4* expression with their consequent effects on expression of a gene activated by GAL4, we employed internal deletions in the *GAL4* promoter to vary its strength and to measure the effects of reducing GAL4 synthesis on *GAL1* expression under nonrepressing conditions (5% glycerol). *GAL4* expression was evaluated by determining the effect of each deletion on *GAL4*–CAT activity; *GAL1* expression was measured by assaying activity of a *GAL1–lacZ* gene in an isogenic background with the same altered promoters driving wild-type GAL4 synthesis. The results of this analysis show that modest reductions in *GAL4* expression lead to much larger reductions in expression of *GAL1*. In fact, a decrease in *GAL4* expression comparable to that mediated by glucose (≈6-fold, 15% of wild type) appears to cause at least a 40-fold reduction in *GAL1* expression (<2.5% of wild type). The relationship between *GAL4* and *GAL1* expression is best described by a sigmoidal curve (Fig. 4), which is suggestive of cooperativity of GAL4 binding or function at the *GAL1* promoter.

Further evidence that *GAL4* regulation represents a mechanism of major significance for glucose repression was provided by examining the effect of constitutive GAL4 synthesis

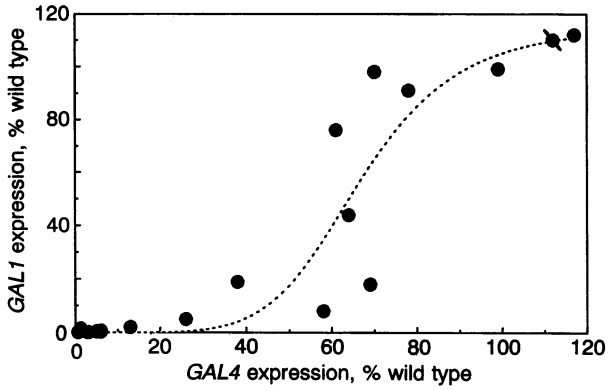

FIG. 4. Effect of reduction of *GAL4* expression on *GAL1* expression. For *GAL4* expression, the CAT activity produced from a *GAL4*–CAT fusion gene carrying various deletions that weaken the *GAL4* promoter is plotted. For *GAL1* expression, the β-galactosidase activity produced from a *GAL1–lacZ* fusion in the same genetic background (YM2632) with *GAL4* expression being driven by the same altered promoters is plotted. All assays were performed using cultures growing exponentially under nonrepressing conditions (YP containing 5% glycerol and 0.1% glucose). Each point represents the average of at least three assays of each enzyme's activity. Standard deviations for all data were ≤22 except for the assay of CAT activity for the point marked ●, which had a standard deviation of 32. Standard deviations for points between 0 and 40% of wild-type *GAL4* activity were ≤13 (CAT assays) and ≤6 (β-galactosidase assays). The curve shown is a computer-generated (NFIT, version 1.0; Island Products, Galveston, TX) idealized Hill plot.

on expression of a *GAL1–lacZ* fusion. An insertion of 4 bp (GGAT) in the center of the conserved CCCC sequence of the upstream MIG1 binding element (site 1) completely relieved control of the *GAL4* promoter by glucose without affecting activity under nonrepressing conditions (Fig. 3, construct H, and Table 2). Expression of the *GAL1* gene during growth on glucose was >50 times higher in the strain with constitutive *GAL4* expression than in the same strain with normal *GAL4* regulation (Table 2). Similar relief of *GAL1* repression was observed when *GAL4* was expressed constitutively due to a mutation in *mig1* (data not shown). The magnitude of the effect is such that there remains only a residual 4-fold effect that must be accounted for by other mechanisms, such as the URS-mediated system of repression (5) or possibly post-translational modifications affecting GAL4 protein function (23).

DISCUSSION

We have determined that expression of the *GAL4* activator gene is repressed modestly by glucose and that this regulation is critical for glucose repression of galactose metabolism. *GAL4* regulation was evident from the activities of *GAL4*–CAT and *GAL4–HIS3* gene fusions integrated by recombination at the *GAL4* locus. The magnitude of the effect is similar to the reduction in GAL4 mRNA levels that Laughon and Gesteland (13) observed in glucose-grown cells. Compelling confirmation of the glucose repression of *GAL4* expression was provided by our ability to select mutants with defects in genes previously shown to be involved in glucose repression (*GRR1*, *SSN6*, and *TUP1*) by using a strain containing a *GAL4–HIS3* gene fusion. Furthermore, all of the genes required for glucose regulation that we tested were also required for *GAL4* regulation (Fig. 2). Thus, *GAL4* is a typical glucose-repressed gene.

Two key results suggest that the modest 5-fold regulation of *GAL4* expression accounts for a substantial amount of the glucose repression of *GAL1*. (*i*) A mutation in the *GAL4* promoter that abolishes its regulation by glucose relieves most of the glucose repression of *GAL1* expression (Table 2). (*ii*) Small reductions in *GAL4* expression are sufficient to account for much greater reductions of *GAL1* expression (Fig. 4). In this experiment, it is significant that when GAL4 levels are reduced by promoter deletions, independently of glucose repression but by an amount similar to that caused by growth on glucose (5- to 6-fold), the resulting reduction of GAL1 expression is of a magnitude comparable to that which we observe to be caused by UAS-mediated glucose repression (≈40-fold) (data not shown).

The relationship between *GAL4* and *GAL1* expression would be most simply explained by cooperative binding of GAL4 to its four binding sites in the *GAL1* promoter. Giniger and Ptashne (24) have established that GAL4 protein binds cooperatively to this promoter *in vivo*. The promoters of other *GAL* genes (e.g., *GAL10*, *GAL2*, and *GAL7*) that are severely repressed by glucose also contain multiple sites for GAL4 binding (1), so *GAL4* repression may affect expression of these genes similarly to *GAL1*. Interestingly, the activity of *GAL80*, which has only one GAL4 binding site, is not repressed significantly by glucose (25).

We propose that the combination of *GAL4* regulation and cooperative GAL4 action constitutes a genetic switch mechanism mediating transition in a two-state system. This is reminiscent of the genetic switch that controls bacteriophage λ development (26). In the derepressed state, the intracellular concentration of GAL4 protein would be sufficient to stabilize binding to multiple adjacent sites in the *GAL1* promoter with the aid of cooperative interactions; in the presence of glucose, the slightly reduced expression of *GAL4* would drop the activator concentration below a narrow threshold level required for occupancy of at least the weaker sites. Alternatively, the effect of *GAL4* regulation could be amplified if, after GAL4 proteins are bound, they then function cooperatively at another level to activate transcription (27).

The experiments of Mylin *et al.* (23) have demonstrated a correlation between GAL4 function and the presence of

Table 2. Effect of constitutive *GAL4* expression on regulation of *GAL1*

	GAL4 expression			*GAL1* expression		
GAL4 regulation	Glycerol	Glucose	Fold decrease	Glycerol	Glucose	Fold decrease
Wild type	6.1 ± 0.8	1.2 ± 0.1	5.3	1194 ± 250	7 ± 1	170
Constitutive	6.4 ± 0.5	5.5 ± 1.4	1.2	1266 ± 95	360 ± 11	4

Strains exhibiting either normal glucose-regulated expression of *GAL4* (strains YM3216 and YM3106) or constitutive expression of *GAL4* (strains YM3747 and YM3756) due to a *Bam*HI linker insertion at position −65 of the *GAL4* promoter (Fig. 3, construct H) were analyzed. All strains are isogenic and are *gal80*. *GAL4* expression was determined by assaying *GAL4*–CAT activity in strains YM3216 and YM3747. *GAL1* expression was determined by assaying *GAL1–lacZ* activity in isogenic strains (YM3106 and YM3756) in which GAL4 synthesis was driven by the same wild-type or mutant promoters that were used to drive CAT synthesis. Assays were performed on exponentially growing cells in YP medium containing either 5% glycerol and 0.1% glucose or 2% glucose as indicated by glycerol or glucose, respectively. Data are expressed as mean ± SEM.

phosphorylated forms of the GAL4 protein in the cell. Addition of glucose to cells growing under inducing conditions caused a rapid shift to the nonphosphorylated form. However, our results suggest such a mechanism can only account for a minor amount of glucose repression of *GAL1* expression since constitutive *GAL4* transcription relieved most of the glucose repression of *GAL1* (Table 2).

Mutational analysis of the *GAL4* promoter allowed the delineation of two nearly identical sequence elements necessary for glucose regulation (Fig. 3). These sites are similar to MIG1 binding sites in the glucose-repressed *SUC2* (invertase) promoter, and mutation of *mig1* relieved repression of *GAL4*. Disruption of site 1 in the *GAL4* promoter completely destroyed regulation; disruption of site 2, the sequence of which differs at only one position from the consensus sequence for MIG1 binding, only moderately affected repression (Fig. 3). This suggests that site 1 mediates stronger binding *in vivo* and is consistent with results of *in vitro* footprinting experiments showing MIG1 binding at site 1 but apparently not to site 2 (30). Thus the function of the downstream site (site 2) may be to stabilize binding of MIG1 at the other site through cooperative interactions. Although the two MIG1 binding sites in *SUC2* are inverted with respect to one another and are separated by 45–50 bp, the *GAL4* sites are directly repeated and are separated by only 10 bp. Therefore, no specific configuration of the sites with regard to orientation or intervening distance seems to be required for repressor function.

The location of the repressor binding sites is unusual. There are no apparent TATA elements in the 38 bp between the downstream element (site 2) and the site of transcription initiation. However, a region just upstream of element 1 is absolutely essential for basal promoter activity and contains two weak TATA-like motifs (data to be presented elsewhere, see Fig. 3). Thus one potential mechanism for regulation is that binding of MIG1 during growth on glucose interferes with the assembly or the activity of the basic transcription apparatus at the TATA box. The positioning of elements suggests MIG1 may repress *GAL4* expression differently than it represses *SUC2*, where it appears more likely that MIG1 competes for binding of an activator to a UAS site overlapping the MIG1 binding site (18). In addition, sequence comparisons (J. Flick and M.J., unpublished data) suggest that MIG1 binds directly to the URS element (5) located between the UAS and TATA box in the *GAL1* promoter. Hence, in the three promoters in which MIG1 is likely to operate, the binding sites appear to reside in different locations. It is interesting that MIG1 appears to operate on both the *GAL4* and *GAL1* promoters. Thus, MIG1 may contribute to regulation of *GAL1* expression at two levels: it regulates the amount of GAL4 activator produced and may modulate GAL4 function at the *GAL1* promoter.

MIG1 has been shown to contain two C_2H_2 zinc-finger motifs that share considerable homology with fingers from three mammalian proteins proposed to be involved in control of mitogenesis and in developmental regulation (18). Two of these, *Egr-1* (*NGFI-A* or *Krox-24*) and *Egr-2* (*Krox-20*), bind to sites similar to those recognized by MIG1 and may be regulators of genes of the mammalian early growth response, including one that encodes a glucose transporter (28). The third gene encodes the Wilms tumor suppressor protein, which probably acts to repress the expression of transforming genes (29). Thus these proteins appear to make up a family of proteins whose DNA binding domains have been highly conserved and whose function is to adapt cells for rapid growth. The finding that MIG1 is involved in regulation of *GAL4*, itself a regulator of transcription, raises the possibility that the Egr or Wilms tumor proteins might also regulate synthesis of DNA binding proteins in mammals. Such systems, in which the regulation of function of one DNA binding protein affects transcription of another, provide the potential for great flexibility and complexity in cellular responses.

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