Genetic Interactions Between *REG1/HEX2* and *GLC7*, the Gene Encoding the Protein Phosphatase Type 1 Catalytic Subunit in *Saccharomyces cerevisiae*

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ABSTRACT

Mutations in *GLC7*, the gene encoding the type 1 protein phosphatase catalytic subunit, cause a variety of abberrant phenotypes in yeast, such as impaired glycogen synthesis and relief of glucose repression of the expression of some genes. Loss of function of the *REG1/HEX2* gene, necessary for glucose repression of several genes, was found to suppress the glycogen-deficient phenotype of the *glc7-1* allele. Deletion of *REG1* in a wild-type background led to overaccumulation of glycogen as well as slow growth and an enlarged cell size. However, loss of *REG1* did not suppress other phenotypes associated with *GLC7* mutations, such as inability to sporulate or, in cells bearing the $glc7^{Y\text{-}170}$ allele, lack of growth at 14°. The effect of *REG1* deletion on glycogen accumulation is not simply due to derepression of glucose-repressed genes, although it does require the presence of *SNF1*, which encodes a protein kinase essential for expression of glucose-repressed genes and for glycogen accumulation. We propose that *REG1* has a role in controlling glycogen accumulation.

IN the yeast *Saccharomyces cerevisiae*, glucose is a favored carbon and energy source whose presence induces expression of some genes while repressing others (GANCEDO 1992; JOHNSTON and CARLSON 1992; TRUMBLY 1992). Nutritional status also controls the accumulation of reserve molecules such as glycogen, a branched polymer of glucose, that accumulates in response to nutrient limitation (LILLIE and PRINGLE 1980). There are intimate connections between glucose-repression mechanisms and the control of glycogen deposition (THOMPSON-JAEGER *et al.* 1991; HARDY *et al.* 1994). For example, *SNF1/CAT1*, which encodes a protein kinase catalytic subunit, is required for the derepression of expression of glucose-repressed genes (CIRIACY 1977; ZIMMERMANN and SCHEEL 1977; CARLSON *et al.* 1981; CELENZA and CARLSON 1986) and for glycogen accumulation (THOMPSON-JAEGER *et al.* 1991; CANNON *et al.* 1994).

Glycogen synthesis in yeast requires several proteins. Glycogen synthase, encoded by two genes, *GSY1* and *GSY2* (FARKAS *et al.* 1991), is responsible for the basic polymerization reaction, and an enzyme encoded by the *GLC3/GHA1* gene (ROWEN *et al.* 1992; THON *et al.* 1992) introduces the branchpoints of the mature glycogen molecule. *GSY2* codes for the more abundant, nutritionally regulated form of glycogen synthase (FARKAS *et al.* 1991). Recently, we identified two other genes, *GLG1* and *GLG2*, that code for self-glucosylating proteins putatively involved in initiation of glycogen biosynthesis (CHENG *et al.* 1995a).

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The regulation of yeast glycogen metabolism involves both transcriptional and posttranslational controls (HARDY *et al.* 1994). The expression of several genes (FARKAS *et al.* 1991; ROWEN *et al.* 1992; THON *et al.* 1992; FRANÇOIS *et al.* 1992), including *GSY2* and *GLC3*, increases with the onset of glycogen accumulation. Phosphorylation of yeast glycogen synthase was one of the first examples of the control of an enzyme by reversible covalent modification (ROTHMAN-DENES and CABIB 1970, 1971). Phosphorylation inactivates the enzyme but full activity can still be elicited by the allosteric activator glucose-6-P. Thus, the −/+ glucose-6-P ratio is often used as a measure of the activation state and, by inference, degree of phosphorylation of glycogen synthase. More recent work has identified three COOH-terminal residues of Gsy2p as possible sites whose phosphorylation leads to inactivation of the enzyme (HARDY and ROACH 1993). However, the enzymes mediating the phosphorylation/dephosphorylation of glycogen synthase remain largely unidentified.

Several protein phosphatases have been implicated in the control of glycogen biosynthesis (PENG *et al.* 1990; FENG *et al.* 1991; POSAS *et al.* 1991; FRANÇOIS *et al.* 1992; HARDY and ROACH 1993; CANNON *et al.* 1994; CLOTET *et al.* 1995), but it is unclear which one actually dephosphorylates glycogen synthase. The strongest case has been made for a *GLC7*-encoded type 1 phosphatase. Some mutant alleles of *GLC7* cause impaired glycogen deposition (CANNON *et al.* 1994; HISAMOTO *et al.* 1994), and one of these, *glc7-1*, was found to be associated with a decreased glycogen synthase activation state (PENG *et al.* 1990; FENG *et al.* 1991; CANNON *et al.* 1994). Mutations that change the putative phosphorylation sites of Gsy2p to Ala to mimic dephosphorylation were found

TABLE 1

Yeast strains

Strain	Genotype	Source
EG328-1A	*MATα trp1 leu2 ura3-52*	K. TATCHELL
EG327-1D	*MATα trp1 leu2 ura3-52 glc7-1*	K. TATCHELL
EG328-2D	*MATα trp1 leu2 ura3-52 gac1::LEU2*	K. TATCHELL
NH-102C	*MATa trp1 leu2 ura3-52 glc7$^{Y\text{-}170}$*	K. MATSUMOTO
F243	*MATa thr4* Mal$^+$	R. C. WEK
DH4[a]	*MATa ura3-52 thr4* Mal$^+$	This study
DH5	*MATα trp1 leu2 ura3-52 snf1::LEU2*	This study
DH6	*MATα trp1 leu2 ura3-52 glc7-1 reg1::(Tn3::URA3)*	This study
DH7	*MATα trp1 leu2 ura3-52 glc7-1 reg1::URA3*	This study
DH8	*MATα trp1 leu2 ura3-52 glc7-1 reg1::LEU2*	This study
DH9[a]	*MATa ura3-52 thr4 glc7$^{Y\text{-}170}$*	This study
DH10[a]	*MATa trp1 leu2 ura3-52 thr4 glc7$^{Y\text{-}170}$ reg1::URA3*	This study
DH11	*MATa trp1 ura3-52 thr4 reg1::URA3*	This study
DH12	*MATα trp1 leu2 ura3-52 reg1::URA3*	This study
DH13	*MATα trp1 leu2 ura3-52 snf1::LEU2 reg1::URA3*	This study
DH14	*MATα trp1 leu2 ura3-52 glc7-1 hsk2::URA3*	This study
DH15	*MATα trp1 leu2 ura3-52 glc7-1 mig1::URA3*	This study
DH17	*MATα trp1 leu2 ura3-52 gac1::LEU2 reg1::URA3*	This study
DH18[b]	*MATα trp1 leu2 ura3-52 glc7-1 reg1::(Tn3::URA3)*	This study
DH19[b]	*MATα trp1 leu2 ura3-52 glc7-1 Mal$^+$*	This study
DH20	*MATa trp1 ura3-52 thr4 glc7-1 reg1::URA3*	This study
DH21	*MATα trp1 leu2 ura3-52 reg1::LEU2 gsy2::URA3*	This study

All DH strains are isogenic to EG328-1A, which is the wild type used in this study; EG327-1D and EG353-1C are isogenic to EG328-1A.

[a] DH4 is the progeny of four backcrosses to the wild-type strain EG328-1A from F243. DH9 and DH10 are the progeny of three backcrosses to the wild-type strain EG328-1A from NH102-1C.

[b] EG328-1A is Mal$^-$. For some experiments, it was necessary to produce a Mal$^+$ strain by mating and backcrossing with F243 (see text). This produced DH19, which is Mal$^+$ and DH18, that contains all the genes necessary for maltose metabolism except for *REG1*.

to suppress the glycogen accumulation defect in a *glc7-1* strain (HARDY and ROACH 1993). Deletion of *GAC1*, which encodes a presumed regulatory subunit of type 1 phosphatase (FRANÇOIS *et al.* 1992; STUART *et al.* 1994), also caused a glycogen-deficient phenotype that could be suppressed by mutations affecting Gsy2p phosphorylation sites (HARDY and ROACH 1993). These results are all consistent with the hypothesis that Gsy2p is dephosphorylated *in vivo* by a phosphatase composed of Glc7p and Gac1p. However, the results neither exclude the existence of other Gsy2p phosphatases nor the occurrence of other functions for Glc7p and Gac1p.

Even less well understood are the protein kinases responsible for glycogen synthase phosphorylation. Initial reports had suggested a role for cyclic AMP-dependent protein kinase. While activation of the cyclic AMP pathway clearly causes inactivation of glycogen synthase *in vivo*, this may not be through direct phosphorylation by cyclic AMP-dependent protein kinase (CANNON and TATCHELL 1987; FARKAS *et al.* 1990; PENG *et al.* 1990; POSAS *et al.* 1991; ROWEN *et al.* 1992; HARDY *et al.* 1994). Since a *glc7-1* strain is defective in glycogen accumulation and also has hyperphosphorylated glycogen synthase, we sought suppressors of the glycogen accumulation defect of *glc7-1* cells with the idea of uncovering glycogen synthase kinases. We identified such a suppressor but found that it was a mutation within the *REG1* gene (ENTIAN and ZIMMERMANN 1980; MATSUMOTO *et al.* 1983; NIEDERACHER and ENTIAN 1991) that has been implicated in the glucose repression mechanism.

MATERIALS AND METHODS

Strains, media, and genetic methods: The *S. cerevisiae* strains used are listed in Table 1. For tests of glycogen accumulation, cells were plated on synthetic complete media with glucose or glycerol as carbon source and grown for 2–3 days at 30° before being exposed to iodine vapor for 2 min. Alternatively, cells grown in liquid media were used for enzymatic glycogen measurement (HARDY and ROACH 1993). For growth tests, cells were grown in rich media (YP) with the indicated carbon source. Plasmids were maintained in *Escherichia coli* strain DH5α. Standard methods for yeast genetic analysis (GUTHRIE and FINK 1991) and transformation (ITO *et al.* 1983) were used.

Screen for suppressors of the glycogen-deficient phenotype of *glc7-1* cells: A library composed of randomly disrupted segments of yeast genomic DNA was constructed in the pHSS6 vector (Figure 1) by random transposon (mini-Tn3) insertion as described by CHUN and GOEBL (1996). Purified pHSS6 library DNA was digested with *Not*I to excise linear segments of the yeast DNA that were then used for one-step gene replacement (GUTHRIE and FINK 1991) in haploid *glc7-1* cells (strain EG327-1D) that are defective for glycogen accumulation. Selection was made for Ura$^+$ transformants that should carry randomly disrupted genes tagged with the Tn3 transposon. The Ura$^+$ colonies that also had restored glycogen accu-

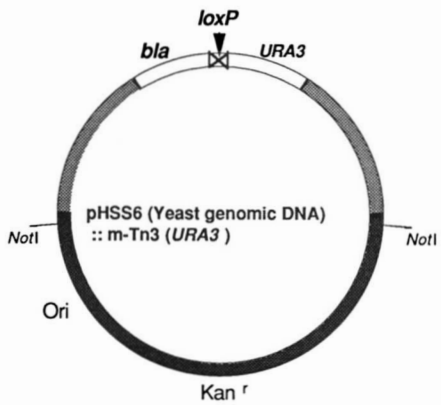

FIGURE 1.—Transposon-tagged yeast genomic DNA fragment in pHSS6 vector. A representative of the library is shown with clear boxes denoting the Tn3 mini-transposon, light shading the yeast genomic DNA and dark shading the pHSS6 vector DNA.

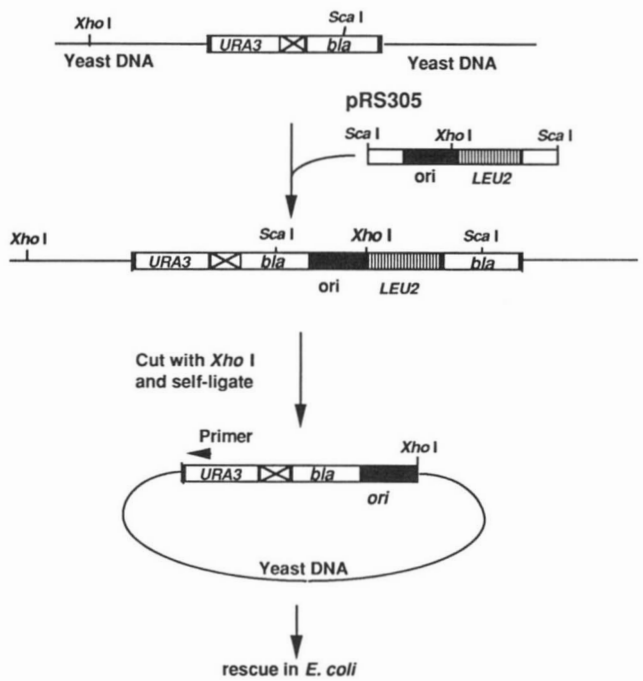

FIGURE 2.—Scheme for rescue of disrupted genes from yeast mutagenized with transposon-tagged libarary.

mulation fell into a single complementation group. To identify the mutated locus, the transposon and flanking yeast genomic DNA were rescued using the strategy described by BURNS *et al.* (1994) as shown in Figure 2. Strain DH6, carrying a Tn-3 insertion, was transformed to Ura^+Leu^+ with pRS305 (SIKORSKI and HEITER 1989) that had been digested with *Sca*I to introduce a bacterial origin of replication. Yeast genomic DNA was then isolated from the transformants, cut with *Xho*I, and ligated with T4 DNA ligase under dilute conditions to favor intramolecular ligation. *E. coli* (DH5α) cells were transformed with the ligation products using the calcium chloride procedure (MANIATIS *et al.* 1982). Rescued plasmid DNAs were purified from bacteria by the alkaline lysis method (BIRNBOIM and DOLY 1979), and the nucleotide sequence flanking the transposon was determined by the dideoxy chain termination method (SANGER *et al.* 1977) using a primer complementary to the 5′ end of the *URA3* gene. Search of the GenBank data base was performed by using the FASTA program (PEARSON and LIPMAN 1988).

Complementation analysis: To test whether the Tn-3 mutants with restored glycogen accumulation fell into the same complementation group, one of the original mutants, which were all *MAT*α, was crossed with a *MATa GLC7* strain (DH4) to change mating type. *MATa* suppressor strains were then crossed with the different *MAT*α mutants isolated from the primary screen. The resulting diploids were tested for their ability to restore glycogen in a *glc7-1* background. Mutant alleles within the same complementation group should be associated with a glycogen-accumulating phenotype in a diploid homozygous for *glc7-1*.

Gene disruptions and plasmid construction: For disruption of *REG1*, PCR was used to generate a DNA fragment that contained *REG1* sequences straddling the chosen marker gene. The *URA3* gene on vector pRS306 (SIKORSKI and HEITER 1989) was used as the template for PCR. The sense (GAGGGCTAGCTTTTGGCTGTTATACGTATAACCACACACCTGGTGAGCAGATTGTACTGAGAGTGC) and antisense (CAGCTTACTTGGATCCTAAAGACGGCACTGATCCACACTACCTGGCATCTGTGCGGTATTTCACAC) primers contain 21 bases at the 3′ end complementary to pRS306 sequences and 45 bases at the 5′ end homologous to 5′ or 3′ ends of *REG1*. The resulting PCR product contained the 5′ sequence (−111 to −66 referred to the open reading frame) and 3′ sequence (3085–3130) of *REG1* at each end of a 1.1-kb sequence containing the *URA3* gene in the middle. This DNA fragment was then used to transform strain EG327-1D to Ura^+, thus replacing the *REG1* gene to generate strain DH7. The gene disruption was confirmed by PCR. A similar strategy was employed to construct a reg1::LEU2 strain, DH8.

The *HXK2* gene was disrupted using pMR226 (K.-D. ENTIAN, University of Tubingen, Germany). After cutting with *Hind*III and *Xba*I, the DNA was used to transform EG327-1D cells to Leu^+. The *MIG1* gene was disrupted using the pJN41 (H. RONNE, Ludwig Institude for Cancer Research, Uppsala, Sweden). The plasmid DNA was cut with *Hind*III and *Xba*I, and used to transform strain EG327-1D to Ura^+. Both disruptions were confirmed by Southern blot analysis.

DNA containing *REG1* in a YCp50 plasmid was kindly provided by MARK JOHNSTON, Washington University, St. Louis. A 4.1-kb *Bam*HI fragment containing the open reading frame was subcloned into the *Bam*HI site of pRS314.

Glycogen synthase and glycogen measurements: Assay of glycogen synthase by the method of THOMAS *et al.* (1968) was as described by HARDY and ROACH (1993). A unit of activity is defined as the amount of enzyme that catalyzes the transfer of 1 μmol of glucose from UDP-glucose to glycogen per minute under conditions of the standard assay. The total activity of glycogen synthase is that measured in the presence of 7.2 mM glucose-6-phosphate. The −/+ glucose-6-P activity ratio is defined as the activity measured in the absence of glucose-6-P divided by the activity measured in its presence. Each measurement was the average of duplicate assays. Glycogen was determined in extracts of cells as described by HARDY and ROACH (1993).

RESULTS

Isolation and genetic characterization of a mutation suppressing the glycogen-deficient phenotype of *glc7-1* cells: Our initial logic was that a screen for second site suppressors of *glc7-1*, a mutation known to impair glycogen synthase activation, might reveal glycogen synthase kinases. We mutagenized strain EG327-1D (*glc7-1*) by

TABLE 2

Glycogen content and maltose growth of yeast strains

Strain	Mutant allele	Glycogen[a] (mg/mg protein)		Growth on maltose
		Logarithmic	Stationary	
EG328-1A	Wild type	0.042	0.827	ND[c]
DH19	*glc7-1*	0.008	0.098	+
DH7	*glc7-1 reg1::URA3*	0.112	0.285	ND
DH18	*glc7-1 reg1::(Tn3)*	0.149	0.358	−
[b]DH18 +*REG1*	*glc7-1 reg1::(Tn3)*	0.011	0.087	+
DH11	*reg1::URA3*	0.485	2.316	ND[c]

[a] Glycogen content was measured in yeast cell extracts as described under MATERIAL AND METHODS. Cells were harvested either during logarithmic growth (5×10^6–10^7 cells/ml) or stationary phase ($\sim 10^8$ cells/ml).
[b] The *REG1* gene was inserted into and expressed from a pRS314 plasmid.
[c] ND, not determined.

random insertion of a *URA3*-marked transposon (mini-Tn3) in the yeast genome (see MATERIALS AND METHODS). We then screened by iodine staining for colonies that had reacquired the ability to accumulate glycogen (Glc^+). All revertants were recessive and fell into a single complementation group (see MATERIALS AND METHODS).

Since diploids homozygous for *glc7-1* do not sporulate (CANNON *et al.* 1994), we crossed five representative mutants to an isogenic wild-type strain EG328-1A (*GLC7 ura3*$^-$). The resulting diploids sporulated and spore clones were tested for glycogen storage and for uracil prototrophy. All glycogen deficient spores in 40 tetrads were Ura^-, demonstrating that suppression of the Glc^- phenotype was tightly linked to the *URA3* marker in the Tn3 transposon. Ura^+ segregated 2^+:2^-, indicating that only one transposon insertion had occurred in each mutant. This was confirmed by Southern analysis of genomic DNA isolated from revertants (data not shown).

Identification of the mutated gene: To determine which gene was disrupted by the mini-Tn3 transposon, we selected one of the suppressor strains, DH6, and followed the general strategy described by BURNS *et al.* (1994). After recovering the Tn3-tagged sequence, it was sequenced using a transposon-specific primer to obtain yeast genomic DNA sequence adjacent to the insertion point. A sequence of ~600 nucleotides was found to include the 5′-noncoding and the beginning of the coding region of the *REG1* gene. The transposon was inserted 23 nucleotides after the ATG codon of the *REG1*. Consistent with this result, we also found that the *URA3* marker in the transposon is tightly linked to *TRP1* (data not shown), which is known to be close to *REG1* on chromosome *4*.

Suppression of the glycogen-deficient phenotype of *glc7-1* is due to loss of function of *REG1*: Maltose inhibits growth of *reg1* mutants (ENTIAN 1980). In crosses of *glc7-1 reg1::Tn3* strains (DH6 and DH18) to the Mal^+ DH4 strain, we found that the Ura^+ and Mal^- phenotypes were always linked, indicating that the disruption of *REG1* correlated with inability to grow on maltose. Furthermore, *REG1* on a plasmid restored growth on maltose to some of the Ura^+ clones (see also Table 2) suggesting that these cells contained all the genes required for maltose metabolism and lacked only *REG1*. As expected, the Mal^- phenotype did not segregate with the *glc7-1* allele, as judged by the glycogen-accumulation phenotype. DH18 is also sensitive to maltose and extrachromosomal expression of *REG1*-restored resistance to growth inhibition by maltose (data not shown). We conclude that defects in growth on maltose as well as suppression of the *glc7-1* glycogen-deficient phenotype were caused by loss of *REG1* function.

Interactions of *REG1*, *GLC7* and *GAC1* in determining glycogen accumulation: Targetted deletion of *REG1* (see MATERIALS AND METHODS) resulted in an allele that caused similar phentoypes to those caused by Tn-3 insertion (Figure 3; Table 2). Loss of *REG1* correlated with three- to fourfold over-accumulation of glycogen during logarithmic growth as compared to wild type, whereas in stationary phase the glycogen levels, though substantially increased compared to *glc7-1* cells, were only about half those of wild type. The *reg1glc7-1* cells were also defective in maltose metabolism (Table 2). Deletion of *REG1* in an otherwise wild-type background led to a significant hyperaccumulation of glycogen, both in logarithmic and stationary phase (Table 2 and Figure 3). While our study was in progress, TU and CARLSON (1995) found evidence for a physical interaction between Reg1p and Glc7p, and in the course of their work also observed that deletion of *REG1* correlated with elevated glycogen stores.

Many of the known controls of glycogen accumulation involve either regulation of the expression of glycogen biosynthetic genes, like *GSY2*, or regulation of the activation state of glycogen synthase (HARDY *et al.* 1994). Thus, we analyzed the glycogen synthase activity in wild-type, *reg1* and *reg1glc7-1* strains. As shown in Figure 4, there was no major difference in the total enzyme activ-

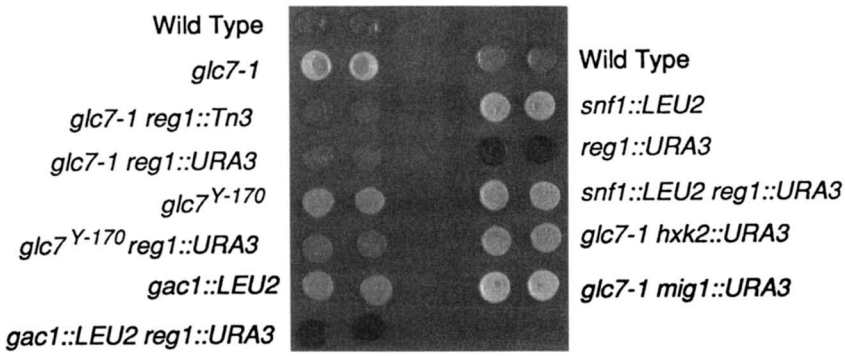

FIGURE 3.—Effect of *REG1* mutation on glycogen accumulation. Cells carrying REG1 mutations, either the original transposon insertion *reg1::Tn3* or the targeted deletion *reg1::URA3,* alone or in combination with the indicated mutations in other relevant genes, were grown on a synthetic complete plate for 72 hr and then stained with iodine vapor.

ity detected in these strains. The activation state of glycogen synthase, as reflected in the −/+ glucose-6-P activity ratio, was significantly reduced in a *glc7-1* strain, as previously reported (PENG 1990; FENG 1991). Disruption of *REG1* in such cells caused an increase in activity ratio but to a level below that of wild-type cells. *REG1* disruption on its own led to a small decrease in activity ratio relative to wild type.

Defects in the *GAC1* gene, which encodes a putative phosphatase regulatory subunit, are also known to cause reduced accumulation of glycogen (FRANÇOIS *et al.* 1992). We therefore tested whether loss of *REG1* function would overcome this deficit as it did for *glc7-1* cells (Figure 3). When DH11 (*reg1::URA3*) was crossed with EG328-2D (*gac1::LEU2*), the resulting tetrads exhibited a $3^+:1^-$ segregation ratio for glycogen storage for most tetrads, suggesting that the *reg1* mutation suppresses the *gac1* glycogen-deficient phenotype. All the spore clones with defective glycogen accumulation were Ura^- and Leu^+, whereas all Ura^+ clones overaccumulated glycogen. Thus, the positive stimulus for glycogen storage accruing from the loss of *REG1* does not require the presence of Gac1p.

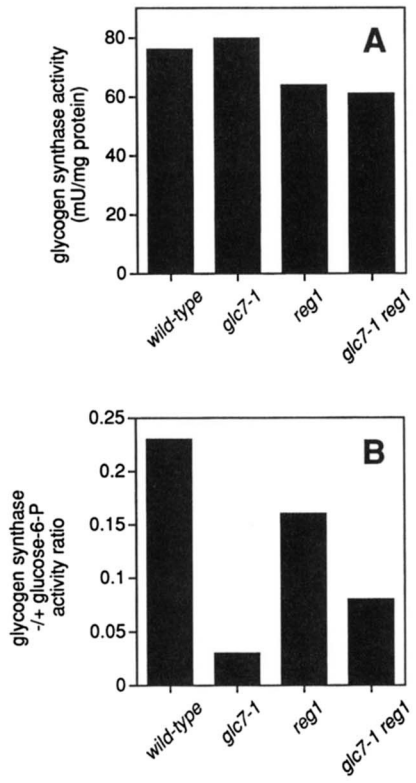

FIGURE 4.—Effect of *REG1* deletion of glycogen synthase activity. Glycogen synthase total activity (A) or activity ratio (B) were measured in extracts of the indicated cells grown to stationary phase. A representative experiment is shown.

Interrelationship between *REG1* and *SNF1* in controlling glycogen accumulation: *REG1* has been proposed to be upstream of *SNF1* in the glucose repression pathway (ENTIAN and ZIMMERMANN 1982; NEIGEBORN and CARLSON 1987; ERICKSON and JOHNSTON 1993, 1994). It was of interest to analyze the epistatic relationship of these two genes as regards glycogen accumulation. Tetrad analysis of a *snf1::LEU2 reg1::URA3* heterozygote indicated a segregation ratio of 2+:2− for the glycogen accumulation phenotype. The glycogen-deficient phenotype cosegregated with *LEU2* but not *URA3* (see also Figure 3). These results indicate that *snf1* mutations are epistatic to *reg1* mutations as regards glycogen accumulation, just as in the glucose repression pathway.

Since mutation of *REG1* prevents glucose repression of some genes (NIEDERACHER and ENTIAN 1987), it is possible that the mechanism of suppression of the *glc7-1* glycogen accumulation defect by *reg1* mutations is due to this effect on gene expression. This possibility seems unlikely for two reasons. First, growth of EG327-1D (*glc7-1*) on glycerol, conditions under which glucose repression is absent, did not restore glycogen storage (data not shown). Second, mutations in two other genes, *HXK2* (MA and BOTSTEIN 1986) and *MIG1* (NEHLIN *et al.* 1991), that are central components of the glucose repression pathway do not suppress the glycogen storage defect of *glc7-1* mutants (Figure 3). We conclude that the suppression of the *glc7-1* defect in glycogen accumulation by *reg1* is due to functions of *REG1* different from those implicated in glucose derepression.

The *glc7-1* mutation suppresses the slow growth and morphological defects of *reg1* cells: Mutations of *REG1* cause a reduced rate of growth (MATSUMOTO *et al.* 1983; TUNG *et al.* 1992). During tetrad analysis of diploid cells

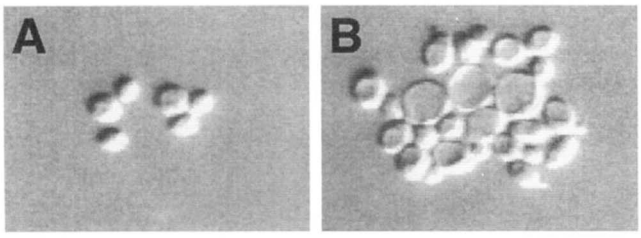

FIGURE 5.—Effect of *REG1* deletion on cell morphology. Wild-type (EG 3281-A) (A) or cells with *REG1* deleted (B) were grown in YPD liquid culture and examined microscopically.

heterozygous for *reg1* and *glc7-1,* we noticed that spores carrying the *reg1* single mutation hyperaccumulated glycogen and formed smaller colonies, whereas spores with the *reg1 glc7-1* double mutation formed normal-sized colonies. We confirmed that *reg1* cells grow significantly more slowly than wild-type cells, with doubling times of 2.3 and 1.6 hr, respectively. The slow growth of *reg1* mutants was partially suppressed by the *glc7-1* mutation (doubling time of 1.9 hr). The *reg1* cells were enlarged in both logarithmic and stationary phase, perhaps indicative of some defect in cell cycle progression (Figure 5). The *glc7-1* mutation suppressed this defect. It is also noteworthy that *gac1* gene disruption had no effect on either the slow growth or the cell morphology phenotype of *reg1* mutation.

Over-accumulation of glycogen has, in our experience, sometimes been associated with slower growth rates and so this property of *reg1* cells might be due to their glycogen hyperaccumulating phenotype. Suppression of slow-growth by *glc7-1* could then be explained by reduced glycogen storage in a *reg1 glc7-1* double mutant. However, we observed that the accumulation of glycogen was greatly reduced in a *reg1 gsy2* double mutant (DH21) without any increase in the growth rate relative to a *reg1* mutant (data not shown). We conclude, therefore, that the slow growth phenotype of *reg1* cells is not simply a consequence of glycogen hyperaccumulation.

***REG1* deletion does not rescue other phenotypes associated with *GLC7* muations:** The type I protein phosphatase in *S. cerevisiae* has been implicated in numerous cellular processes, including sporulation (CANNON *et al.* 1994) and cell cycle control (HISAMOTO *et al.* 1994; BLACK *et al.* 1995). It was of interest to know whether *reg1* could suppress these other phenotypes associated with mutations of *GLC7.* To analyze effects of *REG1* mutation on sporulation, diploids homozygous for *glc7-1* and *reg1* were made by crossing DH20 and DH12. No tetrads were detected by microscopic examination, suggesting that *reg1* does not suppress the sporulation deficiency caused by *glc7-1.* To test for suppression of the G2/M arrest of $glc7^{Y\text{-}170}$ defects by *reg1,* we crossed a *reg1* strain (DH12) to a $glc7^{Y\text{-}170}$ strain (DH9) and isolated independent *reg1* $glc7^{Y\text{-}170}$ strains. These double mutants were grown at 30° on synthetic complete plates to test for glycogen storage by iodine staining and on YPD plates at 14° to test for cold sensitivity. Although *reg1* partially restored glycogen accumulation in $glc7^{Y\text{-}170}$ (Figure 3), it failed to suppress the growth defect of this mutant at 14° (Figure 6). The cell morphology of the double mutant grown at 14° was also examined microscopically and was found to be similar to that associated with a $glc7^{Y\text{-}170}$ single mutant, namely, cells with large buds (HISAMOTO *et al.* 1994; data not shown).

DISCUSSION

The *REG1/HEX2* gene encodes a protein (NIEDERACHER and ENTIAN 1991) initially identified as being necessary for glucose repression of several genes (ZIMMERMANN and SCHEEL 1977; ENTIAN 1980; MATSUMOTO 1981, 1983). Recently, TU and CARLSON (1995) showed that Reg1p interacts physically with Glc7p, leading them to propose that Reg1p is a phosphatase regulatory subunit that targets Glc7p to proteins involved in the glucose repression pathway. TUNG *et al.* (1992) found that *REG1* is identical to *SRN1,* a gene identified as a second site-suppressor of *rna1* mutations that affect mRNA processing (PEARSON *et al.* 1982). *RNA1* has since been found to encode a GTPase-activating protein (GAP) specific for Gsp1p, the budding yeast homologue of the mammalian Ran/TC4 GTPase (BECKER *et al.* 1995). Our results implicate *REG1* in yet another process, glycogen accumulation, and provide evidence for a genetic interaction between the *REG1* gene and *GLC7,* the gene encoding the type 1 protein phosphatase catalytic subunit. Glycogen biosynthesis, which is closely linked to glucose availability, clearly shares some regulatory elements with glucose repression pathways, including *SNF1* (THOMPSON-JAEGER 1991; CANNON *et al.* 1994), *GLC7* (CANNON *et al.* 1994) and *REG1* (this work).

The concept has emerged from study of mammalian protein phosphatases of a common type 1 catalytic subunit that is directed to a wide range of cellular tasks by associated regulatory subunits (HUBBARD and COHEN 1993). A number of potential regulatory subunits have already been identified in *S. cerevisiae,* including Gac1p (FRANÇOIS *et al.* 1992), Glc8p (CANNON *et al.* 1994), Sds22p/Egp1p (HISAMOTO *et al.* 1995; MACKELVIE *et al.* 1995), and Pig1p (C. CHENG, D. HUANG and P. J. ROACH, unpublished data). The suggestion of STUART *et al.* (1994) that Gac1p targets Glc7p toward a function in glycogen metabolism is perhaps one of the clearest examples of this idea to date. The existence of multiple targetting subunits for Glc7p helps explain why different mutations within the *GLC7* gene can cause a variety of phenotypes. For example, *glc7-1* affects glycogen storage but not glucose repression, whereas the *glc7-T152K (cid1-226)* mutant is defective for glucose repression but close to normal for glycogen storage. The $glc7^{Y\text{-}170}$ allele confers cell cycle defects as well as reduced glycogen

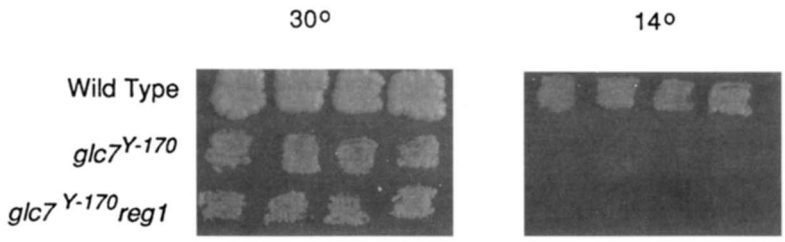

FIGURE 6.—Growth defects associated the *glc7*$^{Y\text{-}170}$ allele are not suppressed by deletion of *REG1*. Cells were grown on YPD plates at the indicated temperature.

deposition (HISAMOTO *et al.* 1994). The *glc7-1* mutation does influence growth since it partially suppresses the slow growth of *reg1* mutants, but this effect most likely reflects disruption of different Glc7p properties than those affected by *glc7*$^{Y\text{-}170}$. Such observations can be explained if subsets of Glc7p regulatory subunit interactions can be independently affected in different alleles of *glc7*.

Though glycogen accumulation and glucose repression are clearly intertwined, there is a degree of independence in the control of the two processes. For example, null mutations in *HXK2* or *MIG1*, both of which relieve glucose repression, do not suppress the glycogen-deficient phenotype of *glc7-1* and a *mig1* mutant has no glycogen accumulation phenotype (HARDY 1995). Similarly, growth of a *glc7-1* strain on glycerol, conditions of derepression, do not overcome the glycogen defect. In addition, we have searched for second-site suppressors of the glycogen-deficient phenotype of *snf1* cells (*ssg* mutants) and have found several mutant strains in which glycogen accumulation is restored without correcting defects linked to glucose repression (D. HUANG and P. J. ROACH unpublished results). Therefore, the downstream factors controlled by *SNF1* and *GLC7* must be at least partly nonoverlapping. This conclusion is consistent with the fact that *SNF1* and *GLC7* act antagonistically to each other in glucose repression whereas both promote glycogen deposition (THOMPSON-JAEGER *et al.* 1991; TU and CARLSON 1994).

Mechanistically, how does Reg1p influence glycogen metabolism, accounting both for the proposal that it is a phosphatase regulatory subunit and that its loss of function suppresses the glycogen defect in *glc7-1* mutants? Considerations of epistasis would formally place *REG1* either downstream of the defect in *glc7-1* or on a separate pathway. Though we cannot totally exclude the former possibility, we prefer the idea that Glc7p acts on a separate pathway, consistent with its proposed role as a glycogen synthase phosphatase. Gac1p.Glc7p may activate glycogen synthase by direct dephosphorylation, while Reg1p.Glc7p acts at some other, as yet unidentified, step to oppose glycogen synthesis. Another possibility considered by TU and CARLSON (1995) is based on the idea of a shared pool of Glc7p molecules interacting with Gac1p and Reg1p. One could hypothesize that loss of Reg1p might lead to a redistribution of Glc7p so as to favor formation of other complexes, including the Gac1p.Glc7p complex, and in this way promote glycogen accumulation. In a *glc7-1* mutant, increased levels of defective catalytic subunit caused by deletion of *REG1* might enable enough interaction with Gac1p to suppress the glycogen-deficient phenotype. However, such a mechanism cannot explain how a *reg1* mutation suppresses the glycogen deficiency of a *gac1* strain, unless there is another regulatory subunit capable of targeting to glycogen. Also, if interaction between Glc7p and Reg1p is impaired in *glc7-T152K* mutants, one would predict an increase in Gac1p.Glc7p formation, similar to that caused by deletion of *REG1*. In fact, *glc7-T152K* cells accumulate glycogen normally (TU and CARLSON 1994).

The relationship of these phosphatases and Snf1p in regulating glycogen metabolism is not clear. We had proposed previously that Snf1p controls the phosphorylation state of glycogen synthase, either inhibiting a protein kinase or activating a phosphatase (HARDY *et al.* 1994). How then does Snf1p relate to Reg1p in controlling glycogen synthesis? First, we have shown that *SNF1* is necessary for *REG1* control of glycogen accumulation since *SNF1* deletion is epistatic to *REG1* mutations as regards glycogen deposition. Indeed, the same epistasis is known to be true for glucose repression (ENTIAN and ZIMMERMANN 1982; NEIGEBORN and CARLSON 1987; ERICKSON and JOHNSTON 1993, 1994). Therefore, Reg1p is either a negative upstream regulator of Snf1p or on a separate pathway controling glycogen. The former model is difficult to sustain if Reg1p acts only as a phosphatase regulatory subunit, since disruption of the Reg1p-Glc7p interaction, as in *glc7-T152K* mutants, should also affect glycogen accumulation, whereas these mutants are normal for glycogen storage. Either Reg1p has some functions independent of Glc7p or else it acts on a separate pathway from Snf1p in controlling glycogen.

The expectation was that suppression of the glycogen storage defect in *glc7-1* mutants would correlate with restoration of glycogen synthase dephosphorylation, as evidenced by elevation of the activity ratio. Though the activity ratio of glycogen synthase in *glc7-1 reg1* double mutants was increased as compared to the low value of the *glc7-1* cell, the activity ratio in a *reg1* mutant, which hyperaccumulates glycogen, was not elevated. We consider two possible explanations. First, the activity ratio as measured *in vitro* may not be an adequate indicator of the effective activity of the enzyme *in vivo*. Thus, if measured under conditions more reflective of the *in*

vivo situation, glycogen synthase activity might have followed more closely the glycogen levels. Also, an enzyme activity measurement reflects an instantaneous value, whereas the level of a stored metabolite is an integration of past events. Second, regulation of multiple proteins might contribute to the control of glycogen biosynthesis. Mutation of *REG1* may influence glycogen synthase, as seen clearly in a *glc7-1* background, as well as other proteins involved in glycogen accumulation. Mutation of *REG1* in a wild-type background might influence glycogen synthesis via one of these other proteins. Included here might be the newly described Glg proteins (CHENG *et al.* 1995a), the branching enzyme encoded by *GLC3* (ROWEN *et al.* 1992; THON *et al.* 1992) or other as yet unidentified regulators of glycogen accumulation. In studies of metabolism, it is not uncommon for multiple steps to contribute to the control of a given pathway with different enzymes having greater or lesser impact depending on the circumstances.

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LITERATURE CITED

BECKER, J., F. MELCHIOR, V. GERKE, F. B. BISCHOFF, H. PONSTING *et al.*, 1995 *RNA1* enocdes a GTPase activating protein specific for Gsp1p, the Ran/TC4 homologue of Saccharomyces cerevisiae. J. Biol. Chem. **270:** 11860–11865.

BIRNBOIM, H. C., and J. DOLY, 1979 A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:** 1513–1523.

BLACK, S., P. D. ANDREWS, A. A. SNEDDON and M. J. R. STARK, 1995 A regulated MET3-GLC7 gene fusion provides evidence of a mitotic role for *Saccharomyces cerevisiae* protein phosphatase I. Yeast **11:** 747–759.

BURNS, N., B. GRIMWADE, P. B. ROSS-MACDONALD, E.-Y. CHOI, K. FINBERG *et al.*, 1994 Large-scale analysis of gene expression, protein localization, and gene disruption in *Saccharomyces cerevisiae.* Genes Dev. **8:** 1087–1105.

CANNON, J. F., and K. TATCHELL, 1987 Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cyclic AMP-dependent protein kinase. Mol. Cell. Biol. **7:** 2653–2663.

CANNON, J. F., J. R. PRINGLE, A. FIECHTER and M. KHALIL, 1994 Characterization of glycogen-deficient glc mutants of *Saccharomyces cerevisiae.* Genetics **136:** 485–503.

CARLSON, M., B. C. OSMOND and D. BOTSTEIN, 1981 Mutants of yeast defective in sucrose utilization. Genetics **98:** 25–43.

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.

CHENG, C., J. MU, I. FARKAS, D. HUANG, M. G. GOEBL *et al.*, 1995a Requirement of self-glucosylating initiator proteins, Glg1p and Glg2p, for glycogen accumulation in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **15:** 6632–6640.

CHUN, K. T., and GOEBL, M. G., 1996 The identification of transposon-tagged mutations in essential genes that affect cell morphology in *Saccharomyces cerevisiae.* Genetics **142:** 39–50.

CIRIACY, M., 1977 Isolation and characterization of yeast mutants defective in intermediary carbon metabolism and in carbon catabolite derepression. Mol. Gen. Genet. **154:** 213–220.

CLOTET, J., F. POSAS, G.-Z. HU, H. RONNE, and J. ARIÑO, 1995 Role of protein phosphatase 2A in the control of glycogen metabolism in yeast. Eur. J. Biochem. **229:** 207–214.

ENTIAN, K.-D., 1980 A defect in carbon catabolite repression associated with uncontrollable and excessive maltose uptake. Mol. Gen. Genet. **179:** 169–175.

ENTIAN, K.-D., and F. K. ZIMMERMANN, 1980 Glycolytic enzymes and intermediates in carbon catabolite repression mutants of *Saccharomyces cerevisiae.* Mol. Gen. Genet. **177:** 345–350.

ENTIAN, K.-D., and F. K. ZIMMERMANN, 1982 New genes involved in carbon catabolite repression and derepression in the yeast *Saccharomyces cerevisiae.* J. Bacteriol. **151:** 1123–1128.

ERICKSON, J. R., and M. JOHNSTON, 1993 Genetic and molecular characterization of *GAL83:* its interaction and similarity with other genes involved in glucose repression in *Saccharomyces cerevisiae.* Genetics **135:** 655–664.

ERICKSON, J. R., and M. JOHNSTON, 1994 Suppressors reveal two classes of glucose repression genes in the yeast *Saccharomyces cerevisiae.* Genetics **136:** 1271–1278.

FARKAS, I., T. A. HARDY, M. G. GOEBL and P. J. ROACH, 1991 Two glycogen synthase isoforms in *Saccharomyces cerevisiae* are coded by distinct genes that are differentially controlled. J. Biol. Chem. **266:** 15602–15607.

FENG, Z., S. F. WILSON, Z.-Y. PENG, K. SCHLENDER, E. M. REIMANN *et al.*, 1991 The yeast *GLC7* gene required for glycogen accumulation encodes a type I protein phosphatase. J. Biol. Chem. **266:** 23796–23801.

FRANÇOIS, J., M. E. VILLANUEVA and H.-G. HERS, 1988 The control of glycogen metabolism in yeast. 1. Interconversion *in vivo* of glycogen synthase and glycogen phosphorylase induced by glucose, a nitrogen source or uncouplers. Eur. J. Biochem. **174:** 551–559.

FRANÇOIS, J. M., S. THOMPSON-JAEGER, J. SKROCH, U. ZELLENKA, W. SPEVAK *et al.*, 1992 GAC1 may encode a regulatory subunit for protein phosphatase type I in *Saccharomyces cerevisiae.* EMBO J. **11:** 87–96.

GANCEDO, J. M., 1992 Carbon catabolite repression in yeast. Eur. J. Biochem. **206:** 297–313.

GUTHRIE, C., and G. R. FINK, 1991 Guide to yeast genetics and molecular biology. Methods Enzymol. **194.**

HARDY, T. A., 1996 The regulation of glycogen synthesis in *Saccharomyces cerevisiae.* Ph.D. Thesis, Indiana University, Indianapolis.

HARDY, T. A., and P. J. ROACH, 1993 Control of yeast glycogen synthase-2 by COOH-terminal phosphorylation. J. Biol. Chem. **268:** 23799–23805.

HARDY, T. A., D. HUANG and P. J. ROACH, 1994 Interactions between cAMP-dependent and SNF1 protein kinases in the control of glycogen accumulation in *Saccharomyces cerevisiae.* J. Biol. Chem. **269:** 27907–27913.

HISAMOTO, N., K. SUGIMOTO and K. MATSUMOTO, 1994 The Glc7 type I protein phophatase of *Saccharomyces cerevisiae* is required for cell cycle progression in G2/M. Mol. Cell. Biol. **14:** 3158–3165.

HISAMOTO N., D. L. FREDERICK, K. SUGIMOTO, K. TATCHELL and K. MATSUMOTO, 1995 The *EGP1* gene may be a positive regulator of protein phosphatase type 1 in the growth control of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **15:** 3767–3776.

HUBBARD, M. J., and P. COHEN, 1993 On target with new mechanism for the regulation of protein phosphorylation. Trend Biol. Sci. **18:** 172–177.

ITO, H., Y. FUKADA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

JOHNSTON, M., and M. CARLSON, 1992 Regulation of carbon and phosphate utilization, pp. 193–281 in *The Molecular and Cellular Biology of the Yeast Saccharomyces cerevisiae: Gene Expression,* Vol. II, edited by E. W. JONES, J. R. PRINGLE and J. R. BROACH. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

LILLIE, S. H., and J. R. PRINGLE, 1980 Reserve carbohydrate metabolism in *Saccharomyces cerevisiae:* responses to nutrient limitation. J. Bacteriol. **143:** 1384–1394.

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.

MACKELVIE, S. H., P. D. ANDREWS and M. J. STARK, 1995 The *Saccharomyces cerevisiae* gene SDS22 encodes a potential regulator of the mitotic function of yeast type 1 protein phosphatase. Mol. Cell. Biol. **15:** 3777–3785.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Clon-*

ing: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MATSUMOTO, K., A. TOH-E and Y. OSHIMA, 1981 Isolation and characterization of dominant mutations resistant to catabolite repression of galactokinase synthesis in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **1:** 83–93.

MATSUMOTO, K., T. YOSHIMATSU and Y. OSHIMA, 1983 Recessive mutations conferring resistance to catabolite repression of galactokinase synthesis in *Saccharomyces cerevisiae.* J. Bacteriol. **153:** 1405–1414.

NEHLIN, J. O., M. CARLBERG and H. RONNE, 1991 Control of yeast GAL genes by MIG1 repressor: a transcriptional cascade in the glucose response. EMBO J. **10:** 3373–3377.

NEIGEBORN, L., and M. CARLSON, 1987 Mutations causing constitutive invertase synthesis in yeast: genetic interactions with snf mutations. Genetics **115:** 247–253.

NIEDERACHER, D., and K.-D. ENTIAN, 1987 Isolation and characterization of the regulatory *HEX2* gene necessary for glucose repression in yeast. Mol. Gen. Genet. **206:** 505–509.

NIEDERACHER, D., and K.-D. ENTIAN, 1991 Characterization of HEX2 protein, a negative regulator element necessary for glucose repression in yeast. Eur. J. Biochem. **200:** 311–319.

PEARSON, W. R., and D. J. LIPMAN, 1988 Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:** 2444–2448.

PEARSON, N. J., P. C. THORBURN and J. E. HABER, 1982 A suppressor of temperature-sensitive rna mutations that affect mRNA metabolism in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **2:** 571–577.

PENG, Z.-Y., R. J. TRUMBLY and E. M. REIMANN, 1990 Purification and characterization of glycogen synthase from a glycogen deficient strain of *Sacchromyces cerevisiae.* J. Biol. Chem. **265:** 13871–13877.

POSAS, F., J. CLOTET and J. ARIÑO, 1991 *Saccharomyces cerevisiae* gene *SIT4* is involved in the control of glycogen metabolism. FEBS Lett. **279:** 341–345.

ROTHMAN-DENES, L. B., and E. CABIB, 1970 Two forms of yeast glycogen synthase and their role in glycogen accumulation. Proc. Natl. Acad. Sci. USA **66:** 967–974.

ROTHMAN-DENES, L. B., and E. CABIB, 1971 Glucose 6-phosphate dependent and independent forms of yeast glycogen synthetase. Their properties and interconversions. Biochemistry **10:** 1236–1242.

ROWEN, D. W., M. MEINKE and D. C. LAPORTE, 1992 *GLC3* and *GHA1* of *Sacchromyces cerevisiae* are allelic and encode the glycogen branching enzyme. Mol. Cell. Biol. **12:** 22–29.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SEIFERT, H. S., E. Y. CHEN, M. SO and F. HEFFRON, 1986 Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **83:** 163–168.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae.* Genetics **122:** 19–27.

STUART, J. S., D. L. FREDERICK, C. M. VARNER and K. TATCHELL, 1994 The mutant type I protein phosphatase encoded by *glc7–1* from *Saccharomyces cerevisiae* fails to interact productively with the *GAC1* encoded regulatory subunit. Mol. Cell. Biol. **14:** 896–905.

THOMAS, J. A., SCHLENDER, K. K. and LARNER, J., 1968 A rapid assay for UDPglucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. Anal. Biochem. **25:** 486–499.

THOMPSON-JAEGER, S., J. FRANÇOIS, J. P. GAUGHRAN and K. TATCHELL, 1991 Deletion of *SNF1* affects the nutrient response of yeast and resembles mutations which activate the adenylate cyclase pathway. Genetics **129:** 697–706.

THON, V. J., C. VIGNERON-LESENS, T. MARIANNE-PEPIN, J. MONTRENIL, A. DECQ *et al.,* 1992 Coordinate regulation of glycogen metabolism in the yeast *Saccharomyces cerevisiae.* J. Biol. Chem. **267:** 15224–15228.

TRUMBLY, R. J., 1992 Glucose repression in the yeast *Saccharomyces cerevisiae.* Mol. Microbiol. **6:** 15–21.

TU, J., and M. CARLSON, 1994 The GLC7 type I protein phosphatase is required for glucose repression in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **14:** 6789–6796.

TU, J., and M. CARLSON, 1995 REG1 binds to protein phosphatase type 1 and regulates glucose repression in *Saccharomyces cerevisiae.* EMBO J. **14:** 5939–5946.

TUNG, K.-S., L. L. NORBECK, S. L. NOLAN, N. S. ATKINSON and A. K. HOPPER, 1992 *SRN1,* a yeast gene involved in RNA processing, is identical to *HEX2/REG1,* a negative regulator in glucose repression. Mol. Cell. Biol. **12:** 2673–2680.

ZIMMERMANN, F. K., and I. SCHEEL, 1977 Mutants of *Saccharomyces cerevisiae* resistant to carbon catabolite repression. Mol. Gen. Genet. **154:** 75–82.

ZIMMERMANN, F. K., I. KAUFMANN, H. RASEMBERG and P. HAUSMANN, 1977 Genetics of carbon catabolite repression in *Saccharomyces cerevisiae:* genes involved in the derepression process. Mol. Gen. Genet. **154:** 95–103.

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