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Two Systems of Glucose Repression of the *GAL1* Promoter in *Saccharomyces cerevisiae*

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Expression of the *GAL1* gene in *Saccharomyces cerevisiae* is strongly repressed by growth on glucose. We show that two sites within the *GAL1* promoter mediate glucose repression. First, glucose inhibits transcription activation by GAL4 protein through UAS_G. Second, a promoter element, termed URS_G, confers glucose repression independently of GAL4. We have localized the URS_G sequences responsible for glucose repression to an 87-base-pair fragment located between UAS_G and the TATA box. Promoters deleted for small (20-base-pair) segments that span this sequence are still subject to glucose repression, suggesting that there are multiple sequences within this region that confer repression. Extended deletions across this region confirm that it contains at least two and possibly three URS_G elements. To identify the gene products that confer repression upon UAS_G and URS_G, we have analyzed glucose repression mutants and found that the *GAL83*, *REG1*, *GRR1*, and *SSN6* genes are required for repression mediated by both UAS_G and URS_G. In contrast, *GAL82* and *HXK2* are required only for UAS_G repression. A mutation designated *urr1-1* (URS_G repression resistant) was identified that specifically relieves URS_G repression without affecting UAS_G repression. In addition, we observed that the *SNF1*-encoded protein kinase is essential for derepression of both UAS_G and URS_G. We propose that repression of UAS_G and URS_G is mediated by two independent pathways that respond to a common signal generated by growth on glucose.

Expression of the *GAL* genes in *Saccharomyces cerevisiae*, required for metabolism of the sugar galactose, is stringently regulated by the carbon source (for a recent review, see reference 22). Growth on galactose results in a 1,000-fold induction of *GAL* gene transcription that is mediated by the products of two regulatory genes, *GAL4* and *GAL80*. In yeasts growing without galactose, the GAL4 protein binds to DNA upstream of each *GAL* gene but is prevented from activating transcription by interaction with the GAL80 protein. The addition of galactose relieves GAL80-mediated inhibition of GAL4, thus allowing GAL4 to activate transcription.

Superimposed upon this well-characterized induction mechanism of *GAL* gene expression is the global regulatory circuit of carbon catabolite repression, which represses expression of many genes involved in carbohydrate metabolism and respiration during growth on glucose (1, 9, 15, 17, 22). This metabolic regulation allows yeasts growing in the presence of multiple carbon sources to utilize selectively the preferred carbon source, glucose, prior to the remaining sugars.

The mechanism(s) by which growth on glucose causes repression of the *GAL* genes and other genes subject to glucose repression is unknown. Genetic analysis of glucose repression has identified several genes involved in this regulatory system. Mutations in *HXK2*, which encodes hexokinase isozyme PII that catalyzes phosphorylation of glucose, the first step in glycolysis, result in a loss of glucose repression of the *GAL*, *SUC*, and *MAL* genes (14, 27, 52). Mutations in two other genes, *REG1* and *GRR1*, result in a similar pleiotropic loss of glucose repression (3, 32, 35). In contrast, mutations in *GAL82* and *GAL83* were reported to relieve repression of only the *GAL* genes (31, 32).

Derepression of glucose-repressed genes is also under coordinate regulation. Six *SNF* genes are required for the derepression of genes subject to glucose repression (10, 34). The best characterized of these, *SNF1*, encodes a protein kinase (12). Mutations in the *SSN6* gene suppress *snf1* mutations and also result in a loss of glucose repression of the *SUC2*, *GAL*, and *MAL* genes (11, 38). The pleiotropic loss of repression caused by mutations in some of these regulatory genes suggests that a common mechanism of glucose repression is shared by the different gene systems.

We are interested in understanding the mechanism of glucose repression of the *GAL* genes and its relation to repression acting on other genes. Here, we present evidence that there are at least two independent pathways of glucose repression of *GAL1* gene expression: one inhibits the ability of GAL4 protein to activate transcription; the other represses transcription through an upstream repression sequence (URS_G) located between the GAL4-binding sites and the TATA box. In addition, we have determined which regulatory genes are required for these two pathways of repression.

MATERIALS AND METHODS

Yeast strains. The yeast strains used in this study are listed in Table 1. Strain construction followed standard methods for genetic crosses, sporulation, and tetrad dissection (42). Segregation of the glucose repression mutations was followed by an assay of *lacZ* activity in yeasts containing pRY181, a *GAL1-lacZ* fusion gene, as described by Yocum et al. (50). The *gal82* and *gal83* mutations were introduced into our S288C background by four and two serial backcrosses, respectively. The *reg1-1966* null mutation was generated by replacement of the entire coding region of *REG1* with a fragment containing the *LEU2* gene (K.-D. Entain, personal communication, and our unpublished results). The *grr1-1829* null mutation was generated by the replacement of amino acids 601 to 1151 of the *GRR1* coding region with a fragment containing the *LEU2* gene (unpublished results).

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TABLE 1. Yeast strains

Strain	Genotype[a]	Source (reference)
YM2061	*MATa lys2-801 met*	This laboratory
YM2220	*MATa lys2-801*::pBM1323 *met Δgal80-534*	This laboratory (51)
YM2475	*MATa lys2-801*::pBM1436 *met*	This laboratory
YM2526	*MATα lys2-801*::pBM1395 *tyr1-501*	This laboratory
YM2416	*MATα lys2-801*::pBM1395 *Δsnf1-3*	MCY1551 (12)
YM2422	*MATα lys2-801*::pBM1323 *Δgal80-534 Δsnf1-3*	MCY1551 (12)
YM2865	*MATa lys2-801*::pBM1499 *met*	This laboratory
YM2866	*MATα lys2-801*::pBM1499 *tyr1-501 Δgal80-534*	This laboratory
YM3117	*MATa lys2-801*::pBM1436 *gal82*	YG4-8D (32)
YM3120	*MATa lys2-801*::pBM1499 *Δgal80-534 gal82*	YG4-8D (32)
YM3121	*MATα lys2-801*::pBM1436 *Δgal80-534 gal83*	CR11-1B (31)
YM3122	*MATα lys2-801*::pBM1499 *Δgal80-534 gal83*	CR11-1B (31)
YM3206	*MATα lys2-801*::pBM1436 *met tyr1-501 Δreg1-1966*	This laboratory
YM3205	*MATa lys2-801*::pBM1499 *met Δgal80-534 Δreg1-1966*	This laboratory
YM3127	*MATa lys2-801*::pBM1436 *met Δgrr1-1829*	This laboratory
YM3128	*MATa lys2-801*::pBM1499 *met Δgal80-534 Δgrr1-1829*	This laboratory
YM3131	*MATa lys2-801*::pBM1436 *Δhxk2-202*	DBY2236 (27)
YM3134	*MATα lys2-801*::pBM1499 *Δgal80-534 Δhxk2-202*	DBY2236 (27)
YM2964	*MATa lys2-801*::pBM1436 *met Δssn6-6*	pJS22 (38)
YM2961	*MATa lys2-801*::pBM1499 *tyr1-501 Δgal80-534 Δssn6-6*	pJS22 (38)
YM2809	*MATα lys2-801*::pBM1436 *tyr1-501 urr1-1*	This laboratory
YM3209	*MATα lys2-801*::pBM1499 *met Δgal80-534 urr1-1*	This laboratory

[a] All strains also carry *ura3-52 Δhis3-200 ade2-101 LEU2*::pRY181(*GAL1/lacZ*)

The *ssn6-6* null mutation was constructed by disruption of the *SSN6* gene with *URA3* by using a restriction fragment from plasmid pJS22 (38). Strains containing the *hxk2-202* and *snf1-3* null deletion mutations in the S288C background were crossed with our wild-type strains to introduce the appropriate genetic markers.

Growth conditions. Yeast cells were grown for RNA preparation on SD medium containing 0.67% yeast nitrogen base without amino acids; 0.5% $(NH_4)_2SO_4$; 20 μg each of adenine, uracil, tryptophan, histidine, and methionine per ml; 30 μg each of tyrosine and lysine per ml; 5% glycerol; and 0.1% glucose (0.1% glucose was insufficient to induce glucose repression [data not shown]). Where indicated, 2% galactose, 2% glucose, or 2% galactose plus 2% glucose was added. The media were inoculated with 5×10^5 to 5×10^6 cells, which were then grown at 30°C to an optical density at 600 nm of 0.8 to 1.2 (12 to 18 h). Yeasts for the experiments analyzing *SNF1* function (see Fig. 8 and 9) were grown to early log phase (optical density at 600 nm, 1.0) in medium containing 2% glucose, harvested, and suspended in prewarmed medium containing 0.1% glucose. Incubation continued at 30°C with shaking, and cultures were maintained at an optical density at 600 nm of <2.0 by dilution with the same medium. Samples were removed at the times indicated for RNA preparation.

Plasmids. Standard procedures for the manipulation of plasmid DNA and transformation into bacteria were followed (28). Plasmid pBM1323 (Fig. 1A) contains the 143-base-pair (bp) *Rsa*I (position −393)-to-*Alu*I (position −250) *GAL1* fragment from pBM261 (23) to which *Eco*RI linkers were added (nucleotide +1 of the *GAL1* sequence is the major transcription start site). This fragment, containing the four GAL4 protein-binding sites (7, 19, 40), was joined to an *Eco*RI-*Bam*HI fragment of *HIS3* (extending from positions −94 to +1318) from YIp55-Sc3386 (provided by K. Struhl) that contains the *HIS3* TATA box and coding region (43). To the *Bam*HI site was joined the 2,162-bp *Bam*HI-*Sma*I fragment from YCp50 containing the *URA3* gene (37). The *Bam*HI site at this junction was destroyed by treatment with *Bam*HI, Klenow enzyme, and deoxyribonucleotides and religation. The *Sma*I site was joined to the 2,220-bp *Pvu*II-*Aat*II fragment from YIp5 that contains the bacterial origin of replication and ampicillin resistance gene (46). The *Aat*II site was rendered blunt by treatment with mung bean nuclease and joined to the *Eco*RV site of a 1.5-kilobase (kb) *Eco*RV-*Cla*I fragment from YIp333 that contains the transcription termination site from the 3′ end of the *LYS2* gene (Fig. 1, filled bricks) (16). The *Cla*I site of the *LYS2* fragment was joined to the 5′ *Eco*RI site of the *GAL1* fragment with a *Bam*HI linker, which destroyed the *Cla*I site but maintained the *Eco*RI site. Plasmid pBM1395 contains the same 6.5-kb *Bam*HI-*Kpn*I vector fragment as pBM1323, but UAS_G was replaced by a 280-bp *Hin*cII fragment of YEp13 modified with *Bam*HI linkers that contains the UAS of the *LEU2* promoter (UAS_L, positions −125 to −405 from ATG of *LEU2*) (2, 29). The UAS_L was joined to a 0.92-kb *Eco*RI-*Kpn*I fragment from plasmid pBM1370 (described below) which contains the *GAL1* promoter sequences (lacking GAL4 protein-binding sites) from positions −214 to +50 fused to the *HIS3* sequences from positions +19 to +642.

Plasmids pBM1499 and pBM1436 were derived from pBM1323 and pBM1395, respectively, by the insertion of a 1.2-kb *Bgl*II-*Hpa*I fragment (treated with Klenow enzyme and deoxyribonucleotides) from YIp333 into their *Nru*I sites. This fragment contains the sequences spanning approximately 1 to 2.2 kb downstream from the 3′ end of the *LYS2* gene (Fig. 1, open bricks) (5).

Plasmid pBM1527 (see Fig. 3B) was derived from pBM1436 and contained *GAL1* sequences from positions −214 to −97 joined to a fragment of the *HIS3* gene from positions −52 to +1318 (Sc3305, provided by K. Struhl) with a *Bam*HI linker. Plasmid pBM1535 (see Fig. 3C) was derived from pBM1436 by deletion of *GAL1* sequences from positions −214 to −100. The *GAL1* sequences remaining in pBM1535 were from positions −100 to +50.

Deletions and mutagenesis. Plasmid p10GH (26) contains a fusion of the *GAL1* promoter (positions −214 to +56) to *HIS3* coding sequences (positions −8 to +1318). A *Sac*I restriction site was introduced at the junction of *GAL1* and *HIS3* sequences by site-directed mutagenesis. Briefly, the

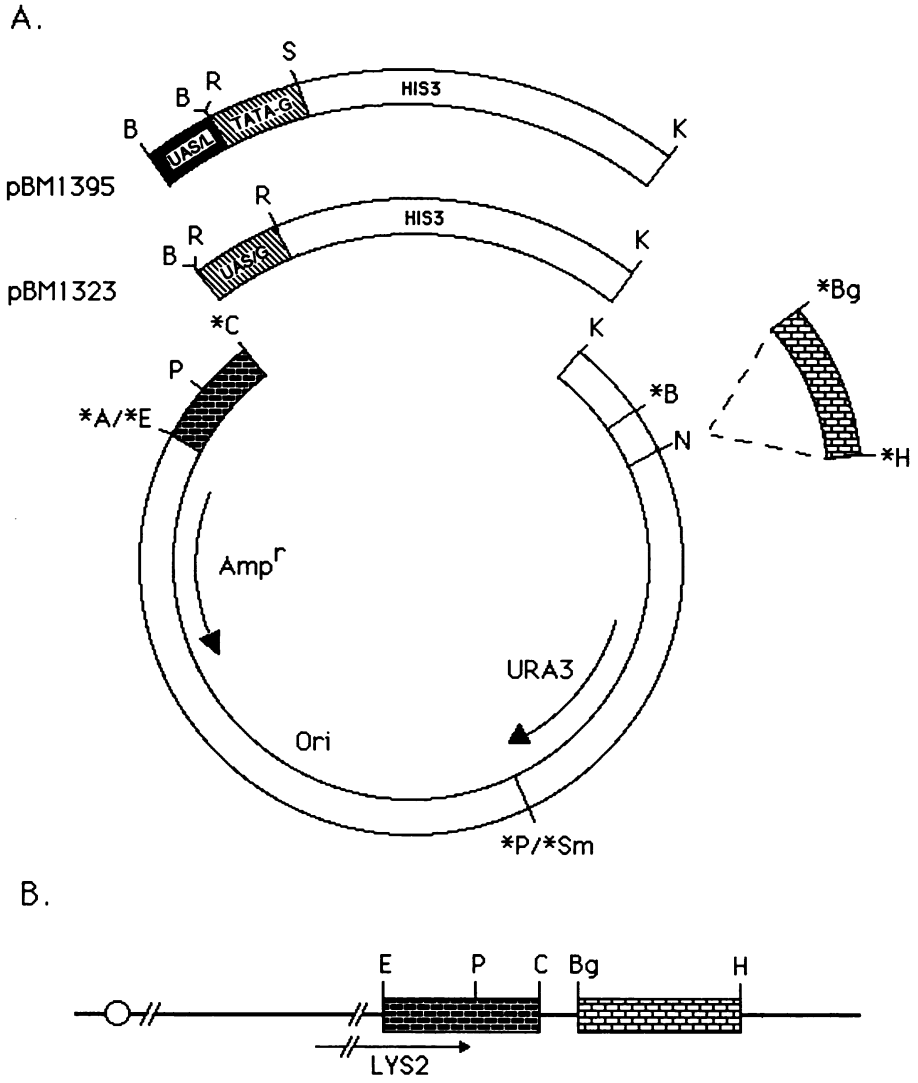

FIG. 1. (A) Restriction site map of pBM1395 and pBM1323 plasmids from which all plasmids were derived. The *LEU2* UAS is identified by the shaded segment, and *GAL* sequences have diagonal stripes. pBM1436 and pBM1499 were derived from pBM1395 and pBM1323, respectively, by insertion into the *Nru*I site of a *Bgl*II-*Hpa*I fragment (open bricks) as diagrammed. (B) Schematic diagram of yeast chromosome 2 showing the sites of integration of all plasmids used in this study. The restriction fragment containing the *LYS2* termination signals and the *Pvu*II site used to direct integration is shown as filled bricks. The downstream restriction fragment providing a site for homologous recombination used in the second genetic selection for loss of vector sequences (see Materials and Methods) is shown as open bricks. pBM1395 is 7.7 kb in size, and pBM1323 is 7.4 kb. Restriction sites: A, *Aat*II; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RV; H, *Hpa*I; K, *Kpn*I; N, *Nru*I; P, *Pvu*II; R, *Eco*RI; S, *Sac*I; Sm, *Sma*I. *, Sites destroyed during construction of the plasmid.

0.92-kb *Eco*RI-*Kpn*I fragment of plasmid p10GH was cloned between the *Eco*RI and *Kpn*I sites of M13mp18 and hybridized to an oligonucleotide (5′ GTCATCTTTGAGCTCTCCT TGAC 3′) that is complementary to sequences at the *GAL1-HIS3* junction and included a *Sac*I site. The desired mutant, pBM1370, was recovered as described by Kunkel (24). The internal deletions of the *GAL1* promoter shown below (see Fig. 4) were created by site-directed mutagenesis of single-stranded pBM1370 DNA by using the following oligonucleotides: pBM1632, 5′ TTGATTCGTTTCTCTAGACTCAG GAATTCC 3′; pBM1633, 5′ TTATCATCCTTCTCTAGA TCTTCATTTGAA 3′; pBM1634, 5′ TAAGGCTAAATC TCTAGACTCTATGGTTGT 3′; pBM1635, 5′ CTGAT TAATTCTTCTAGACTAAACTAATCG 3′; pBM1636, 5′ GATCAAAAATCTTCTAGATCACCCCAGAAA 3′; and pBM1637, 5′ CATTTATATACTTCTAGACTCATCGCT TCG 3′. Each oligonucleotide looped out approximately 20 bp of the *GAL1* DNA sequence by annealing with 10 bp of complementary sequences at both the 5′ and 3′ ends of the oligonucleotide (the exact nucleotides deleted from the promoter are given below [see Fig. 4]). The mismatched oligonucleotide sequence inserted 10 bp (A/G A/G TCTAGA A/G A/G) containing an *Xba*I restriction site. After isolation and characterization of the mutant bacteriophage, the 0.25-kb *Eco*RI-*Sac*I fragment then replaced the corresponding fragment in pBM1436. The extended deletions shown below (see Fig. 5) were constructed from the internal deletions (see Fig. 4) by joining the *Eco*RI-*Xba*I fragment from a more-5′ deletion with the *Xba*I-*Sac*I fragment from a more-3′ deletion. The resulting *Eco*RI-*Sac*I fragment was then subcloned

into pBM1436, replacing the parental fragment. The DNA sequence in each promoter deletion mutant was confirmed to be correct.

Mutant isolation. Our criterion for mutants defective specifically in glucose repression acting upon the *GAL1* URS (see Results) was that they should retain repression acting to inhibit GAL4 activation via the UAS_G. As a result of glucose repression of *HIS3* expression acting through the URS_G in pBM1395 (Fig. 1A), a *his3-200* strain containing this plasmid formed tiny colonies (0.2-mm diameter after 2 days of incubation at 30°C) on SD plates lacking histidine and containing 10 mM 3-amino-1,2,4-triazole (an inhibitor of the *HIS3*-encoded enzyme) and >0.5% glucose. Large colonies formed on these plates if they contained <0.1% glucose. To isolate mutants defective in repression, approximately 10^4 cells from separate cultures were plated on the selective media described above and mutagenized by placing 2 μl of ethyl methanesulfonate near the edge of the plate. After incubation for 2 days at 30°C, a single large (>1 mm) His^+ colony from each plate was purified and retested for rapid growth on the selective media. Of 47 isolates, 29 selected for further characterization were also defective in glucose repression that acts via the GAL4 protein, as determined by assaying *GAL1* expression with a *GAL1-lacZ* fusion plasmid. Our preliminary analysis identified both a *reg1* mutant and a *grr1* mutant among this group. Of the remaining 18 isolates, 8 were specifically defective in glucose repression mediated by the URS_G, as determined by Northern (RNA) blot analysis of pBM1436 expression. Further genetic analysis of one such mutant, designated *urr1-1* (URS repression resistant), showed that it was not allelic to *GAL82*, *GAL83*, *REG1*, *GRR1*, *HXK2*, or *SSN6* mutations (unpublished data).

Plasmid integration and copy number determination. Yeasts treated with lithium acetate (21) were transformed to $URA3^+$ with plasmid DNA linearized with the *Pvu*II enzyme. Cleavage at the *Pvu*II site of all plasmids used in this study directs integration at a site near the 3′ end of the *LYS2* gene (Fig. 1B, filled bricks) (5, 16). This arrangement placed the hybrid promoter on the plasmid approximately 300 bp downstream of the *LYS2* transcription termination signal. Yeasts transformed with plasmid pBM1323 or pBM1395 (Fig. 1A) were analyzed by Southern blotting to identify transformants containing a single integrated copy of the plasmid. To eliminate the need to analyze all transformants by Southern blotting, the vector backbone of plasmids pBM1323 and pBM1395 was modified by insertion of a DNA fragment containing sequences just downstream of the genomic site of integration (Fig. 1, open bricks) to create pBM1499 and pBM1436, respectively. This modification allowed the use of a genetic selection to resolve tandem duplications of the integrated plasmids. Integration of pBM1436 or pBM1499 created two small duplications of the chromosome that included (i) the fragment used for integration (Fig. 1, filled bricks) and (ii) the fragment adjacent to the site of integration (Fig. 1, open bricks). These URA^+ transformants were then plated on medium containing 5-fluoroorotic acid to select for loss of the *URA3* gene by homologous recombination between the duplicated sequences (6). Recombination between the duplicated sequences at the site of integration (Fig. 1, filled bricks) resulted in the loss of all plasmid sequences; recombination between the second duplication of sequences 3′ to the site of integration (Fig. 1, open bricks) resulted in a loss of all copies of the *URA3* gene and vector sequences but caused a single copy of the *HIS3* gene construction to be retained. These two recombinational events could be easily distinguished by testing 5-fluoroorotic acid-resistant colonies for the His^+ phenotype. Southern blot analysis of several recombinants verified that they contained the expected structure (data not shown).

RNA isolation and Northern blots. Total RNA was isolated from yeasts as described by Elder et al. (13). For Northern blot analysis, 20 μg of total RNA in water was dried and suspended in 25 μl of 10 mM Na_2HPO_4-NaH_2PO_4 (pH 6.5) containing 1 M glyoxal and 50% dimethyl sulfoxide and incubated at 50°C for 1 h (47). After the addition of 1/10 of a volume of loading buffer (10 mM Na_2HPO_4-NaH_2PO_4 [pH 6.5], 0.2% [each] bromphenol blue and xylene cynol, 50% glycerol), the samples were loaded on a 15-cm 1.2% agarose gel in 10 mM Na_2HPO_4-NaH_2PO_4 (pH 6.5) and subjected to electrophoresis at 22 to 50 V for approximately 350 V · h. The gel was then washed in 50 mM NaOH for 20 min, neutralized in 250 mM Na_2HPO_4-NaH_2PO_4 (pH 6.5) for 30 min, and finally equilibrated in 10 mM Na_2HPO_4-NaH_2PO_4 (pH 6.5) for 45 min. RNA was transferred to a GeneScreen membrane (Du Pont Co.) by capillary diffusion in 25 mM Na_2HPO_4-NaH_2PO_4 (pH 6.5) for 8 to 18 h and cross-linked to the membrane by using a UV Stratagene 2400 apparatus as recommended by the manufacturer. Hybridization was carried out in a solution containing 50% formamide, 5× SSPE (1× SSPE is 150 mM NaCl, 10 mM Na_2HPO_4-NaH_2PO_4 [pH 7.4], and 1 mM EDTA), 0.2% sodium dodecyl sulfate, and 100 μg of salmon sperm DNA per ml. Approximately 10^7 cpm of riboprobe (see below) was added to each hybridization and incubated for 12 to 18 h at 58°C. Membranes were washed three times at 65°C in 0.1× SSPE plus 0.2% sodium dodecyl sulfate for 10 min, once in 50% formamide–1× SSPE plus 0.2% sodium dodecyl sulfate for 30 min at 65°C, and finally in 0.1× SSPE for 10 min at room temperature and exposed to film for 1 to 8 h.

Riboprobes. ^{32}P-labeled antisense RNA probes (riboprobes) for *HIS3* and *LEU2* RNA were synthesized with T7 RNA polymerase by an in vitro runoff transcription reaction essentially as described by Selleck and Majors (39), except that [α-^{32}P]CTP (800 Ci/mmol [Amersham]) was substituted for radioactive UTP. The template for the *HIS3* probe was pBM1034, which contains a fragment of *HIS3* from positions −8 to +642 modified with *Bam*HI linkers inserted into the pT7-4 riboprobe vector (obtained from S. Tabor). The template for the *LEU2* probe was pBM1117, which contains a 0.5-kb *Eco*RI-*Cla*I fragment from the *LEU2* gene in YEp13 inserted in the pT7-3 vector (S. Tabor). Template pBM1034 was digested with *Bgl*II prior to RNA synthesis, which produced a runoff transcript complementary to positions +419 to +642 of the *HIS3* RNA. pBM1117 was digested with *Cla*I to produce a runoff transcript complementary to positions +162 to +648 of the *LEU2* RNA.

RESULTS

We considered two possible mechanisms for glucose repression of *GAL1* gene expression: (i) inhibition of the ability of GAL4 protein to activate transcription via UAS_G and (ii) direct repressor binding within the *GAL1* promoter, which would block transcription activation. To distinguish between these mechanisms, two hybrid *GAL1* promoters were constructed: one whose only *GAL1* sequences were the GAL4 protein-binding sites (UAS_G), and a second promoter containing a different UAS whose only *GAL1* sequences came from the region between UAS_G and the translation initiation site.

Transcription activation by UAS_G is repressed by glucose. To assess glucose repression acting through the GAL4

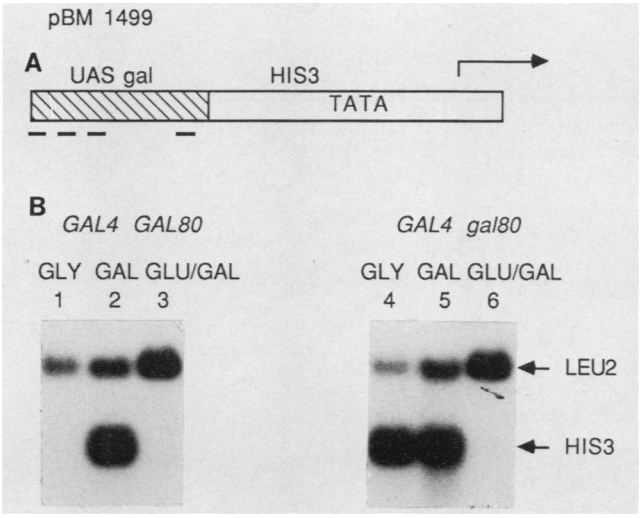

FIG. 2. (A) Structure of the UAS_G-*HIS3* fusion promoter contained in plasmid pBM1499. The underlined areas represent the locations of the binding sites of the GAL4 protein. The UAS_G (positions −393 to −250) is joined to the *HIS3* promoter at position −94 from the start site of transcription. (B) *HIS3* expression from the hybrid promoter in pBM1499. Total RNA was prepared from a *GAL80* strain (YM2865, lanes 1 to 3) or from a *gal80* strain (YM2866, lanes 4 to 6) grown on the carbon sources indicated and probed for *HIS3* and *LEU2* RNA as described in Materials and Methods. Carbon sources: GLY, 5% glycerol plus 0.1% glucose; GAL, 2% galactose; GLU/GAL, 2% glucose plus 2% galactose.

protein, a hybrid promoter which joined the UAS_G to the non-glucose-repressed *HIS3* gene was constructed (pBM1499, Fig. 2). The UAS_G in this hybrid promoter contained four GAL4 protein-binding sites and only minimal flanking sequences. The *HIS3* gene retains the TATA box and some flanking sequences of the promoter but is lacking the *HIS3* UAS (43). Expression of *HIS3* from this hybrid promoter was under *GAL4* and *GAL80* control: in a *GAL80* strain, *HIS3* expression required galactose (Fig. 2, lanes 1 and 2) and was repressed in cells grown on galactose plus glucose (lane 3); in a *gal80* strain, *HIS3* expression did not require galactose (lane 4) but remained completely repressed by glucose (lane 6). We conclude from these results that transcription activation mediated by *GAL4* is repressed by glucose independently of other *GAL1* promoter elements and that GAL80 protein is not essential for this repression. These data confirm an earlier experiment (49) and agree with previous work which concluded that inducer exclusion is unlikely to be a primary mechanism of glucose repression (48, 51).

The *GAL1* promoter contains a URS repressed by glucose. To determine whether the *GAL1* promoter is subject to repression independently of the repression acting on UAS_G, a hybrid *GAL1* promoter that did not require *GAL4* was constructed (pBM1436, Fig. 3A). The *GAL1* promoter was placed under control of the LEU3 activator protein by replacing the UAS_G with the UAS from the *LEU2* gene (UAS_L) (8, 29). This hybrid promoter was then fused to *HIS3* coding sequences. *HIS3* was expressed from pBM1436 in yeast cells grown in 5% glycerol plus 0.1% glucose without galactose (Fig. 3A, lane 1) and in a *gal4* mutant (data not shown), demonstrating that this hybrid promoter is not regulated by *GAL4* and *GAL80*. However, in yeasts grown in 5% glycerol plus 2% glucose, expression of *HIS3* from

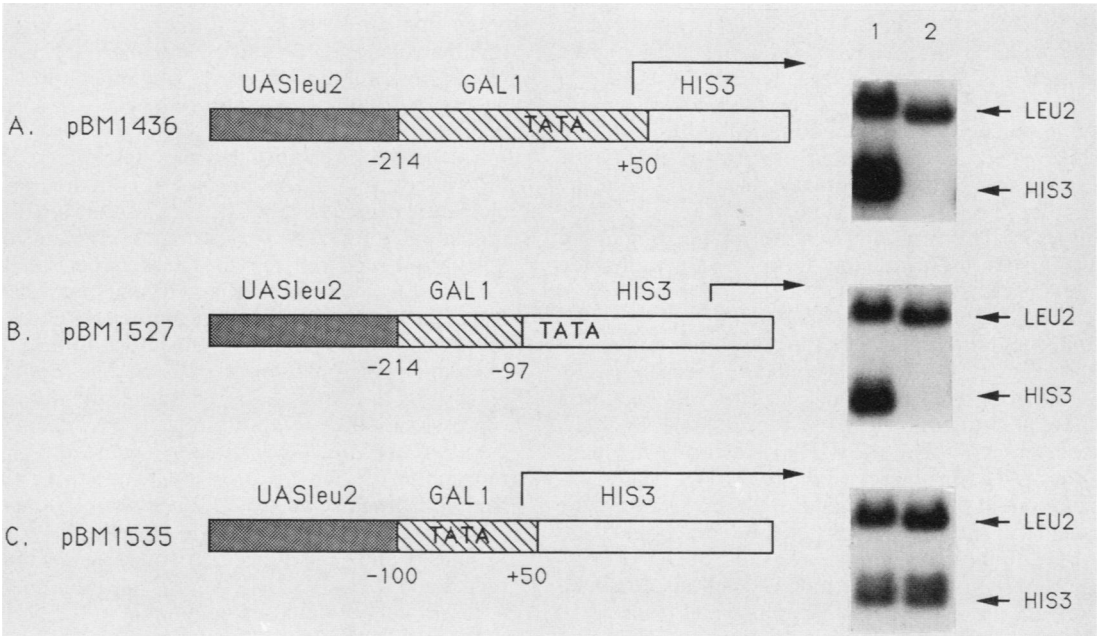

FIG. 3. (A) Schematic diagram of the hybrid promoter in pBM1436 that contains the UAS_L (shaded box), the *GAL1* sequences from between the UAS_G and the ATG codon (striped box), and the *HIS3* coding sequences (open box); (B) pBM1527, derivative of pBM1436 in which the TATA box and RNA start site are provided by *HIS3*; (C) pBM1535, derivative of pBM1436 that deletes the *GAL1* sequences from positions −214 to −100. Numbers refer to the *GAL1* sequences only. Expression was assayed in yeast cells after transformation into YM2061 (*GAL4 GAL80*). Total RNA was prepared from yeast cells grown on 5% glycerol and 0.1% glucose in the absence (lanes 1) or presence (lanes 2) of 2% glucose and probed for *HIS3* and *LEU2* RNA.

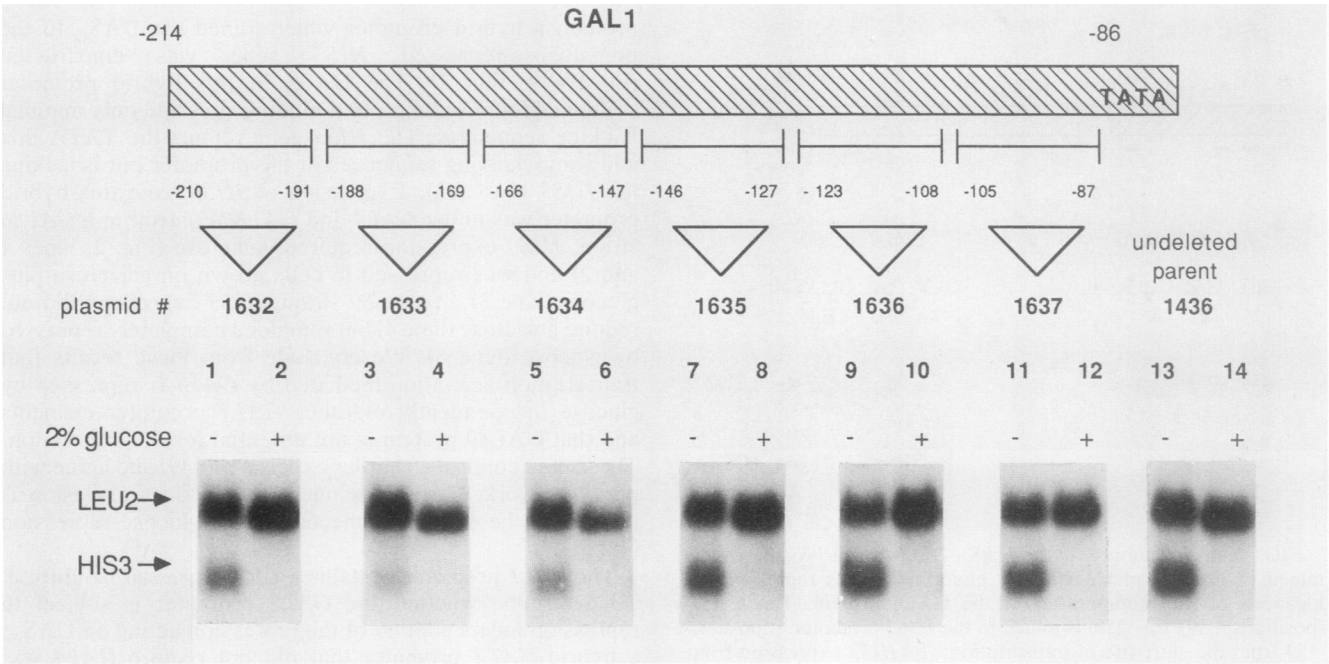

FIG. 4. Diagram of internal deletions of the *GAL1* promoter region from positions −214 to −86. The numbers below each deletion interval indicate the exact *GAL1* sequences that are deleted from each plasmid and replaced by linker DNA containing an *Xba*I restriction site. Each deletion derivative replaced the corresponding parental fragment of pBM1436 to generate plasmids pBM1632 to pBM1637 (lanes 1 to 12) (see Materials and Methods). Expression of each promoter deletion was assayed in yeast cells after transformation into YM2061. Total RNA was prepared from yeast cells grown on 5% glycerol and 0.1% glucose in the absence (−) or presence (+) of 2% glucose and probed for *HIS3* and *LEU2* RNA.

pBM1436 appeared completely repressed (Fig. 3A, lane 2). This repression must be due to the *GAL1* promoter sequences, because expression of the endogenous *LEU2* gene was not repressed by growth on glucose and functioned as an internal control in all experiments. We have obtained similar results with two other hybrid *GAL1* promoters that contain non-glucose-regulated UAS elements (data not shown). We have designated this *GAL1* promoter element the URS_G.

To further localize the URS_G element, the *GAL1* promoter was divided in half to create two additional hybrid promoters. pBM1527 (Fig. 3B) contained 117 bp of the *GAL1* promoter (−214 to −97) from between the UAS_G and the TATA box; the TATA box and mRNA start site were provided by *HIS3*. This promoter was still subject to glucose repression (Fig. 3B), indicating that these sequences include the URS_G element. By contrast, the hybrid promoter in pBM1535, which contained *GAL1* sequences from positions −100 to +50, including the TATA box, was not subject to glucose repression (Fig. 3C). We conclude that the *GAL1* promoter sequences from positions −214 to −97 contain a URS_G element able to confer glucose repression on a heterologous promoter and that the URS_G functions independently of other *GAL1* promoter elements.

Deletion analysis of the URS_G. To locate more precisely the URS_G element, a series of small internal deletions were generated by site-directed mutagenesis of the *GAL1* sequences in pBM1436. These promoter deletions removed approximately 20 bp of the *GAL1* sequence and inserted 10 bp of DNA containing an *Xba*I restriction site. Glucose repression of each promoter deletion was assessed by measuring *HIS3* RNA levels in yeasts grown in 5% glycerol plus either 0.1% glucose or 2% glucose (Fig. 4). Surprisingly, all of the mutant promoters remained strongly repressed by growth on 2% glucose. This result suggests that there are multiple URS_G elements within this region and that each deletion leaves at least one functional element intact.

Three promoter deletions in the interval between positions −210 and −147 (Fig. 4, pBM1632, pBM1633, and pBM1634) caused reduced expression under nonrepressing conditions when compared with expression by the undeleted parent (compare lanes 1, 3, and 5 with lane 13). This effect may result from disruption of an undefined promoter element necessary for efficient expression of the *GAL1* promoter (see below).

To test the possibility that the region from positions −214 to −87 of the *GAL1* promoter contains multiple URS_G elements, progressively larger deletions were constructed by recombining the 5′- and 3′-terminal fragments of the internal deletions described above. These larger deletions (Fig. 5) extended from both the 5′ and 3′ ends as well as internally. Repression of each deleted promoter by glucose was determined in yeast cells and is shown in Fig. 5. Deletions extending from position −210 in the 3′ direction to positions −168 (Fig. 5, line 2) and −146 (line 3) did not relieve glucose repression of the promoter; however, a deletion extending to position −126 (line 4) resulted in a substantial loss of glucose repression of *HIS3* expression. Therefore, the sequences from positions −146 to −126 appear to contain a URS_G element. Deletions which extended to positions −107 (Fig. 5, line 5) and −86 (line 6) resulted in a complete loss of repression.

The effects of deletions extending in the 5′ direction from position −86 of the *GAL1* promoter were somewhat more complex. Deletions of sequences to position −124 (Fig. 5, line 7) and to position −147 (line 8) did not relieve glucose repression even though the interval containing a URS_G element (positions −146 to −126) was removed. This suggests that the sequences remaining in the deletion shown in

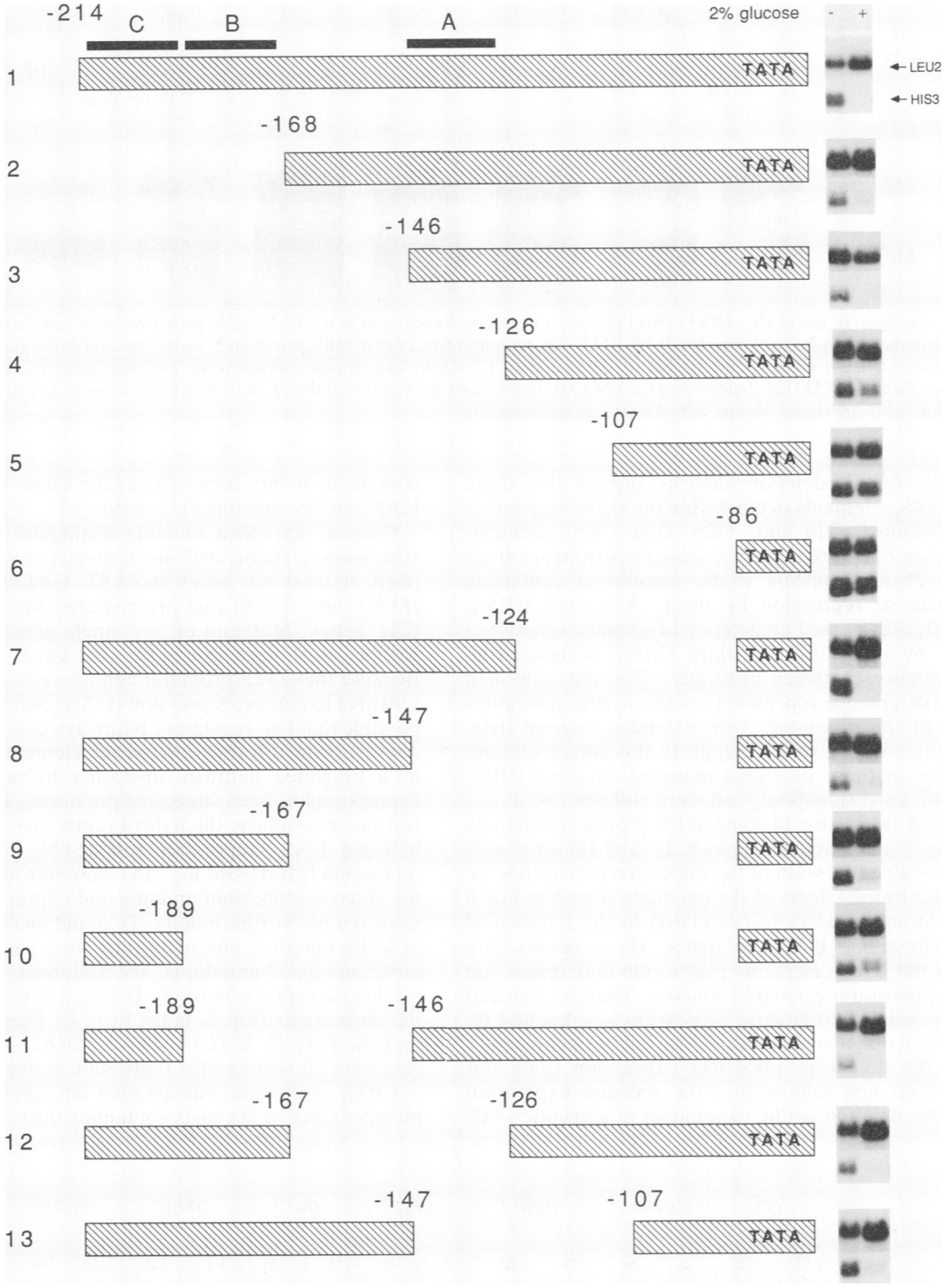

FIG. 5. Diagram of extended deletions of the *GAL1* promoter region from positions −214 to −86. These deletions were constructed by recombining the appropriate 5′- and 3′-terminal fragments of the internal deletions in Fig. 4. The resulting fragment containing the deletion of *GAL1* sequences replaced the corresponding fragment of pBM1436 (see Materials and Methods). The striped boxes and the numbers represent the *GAL1* sequences retained in each promoter deletion. Bars A, B, and C above line 1 indicate the approximate locations of URS_G elements. Expression of each promoter deletion was assayed in yeast cells after transformation into YM2061. Total RNA was prepared from yeast cells grown on 5% glycerol and 0.1% glucose in the absence (−) or presence (+) of 2% glucose and probed for *HIS3* and *LEU2* RNA.

line 8 (from positions −214 to −147) must contain an additional URS_G element(s). A deletion extending further, to position −167 (line 9), resulted in very slight resistance to glucose repression, and a deletion extending to position −189 (line 10), resulted in significant resistance to glucose repression, indicating disruption of a URS_G element in the interval from positions −166 to −188. In addition, the sequences between positions −214 and −189 appeared to function as a URS_G element, as they conferred partial repression to *HIS3* expression (compare lines 6 and 10).

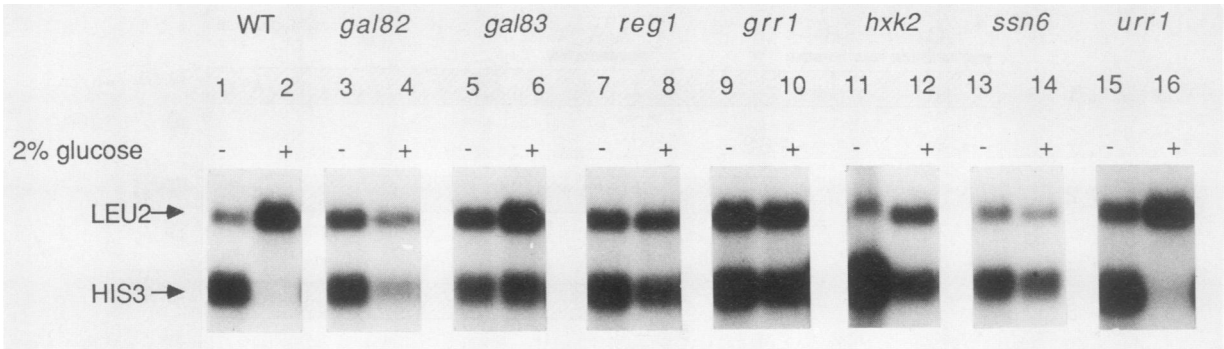

FIG. 6. Expression of the UAS_G-*HIS3* hybrid promoter in glucose repression mutants. Plasmid pBM1499 was transformed into the glucose repression mutants shown in lanes 3 to 16. Total RNA was prepared from yeast cells grown on 5% glycerol and 0.1% glucose in the absence (−) or presence (+) of 2% glucose and probed for *HIS3* and *LEU2* RNA. The strains assayed were YM2866 (lanes 1 and 2; WT, wild type), YM3120 (lanes 3 and 4), YM3122 (lanes 5 and 6), YM3205 (lanes 7 and 8), YM3128 (lanes 9 and 10), YM3134 (lanes 11 and 12), YM2961 (lanes 13 and 14), and YM3209 (lanes 15 and 16). All strains carry *gal80*.

The results of this deletion analysis suggest that three functional URS_G elements occur within the *GAL1* sequences between positions −214 and −126. One URS_G element, designated A in Fig. 5, requires the sequences from positions −146 to −126 and appears to be capable of conferring complete glucose repression by itself. A second URS_G, designated B, is disrupted by deletion of sequences between positions −189 and −167. The third URS_G, designated C, requires sequences between positions −214 and −189 and confers partial glucose repression on the hybrid promoter. Expression of the promoters with extended internal deletions (Fig. 5, lines 11 to 13) supports this interpretation, because none of these deletions removed all three URS_G elements and, correspondingly, all were still repressed.

Derepressed expression of some of the promoter deletions shown in Fig. 5 (most notably, lines 8, 11, and 12) is reduced if compared with expression of the undeleted parent (line 1). This is similar to the effects of the deletions shown in Fig. 4 (lanes 1 to 6) and appears to be related to the removal of sequences containing URS_G elements. These effects may suggest that the URS_G elements play a role in derepression as well as repression regulated by glucose. That this reduced expression is related to glucose repression is suggested by our observation that a *grr1* mutation (see below) significantly suppresses the defect in pBM1633 expression (data not shown). We do not believe that the reduced expression caused by this deletion is due to removal of a cryptic UAS contained in this region, because these sequences lacked UAS activity (unpublished data).

Glucose repression mutants distinguish two pathways of repression. Genetic analysis of glucose repression has identified at least six genes (*GAL82*, *GAL83*, *REG1*, *GRR1*, *HXK2*, and *SSN6*) that are required for repression of the *GAL* genes. Mutation of any single gene results in only a partial loss of repression of the *GAL* genes (3, 27, 32, 35, 38). Because the *GAL1* promoter contains two types of elements sensitive to glucose repression (UAS_G and URS_G), it seemed possible that two regulatory pathways that function independently to confer repression to each element exist. The notion of a branched pathway operating to repress *GAL* gene expression has been suggested previously by others (32). To test this possibility, the hybrid promoters which distinguish between UAS_G repression (pBM1499, Fig. 2) and URS_G repression (pBM1436, Fig. 3A) were introduced into each of the glucose repression mutants and expression of *HIS3* was analyzed on Northern blots. To avoid analyzing phenotypes of leaky mutants, null mutations were used (except for the *gal82* and *gal83* mutations; see Materials and Methods).

Glucose repression of the UAS_G was defective in each of the six mutants (Fig. 6, lanes 3 to 14). These results indicate that the *GAL82*, *GAL83*, *REG1*, *GRR1*, *HXK2*, and *SSN6* genes are all required for repression acting upon the UAS_G.

URS_G repression was assayed and appeared to be completely defective in yeasts containing the *reg1*, *grr1*, and *ssn6*

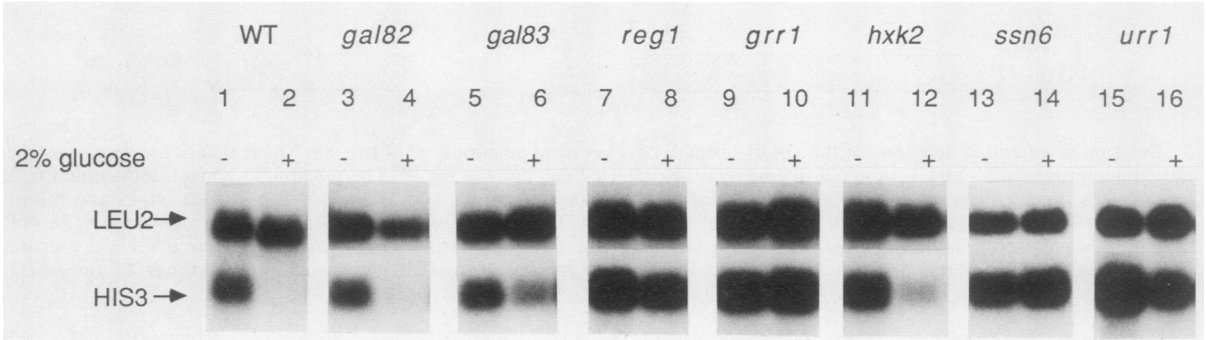

FIG. 7. Expression of UAS_L-URS_G hybrid promoter in glucose repression mutants. pBM1436 was transformed into the glucose repression mutants shown in lanes 3 to 16. Total RNA was prepared from yeast cells grown on 5% glycerol and 0.1% glucose in the absence (−) or presence (+) of 2% glucose and probed for *HIS3* and *LEU2* RNA. The strains assayed were YM2475 (lanes 1 and 2; WT, wild type), YM3117 (lanes 3 and 4), YM3121 (lanes 5 and 6), YM3206 (lanes 7 and 8), YM3127 (lanes 9 and 10), YM3131 (lanes 11 and 12), YM2964 (lanes 13 and 14), and YM2809 (lanes 15 and 16).

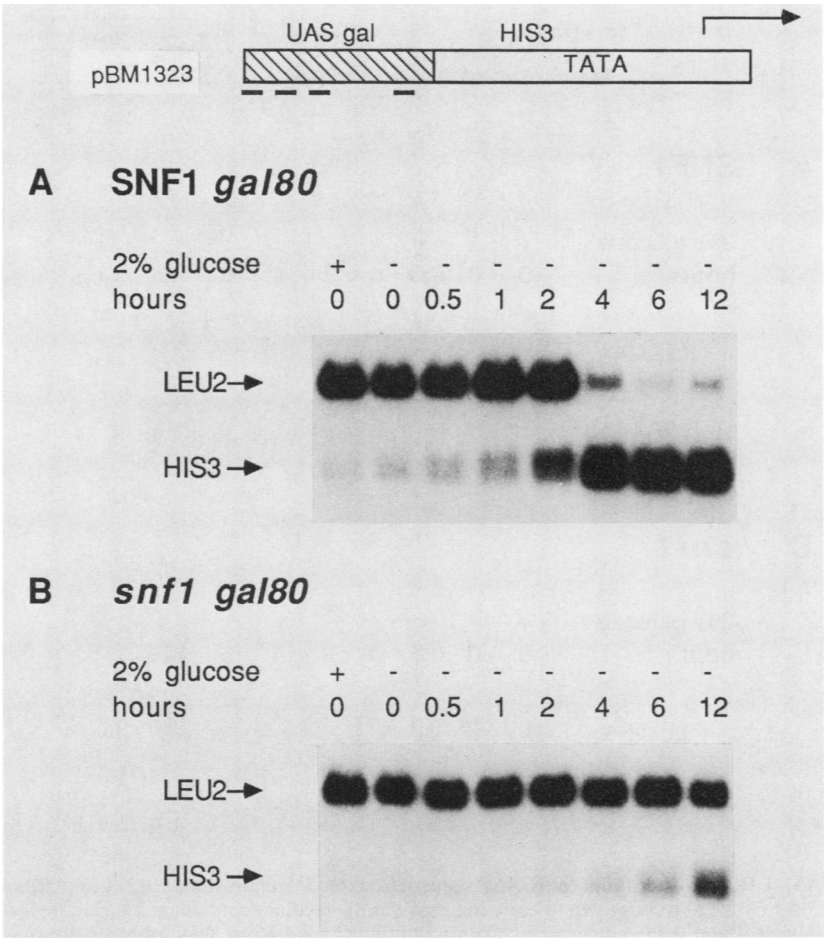

FIG. 8. Expression of UAS_G-*HIS3* hybrid promoter (UAS_G, positions −393 to −250, fused to position −94 of *HIS3*) in an *snf1* mutant. (A) An *SNF1 gal80* strain containing pBM1323 (YM2220) was grown to early log phase in SD medium containing 2% glucose, harvested, and suspended in SD medium containing 5% glycerol and 0.1% glucose. Incubation continued, and RNA was prepared from samples taken at the times indicated. (B) RNA was prepared from *snf1 gal80* yeast containing pBM1323 (YM2422) exactly as for panel A. Total RNA (20 μg) from each time point was loaded in each lane and probed for *HIS3* and *LEU2* RNA.

mutations (Fig. 7, lanes 7 to 10, 13, and 14) and partially defective in yeasts containing the *gal83* mutation (lanes 5 and 6). In contrast, the *gal82* mutation had no apparent effect (Fig. 7, lanes 3 and 4), and the *hxk2* mutation had only a slight effect on URS_G repression (lanes 11 and 12). The effects of the glucose repression mutations on UAS_G and URS_G repression, taken together, suggest that the *GAL83*, *REG1*, *GRR1*, and *SSN6* genes are required for repression acting upon both the UAS_G and URS_G, whereas the *HXK2* and *GAL82* genes are required primarily for UAS_G repression and appear dispensable for URS_G repression.

URS_G-specific mutants. Because mutations specifically relieving UAS_G repression have been identified, we sought mutants that are defective only in URS_G repression. Isolation of mutants of this class would be important for analysis of the URS_G system of repression. By using the hybrid promoter in pBM1395 (Fig. 1A), mutants that were defective in URS_G repression (see Materials and Methods) were selected. Among these mutants, we identified both a *reg1* and a *grr1* mutation, which relieve UAS_G as well as URS_G repression. Eight mutations that relieve repression of only the URS_G were identified. One such mutant (designated *urr1-1* [URS_G repression resistant]) has been further characterized and defines a gene that is not *GAL82*, *GAL83*, *REG1*, *GRR1*, *HXK2*, or *SSN6* (data not shown). The *urr1-1* mutation had no significant effect on repression acting on the UAS_G (Fig. 6, lanes 15 and 16) but clearly resulted in defective URS_G repression (Fig. 7, lanes 15 and 16).

SNF1 is required for UAS_G and URS_G derepression. Mutations in the *SNF1* gene result in greatly reduced expression of the *GAL* genes and other genes subject to glucose repression (10, 38). Mutations in *snf1* do not affect expression solely through a reduction of inducer levels, because *gal80* mutations do not suppress the defect in expression of the *GAL1* promoter (unpublished results). Therefore, *SNF1* may act more directly on the activity of the GAL4 protein or other regulatory elements of the *GAL1* promoter. To analyze in more detail the defects in glucose derepression of an *snf1* mutant, expression of the hybrid promoters containing the UAS_G (pBM1323) and URS_G (pBM1395) was analyzed.

Because *snf1* mutants grow poorly on 5% glycerol plus 0.1% glucose, expression of *HIS3* from pBM1323 was determined during derepression after growth on 2% glucose. Under these conditions, yeasts that carry *SNF1 gal80* began to derepress the UAS_G in approximately 2 h, and expression continued to increase for 2 to 4 h (Fig. 8A). Under identical conditions, *snf1 gal80* yeasts were severely defective in derepression of the UAS_G. Only a small increase in *HIS3*

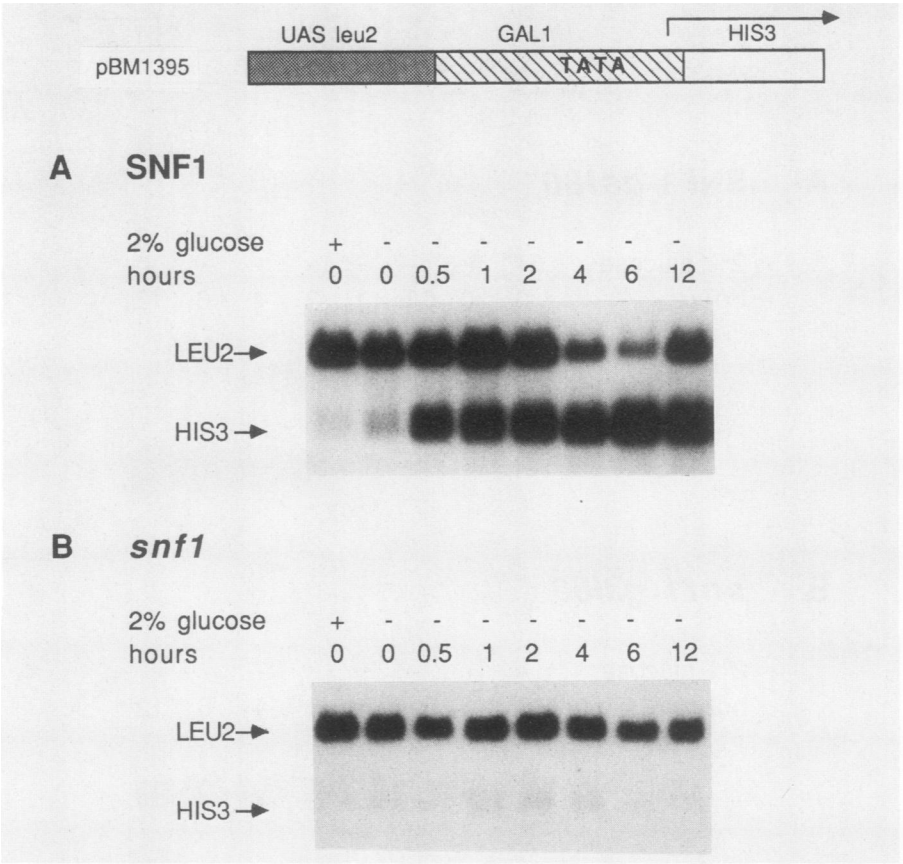

FIG. 9. Expression of UAS_L-URS_G hybrid promoter (*GAL1* sequences extend from positions −214 to +50) in a *snf1* mutant. (A) *SNF1* yeast cells containing pBM1395 (YM2526) were grown to early log phase in SD medium containing 2% glucose, harvested, and suspended in SD medium containing 5% glycerol and 0.1% glucose. Incubation continued, and RNA was prepared from samples taken at the times indicated. (B) RNA was prepared from *snf1* yeast containing pBM1395 (YM2416) exactly as for panel A. Total RNA (20 μg) from each time point was loaded in each lane and probed for *HIS3* and *LEU2* RNA.

expression was seen after 6 and 12 h of derepression (Fig. 8B). The *snf1* mutation therefore severely reduces the ability of GAL4 protein to activate transcription via UAS_G.

Surprisingly, the increasing expression of the *HIS3* gene in *SNF1* yeasts correlated with diminished expression of the endogenous *LEU2* gene used as our internal control, an effect that was reproducible (Fig. 8A). It is possible that high-level gene expression stimulated by *GAL4* during glucose derepression "squelches" expression of other genes (18).

The effect of the *snf1* mutation on the expression of pBM1395, containing the URS_G, was also determined during derepression after growth on glucose. Yeasts that are *SNF1* began to derepress the URS_G within 30 min after removal of glucose and were fully derepressed after 1 h (Fig. 9A). *snf1* yeasts failed to derepress the URS_G within 12 h after the removal of glucose (Fig. 9B). These results strongly suggest that *SNF1* is required to relieve repression mediated by the URS_G.

DISCUSSION

We have found two independent mechanisms of glucose repression acting on the *GAL1* promoter: one functioned through GAL4 protein-binding sites to reduce activation, presumably reflecting repression of GAL4 function; the other, designated URS_G, repressed *GAL1* expression through promoter sequences between UAS_G and the TATA box.

To assay glucose repression that occurs specifically via the GAL4 protein, we replaced the UAS of the *HIS3* gene with UAS_G. Previously, a 365-bp restriction fragment of the *GAL1* promoter (*Dde*I to *Sau*3A, −462 to −97) containing the UAS_G was used to replace the UASs of both the *HIS3* and *CYC1* genes to study regulation by *GAL4* (20, 44). However, since we have determined that this fragment contains URS_G elements in addition to the GAL4 protein-binding sites, we analyzed the function of a fragment containing only UAS_G. Expression activated by this fragment was regulated by *GAL4* and *GAL80* and was severely repressed during growth in the presence of glucose. Therefore, repression of the UAS_G is independent of URS_G elements within the *GAL1* promoter. In addition, since *gal80* yeasts do not require galactose for induction, repression of *GAL4* activity by glucose is not simply a consequence of inducer exclusion, a conclusion suggested previously by others (48, 51).

The mechanism of repression of the UAS_G has yet to be determined, but it seems clear from in vivo footprinting data that binding of GAL4 protein to the UAS_G is lost in glucose-grown cells (19, 40). This is consistent with the absence of detectable GAL4 protein in glucose-grown cells (33) and may reflect the slight reduction of *GAL4* transcrip-

tion (25) or could be due to a posttranslational modification similar to glucose inactivation of the *GAL2*-encoded galactose permease (30, 36). Recently it has been shown that the appearance of a phosphorylated form of GAL4 protein is correlated with expression of the *GAL* genes. The addition of glucose causes repression of *GAL* gene expression and a concomitant dephosphorylation of GAL4 protein (33). Phosphorylation of GAL4 protein may prove to be a key step in the regulation of transcription activation.

Previous studies have suggested the possibility of direct repressor binding within the *GAL1* promoter as a mechanism of glucose repression (45, 49). However, the repression acting upon UAS_G activity complicates attempts to analyze this potential mechanism in the context of the intact *GAL1* promoter. We assayed repression of the *GAL1* promoter independently of repression acting to inhibit GAL4 protein activity by replacing the UAS_G with the UAS_L from *LEU2*, a gene not repressed by glucose (Fig. 2 to 9). Expression of such hybrid promoters (containing *GAL1* sequences from positions −214 to +50 [Fig. 3A] or from −214 to −97 [Fig. 3B]) was strongly repressed by growth on glucose, indicating that these sequences contain a glucose-repressible URS. From an enzyme assay of a URS_G-containing *lacZ* fusion, we estimated that glucose repression of this hybrid promoter was 30-fold (data not shown).

Deletion analysis was used to define more precisely the sequences required for URS_G repression. The failure of small internal deletions that span the region from positions −214 to −86 to disrupt URS_G function suggests that more than one URS_G element is present (Fig. 4). Analysis of larger promoter deletions supports this conclusion (Fig. 5). A fragment containing the right half of the region (positions −146 to −86) confers repression (Fig. 5, line 3), as does a fragment consisting of the left half (positions −214 to −147, line 8). Therefore, at least two URS_G elements, each sufficient for repression, lie in this region of the *GAL1* promoter. The element most clearly defined by these data (designated A) appears to reside between positions −146 and −126 (Fig. 5, compare lines 3 and 4). One or possibly two elements (designated B and C) are present in the interval from positions −214 to −167.

Comparison of the sequences of the three URS_G intervals reveals four degenerate repetitive motifs. One direct repeat containing 9 identical residues (of 10) occurs within URS_G intervals C and B and contains the sequences −197 TCAAATGAAC −188 and −184 TCAAATTAAC −175. The three other repeats are inverted relative to one another and occur within URS_G elements C and A (−209 CCCCA CAAA −201 and −136 TTTCTGGGG −129, 8 of 9 residues identical), B and A (−180 ATTAACAACC −171 and −129 GGTAATTAAT −120, 8 of 10 residues identical), and finally, B and A (−162 ATAATGC −156 and −143 GCCT TAT −137, 6 of 7 residues identical). The functional significance of these repeated sequences, if any, has yet to be determined. It is possible that the three URS_G elements respond to unrelated cellular factors acting in parallel and need not share sequence homology.

We imagine two mechanisms by which URS_G could cause glucose repression of *GAL1* expression. First, the URS_G might contain binding sites for a repressor protein(s) that blocks function of the activator protein or TATA box factor. Second, it is possible that proper spacing between the UAS_L and the *GAL1* TATA box is required for glucose repression. We currently do not favor this second possibility, because some hybrid promoters with very similar spacing between UAS_L and the TATA box nevertheless differ significantly in their levels of repression by glucose (Fig. 5, lines 4 and 9 and lines 5 and 10). However, we cannot at this time completely rule out spacing as a partial determinant of URS_G repression. To date, evidence for specific protein binding within the URS_G region in glucose-grown cells is lacking (39, 41).

Resolution of glucose repression of *GAL1* into two components raises the issue of whether a common mechanism acts upon both the UAS_G and URS_G. We think it unlikely that a single mechanism could directly inhibit both *GAL4* activity and utilization of the *GAL1* promoter when these regulatory elements are dissociated into heterologous promoters. The possibility that independent pathways exist for UAS_G and URS_G repression is strengthened by our observation that several genes required for glucose repression of the *GAL* genes have different effects on UAS_G and URS_G. Repression of both UAS_G and URS_G is almost completely defective in *reg1*, *grr1*, and *ssn6* mutants (Fig. 6 and 7). By contrast, the *hxk2* and *gal82* mutations have little or no effect on URS_G repression and the *gal83* mutation results in only a partial loss of URS_G repression. The phenotypes of the *gal82* and *gal83* mutants, however, should be interpreted cautiously, as they are not completely isogenic to our S288C background and the natures of the mutations are unknown.

It is striking that the *hxk2* null mutation has only a minor effect on URS_G repression but appears completely defective in UAS_G repression. *HXK2* encodes a hexokinase isozyme (PII) that catalyzes the phosphorylation of glucose, an early step in glucose utilization that is thought to be involved in producing the signal for glucose repression (14). Perhaps *HXK2* produces a signal required for UAS_G repression and repression of other glucose-repressed genes (e.g., *SUC2* and *MAL*) that is not essential for the URS_G pathway of repression.

Evidence supporting the independence of the URS_G pathway was provided by a genetic selection for mutants resistant to glucose repression based solely upon URS_G function. From this selection, we obtained a mutation, designated *urr1-1*, that substantially relieved URS_G repression (Fig. 7) but had no significant effect on UAS_G repression (Fig. 6). In addition, we obtained *reg1* and *grr1* mutants defective in both UAS_G repression and URS_G repression. The facts that known glucose repression mutants relieve URS_G-mediated repression (Fig. 7) and that we recovered known mutants from a genetic selection based solely on URS_G function provide compelling evidence for the physiological relevance of the URS_G.

Our findings resolve the genes required for glucose repression of *GAL1* into three functional classes, summarized in a model shown in Fig. 10. This working model simply serves to illustrate that glucose repression of *GAL1* expression is a branched pathway: one class of genes (e.g., *GRR1*) operates through both pathways; other genes (e.g., *HXK2* and *URR1*) are specific for either pathway.

The derepression of genes repressed by glucose requires the *SNF1* gene, which encodes a protein kinase. Mutations in *SNF1* result in greatly reduced expression of the *GAL* genes as well as other genes subject to glucose repression (10, 12). We determined that hybrid promoters containing either UAS_G or URS_G are severely impaired in the ability to derepress after a shift to nonrepressing medium in a *snf1* mutant (Fig. 8 and 9). This suggests that the *SNF1* kinase functions early in the regulatory pathway to inhibit repression at a step common to both systems of repression. We have therefore placed *SNF1* before the branch point in our model (Fig. 10). The facts that *ssn6* mutations relieve glucose repression of both the UAS_G and URS_G and suppress

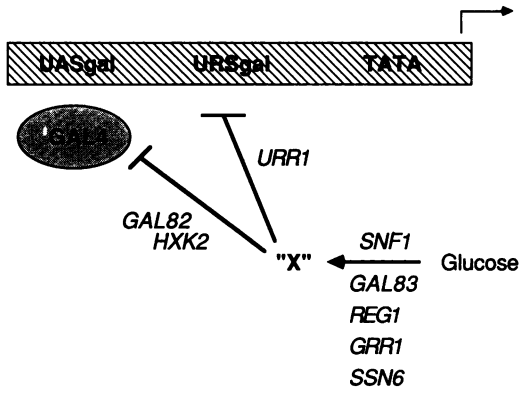

FIG. 10. Model for glucose regulation of the *GAL1* promoter. The data from this paper are most easily explained by proposing that glucose is converted to an intracellular signal, "X," which can trigger repression at both UAS_G and URS_G. The products of the *GAL83*, *REG1*, *GRR1*, and *SSN6* genes function early in the pathway and are required for the synthesis or maintenance of this signal. A branch point that splits glucose repression into two pathways that respond to the same signal is proposed. One pathway represses UAS_G activity; the other confers repression on the URS_G. The UAS_G-specific pathway requires the products of the *HXK2* and *GAL82* genes plus the "early" genes but is independent of the function of the *URR1* gene product. URS_G repression requires the *URR1* gene product and the early genes but is independent of the function of the *GAL82* and *HXK2* genes. *SNF1* activity is required for derepression of both UAS_G and URS_G and therefore must act early in the pathway to antagonize the activity of the early genes.

snf1 defects in *SUC2* and *GAL10* expression (11, 38) support this placement.

In summary, our results identify two apparently different mechanisms of glucose repression acting upon the *GAL1* promoter: one acting to inhibit GAL4-mediated transcription activation of UAS_G, the other acting upon URS_G elements between the UAS_G and the TATA box to repress transcription. In addition, a third mechanism of glucose repression acts to reduce the level of the inducer of the *GAL* genes (4, 30, 36). It is apparent that glucose repression is a highly evolved regulatory mechanism that operates to reduce gene expression in several ways. To understand glucose repression, it will be necessary to study each mechanism separately. Because most previous studies of glucose repression have not recognized the existence of multiple regulatory mechanisms, their conclusions may apply only to events occurring early in the pathway of repression. Our analysis should make it possible to identify and characterize the regulators responsible for specific repression via UAS_G and URS_G.

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LITERATURE CITED

1. **Adams, B. G.** 1972. Induction of galactokinase in *Saccharomyces cerevisiae*: kinetics of induction and glucose effects. J. Bacteriol. **111**:308–315.
2. **Andreadis, A., Y. Hsu, G. B. Kohlhaw, and P. Schimmel.** 1982. Nucleotide sequence of yeast *LEU2* shows 5'-noncoding region has sequences cognate to leucine. Cell **31**:319–325.
3. **Bailey, R. B., and A. Woodword.** 1984. Isolation and characterization of a pleiotropic glucose repression resistant mutant of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **193**:507–512.
4. **Bajwa, W., T. E. Torchia, and J. E. Hopper.** 1988. Yeast regulatory gene *GAL3*: carbon regulation; UAS_{Gal} elements in common with *GAL1*, *GAL2*, *GAL7*, *GAL10*, *GAL80*, and *MEL1*; encoded protein strikingly similar to yeast and *Escherichia coli* galactokinases. Mol. Cell. Biol. **8**:3439–3447.
5. **Barnes, D. A., and J. Thorner.** 1986. Genetic manipulation of *Saccharomyces cerevisiae* by use of the *LYS2* gene. Mol. Cell. Biol. **6**:2828–2838.
6. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
7. **Bram, R. J., and R. D. Kornberg.** 1985. Specific protein binding to far upstream activating sequences in polymerase II promoters. Proc. Natl. Acad. Sci. USA **82**:43–47.
8. **Brisco, P. R., T. S. Cunningham, and G. B. Kohlhaw.** 1987. Cloning, disruption and chromosomal mapping of yeast *LEU3*, a putative regulatory gene. Genetics **115**:91–99.
9. **Carlson, M.** 1987. Regulation of sugar utilization in *Saccharomyces* species. J. Bacteriol. **169**:4873–4877.
10. **Carlson, M., B. C. Osmond, and D. Botstein.** 1981. Mutants of yeast defective in sucrose utilization. Genetics **98**:25–40.
11. **Carlson, M., B. C. Osmond, L. Neigeborn, and D. Botstein.** 1984. A suppressor of *SNF1* mutations causes constitutive high-level invertase synthesis in yeast. Genetics **107**:19–32.
12. **Celenza, J. L., and M. Carlson.** 1986. A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science **233**:1175–1180.
13. **Elder, R. T., E. Y. Loh, and R. W. Davis.** 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80**:2432–2436.
14. **Entian, K.-D.** 1980. Genetic and biochemical evidence for hexokinase PII as a key enzyme involved in carbon catabolite repression in yeast. Mol. Gen. Genet. **178**:633–637.
15. **Entian, K.-D.** 1986. Glucose repression: a complex regulatory system in yeast. Microbiol. Sci. **3**:366–371.
16. **Fleig, U. N., R. D. Pridmore, and P. Philippsen.** 1986. Construction of *LYS2* cartridges for use in genetic manipulations of *Saccharomyces cerevisiae*. Gene **46**:237–245.
17. **Gancedo, J. M., and C. Gancedo.** 1986. Catabolite repression mutants of yeast. FEMS Microbiol. Rev. **32**:179–187.
18. **Gill, G., and M. Ptashne.** 1988. Negative effect of the transcriptional activator GAL4. Nature (London) **334**:721–724.
19. **Giniger, E., S. M. Varnum, and M. Ptashne.** 1985. Specific DNA binding of GAL4, a positive regulatory protein of yeast. Cell **40**:767–774.
20. **Guarente, L., R. R. Yocum, and P. Gifford.** 1982. A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. Proc. Natl. Acad. Sci. USA **79**:7410–7414.
21. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
22. **Johnston, M.** 1987. A model fungal gene regulatory mechanism: the *GAL* genes of *Saccharomyces cerevisiae*. Microbiol. Rev. **51**:458–476.
23. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1440–1448.
24. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.

25. **Laughon, A., and R. F. Gesteland.** 1982. Isolation and preliminary characterization of the *GAL4* gene, a positive regulator of transcription in yeast. Proc. Natl. Acad. Sci. USA **79**:6827–6831.
26. **Lorch, Y., and R. D. Kornberg.** 1985. A region flanking the *GAL7* gene and a binding site for *GAL4* protein as upstream activating sequences in yeast. J. Mol. Biol. **186**:821–824.
27. **Ma, H., and D. Botstein.** 1986. Effects of null mutations in the hexokinase genes of *Saccharomyces cerevisiae* on catabolite repression. Mol. Cell. Biol. **6**:4046–4052.
28. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Martinez-Arias, A., H. J. Yost, and M. J. Casadaban.** 1984. Role of an upstream regulatory element in leucine repression of the *Saccharomyces cerevisiae leu2* gene. Nature (London) **307**:740–742.
30. **Matern, H., and H. Holzer.** 1977. Catabolite inactivation of the galactose uptake system in yeast. J. Biol. Chem. **252**:6399–6402.
31. **Matsumoto, K., A. Toh-e, and Y. Oshima.** 1981. Isolation and characterization of dominant mutations resistant to carbon catabolite repression of galactokinase synthesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1**:83–93.
32. **Matsumoto, K., T. Yoshimatsu, and Y. Oshima.** 1983. Recessive mutations conferring resistance to carbon catabolite repression of galactokinase synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **153**:1405–1414.
33. **Mylin, L. M., J. P. Bhat, and J. E. Hopper.** 1989. Regulated phosphorylation and dephosphorylation of GAL4, a transcriptional activator. Genes Dev. **3**:1157–1165.
34. **Neigeborn, L., and M. Carlson.** 1984. Genes affecting the regulation of *SUC2* gene expression by glucose repression in *Saccharomyces cerevisiae*. Genetics **108**:845–858.
35. **Neigeborn, L., and M. Carlson.** 1987. Mutations causing constitutive invertase synthesis in yeast: genetic interactions with *snf* mutations. Genetics **115**:247–253.
36. **Ramos, J., and V. P. Cirillo.** 1989. Role of cyclic-AMP-dependent protein kinase in catabolite inactivation of the glucose and galactose transporters in *Saccharomyces cerevisiae*. J. Bacteriol. **171**:3545–3548.
37. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60**:237–243.
38. **Schultz, J., and M. Carlson.** 1987. Molecular analysis of *SSN6*, a gene functionally related to the *SNF1* protein kinase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:3637–3645.
39. **Selleck, S. B., and J. Majors.** 1987. Photofootprinting *in vivo* detects transcription-dependent changes in yeast TATA boxes. Nature (London) **325**:173–177.
40. **Selleck, S. B., and J. E. Majors.** 1987. In vivo DNA-binding properties of a yeast transcription activator protein. Mol. Cell. Biol. **7**:3260–3267.
41. **Selleck, S. B., and J. Majors.** 1988. *In vivo* "photofootprint" changes at sequences between the yeast *GAL1* upstream activating sequence and "TATA" element require activated GAL4 protein but not a functional TATA element. Proc. Natl. Acad. Sci. USA **85**:5399–5403.
42. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Laboratory course manual for methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. **Struhl, K.** 1982. The yeast *his3* promoter contains at least two distinct elements. Proc. Natl. Acad. Sci. USA **79**:7385–7389.
44. **Struhl, K.** 1984. Genetic properties and chromatin structure of the yeast *GAL* regulatory element: an enhancer-like sequence. Proc. Natl. Acad. Sci. USA **81**:7865–7869.
45. **Struhl, K.** 1985. Negative control at a distance mediates catabolite repression in yeast. Nature (London) **317**:822–824.
46. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
47. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **9**:5201–5205.
48. **Torchia, T. E., R. W. Hamilton, C. L. Cano, and J. E. Hopper.** 1984. Disruption of regulatory gene *GAL80* in *Saccharomyces cerevisiae*: effects on carbon-controlled regulation of the galactose/melibiose pathway genes. Mol. Cell. Biol. **4**:1521–1527.
49. **West, R. W., Jr., R. R. Yocum, and M. Ptashne.** 1984. *Saccharomyces cerevisiae GAL1-GAL10* divergent promoter region: location and function of the upstream activating sequence UAS_G. Mol. Cell. Biol. **4**:2467–2478.
50. **Yocum, R. R., S. Hanley, R. West, Jr., and M. Ptashne.** 1984. Use of *lacZ* fusions to delimit regulatory elements of the inducible divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1985–1998.
51. **Yocum, R. R., and M. Johnston.** 1984. Molecular cloning of the *GAL80* gene from *Saccharomyces cerevisiae* and characterization of a *gal80* deletion. Gene **32**:75–82.
52. **Zimmermann, F. K., and I. Scheel.** 1977. Mutants of *Saccharomyces cerevisiae* resistant to carbon catabolite repression. Mol. Gen. Genet. **154**:75–82.