Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5922–5926, July 1992
Genetics

Multiple mechanisms mediate glucose repression of the yeast *GAL1* gene

(*GAL4/GAL80*/gene regulation/*Saccharomyces cerevisiae*/*SNF1*)

MARC S. LAMPHIER* AND MARK PTASHNE

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

Contributed by Mark Ptashne, March 20, 1992

ABSTRACT **Several mechanisms contribute to the glucose repression of the *GAL1* gene in *Saccharomyces cerevisiae*. We show that one mechanism involves the transcriptional down-regulation of the *GAL4* gene and a second requires the *GAL80* gene. We also examine the contribution of cis-acting negative elements in the *GAL1* promoter to glucose repression. In an otherwise wild-type strain disruption of any one of these three mechanisms alleviates repression of *GAL1* only 2- to 4-fold. However, in the absence of the other two mechanisms the transcriptional down-regulation of *GAL4* is sufficient to repress *GAL1* expression 40- to 60-fold and the *GAL80*-dependent mechanism is sufficient to repress *GAL1* expression 20- to 30-fold. These first two mechanisms constitute a functionally redundant system of repression and both must be disrupted in order to abolish glucose repression of *GAL1*. In contrast, negative elements in the *GAL1* promoter are effective in repressing *GAL1* expression 2- to 4-fold in glucose medium only when at least one of the other two mechanisms of repression is present. Thus, glucose repression of *GAL1* is mediated primarily by the first two mechanisms, whereas the third mechanism supplements repression severalfold.**

Addition of galactose to cultures of the yeast *Saccharomyces cerevisiae* growing in medium with glycerol induces expression of the *GAL* genes at least 1000-fold. If glucose is added in addition to galactose the *GAL* genes are induced to only 1% of the levels elicited by galactose alone, a phenomenon called glucose repression (reviewed in ref. 1). Activation of the *GAL* genes requires GAL4, a positive regulatory protein that binds to sites comprising the upstream activation sequences of the *GAL* genes (UAS_{gal}) (2, 3). Regulation by galactose is mediated by GAL80, a negative regulatory protein that associates with GAL4 in the absence of galactose to form a transcriptionally inactive complex. Repression by GAL80 is relieved when cells are grown in medium with galactose (4–8).

The mechanisms by which glucose represses expression of the *GAL* genes are more complex (1, 9–13). Several reports have suggested that the negative regulator GAL80 may play a role in mediating this repression (9, 10, 14–16), but other reports have shown that deleting the *GAL80* gene does not significantly affect glucose repression (17, 18). Several negative elements (upstream repression sequences, URS_{gal}) located between the UAS_{gal} and the TATA box of the *GAL1* promoter have been shown to mediate some glucose repression (11, 13, 19). Footprinting studies performed *in vivo* have shown that the UAS_{gal} is not protected by GAL4 when cells are grown in glucose medium (3, 20), suggesting that some of the effects of glucose on *GAL* gene expression may be due to a reduction in the concentration of cellular GAL4 and/or an inhibition in its DNA-binding activity. Consistent with the former possibility, it has been shown that the *GAL4* gene is weakly down-regulated in glucose medium (12, 21) and that small changes in *GAL4* expression can have a large effect on the glucose-repressed levels of *GAL1* expression (12). It has also recently been shown that the transcriptional repressor MIG1 binds to and weakly represses the activities of both the *GAL4* and *GAL1* promoters in glucose (13). Other recent reports have suggested that the phosphorylation of GAL4 may regulate its activity in glucose (22–24), although it has been shown that one predominant phosphorylation of GAL4 is not required for, and may be a consequence of, transcriptional activation (25).

The *SNF1* gene encodes a protein kinase that is required for the expression of many glucose-repressible genes in yeast (26, 27). In an *snf1* strain the *GAL1* gene is induced in galactose to <1% of its normal levels, possibly because mutation of *SNF1* causes the constitutive repression of *GAL1* expression through all, or some subset, of the mechanisms that mediate glucose repression. This defect in expression may be due in part to repression mediated by negative elements in the *GAL1* promoter (11) and the down-regulation of *GAL4* transcription (12).

In this paper we show that at least three mechanisms mediate glucose repression of the *GAL1* gene. One mechanism involves a relatively weak down-regulation of the *GAL4* gene, a second mechanism requires the *GAL80* gene, and a third mechanism requires negative elements in the *GAL1* promoter. The first two mechanisms constitute a functionally redundant system of glucose repression: either is sufficient to effectively repress *GAL1* and both must be disrupted before any significant defect in the glucose repression of *GAL1* is observed. Disruption of both of these mechanisms also alleviates the dependence of *GAL1* expression on *SNF1*. In contrast, the third mechanism has no obvious effect on *GAL1* in the absence of other mechanisms of glucose repression but can supplement repression 2- to 4-fold when at least one other mechanism is present.

MATERIALS AND METHOD

Yeast Strains and β-Galactosidase Assays. The genotypes of yeast strains are described in the legends to tables. Yeast cells were made competent for transformation by treatment with lithium acetate (28). *GAL4* effector constructs were integrated at the *LEU2* locus by transformation following digestion of the effector plasmid with *Bst*EII or *Kpn* I, LR1Δ50Δ2μm was integrated at the *URA3* locus by transformation following digestion with *Apa* I. Copy number was determined by Southern analysis. Disruption of the *GAL80* loci of MLY220B and MLY92Δ50 was achieved by transformation with a fragment containing the *GAL80* gene disrupted by replacing an internal *Bgl* II fragment with the *HIS3* gene.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: UAS_{gal}, upstream activation sequences of the *GAL* gene; URS_{gal}, upstream repression sequences of the *GAL* gene.

*Present address: Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamadaoka, Suita-shi, Osaka, 565, Japan.

Conversion of MLY220B to *SNF1*$^+$ was achieved by transformation with a fragment containing the entire *SNF1* gene. *SNF1* transformants were selected based on their ability to grow on both sucrose and galactose. For β-galactosidase assays, cells were grown at 30°C in either rich (YEP) or defined (SD) medium (29) supplemented with 2% (wt/vol) glucose, and then diluted into medium containing 5% (vol/vol) glycerol, 2% (wt/vol) galactose, and/or 2% glucose, incubated for at least 12 hr and harvested at OD_{660} 0.4–0.8. β-Galactosidase assays were performed in triplicate as reported previously (30, 31). Standard deviations were <15% except where noted.

Plasmids. DNA manipulations were performed by standard methods (32). LR1Δ50 was constructed similarly to plasmids described previously (33) and lacks *GAL1* promoter sequence between −169 and −129 relative to the transcription start site. A derivative of LR1Δ50 that could be integrated into the chromosome (LR1Δ50Δ2μm) was generated by removing an *Eco*RI fragment containing 2-μm replicating sequences. pMA448 was described previously (4). We created derivatives of pMA448 in which *GAL4* was expressed from the heterologous *PPR1* or *HIS4* promoters as follows. pML283 (P_{PPR1}–*GAL4*) was constructed by replacing *GAL4* promoter and 5′ coding sequences in pMA448 with a 1.5-kilobase (kb) *Bam*HI–*Hin*dIII fragment containing *PPR1* promoter sequence (34, 35) up to base pair (bp) −25 (relative to the ATG start codon) and a *Hin*dIII–*Xho* I fragment containing *GAL4* sequence from bp −15 to +218. The *PPR1* promoter fragment was provided by Liam Keegan (University of Basel, Basel) and contains a *Hin*dIII restriction site introduced at bp −25 by site-directed mutagenesis. The *Hin*dIII–*Xho* I *GAL4* fragment was obtained from the plasmid P_{ADH}GAL4 (36). pML285 replaces *GAL4* sequence in pMA448 with a *Bam*HI–*Hin*dIII fragment containing the *HIS4* promoter sequence (37) to bp −48 and the *GAL4* *Hin*dIII–*Xho* I fragment described above. The *HIS4* promoter fragment consists of *HIS4* UAS from bp −216 to −171, joined to the *HIS4* initiation region from bp −134 to −48. A *Hin*pI site at bp −48 was joined to the *Hin*dIII site of the *GAL4* fragment via an 8-bp *Cla* I–*Hin*dIII linker fragment from pBR322. The *lacZ* fusions pML282/G4, pML282/P1, and pML282/H4 were constructed by replacing the *Bam*HI–*Xho* I fragment of the 2-μm replicating plasmid $P_{ADH}GAL4_{(1-74)}$ (36) with the *Bam*HI–*Xho* I fragments from pMA448, pML283, and pML285, respectively. pML255 was constructed by inserting the *Bam*HI–*Xho* I fragment of pML285 and a *Xho* I–*Sal* I fragment containing an activation domain encoded by *Escherichia coli* genomic DNA from the plasmid B3 (38) into the yeast vector YIP5. pG4ACL was constructed by inserting a *Bam*HI fragment containing the *GAL4* gene into the *Bam*HI site of the *ARS–CEN* plasmid A75p9 provided by Andrew Murray (University of California, San Francisco).

RESULTS

Role of *GAL4* Transcriptional Regulation. Table 1 shows that glucose repression of *GAL1* is not abolished when *GAL80* is mutated and sequences containing negative glucose repression elements in the *GAL1* promoter are eliminated. Mutation of *GAL80* resulted in the galactose-independent expression of *GAL1* but relieved glucose repression only severalfold. It had been reported that mutation of *GAL80* had no effect on glucose repression (17, 18). Removal of a 40-bp region between the UAS_{gal} and TATA box of *GAL1* (LR1Δ50) also alleviated glucose repression severalfold. We found that larger deletions within the *GAL1* promoter did not further alleviate repression (data not shown). We therefore used *gal80* yeast strains containing this internally deleted derivative of *GAL1–lacZ* (LR1Δ50) in order to investigate the possible role of *GAL4* transcriptional down-regulation in mediating glucose repression of *GAL1*.

Table 1. Effect of mutating *GAL80* and of deleting sequences in the *GAL1* promoter on *GAL1* glucose repression

GAL80	*GAL1* promoter	β-Galactosidase activity			
		Gal	Gal + Glc	Glc	Glycerol
+	pRY131	2400	15	0	0
+	LR1Δ50	2800	55	0	0
−	pRY131	3300	40	25	3600
−	LR1Δ50	3400	95	80	4060

β-Galactosidase activities of the full-length *GAL1* promoter fused to *lacZ* (pRY131; ref. 31) or of a derivative with the sequence between −169 and −129 (relative to the start site of *GAL1* transcription) deleted (LR1Δ50) were assayed in the yeast strains YM608 (*MATα, ura3-52, his3-200, ade2-101, lys2-801, trpl-901*) and YM704 (*MATα, gal80, ura3-52, his3-200, ade2-101, lys2-801, trpl-901, tyr*) following growth in SD medium supplemented with 2% galactose (Gal), 2% galactose and 2% glucose (Gal + Glc), 2% glucose (Glc), or 5% glycerol. Yeast strains were provided by Mark Johnston (Washington University School of Medicine, St. Louis).

Table 2 shows that in the above-mentioned strain the normal transcriptional regulation of *GAL4* is required to achieve full glucose repression of *GAL1*. We assayed the regulation of *GAL1* in *gal80* strains containing either the native *GAL4* gene or *GAL4* expressed from the heterologous *HIS4* or *PPR1* promoter. In addition we fused each *GAL4* expression construct to *lacZ* to compare its relative expression level. We observed (Table 2) that when *GAL4* was expressed from its own promoter, *GAL1* was repressed ≈75-fold in medium containing glucose. The *GAL4* promoter itself was down-regulated 3- to 5-fold in glucose medium, similar to estimates reported elsewhere (12, 21). In contrast, when *GAL4* was expressed from either the *HIS4* or the *PPR1* promoter, little or no repression of *GAL1* was observed. Neither heterologous promoter was repressed in glucose. The *HIS4* promoter was significantly stronger than *GAL4*, but the *PPR1* promoter was expressed at levels similar to those of *GAL4*. These results suggest that the down-regulation of the *GAL4* promoter is required for normal glucose repression of *GAL1* in a *gal80* strain. Similar results have been reported by another group (12).

Table 2. Effect of *GAL4* transcriptional regulation on *GAL1* glucose repression

Effector promoter	Activity of *GAL1* promoter			Activity of *GAL4* expression construct		
	Glycerol	Glucose	Ratio	Glycerol	Glucose	Ratio
P_{GAL4}	3000	40	75	3.4	0.9	3.8
P_{HIS4}	3200	2100	1.5	14	16	0.9
P_{PPR1}	2600	900	2.9	2.3	3.3	0.7

Activity of the integrated *GAL1* target (LR1Δ50) in MLY490C (*MATα, Δgal4-542, gal80-538, ura3-52, his3-200, ade2-101, lys2-801, trpl-901, leu2-3,112*, Met$^-$) also integrated with the native *GAL4* promoter (P_{GAL4}) or with the heterologous *HIS4* (P_{HIS4}) or *PPR1* (P_{PPR1}) promoter–*GAL4* fusions, carried on plasmids pMA448 (4), pML285, and pML283, respectively. *HIS4* encodes an enzyme required for the biosynthesis of histidine and is induced in response to amino acid starvation (39). *PPR1* encodes an activator required for the expression of uracil biosynthetic enzymes, and its regulation has not been studied (34, 35). Activities of the P_{GAL4}, P_{HIS4}, and P_{PPR1} promoters fused to *lacZ* were assayed in MLY490C transformed with the 2-μm replicating plasmids pML282/G4, pML282/H4, and pML282/P1, respectively. These constructions fuse the amino-terminal 74 amino acids of GAL4 to LacZ. Cells were grown in SD medium with 5% glycerol (GLY) or 2% glucose (GLU). Standard deviations were <40% for assays of *lacZ* fusions to the *GAL4* and *PPR1* promoters and <15% for all other assays.

Table 3. Effect of increasing *GAL4* gene dosage on *GAL1* regulation

No. of *GAL4* copies	Activity		Ratio
	Glycerol	Glucose	
One	3800	75	51
Two	3900	520	7.5
Three	3900	1300	3.0
One plus pG4ACL	3400	740	4.6
One plus pLPK8	3600	2800	1.3

The parental yeast strain MLY530 (*MATα, gal80-538, URA3*::*LR1Δ50Δ2μm, his3-200, ade2-101, trpl-901, leu2-3,112*) was integrated with one copy or two copies of *GAL4* (pMA448) or transformed with an *ARS–CEN* (pG4ACL) or 2-μm (pLPK8, ref. 36) vector carrying the *GAL4* gene. β-Galactosidase activity was assayed following growth in SD medium with 5% glycerol or 2% glucose.

The data in Table 3 further support the idea that the observed weak down-regulation of *GAL4* is essential for full glucose repression of *GAL1* in a *gal80* strain. When we increased the gene dosage of *GAL4* 2- and 3-fold, glucose repression of *GAL1* was substantially alleviated. Glucose repression was also alleviated when *GAL4* gene copy number was increased using a low-copy-number *ARS–CEN* vector, and was abolished when *GAL4* was expressed on a multicopy plasmid in a *gal80* strain, as reported previously (14). However, the transcriptional down-regulation of *GAL4* may not account entirely for the glucose repression of *GAL1* (LR1Δ50) in a *gal80* strain, since we observed that *GAL1* was weakly repressed in glucose when *GAL4* was expressed from either of the nonrepressed heterologous promoters (Table 2; also see *Discussion*).

A *GAL80*-Dependent Mechanism of Glucose Repression. Table 4 shows that there is a mechanism of glucose repression that requires *GAL80*. When we assayed regulation of *GAL1* in a *gal80* strain, glucose repression of *GAL1* was dependent upon the normal transcriptional regulation of *GAL4*, as observed previously (Table 2). However, when we assayed glucose repression in a *GAL80*$^+$ strain, *GAL1* was strongly repressed regardless of which promoter expressed *GAL4*. Thus, glucose repression of *GAL1* is mediated by two functionally redundant mechanisms, one dependent upon the normal regulation of *GAL4* and one dependent upon *GAL80*. Under the conditions typically used to assay *GAL* gene regulation (i.e., 2% galactose and/or 2% glucose), either mechanism is sufficient to effectively repress *GAL1*, and both must be eliminated to significantly alleviate glucose repression of *GAL1*.

Disrupting Two Mechanisms of Glucose Repression Alleviates the Effects of Mutating *SNF1*. Table 5 shows that deletion of *GAL80* and disruption of *GAL4* transcriptional regulation alleviates the defect in *GAL1* expression caused by mutation

Table 4. Effect of *GAL80* on glucose repression of *GAL1*

GAL4 effector construct	*GAL80* allele	Activity		Ratio
		Gal	Gal + Glc	
P_{GAL4}–*GAL4*	–	2040	45	45
P_{HIS4}–*GAL4*	–	2130	1650	1.3
P_{PPR1}–*GAL4*	–	1770	680	2.6
P_{GAL4}–*GAL4*	+	2145	25	86
P_{HIS4}–*GAL4*	+	1860	65	29
P_{PPR1}–*GAL4*	+	2095	50	42

The parental yeast strain MLY92Δ50 (*MATα, Δgal4-537, URA3*::*LR1Δ50Δ2μm, leu2-3,112, his3-200*) was integrated with either pMA448 (P_{GAL4}–*GAL4*), pML285 (P_{HIS4}–*GAL4*), or pML283 (P_{PPR1}–*GAL4*). Isogenic *gal80*$^-$ derivatives of these strains were created as described in *Materials and Methods*. β-Galactosidase activities were assayed following growth in YEP medium with 2% galactose (Gal) or 2% galactose and 2% glucose (Gal + Glc).

Table 5. Effect of *SNF1* on galactose-induced *GAL1* expression

GAL4 effector	*GAL80* allele	Activity	
		SNF1$^+$	*snf1*
P_{GAL4}–*GAL4*	+	605	5
	–	810	15
P_{PPR1}–*GAL4*	+	650	70
	–	1050	1110

The parental strain MLY220 (*MATα, Δgal4-542, URA3*::*LR1Δ50Δ2μm, his3-200, ade2-101, ade1, lys2-801, leu2-3,112, snf1-28*) was integrated with either pMA448 (P_{GAL4}–*GAL4*) or pML283 (P_{PPR1}–*GAL4*). Southern analysis revealed MLY220 that was integrated with one copy of pMA448 or three copies of pML283. Isogenic *gal80*$^-$ and *SNF1*$^+$ derivatives of these strains were created as described in *Materials and Methods*. Cells were grown first in YEP medium with 2% glucose (*snf1* strains cannot utilize galactose) and then reinoculated into YEP medium 2% galactose, incubated for 12 hr, and then assayed for β-galactosidase activity.

of *SNF1*. We found that in a strain containing the wild-type *GAL4* and *GAL80* alleles, mutation of *SNF1* caused a severe defect in the galactose-induced expression of *GAL1*, consistent with earlier reports (26, 40). This defect was not alleviated by mutating *GAL80* (11, 40) or by substituting the heterologous *PPR1* promoter for the native *GAL4* promoter but was completely alleviated by changing both the *GAL80* and *GAL4* alleles simultaneously. Thus the same two functionally redundant mechanisms that mediate the effects of glucose repression also mediate the effects of mutating *SNF1* on *GAL1* expression.

Contribution of *GAL1* Cis-Acting Sequences. Table 1 showed that deleting a 40-bp region between the UAS_{gal} and the TATA box in the *GAL1* promoter partially alleviated glucose repression in a strain containing a mutant allele of *GAL80* but the wild-type allele of *GAL4*. Table 6 summarizes experiments in which we tested the abilities of negative elements in the *GAL1* promoter to mediate glucose repression independent of other mechanisms of repression. We observed that when *GAL80* was mutated and *GAL4* was expressed from the heterologous *PPR1* or *HIS4* promoter, glucose had little or no effect on the expression of either the intact or the internally deleted derivative of *GAL1*. Thus, negative elements in the *GAL1* promoter are not sufficient to significantly repress *GAL1* expression in glucose medium in the absence of other mechanisms of glucose repression. Glucose repression of the intact *GAL1* promoter was partially restored when *GAL4* was replaced by a weakly activating

Table 6. Contribution of *GAL1* promoter sequences to glucose repression

GAL4 effector	β-Galactosidase activity			
	pRY131		LR1Δ50	
	Glycerol	Glucose	Glycerol	Glucose
P_{GAL4}–*GAL4*	3240	20	3670	85
P_{PPR1}–*GAL4*	2480	830	1860	950
P_{HIS4}–*GAL4*	3140	2900	3420	3160
P_{HIS4}–$GAL4_{1-147}$–*B3*	335	60	580	495

Activity of the intact *GAL1* promoter (pRY131; ref. 31) and a derivative with the sequence between −169 and −129 deleted (LR1Δ50) were transformed into derivatives of MLY490C (Table 2) integrated with the wild-type *GAL4* clone (P_{GAL4}–*GAL4*), *GAL4* expressed from the *HIS4* (P_{HIS4}–*GAL4*) or *PPR1* (P_{PPR1}–*GAL4*) promoter, or the weak activator B3 expressed from the *HIS4* promoter (P_{HIS4}–$GAL4_{1-147}$–*B3*). B3 consists of the binding domain (amino acids 1–147) of GAL4 and an activating domain encoded by *E. coli* genomic sequence (38). These effector constructs were carried on the plasmids pMA448, pML283, pML285, and pML255, respectively. β-Galactosidase activities were assayed following growth in SD medium with 5% glycerol or 2% glucose.

derivative of GAL4 (B3) expressed from the *HIS4* promoter. Therefore negative elements in this 40-bp region of *GAL1* appear to be capable of repressing low levels, but not high levels, of *GAL1* expression.

DISCUSSION

The results show that glucose repression of *GAL1* is mediated through at least three different mechanisms. One effect of glucose is to down-regulate *GAL4* expression 3- to 5-fold, and in a *gal80* strain this relatively small change in *GAL4* expression can lead to a very large reduction in the glucose-repressed levels of *GAL1* expression. Similar results have been reported elsewhere (12). It has also been reported that a 2-fold increase in the gene dosage of *LAC9*, a homolog of *GAL4* from *Kluyveromyces lactis*, relieves glucose repression of its target genes (41). This sensitive response of *GAL1* to small changes in *GAL4* expression may reflect the cooperative binding of GAL4 to multiple weak binding sites in the UAS_{gal} (42) and/or possibly the selective inhibition of GAL4 activity at lower expression levels due to protein or mRNA degradation, dissociation of GAL4 monomers, interaction of GAL4 with a negative factor(s), or some other process. We do not favor the idea that cooperative binding of GAL4 is responsible for amplifying the effect of *GAL4* transcriptional down-regulation; some promoters containing a single synthetic strong GAL4 binding site, to which GAL4 cannot bind cooperatively, are also strongly repressed in glucose in a *GAL4, gal80* strain (M.S.L., unpublished observations). It is possible that the strong repression of *GAL1* observed in a *gal80* strain is the result both of the reduction of *GAL4* transcriptional levels and of another, posttranscriptional mechanism of glucose repression that is effective primarily at lower GAL4 concentrations.

Our findings may reconcile earlier reports that on one hand had suggested a role for *GAL80* in mediating glucose repression (9, 10, 14–16) and on the other hand had shown that deleting *GAL80* had little effect on glucose repression (17, 18). We observe that mutating *GAL80* affects glucose repression only in a strain in which the transcriptional regulation of *GAL4* is also defective. This result is analogous to those obtained by Matsumoto *et al.* (9, 10) and Nehlin *et al.* (13). Matsumoto *et al.* (9, 10) observed that mutating *GAL80* had an effect on the glucose repression of *GAL1* only in a strain that contained any of several other unlinked glucose repression mutants—*reg1, gal82*, or *gal83*. These latter mutations have recently been shown to alleviate the transcriptional down-regulation of *GAL4* in glucose (12). Nehlin *et al.* (13) have shown that the transcriptional repressor MIG1 binds to and weakly represses both the *GAL4* and *GAL1* promoters. Mutation of *MIG1* by itself had little effect on the glucose repression of *GAL1*, but mutation of *MIG1* and *GAL80* together resulted in the virtual elimination of the glucose repression of *GAL1*.

We do not know exactly what role GAL80 may play in mediating glucose repression. Possibly glucose could inhibit the process by which GAL80 repression of GAL4 activity is relieved in galactose. It is known that glucose inhibits the activity of the galactose permease (43, 44), transcription of the gene encoding this permease, *GAL2* (45), and transcription of a gene required for the rapid induction of the *GAL* genes, *GAL3* (46). However, as has been noted previously (18), the glucose-induced decrease in galactose permease activity would not be sufficient to significantly inhibit the uptake of 2% galactose, the concentration typically used in yeast media. Furthermore, the *GAL2* and *GAL3* genes are themselves regulated by GAL4 and GAL80 and it is likely that their repression in glucose is a consequence, rather than a cause, of the *GAL80*-dependent effect of glucose. Possibly growth in glucose results in the inhibition of the activity of some other component of the galactose induction pathway or renders *GAL4* and/or *GAL80* insensitive to the galactose induction signal.

Mutation of *SNF1* causes a severe defect in *GAL1* expression in strains in which either *GAL4* transcriptional regulation or the *GAL80* gene are intact but has no effect on *GAL1* expression when both are disrupted. Thus, mutation of *SNF1* affects *GAL1* expression through the same functionally redundant mechanisms that mediate glucose repression, consistent with the idea that mutation of *SNF1* causes the constitutive glucose repression of yeast genes. Mutation of *SNF1* causes a reduction in *GAL4* transcriptional levels (12).

A third mechanism of glucose repression requires negative elements located in the *GAL1* promoter (11, 19). We show that sequences in a 40-bp region between the UAS_{gal} and the TATA box contribute 2- to 4-fold to the glucose repression of *GAL1* in yeast strains in which at least one other mechanism of glucose repression is present. Deletion of this 40-bp region removes a negative element designated URS_A (11) or O_6 (19), which has recently been shown to bind MIG1 (13), but leaves several other negative elements, URS_B, URS_C (11), and O_5 (19), intact. However, we did not observe any additional relief from glucose repression when these latter sites were deleted in addition to URS_A/O_6 (data not shown). *GAL1* negative elements are apparently effective in repressing *GAL1* only when its promoter activity is weak—e.g., when expression has been partially repressed by one of the other mechanisms of glucose repression, when a weaker derivative of *GAL4* is used (Table 6), or when the UAS_{gal} are replaced by weak heterologous UAS, such as *LEU2* (11). These results suggest that the first two mechanisms of glucose repression, the *GAL4*- and *GAL80*-dependent mechanisms, are primarily responsible for repressing *GAL1* expression in glucose medium and that negative elements located primarily in a 40-bp region between the UAS_{gal} and the TATA box act to supplement repression 2- to 4-fold.

We thank Mark Johnston and David Griggs for communication of results prior to publication and for yeast strains, H. Himmelfarb for critical reading of manuscript, members of the Ptashne laboratory for valuable advice, and Dr. T. Taniguchi of Osaka University for supporting the final stages of this research. This work was initially supported by a grant from the National Institutes of Health and subsequently by a grant from the Human Frontier Sciences Program Organization to M.S.L.

1. Johnston, M. (1987) *Microbiol. Rev.* **51,** 458–476.
2. Bram, R. J. & Kornberg, R. D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 43–47.
3. Giniger, E., Varnum, S. & Ptashne, M. (1985) *Cell* **40,** 767–774.
4. Ma, J. & Ptashne, M. (1987) *Cell* **50,** 137–142.
5. Ma, J. & Ptashne, M. (1988) *Cell* **55,** 443–446.
6. Johnston, S. A., Salmeron, J. M. & Dincher, S. S. (1987) *Cell* **50,** 143–146.
7. Lue, N. F., Chasman, D. I., Buchman, A. R. & Kornberg, R. D. (1987) *Mol. Cell. Biol.* **4,** 260–267.
8. Salmeron, J. M., Leuther, K. & Johnston, S. A. (1990) *Genetics* **125,** 21–27.
9. Matsumoto, K., Toh-e, A. & Oshima, Y. (1981) *Mol. Cell. Biol.* **1,** 83–93.
10. Matsumoto, K., Yoshimatsu, T. & Oshima, Y. (1983) *J. Bacteriol.* **153,** 1405–1414.
11. Flick, J. & Johnston, M. (1990) *Mol. Cell. Biol.* **10,** 4757–4769.
12. Griggs, D. & Johnston, M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8597–8601.
13. Nehlin, J., Carlberg, M. & Ronne, H. (1991) *EMBO J.* **10,** 3373–3377.
14. Johnston, S. A. & Hopper, J. E. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6971–6975.
15. Guarente, L., Yocum, R. R. & Gifford, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7410–7414.
16. Nogi, Y., Shimada, H., Matsuzaki, Y., Hashimoto, H. & Fukasawa, T. (1984) *Mol. Gen. Genet.* **195,** 29–34.

17. Torchia, T. E., Hamilton, R., Cano, C. & Hopper, J. (1984) *Mol. Cell. Biol.* **4,** 1521–1527.
18. Yocum, R. R. & Johnston, M. (1984) *Gene* **32,** 75–82.
19. Finley, R. L., Chen, S., Ma, J., Byrne, P. & West, R. W. (1990) *Mol. Cell. Biol.* **10,** 5663–5670.
20. Selleck, S. B. & Majors, J. M. (1987) *Mol. Cell. Biol.* **7,** 3260–3267.
21. Laughon, A. & Gesteland, R. F. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6827–6831.
22. Mylin, L. M., Bhat, J. P. & Hopper, J. E. (1989) *Genes Dev.* **3,** 1157–1165.
23. Mylin, L. M., Johnston, M. & Hopper, J. E. (1990) *Mol. Cell. Biol.* **10,** 4623–4629.
24. Long, R., Mylin, L. & Hopper, J. (1991) *Mol. Cell. Biol.* **11,** 2311–2314.
25. Sadowski, I., Niedbala, D., Wood, K. & Ptashne, M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10510–10514.
26. Carlson, M., Osmond, B. C. & Botstein, D. (1981) *Genetics* **98,** 25–40.
27. Celenza, J. & Carlson, M. (1986) *Science* **233,** 1175–1180.
28. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
29. Sherman, F., Fink, G. & Lawrence, C. (1983) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
30. Miller, J. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
31. Yocum, R., Hanley, S., West, R. & Ptashne, M. (1984) *Mol. Cell. Biol.* **4,** 1985–1998.
32. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
33. West, R., Yocum, R. & Ptashne, M. (1984) *Mol. Cell. Biol.* **4,** 2467–2478.
34. Kammerer, B., Guyonvarch, A. & Hubert, J. (1984) *J. Mol. Biol.* **180,** 239–250.
35. Losson, R., Fuchs, R. & Lacroute, F. (1985) *J. Mol. Biol.* **185,** 65–81.
36. Silver, P., Keegan, L. & Ptashne, M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5951–5955.
37. Donahue, T., Farabaugh, P. & Fink, G. (1982) *Gene* **18,** 47–59.
38. Ma, J. & Ptashne, M. (1987) *Cell* **51,** 113–119.
39. Jones, E. & Fink, G. (1983) in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*, eds. Strathern, J., Jones, E. & Broach, J. R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 181–299.
40. Schultz, J. & Carlson, M. (1987) *Mol. Cell. Biol.* **7,** 3637–3643.
41. Kuger, P., Godecke, A. & Breunig, K. (1990) *Nucleic Acids Res.* **18,** 745–751.
42. Giniger, E. & Ptashne, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 382–386.
43. Holzer, H. (1976) *Trends Biochem. Sci.* **1,** 178–181.
44. Matern, H. & Holzer, H. (1977) *J. Biol. Chem.* **252,** 6399–6402.
45. Tschopp, J., Emr, D., Field, C. & Schekman, R. (1986) *J. Bacteriol.* **166,** 313–318.
46. Bajwa, W., Torchia, T. & Hopper, J. (1988) *Mol. Cell. Biol.* **8,** 3439–3447.