MOLECULAR AND CELLULAR BIOLOGY, May 1995, p. 2646–2653 Vol. 15, No. 5
0270-7306/95/$04.00+0

The *GCR1* Requirement for Yeast Glycolytic Gene Expression Is Suppressed by Dominant Mutations in the *SGC1* Gene, Which Encodes a Novel Basic-Helix-Loop-Helix Protein

KAYOKO NISHI,[1] CHANG SEO PARK,[1] ALAN E. PEPPER,[1] GREG EICHINGER,[2] MICHAEL A. INNIS,[2] AND MICHAEL J. HOLLAND[1]*

Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616-8635,[1] and Cetus Corporation, Emeryville, California 94608[2]

Received 4 August 1994/Returned for modification 12 September 1994/Accepted 3 February 1995

The *GCR1* gene product is required for maximal transcription of yeast glycolytic genes and for growth of yeast strains in media containing glucose as a carbon source. Dominant mutations in two genes, *SGC1* and *SGC2*, as well as recessive mutations in the *SGC5* gene were identified as suppressors of the growth and transcriptional defects caused by a *gcr1* null mutation. The wild-type and mutant alleles of *SGC1* were cloned and sequenced. The predicted amino acid sequence of the *SGC1* gene product includes a region with substantial similarity to the basic-helix-loop-helix domain of the Myc family of DNA-binding proteins. The *SGC1-1* dominant mutant allele contained a substitution of glutamine for a highly conserved glutamic acid residue within the putative basic DNA binding domain. A second dominant mutant, *SGC1-2*, contained a valine-for-isoleucine substitution within the putative loop region. The *SGC1-1* dominant mutant suppressed the *GCR1* requirement for enolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, and pyruvate kinase gene expression. Expression of the yeast enolase genes was reduced three- to fivefold in strains carrying an *sgc1* null mutation, demonstrating that *SGC1* is required for maximal enolase gene expression. Expression of the enolase genes in strains carrying *gcr1* and *sgc1* double null mutations was substantially less than observed for strains carrying either null mutation alone, suggesting that *GCR1* and *SGC1* function on parallel pathways to activate yeast glycolytic gene expression.

GCR1 is a positive regulatory gene required for maximal expression of many yeast glycolytic genes (14). The steady-state levels of glycolytic mRNAs are reduced 5- to 50-fold in strains carrying a *gcr1* null mutation, indicating that the *GCR1* gene product affects transcription of glycolytic genes (23). Analysis of total yeast cellular proteins by two-dimensional electrophoresis showed that the steady-state levels of a large number of yeast proteins are affected by a *gcr1* null mutation, suggesting that *GCR1* encodes a global regulator of yeast gene expression (23). GCR1 protein binds to a CTTCC sequence motif (3, 24) located within the upstream regulatory sequences of glycolytic genes that are regulated by *GCR1*. The importance of GCR1 binding sites for activation of transcription has been directly demonstrated. Specifically, two GCR1 binding sites within the upstream activation sequences (UASs) of the yeast *TPI1* gene (31) and a GCR1 binding site within the UASs of the yeast *ENO2* gene (36) are required for *GCR1*-dependent activation of transcription.

GCR1 binding sites are usually located adjacent to binding sites for the abundant DNA-binding protein RAP1. For the *ENO1*, *ENO2*, *TPI1*, *PYK1*, *PGK1*, and *TDH3* genes, the CTTCC sequence motif is required along with an adjacent RAP1 binding site for maximal UAS activity (5, 8, 13, 31, 36). These observations suggest that GCR1 and RAP1 proteins cooperate to activate gene expression. The *GCR2* gene product is also required for maximal expression of yeast glycolytic genes (33). Using a two-hybrid assay, Uemura and Jigami obtained evidence that the GCR1 and GCR2 proteins interact with each other, suggesting that a GCR1-GCR2 complex may be responsible for transcriptional activation (34).

* Corresponding author. Phone: (916) 752-8378. Fax: (916) 752-3516.

To further define the mechanism of *GCR1*-dependent activation of gene expression, suppressors of the requirement for *GCR1* were isolated and characterized. We show here that dominant, gain-of-function mutations in a gene designated *SGC1* (suppressor of *gcr1*) suppressed the growth, transcription, and sporulation defects caused by a *gcr1* null mutation. We also show that the *SGC1* gene product contains a region of strong amino acid sequence similarity to the basic-helix-loop-helix domain of the Myc family of DNA-binding proteins and that two dominant *SGC1* mutations reside within the basic-helix-loop-helix domain.

MATERIALS AND METHODS

Strains and growth conditions. All yeast strains used are listed in Table 1. *Saccharomyces cerevisiae* S173-6B and S173-29A were provided by F. Sherman, University of Rochester, Rochester, N.Y. Other yeast strains used for the analyses described here were constructed as described below. Two *gcr1* null mutations were used in the analyses. The *gcr1-1* mutation (14) contains a single base pair insertion mutation within the *GCR1* coding sequences (23). A second *gcr1* null mutation, *gcr1*Δ::*URA3*, contains a deletion of 90% of the *GCR1* coding sequences and an insertion of a functional *URA3* gene at the site of the deletion mutation (23). Both null mutations are stable and exhibit the same phenotypes (23).

YPd medium contained 1% yeast extract, 2% Bacto Peptone (Difco Laboratories), and 2% glucose. YPgl was identical to YPd except that 2% glycerol and 2% lactate were used as carbon sources. YNBd medium contained 0.67% yeast nitrogen base without amino acids (Difco Laboratories) and 2% glucose, supplemented with 20 μg of amino acids per ml as indicated. YNBgl medium was identical to YNBd except that 2% glycerol and 2% lactate were used as carbon sources. Yeast strains were grown at 30°C and harvested in early log growth phase (A_{660} = 0.5).

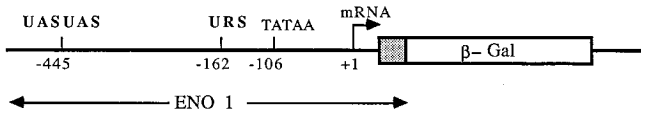
Diploid cells were generated by mating of haploid strains of opposite mating types and selected on plates by using complementing auxotrophic markers or by microdissection of zygotes. Sporulation of diploid strains was performed by patching cells on agar plates containing 1% potassium acetate. Yeast transformations were performed by the alkaline cation procedure described by Ito et al. (25).

TABLE 1. Strains used in this study

Strain	Genotype	Source
S173-6B	**a** *GCR1 SGC1 leu2-3 leu2-112 his3-1 trp1-289 ura3-52*	F. Sherman
S173-29A	α *GCR1 SGC1 ade2 leu2-3 leu2-112 his3-1 trp1-289*	F. Sherman
BK11	**a** *GCR1 SGC1 his3-1 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
NPT7	**a** *gcr1*Δ::*URA3 SGC1 leu2-3 leu2-112 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
NPT13	α *gcr1*Δ::*URA3 SGC1 ade2 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
TY100	**a** *gcr1-1 SGC1 eno1*Δ::*URA3 leu2-112 his3-1 trp1-289 ura3-52*	T. Yokoi
TY11A-1	**a** *gcr1-1 SGC1 leu2-3 leu2-112 his3-1 trp1-289 ura3-52*	T. Yokoi
TY690	α *gcr1*Δ::*URA3 SGC1 ade2 leu2-3 leu2-112 his3-1 trp1-289 ura3-52*	T. Yokoi
KSM11-74	**a** *gcr1*Δ::*URA3 SGC1-1 leu2-3 leu2-112 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
KSM13-2	α *gcr1*Δ::*URA3 SGC1-2 ade2 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
KSM13-110	**a** *gcr1*Δ::*URA3 SGC2-1 ade2 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
KSM8-42	α *gcr1*Δ::*URA3 sgc3-1 ade2 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
KSM108-42	α *gcr1*Δ::*URA3 sgc4-1 ade2 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
KSM4-22	**a** *gcr1*Δ::*URA3 sgc5-1 leu2-3 leu2-112 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
CPY3	**a** *gcr1-1 SGC1-1 leu2-3 leu2-112 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
CPY5	**a** *GCR1 SGC1-1 leu2-3 leu2-112 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
CPY9	α *gcr1-1 SGC1-2 ade2 trp1-289 ura3-52 ENO1*::pENOlac-2	This work
CPY15	**a** *gcr1-1 sgcl*Δ::*HIS3 leu2-3 leu2-112 his3-1 trp1-289 ura3-52*	This work
CPY17	**a** *GCR1 sgc1*Δ::*HIS3 leu2-3 leu2-112 his3-1 trp1-289 ura3-52*	This work
CPY1-1A	α *GCR1 SGC1 leu2-3 leu2-112 his3-1 trp1-289 ura3-52*	This work
CPY18-5B	α *GCR1 SGC1-1 leu2-3 leu2-112 his3-1 trp1-289 ura3-52*	This work

The parental strains NTP7 and NTP13 were constructed as follows. Strain BK11 was generated by transformation of strain S173-6B with a DNA fragment containing a functional yeast *LEU2* gene followed by transformation with the integration plasmid pENOlac-2 (described below). Transformants carrying pENOlac-2 integrated at the *ENO1* locus of strain BK11 were identified by Southern blotting analysis. This latter strain was crossed with strain TY690 and sporulated. Haploid strains NTP7 and NPT13 were identified after tetrad analysis.

Plasmid construction. Plasmid pENOlac-2 was constructed from the yeast integrating vector peno46/*HIS3*, which carries the complete *ENO1* structural gene and a selectable yeast *HIS3* gene (29). Plasmid peno46/*HIS3* was digested with *Hin*dIII to remove *ENO1* coding sequences extending from codon 45 to the translational termination codon. *Hin*dIII cohesive termini were filled in with the Klenow fragment of DNA polymerase I. A 3.4-kb *Bam*HI-*Pvu*I fragment from plasmid pMC1403 (12), containing the *Escherichia coli lacZ* coding sequence, was treated with the Klenow fragment of DNA polymerase I and ligated into the *ENO1* coding sequence of *Hin*dIII-digested peno46/*HIS3* to generate plasmid pENOlac-2. The ligation junction between the blunt-end *Bam*HI site in *lacZ* and the *Hin*dIII site at codon 45 of *ENO1* created an in-frame translational fusion between the first 45 codons of *ENO1* and the *lacZ* structural gene (Fig. 1). Plasmid pENOlac-2 was integrated at the *ENO1* locus in strains BK11, NPT7, and NPT13. Expression of the *ENO1-lacZ* gene fusion in strains BK11 (*GCR1*) and NPT13 (*gcr1*Δ::*URA3*) grown in YNB medium containing 2% glucose was evaluated by using the *o*-nitrophenylgalactopyranoside (ONPG) β-galactosidase assay (29). Expression was 7.5-fold lower in strain NPT13 than in strain BK11 (Fig. 1).



Strain	gcr allele	Growth phenotype in glucose medium	β-Galactosidase activity (units)
BK11	*GCR1*	+	2445 ± 950
NPT13	*gcr1 Δ ::URA3*	–	325 ± 61

FIG. 1. Expression of an *ENO1-lacZ* gene fusion in strains carrying a wild-type *GCR1* gene or a *gcr1*Δ::*URA3* null mutation. The 5′ regulatory sequences and the first 45 codons of the yeast *ENO1* gene were fused in frame to the *E. coli lacZ* gene on the integration plasmid pENOlac-2 as described in Materials and Methods. This plasmid was integrated at the *ENO1* locus in strain BK11 carrying a wild-type *GCR1* gene and strain NPT13 carrying a *gcr1* null mutation. β-Galactosidase (β-Gal) activity (micromoles of ONPG consumed per minute per milligram of protein) was measured in whole-cell extracts prepared from cells grown in YNB medium containing 2% glucose as previously described (6). URS, upstream repression sequence.

Mutagenesis and isolation of *gcr1* suppressor mutants. The parental strains NPT7 and NTP13 were mutagenized to 50 and 80% lethality with ethyl methanesulfonate (EMS) by standard methods (26). Approximately 10^7 viable cells from each mutagenesis were plated on YPd selective medium. The recovery rate of glucose-positive colonies was 0.7×10^{-5} to 0.3×10^{-5}. Glucose-positive colonies were replica plated onto nitrocellulose filters. Immobilized cells were permeabilized by immersing the filter in liquid nitrogen. β-Galactosidase activity was visualized by using an in situ X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) assay (6). X-Gal-positive colonies were verified by using the ONPG β-galactosidase assay (29). The recovery rate of glucose-positive, X-Gal-positive mutants was approximately 8.8×10^{-7}.

Mutants that failed to mate, sporulate, or grow on nonfermentable carbon sources were discarded. Glucose-positive, X-Gal-positive mutants were mated to parental strain NTP7 or NPT13. Five dominant mutants and 13 recessive mutants were identified and further analyzed. These diploid strains were sporulated and subjected to tetrad analysis. Haploids (**a** and α) carrying each of the mutations were identified and used for complementation analysis. For the recessive mutants, pairwise crosses were performed with all of the mutants. The resulting diploid strains were then tested for growth in a medium containing glucose and for elevated β-galactosidase activity. The 13 recessive mutants fell into three complementation groups designated *sgc3*, *sgc4*, and *sgc5*. For the dominant mutants, pairwise crosses were performed with all of the mutants. The diploids were sporulated and subjected to tetrad analysis. Spores were then tested for the ability to grow in a medium containing glucose and for elevated β-galactosidase activity. The five dominant mutants fell into two complementation groups designated *SGC1* and *SGC2*. To test complementation among the dominant and recessive mutants, haploid strains carrying *SGC1* or *SGC2* dominant mutations were crossed with haploid strains carrying *sgc3*, *sgc4*, or *sgc5* recessive mutations, sporulated, and subjected to tetrad analysis. This analysis showed that neither of the dominant mutations were alleles of the recessive mutations. Representative haploid strains from each complementation group were backcrossed twice with strain NPT7 or NPT13 and subjected to tetrad analysis. In all cases, the glucose-positive X-Gal-positive phenotype cosegregated as a single mutation. Haploid strains carrying the *SGC1-1*, *SGC2-1*, *sgc3-1*, *sgc4-1*, and *sgc5-1* mutations were designated KSM11-74, KSM13-110, KSM8-42, KSM108-42, and KSM4-22, respectively. The growth rates of these latter strains as well as strains BK11 and NPT13 were determined in YP medium containing 2% glucose, 1% Casamino Acids, and 20 μg of required amino acids and uracil per ml.

Strain CPY3 was generated by transformation of strain KSM11-74 with a *Sal*I fragment containing the *gcr1-1* null mutation. Strain CPY5 was generated by transformation of strain KSM11-74 with a *Sal*I fragment containing the wild-type *GCR1* gene. In each case, replacement of the *gcr1*Δ::*URA3* gene disruption in strain KSM11-74 with DNA fragments containing either the *gcr1-1* or *GCR1* gene was selected on the basis of resistance to fluoro-orotic acid (5-FOA). Strain CPY18-5B was derived from a cross between strains CPY1-1A and CPY3.

Isolation of the *SGC1* structural gene. Chromosomal DNA was isolated from strain KSM11-74 carrying the *SGC1-1* dominant mutation. The DNA was partially digested with *Sau*3A and size fractionated by sucrose gradient density

centrifugation. *Sau*3A fragments (4 to 7 kb) were ligated into the *Bam*HI cloning site of plasmid pCZTLC1, a pBR322-based plasmid vector containing a selectable yeast *LEU2* gene, a *TRP1* autonomous replication sequence, and the centromere from chromosome 11. Yeast strain NPT7 was transformed with this gene library. Transformants were selected for leucine prototrophy and the ability to grow on plates containing glucose as a carbon source. Leu^+ glucose-positive transformants were screened for elevated β-galactosidase activity by using the in situ X-Gal assay described above. Two independent transformants exhibiting the *SGC1-1* dominant phenotype were recovered. Plasmid DNAs, designated pSGC1-1A and pSGC1-1B, were isolated from these transformants. Yeast strain NTP7 was retransformed with these plasmids to confirm the plasmid-dependent glucose-positive X-Gal-positive phenotype. Restriction endonuclease cleavage maps of plasmids pSGC1-1A and pSGC1-1B were generated by standard methods.

The wild-type *SGC1* gene as well as genes corresponding to the *SGC1-2*, *SGC1-3*, and *SGC1-4* dominant mutations were isolated by plasmid gap repair. Plasmid pSGC1-1A was digested with *Xho*I to remove sequences that included the entire *SGC1-1* gene. Purified gapped plasmid DNA was used to transform yeast strain NPT7 as well as strains carrying the *SGC1-2*, *SGC1-3*, and *SGC1-4* mutations to leucine prototrophy. Plasmid DNA was recovered from Leu^+ transformants and subjected to restriction endonuclease mapping to verify gap repair. Plasmids carrying the dominant mutant alleles conferred the glucose-positive X-Gal-positive phenotype on strain NPT7. The nucleotide sequences of the wild-type *SGC1* gene and the *SGC1-1*, *SGC1-2*, *SGC1-3*, and *SGC1-4* mutant alleles were determined by the dideoxynucleotide sequencing method using oligonucleotide primers (United States Biochemicals).

Construction and characterization of strains carrying an *sgc1* null mutation. To construct an *sgc1* null mutation, the DNA sequence extending from an *Sst*I site located upstream from the *SGC1* gene to an *Xho*I site located downstream from the *SGC1* gene was excised from plasmid pSGC1-1A and subcloned into plasmid pUC18. This latter plasmid, designated pSG62, was digested with *Bgl*II and *Nco*I to remove *SGC1* coding sequences extending from codons 11 to 208, filled in with the Klenow fragment of DNA polymerase I, and religated in the presence of *Bam*HI linker DNA to create a unique *Bam*HI site. A *Bam*HI fragment containing a functional yeast *HIS3* gene was then ligated into the novel *Bam*HI site. This latter plasmid was digested with *Sst*I and *Sca*I, and a fragment containing the *sgc1*Δ::*HIS3* deletion/insertion mutation was isolated and used to transform a diploid strain derived by mating strains S173-6B and TY690 to histidine prototrophy. Chromosomal DNA was isolated from 12 His^+ transformants, digested with *Bam*HI, and subjected to Southern blotting analysis using a hybridization probe corresponding to *SGC1* coding sequences. Five of the transformants were heterozygous for the *sgc1*Δ::*HIS3* gene disruption. These diploid strains were sporulated and subjected to tetrad analysis to recover strain CPY17 carrying the *sgc1*Δ::*HIS3* gene disruption. Strain CPY15 carrying the *sgc1*Δ::*HIS3* gene disruption and a *gcr1-1* null mutation was generated by transforming strain TY11A-1 with the *Sst*I-*Sca*I fragment containing the *sgc1*Δ::*HIS3* deletion/insertion mutation.

To generate a diploid strain homozygous for the *sgc1* null mutation, yeast strain CPY17 carrying the *sgc1*Δ::*HIS3* null mutation was converted to a homozygous diploid strain by transformation with plasmid pBA134 (provided by Ira Herskowitz, University of California, San Francisco) containing a functional yeast *HO* gene and a selectable yeast *URA3* gene. Ectopic expression of the *HO* gene in a heterothallic yeast strain results in a mixed population of **a** and α haploid cells which form diploids in culture. Four putative diploids were plated on 5-FOA plates, and cells that had lost plasmid pBA134 were recovered. Diploid strains were generated in the same way with strain S173-6B, which carries a wild-type *SGC1* gene. Diploids derived from CPY17 and S173-6B were tested for the ability to sporulate. S173-6B diploids sporulated and yielded viable spores. CPY17 diploids failed to sporulate. Strain CPY1-1A was recovered after tetrad analysis of spores derived from S173-6B diploids.

Northern (RNA) blot analysis. Total yeast cellular RNA was isolated by glass bead disruption of cells isolated from 5-ml log-phase cultures (A_{660} = 0.6) grown in YNBd or YNBgl medium. Approximately 5 μg of total cellular RNA was electrophoresed in 1.2% formaldehyde agarose gels and transferred to nylon membranes. Hybridizations and washes of the Nytran membranes were performed according to the procedure recommended by manufacturer (Schleicher & Schuell) for oligonucleotides. Synthetic oligonucleotide hybridization probes were oligo-ENO1-1 (3′ CTTTTGGATCGATAAAAAGTATTTTTTGGTT CGTTGACGAATAGTTGTG 5′), a 49-mer complementary to nucleotides +1 to +23 of the *ENO1* transcript (the remaining sequences in this probe are complementary to *ENO1* sequences located upstream from the transcriptional initiation site) (16); oligo-ENO2-1 (3′ GGTCGTTGATTATGATATTGTATG TTATTAT 5′), a 32-mer complementary to nucleotides +5 to +36 of *ENO2* mRNA (15); oligo-PGK1-1 (3′ CCAGCCAAAGCTGAACGGTGTTGCACGA CGGCCAA 5′) and oligo-PGK1-2 (3′ GCAGTAGTAACCACCACCACTGT GACGGTGACAGC 5′), two 35-mers complementary to yeast *PGK1* mRNA (20); oligo-PGM1 (3′ CTGTGCCAGTTAGGCTTACCTTGCTTTTCT), a 30-mer complementary to yeast *PGM1* mRNA (30); oligo-PYK1 (3′ CTTCTTA ACATGGGTCCATCTGGTAACCGG 5′), a 30-mer complementary to yeast *PYK1* mRNA (9); oligo-TPI1-1 (3′ TCTTCGGTGTTCAGTGACAGCCACGA GTTTTGCGG 5′) and oligo-TPI1-2 (3′ AACCGAAGGTTCAACCCACTGTT CCGACGGTCGCT 5′), two 35-mers complementary to yeast *TPI1* mRNA (1); oligo-SSA1-1 (3′ TCATTAGTTCATAATGTTCTTTGT 5′), a 24-mer complementary to *SSA1* mRNA (32); and oligo-ACT-1 (3′ GCCAAGACCATACAC ATTTCGGCCAAAACG 5′), a 30-mer complementary to yeast actin mRNA (19). Oligonucleotides were 5′ labeled with T4 polynucleotide kinase and [^{32}P] ATP (3,000 Ci/mmol; Amersham).

SGC1 mRNA was detected with nick-translated hybridization probes corresponding to the 1.3-kb *Sau*3A-*Pst*I fragment, the 1.2-kb *Pst*I-*Sst*I fragment, and the 2.6-kb *Sst*I-*Sau*3A fragment derived from plasmid pSGC1-1A. Glyceraldehyde-3-phosphate dehydrogenase mRNA was detected with a nick-translated hybridization probe corresponding to the 2.1-kb *Hin*dIII fragment from plasmid p*gap*491 (21) which includes the entire coding region from the glyceraldehyde-3-phosphate dehydrogenase gene *TDH3*.

The levels of mRNAs, detected after Northern blotting analysis, were quantitated with a scanning laser densitometer (Molecular Dynamics model 300A). mRNA levels were normalized to the level for yeast actin mRNA, which served as an internal control.

Western blot (immunoblot) analysis. Expression of *ENO1* and *ENO2* was measured by Western blotting analysis using a yeast enolase polyclonal antibody as previously described (15). Whole-cell extracts were prepared by glass bead disruption of cells isolated from 5-ml log-phase cultures grown in YNBgl or YNBd (A_{660} = 0.6). The secondary antibody was peroxidase-conjugated goat anti-rabbit immunoglobulin G (Calbiochem), which was detected by the enhanced chemiluminescence method as described by the manufacturer (Amersham). The levels of enolase polypeptides were quantitated with a scanning laser densitometer (Molecular Dynamics model 300A).

RESULTS

Isolation of suppressors of a *gcr1* null mutation. We showed previously that a small deletion mutation that removed an ABF1 binding site within the 5′ regulatory sequences of the enolase gene *ENO2* rendered *ENO2* expression independent of the *GCR1* gene product (7). This observation raised the possibility that certain *trans*-acting mutations also suppress the *GCR1* requirement for glycolytic gene expression. Strains carrying a *gcr1* null mutation are viable but display three distinct phenotypes. First, expression of many glycolytic genes including the two yeast enolase genes *ENO1* and *ENO2* is reduced up to 50-fold in cells grown in a medium containing glycerol plus lactate and approximately 10-fold in cells grown in a medium containing glucose (2, 14, 23). Second, *gcr1* strains grow poorly in a medium containing glucose but grow at near wild-type rates in a medium containing glycerol plus lactate (14). Finally, *gcr1* homozygous diploids fail to sporulate (37). To isolate suppressor mutants, strains NPT13 and NPT7 containing a *gcr1* null mutation and an *ENO1-lacZ* gene fusion integrated at the *ENO1* locus were mutagenized with EMS and selected for growth on plates containing YP medium and 2% glucose. Glucose-positive colonies (391 mutants) were screened for elevated expression of the *ENO1-lacZ* gene fusion by using a colony X-Gal assay. Approximately 7% (27 mutants) of the glucose-positive colonies exhibited elevated β-galactosidase activity compared with strains NPT13 and NPT7. Eighteen of these latter mutants which were able to mate, sporulate, and grow on nonfermentable carbon sources were further analyzed. Five of the mutants were dominant over wild type, and 13 of the mutants were recessive to wild type. These mutants were placed into complementation groups as described in Materials and Methods. The 18 mutants fell into five complementation groups (Table 2). Representative mutants from each complementation group were backcrossed twice to the unmutagenized parental strains and subjected to tetrad analysis. For each mutant, the glucose-positive X-Gal-positive phenotype segregated as a single mutation. These latter alleles, designated *SGC1-1*, *SGC2-1*, *sgc3-1*, *sgc4-1*, and *sgc5-1*, were used for the analyses described below.

Properties of the suppressor mutant strains. Growth rates were determined for representative mutant strains grown in a complex medium containing glucose (see Materials and Methods). As expected, all of the mutant strains grew more rapidly

TABLE 2. Complementation analysis of mutations that suppress a *gcr1* null mutation

Complementation group	Dominant/recessive	No. of alleles
SGC1	Dominant	4
SGC2	Dominant	1
sgc3	Recessive	6
sgc4	Recessive	5
sgc5	Recessive	2

TABLE 3. Doubling times of strains carrying suppressors of a *gcr1* null mutation and grown on YP containing glucose

Strain	Genotype	Doubling time (h)
KSM11-74	*gcr1*Δ::*URA3 SGC1-1*	2.2
KSM13-110	*gcr1*Δ::*URA3 SGC2-1*	2.7
KSM8-42	*gcr1*Δ::*URA3 sgc3-1*	3.5
KSM108-42	*gcr1*Δ::*URA3 scg4-1*	4.3
KSM4-22	*gcr1*Δ::*URA3 sgc5-1*	3.3
BK11	*GCR1 SGC1*	1.5
NPT13	*gcr1*Δ::*URA3 SGC1*	>8.0

than the parental strain NPT13 (Table 3). The highest growth rate was observed for a strain carrying the *SGC1-1* mutation.

Expression of the yeast enolase genes in strains carrying a *gcr1* null mutation and each of the suppressor mutations was analyzed by Northern blotting using hybridization probes specific for *ENO1* and *ENO2* mRNAs, respectively (Fig. 2A). *ENO1* mRNA was elevated in strains carrying each of the suppressor mutations. Interestingly, the *ENO1* mRNA level observed for a strain carrying the *SGC1-1* mutation was approximately 10-fold higher than that observed for the control strain carrying a wild-type *GCR1* gene. The *ENO2* mRNA levels were elevated in strains carrying the *SGC1-1*, *SGC2-1*, and *sgc5-1* mutations but were not significantly elevated in strains carrying the *sgc3-1* or *sgc4-1* mutation.

Northern blotting analysis was also performed to evaluate the steady-state level of glyceraldehyde-3-phosphate dehydrogenase mRNA in strains carrying a *gcr1* null mutation and each of the *SGC1* mutations (Fig. 2A). Glyceraldehyde-3-phosphate dehydrogenase mRNA (23) and enzymatic activity (14) are reduced in strains carrying a *gcr1* null mutation. There are three glyceraldehyde-3-phosphate dehydrogenase structural genes (*TDH1*, *TDH2*, and *TDH3*) per haploid yeast genome (22). The hybridization probe used in this analysis cross-hybridizes with all three mRNAs; however, *TDH3* encodes 50 to 60% of the cellular glyceraldehyde-3-phosphate dehydrogenase enzymatic activity (27). Glyceraldehyde-3-phosphate dehydrogenase mRNA levels were elevated in *gcr1* strains carrying the *SGC1-1*, *SGC2-1*, or *sgc5-1* mutation but were not significantly increased in *gcr1* strains carrying the *sgc3-1* or *sgc4-1* mutation (Fig. 2A). These results and those described above for the enolase genes suggest that the *SGC1-1*, *SGC2-1*, and *sgc5-1* mutations are global suppressors of the growth and transcriptional defects caused by the *gcr1* null mutation. In contrast, the *sgc3-1* and *sgc4-1* mutations appear to affect only transcription of the *ENO1* gene.

The effect of the *SGC1-1* mutation on expression of four additional glycolytic genes was evaluated in a strain carrying a *gcr1* null mutation by Northern blotting analysis (Fig. 2B). Relative mRNA levels were determined by densitometry and normalized to the level of yeast actin mRNA, which is not regulated by *GCR1* or *SGC1*. The steady-state levels of mRNAs encoded by *PGK1* (3-phosphoglycerate kinase), *PGM1* (phosphoglycerate mutase), *PYK1* (pyruvate kinase), and *TPI1* (triose phosphate isomerase) were 10-, 50-, 12-, and 12-fold, respectively, lower in a strain carrying a *gcr1* null mutation than in a strain carrying a wild-type *GCR1* gene (Fig. 2B). The effects of the *gcr1* null mutation on expression of these genes determined by Northern blotting (Fig. 2B) were comparable to those determined by Clifton and Fraenkel (14) using enzymatic activity assays with extracts prepared from cells grown under similar growth conditions. The steady-state levels of mRNAs encoded by *PGK1*, *PGM1*, and *PYK1* were 4.5-, 6-, and 5-fold, respectively, higher in a *gcr1* strain carrying the *SGC1-1* dominant mutation than in the strain carrying the wild-type *SGC1* gene (Fig. 2B). In contrast, the steady-state level of *TPI1* mRNA increased less than twofold in a *gcr1* strain carrying the *SGC1-1* dominant mutation (Fig. 2B).

Finally, the sporulation efficiency of diploid strains that are homozygous for a *gcr1* null mutation and heterozygous for the *SGC1-1* or *SGC2-1* mutation was similar to that of a strain carrying wild-type *GCR1* alleles, demonstrating that these dominant mutations also suppress the sporulation deficiency caused by the *gcr1* null mutation.

Isolation of the wild-type and mutant alleles of the *SGC1* structural gene. The *SGC1-1* mutant allele was cloned by transformation of strain NPT7 with a centromere-based plasmid library of genomic DNA isolated from a strain carrying the *SGC1-1* dominant mutation. Transformants were selected for the plasmid *LEU2* marker and growth on plates containing YNB medium and 2% glucose. Glucose-positive transformants were screened for elevated synthesis of β-galactosidase activity from an *ENO1-lacZ* gene fusion integrated at the *ENO1* locus of strain NPT7. Two independent plasmids (pSGC1-1A and pSGC1-1B; Fig. 3A) containing the putative *SGC1-1* structural gene were isolated. Restriction endonuclease mapping re-

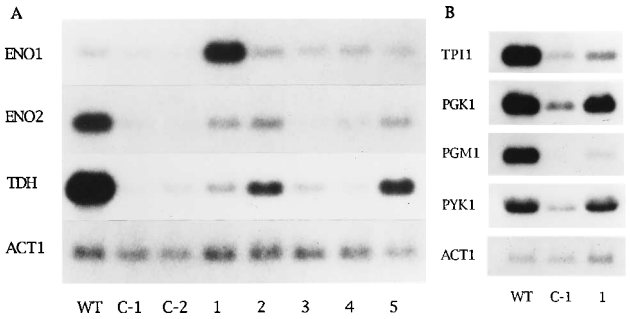


FIG. 2. Northern blot analysis of glycolytic mRNAs in strains carrying suppressors of a *gcr1* null mutation. (A) Total cellular RNA was isolated from strains grown in YNB medium containing 2% glucose. Lanes: WT, strain S173-6B carrying a wild-type *GCR1* gene; C-1, strain NPT7 carrying a *gcr1* null mutation; C-2, strain NPT13 carrying a *gcr1* null mutation; 1, strain KSM11-74 carrying a *gcr1* null mutation and the *SGC1-1* mutation; 2, strain KSM13-110 carrying a *gcr1* null mutation and the *SGC2-1* mutation; 3, strain KSM8-42 carrying a *gcr1* null mutation and the *sgc3-1* mutation; 4, strain KSM108-42 carrying a *gcr1* null mutation and the *sgc4-1* mutation; and 5, strain KSM4-22 carrying a *gcr1* null mutation and the *sgc5-1* mutation. Hybridization probes were as described in Materials and Methods. Northern blots with yeast ENO1, ENO2, and glyceraldehyde-3-phosphate dehydrogenase (TDH) hybridization probes are indicated. Yeast actin mRNA (ACT1), which is not regulated by the *GCR1* gene, served as an internal control. (B) Total cellular RNA was isolated from strains grown in YNB medium containing 2% glucose. Lanes: WT, strain S173-6B carrying a wild-type *GCR1* gene; C-1, strain NPT7 carrying a *gcr1* null mutation; and 1, strain KSM11-74 carrying a *gcr1* null mutation and the *SGC1-1* mutation. Hybridization probes were as described in Materials and Methods. Northern blots with yeast TPI1, PGK1, PGM1, and PYK1 hybridization probes are indicated. Yeast actin mRNA (ACT1), which is not regulated by the *GCR1* gene, served as an internal control.

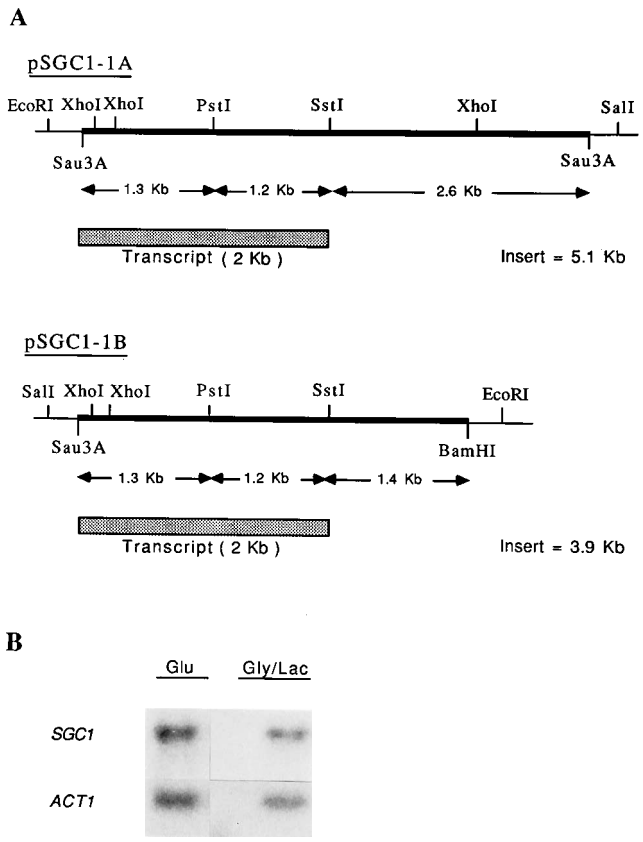


FIG. 3. (A) Restriction endonuclease maps of the yeast genomic DNA inserts in two independent plasmid isolates, pSGC1-1A and pSGC1-1B, carrying the *SGC1-1* gene. The restriction maps are aligned to indicate regions of overlap between the two cloned segments of yeast genomic DNA. Northern blotting analysis was performed with total cellular RNA isolated from strain S173-6B, using hybridization probes corresponding to the 1.3-kb *Sau*3A-*Pst*I fragment, the 1.2-kb *Pst*I-*Sst*I fragment, and the 2.6-kb *Sst*I-*Sau*3A fragment from plasmid pSGC1-1A. The approximate position of a 2-kb *SGC1* transcript is indicated. (B) Northern blot analysis of total cellular RNA isolated from strain S173-6B grown in YNB medium containing 2% glucose (Glu) or 2% glycerol plus 2% lactate (Gly/Lac) as the carbon source. Equal amounts of total cellular RNA (5 μg) were applied to each lane of the gel. Hybridization probes were the 1.3-kb *Sau*3A-*Pst*I fragment from plasmid pSGC1-1A (*SGC1*) and an oligonucleotide complementary to yeast actin mRNA (*ACT1*).

```
   1 GAGCTCACTTCATTGCGCTGTTACTCCCCTAACAACGTGGCCTCTCTCCATTCGGTAAGG     60
  61 AATCAAATTTTGTTCTAGAATGTTCATGCTGCTGCGGTCATTTCTTACCCTAGTAGTAAT    120
 121 TCATTGTGCCCACGTACGAACCGTACAATGGGATTCTGCCCTGCGCACGTTCACAGTTCG    180
 181 CCAAATAACAATTCTTATTTTCATTTCTTTTTCACAGTGTAGATGATACCGTGTCTGCGA    240
 241 CATTTCCATTCTTAGTAGAAATGCAAAACGCAACGTTTGCTATTAATTTTTTTCACCTCT    300
 301 GTTTCCTTTTTTTTTCTCACCTCAACAATCAAATGTACTTATATAAACTTCTTTTGTTAT    360
 361 CTCCAAAATTTAAACTTATTTACATCCTTTTTCACTTCATATATCAATTCATGGGAAAGT    420
 421 AAAGCTATCTTAATTACATCCTTATTTTATATTTCCCAACCATCAACCTTTATTCTTTAC    480
 481 ATATTTCTCTCTTTTCTCATTTTCTTTCTTATTAAGCGTAAAGCACCACAAGCAGTACCG    540
 541 ATCTAAAGTCTCTCTACTTTACTATTATTTTTTTTTTTTGTTCACTTCATAAACTTTTAG    600
 601 TGCAAAAATAAAAATAAAGACAAATAACACACTATCAAATCTCTTCAAGTTTAACAATTA    660

 661 TGAACTCTATTTTAGACAGAAATGTTAGATCTAGCGAAACTACTTTAATTAAACCTGAAT    720
   1 M  N  S  I  L  D  R  N  V  R  S  S  E  T  T  L  I  K  P  E     20

 721 CTGAATTTGATAATTGGTTGTCGGATGAAAATGACGGAGCTAGTCATATCAACGTCAACA    780
  21 S  E  F  D  N  W  L  S  D  E  N  D  G  A  S  H  I  N  V  N     40

 781 AGGACTCCTCGTCAGTTCTTTCTGCATCTTCTTCCACATGGTTCGAACCATTGGAAAACA    840
  41 K  D  S  S  S  V  L  S  A  S  S  S  T  W  F  E  P  L  E  N     60

 841 TTATCTCCTCTGCATCCAGCTCCTCGATAGGCTCTCCAATCGAAGACCAGTTTATATCTT    900
  61 I  I  S  S  A  S  S  S  S  I  G  S  P  I  E  D  Q  F  I  S     80

 901 CCAACAACGAGGAATCTGCTCTTTTTCCAACAGATCAGTTTTTCAGTAATCCTTCCTCAT    960
  81 S  N  N  E  E  S  A  L  F  P  T  D  Q  F  F  S  N  P  S  S    100

 961 ACTCGCATTCTCCCGAGGTTAGCAGCTCGATAAAAAGAGAAGAGGATGACAATGCCCTTT   1020
 101 Y  S  H  S  P  E  V  S  S  S  I  K  R  E  E  D  D  N  A  L    120

1021 CGTTGGCAGATTTTGAACCGGCTTCTTTGCAATTAATGCCTAACATGATAAATACTGATA   1080
 121 S  L  A  D  F  E  P  A  S  L  Q  L  M  P  N  M  I  N  T  D    140

1081 ATAATGACGATAGTACCCCACTTAAGAATGAAATCGAGCTAAACGACTCGTTTATAAAAA   1140
 141 N  N  D  D  S  T  P  L  K  N  E  I  E  L  N  D  S  F  I  K    160

1141 CAAATCTAGATGCTAAGGAAACGAAAAAGAGGGCTCCAAGAAAAAGACTGACCCCCTTCC   1200
 161 T  N  L  D  A  K  E  T  K  K  R  A  P  R  K  R  L  T  P  F    180
                                                                ---
1201 AAAAGCAAGCTCACAACAAGATTGAAAAACGCTACAGAATAAACATCAACACAAAGATTG   1260
 181 Q  K  Q  A  H  N  K  I  E  K  R  Y  R  I  N  I  N  T  K  I    200
      ---------------BASIC---------------------  ------------HELIX I-----
1261 CAAGACTGCAGCAGATTATCCCATGGGTAGCAAGTGAACAAACAGCTTTCGAAGTAGGTG   1320
 201 A  R  L  Q  Q  I  I  P  W  V  A  S  E  Q  T  A  F  E  V  G    220
      ----------------------
1321 ATTCTGTAAAAAAACAGGACGAAGACGGCGCAGAAACTGCCGCTACTACTCCTCTTCCAT   1380
 221 D  S  V  K  K  Q  D  E  D  G  A  E  T  A  A  T  T  P  L  P    240

1381 CTGCCGCTGCTACAAGCACGAAGCTAAATAAAAGCATGATCCTAGAAAAAGCTGTTGACT   1440
 241 S  A  A  A  T  S  T  K  L  N  K  S  M  I  L  E  K  A  V  D    260
                                     ----------------HELIX II---------
1441 ATATTCTATATCTACAAAATAACGAACGACTATACGAAATGGAAGTTCAAAGGTTGAAAA   1500
 261 Y  I  L  Y  L  Q  N  N  E  R  L  Y  E  M  E  V  Q  R  L  K    280
      -------------
1501 GTGAAATCGACACTTTGAAACAAGACCAAAAATAAAAGCACGCTTGCTTAAACACCAATA   1560
 281 S  E  I  D  T  L  K  Q  D  Q  K  *                           291

1561 TTATAAAATTACGATAGAAATTCCTTTCTCCTCTTTTGTTTATGCGACATTTTTTTCTTT   1620
1621 CTTAGCGAAGTACTTTAAAAAACAAGCCCAAAAAATAACAAGGCCATAACATAGCAGACA   1680
1681 AGAATACAAAAAAAAAAAAAGAAGTTGGAAAAAACATACAAAAAAAAAA              1728
```

FIG. 4. Nucleotide sequence of the yeast *SGC1* structural gene. The nucleotide sequence of 1,728 bp of genomic DNA was determined as described in Materials and Methods. The predicted amino acid sequence of a 291-codon open reading frame is indicated below the nucleotide sequence. The locations of regions of the *SGC1* nucleotide sequence that are similar to the basic, helix I, and helix II regions of the Myc family of DNA-binding proteins are indicated above the nucleotide sequence.

vealed that the two plasmids contained overlapping segments of yeast genomic DNA (Fig. 3A). Northern blotting analysis was performed with probes derived from pSGC1-1A to identify a putative *SGC1-1* transcript. The 1.3-kb *Sau*3A-*Pst*I fragment from pSGC1-1A hybridized to a 2-kb transcript from total cellular RNA (Fig. 3B). The 1.2-kb *Pst*I-*Sst*I fragment from pSGC1-1A also hybridized to a 2-kb transcript from total cellular RNA, whereas the 2.6-kb *Sst*I-*Sau*3A fragment failed to hybridize to a cellular transcript (data not shown). These data show that the *Pst*I site lies within sequences that encode the 2-kb transcript. The data also show that the steady-state levels of the putative *SGC1* transcript are similar in cells grown in a medium containing glucose or glycerol plus lactate (Fig. 3B).

The wild-type *SGC1* gene as well as three additional mutant alleles, *SGC1-2*, *SGC1-3*, and *SGC1-4*, was isolated by plasmid gap repair using pSGC1-1A that was limit digested with *Xho*I. The nucleotide sequence of the wild-type *SGC1* allele extending upstream and downstream from the *Pst*I site is shown in Fig. 4. A 291-codon open reading frame which spans the *Pst*I restriction endonuclease site was identified. The nucleotide sequence of *SGC1-1* differed from the sequence of the wild-type allele by a single base substitution in codon 189 which converts a Glu codon (GAA) to a Gln codon (CAA). The nucleotide sequences of *SGC1-2*, *SGC1-3*, and *SGC1-4* were identical. Each of these mutant alleles contained a single base substitution in codon 210 which converts a valine codon (GUA) to an isoleucine codon (AUA).

The nucleotide and amino acid sequences of *SGC1* were compared with known sequences in the databases. The predicted amino acid sequence of SGC1 extending from residues 181 to 265 contained a match to the basic-helix-loop-helix domain of the Myc family of DNA-binding proteins. An alignment among the basic-helix-loop-helix regions of SGC1 and the yeast CBF1 (10), PHO4 (4, 38), and INO2 (28) proteins is shown in Fig. 5. Interestingly, the mutation in *SGC1-1* converts a highly conserved glutamic acid residue within the basic region to a glutamine residue. The valine-to-isoleucine mutation in *SGC1-2* is located in the loop region immediately downstream from helix I.

***SGC1* is required for maximal transcription of the yeast enolase genes.** To further define the role of *SGC1* in transcrip-

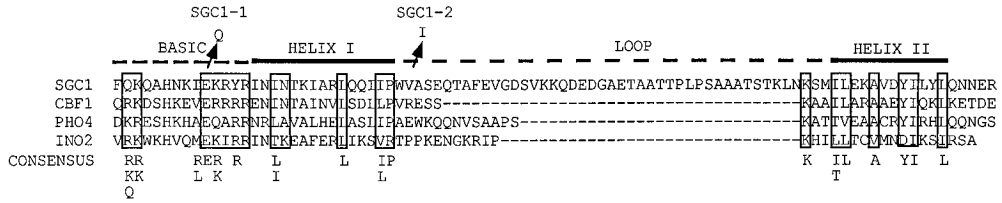


FIG. 5. Amino acid sequence alignment among the basic-helix-loop-helix regions of SGC1 and the yeast proteins CBF1 (10), PHO4 (4, 38), and INO2 (28). Highly conserved amino acids within the basic, helix I, and helix II regions are boxed. The positions of the E189Q mutation in SGC1-1 and the V210I mutation in SGC1-2 are indicated. The dashes indicate that the loop region of SGC1 is larger than that in CBF1, PHO4, or INO2. A consensus amino acid sequence for basic-helix-loop-helix proteins from yeasts and humans is also indicated.

tional regulation of the enolase genes, a strain carrying an *sgc1* null mutation was constructed. *SGC1* coding sequences extending from a *Bgl*II site at codon 10 to an *Nco*I site at codon 208 were removed from plasmid pSG62 and replaced with a *Bam*HI fragment containing a functional *HIS3* structural gene. A strain carrying this gene disruption was constructed as described in Materials and Methods. Strains carrying the *sgc1* null mutation were viable and did not display a significant growth phenotype. Diploid strains homozygous for the *sgc1* null mutation were constructed as described in Materials and Methods. These latter strains failed to sporulate, indicating that the *SGC1* gene is required for sporulation. Northern blotting analysis with a probe for *ENO1* mRNA showed that *ENO1* mRNA levels were reduced approximately fourfold in a strain carrying an *sgc1* null mutation (Fig. 6A). The steady-state level of *PGM1* mRNA was reduced approximately twofold in a strain carrying an *sgc1* null mutation, whereas the steady-state levels of *ACT1*, *TPI1*, and *SSA1* (heat shock protein 70) mRNAs were not significantly affected by the *sgc1* null mutation (Fig. 6).

The yeast *CBF1* and *INO2* genes encode basic-helix-loop-helix DNA-binding proteins (10, 28). CBF1 binds to centromeres and is required for expression of genes involved in methionine biosynthesis (10). INO2 is required for expression of the yeast *INO1* gene. Loss-of-function mutations in *CBF1* or *INO2* cause methionine auxotrophy or an inositol requirement, respectively (28). Strains carrying an *sgc1* null mutation did not display a requirement for methionine or inositol, indicating that SGC1 is not an essential dimerization partner for CBF1 or INO2.

The effects of the *SGC1-1* and *SGC1-2* mutations and the *sgc1* null mutation on enolase gene expression in strains carrying a wild-type *GCR1* gene or a *gcr1* null mutation were evaluated by Western blotting analysis (Fig. 7). The steady-state concentration of the enolase 1 polypeptide was reduced fourfold in strains carrying an *sgc1* null mutation and either a wild-type *GCR1* gene or the *gcr1-1* null mutation. The steady-state concentration of the enolase 2 polypeptide was reduced 30 and 70% in strains carrying an *sgc1* null mutation and either the wild-type *GCR1* gene or the *gcr1-1* null mutation, respectively. These results show that the *SGC1* gene product is required for maximal expression of both enolase genes in the presence or absence of a functional *GCR1* gene. *ENO2* expression increased threefold in strains carrying the *SGC1-1* dominant mutation and a *gcr1-1* null mutation and only slightly in a strain carrying a wild-type *GCR1* gene. *ENO1* expression increased 13- and 4-fold in strains carrying the *SGC1-1* dominant mutation and a *gcr1-1* null mutation or wild-type *GCR1* gene, respectively. Consistent with the Northern blotting analysis shown in Fig. 2A, the level of expression of the *ENO1* gene in a strain carrying the *SGC1-1* and *gcr1-1* mutations was approximately fourfold higher than that observed for a strain carrying wild-type alleles of *SGC1* and *GCR1*.

Finally, enolase gene expression was also elevated in a strain carrying the *SGC1-2* allele and a *gcr1-1* null mutation; however, the effects were less than those observed for the *SGC1-1* allele (Fig. 7).

DISCUSSION

The genetic analyses reported here show that the growth defect and the transcriptional defects for expression of the enolase genes *ENO1* and *ENO2* and the glyceraldehyde-3-phosphate dehydrogenase genes caused by a *gcr1* null mutation can be suppressed by dominant mutations in the *SGC1* and *SGC2* genes as well as recessive mutations in the *SGC5* gene. The *SGC1-1* mutation partially suppressed the transcriptional defect caused by a *gcr1* null mutation for three additional glycolytic genes, *PGK1*, *PGM1*, and *PYK1*, but had only a modest affect (less than twofold) on expression of *TPI1*, which encodes triose phosphate isomerase. Recessive mutations in two additional genes, *SGC3* and *SGC4*, partially suppressed the growth defect of the *gcr1* null mutation but only caused an increase in *ENO1* expression. This latter result suggested that mutations that cause an increase in *ENO1* expression alone can partially suppress the growth defect caused by a *gcr1* null mutation. We recently confirmed this observation by showing that overexpression of *ENO1* in a strain carrying a *gcr1* null mutation partially suppressed the glucose-negative growth phenotype (unpublished results); however, the observed suppression was less than that observed for strains carrying the *SGC1-1* dominant mutation.

The highest level of suppression of the growth and transcrip-

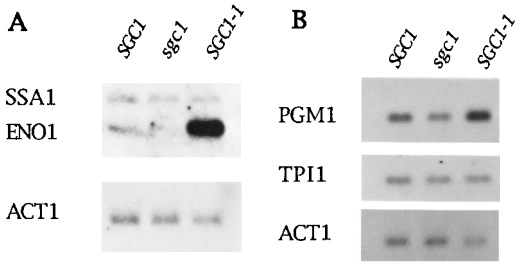


FIG. 6. Total cellular RNA was isolated from the following strains grown in YNB medium containing 2% glucose. Lanes: *SGC1*, strain S173-6B carrying the wild-type *SGC1* gene; *sgc1*, strain CPY17 carrying a *sgc1*Δ::*HIS3* null mutation; and *SGC1-1*, strain CPY5 carrying a *SGC1-1* mutation. (A) Northern blot analysis using oligonucleotide hybridization probes complementary to *ENO1* mRNA (ENO1), *SSA1* mRNA (SSA1), and actin mRNA (ACT1). (B) Northern blot analysis using oligonucleotide hybridization probes complementary to *PGM1* mRNA (PGM1), *TPI1* mRNA (TPI1), and actin mRNA (ACT1).

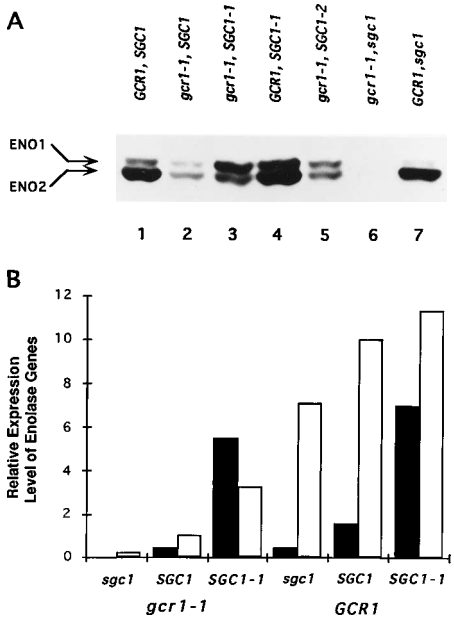


FIG. 7. (A) Western blot analysis of ENO1 and ENO2 polypeptides in strains carrying the *SGC1-1* and *SGC1-2* mutations and the *sgc1* null mutation in the presence or absence of a functional *GCR1* gene. Total cellular protein was isolated from strains grown in YNB medium containing 2% glucose. Lanes: 1, strain S173-6B carrying wild-type *GCR1* and *SGC1* genes; 2, strain TY100 carrying a *gcr1-1* null mutation and a wild-type *SGC1* gene; 3, strain CPY3 carrying a *gcr1-1* null mutation and the *SGC1-1* mutation; 4, strain CPY5 carrying a wild-type *GCR1* gene and the *SGC1-1* mutation; 5, strain CPY9 carrying a *gcr1-1* null mutation and the *SGC1-2* mutation; 6, CPY15 carrying a *gcr1-1* null mutation and a *sgc1* null mutation; and 7, strain CPY17 carrying a wild-type *GCR1* gene and a *sgc1* null mutation. Equal amounts of total cellular protein were applied to each lane of the gel. Western blot analysis was performed as described in Materials and Methods, using a polyclonal antibody which cross-reacts with the enolase 1 (ENO1) and enolase 2 (ENO2) polypeptides. (B) Densitometric quantitation of multiple exposures the Western blot analysis shown in panel A. The relative enolase gene expression levels were measured relative to the level of enolase 2 polypeptide synthesized in strain S173-6B (10 arbitrary units). The filled bars represent enolase 1 polypeptide, and the open bars represent enolase 2 polypeptide.

tional defects caused by a *gcr1* null mutation was observed for the dominant mutant allele *SGC1-1*. This latter mutation and the *SGC2-1* dominant mutant also suppressed the sporulation defect caused by a *gcr1* null mutation. Expression of the enolase genes was reduced three- to fivefold in a strain carrying an *sgc1* null mutation, demonstrating that *SGC1* encodes a novel regulatory factor that is required for maximal expression of the yeast enolase genes. This latter observation mitigates against the possibility that dominant mutations in the *SGC1* gene created a nonphysiological activator of enolase gene expression.

Interestingly, the largest transcriptional effect of the *SGC1-1* mutation was observed for expression of *ENO1*. *ENO1* expression was elevated 13- and 4-fold in strains carrying the *SGC1-1* mutation and either a *gcr1* null mutation or a wild-type *GCR1* gene, respectively. Since expression of the *ENO1* gene in strains grown in a medium containing glucose is repressed approximately 20-fold by an upstream repression sequence (URS) element located between the *ENO1* UAS element and TATAAA box (11, 16), it is tempting to speculate that the *SGC1-1* dominant mutation overrides URS element-dependent repression, permitting higher levels of *ENO1* expression in strains grown in glucose.

How do *GCR1* and *SGC1* cooperate to activate gene expression? A clue to this interaction comes from the observation that enolase gene expression is significantly lower in strains carrying *gcr1* and *sgc1* null mutations than in strains carrying either null mutation alone. Thus, the two regulatory genes appear to function on parallel pathways to activate enolase gene expression. The observation that *TPI1* expression is regulated by *GCR1* but not *SGC1* further suggests that the *SGC1* gene product is not required for the *GCR1*-dependent activation pathway. For those glycolytic genes that are regulated by *GCR1* and *SGC1*, dominant mutations in the *SGC1* gene may potentiate the *SGC1*-dependent pathway, resulting in a reduced requirement for the *GCR1*-dependent pathway. In this regard, the genetic relationship between the *GCR1* and *SGC1* regulatory genes differs from the genetic relationship between the *GCR1* and *GCR2* regulatory genes. *GCR2* is required for maximal expression of all of the yeast glycolytic genes that are regulated by *GCR1* (33). In contrast to the results described above for the *gcr1* and *sgc1* null mutations, however, enolase gene expression was reduced in strains carrying *gcr1* and *gcr2* null mutations to the same levels observed for a strain carrying a *gcr1* null mutation alone (33), suggesting that the *GCR1* and *GCR2* gene products function on the same regulatory pathway.

An analysis of the amino acid sequence of the *SGC1* gene product revealed a strong match to the basic-helix-loop-helix domain of the Myc family of DNA-binding proteins. This observation suggests that *SGC1* encodes a DNA-binding protein. The positions of the dominant gain-of-function mutations are interesting in this regard. The mutation in *SGC1-1* converts a glutamic acid residue to a glutamine residue (E189Q). A homologous glutamic acid residue is conserved within the basic DNA binding domains of all basic-helix-loop-helix proteins described to date. X-ray structure analysis showed that the homologous glutamic acid residue in the basic-helix-loop-helix protein Max participates in DNA binding by contacting the C or A residue of the E-box binding site (CACGTG) for Max (17). Amino acid substitution mutations at the homologous glutamic acid residue in the yeast basic-helix-loop-helix protein PHO4 (E9) have been constructed and analyzed (18). Substitution mutations, including E9D, E9N, and E9L, abolished PHO4 DNA binding in vitro (18). In contrast, a glutamine-for-glutamic acid (E9Q) mutation analogous to the *SGC1-1* mutation did not abolish PHO4 DNA binding (18). These observations raise the possibility that the dominant mutation in *SGC1-1* caused an alteration in the DNA binding properties of a putative SGC1 homodimer or SGC1-containing heterodimeric DNA-binding protein. The isoleucine-for-valine substitution mutation in *SGC1-2* is more difficult to speculate about since loop regions among basic-helix-loop-helix proteins are variable in length and amino acid sequence. Although the *SGC1-2* allele was a weaker suppressor than *SGC1-1*, it is conceivable that the *SGC1-1* and *SGC1-2* mutations caused similar gains of function since certain mutations in the loop region of E47 result in altered DNA binding site selection (35).

Regulatory sequences that are required for and sufficient for *GCR1*-dependent activation of *ENO2* expression were previously mapped between positions −486 and −390 relative to the transcriptional initiation site (36). Overlapping binding sites for ABF1 and RAP1 were mapped within these regulatory sequences between positions −486 and −450 (7). A GCR1 binding site and a second RAP1 binding site were mapped between positions −420 and −390 (36). Sequences between positions −450 and −420 are required together with sequences between positions −420 and −390 for high-level *GCR1*-dependent *ENO2* expression (36). *ENO2* regulatory sequences between positions −486 and −390 lack a sequence corresponding to the E-box binding site consensus (CACGTG) for basic-

helix-loop-helix DNA-binding proteins, suggesting that SGC1 protein may not interact directly with these latter regulatory sequences. Mapping of putative SGC1 binding site(s) within the *ENO2* 5′ flanking sequences using purified recombinant SGC1 protein is in progress.

ACKNOWLEDGMENTS

We thank Teresa Yokoi for invaluable technical assistance and Craig Martens for assistance with DNA sequence analyses.

This research was supported by Public Health Service grant GM30307 from the National Institutes of Health.

REFERENCES

1. **Alber, T., and G. Kawasaki.** 1982. Nucleotide sequence of the triose phosphate isomerase gene of Saccharomyces cerevisiae. J. Mol. Appl. Genet. **1:**419–434.
2. **Baker, H. V.** 1986. Glycolytic gene expression in *Saccharomyces cerevisiae*: nucleotide sequence of *GCR1*, null mutants, and evidence for expression. Mol. Cell. Biol. **6:**3774–3784.
3. **Baker, H. V.** 1991. GCR1 of *Saccharomyces cerevisiae* encodes a DNA binding protein whose binding is abolished by mutations in the CTTCC sequence motif. Proc. Natl. Acad. Sci. USA **88:**9443–9447.
4. **Berben, G., M. Legrain, V. Gilliquet, and F. Hilger.** 1990. The yeast regulatory gene PHO4 encodes a helix-loop-helix motif. Yeast **6:**451–454.
5. **Bitter, G. A., K. K. Chang, and K. M. Egan.** 1991. A multi-component upstream activation sequence of the Saccharomyces cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene promoter. Mol. Gen. Genet. **231:**22–32.
6. **Breeden, L., and K. Nasmyth.** 1985. Regulation of the yeast HO gene. Cold Spring Harbor Symp. Quant. Biol. **50:**643–650.
7. **Brindle, P. K., J. P. Holland, C. E. Willett, M. A. Innis, and M. J. Holland.** 1990. Multiple factors bind the upstream activation sites of the yeast enolase genes *ENO1* and *ENO2*: ABFI protein, like repressor activator protein RAP1, binds *cis*-acting sequences which modulate repression or activation of transcription. Mol. Cell. Biol. **10:**4872–4885.
8. **Buchman, A. R., N. F. Lue, and R. D. Kornberg.** 1988. Connections between transcriptional activators, silencers, and telomeres as revealed by functional analysis of a yeast DNA-binding protein. Mol. Cell. Biol. **8:**5086–5099.
9. **Burke, R. L., O. P. Tekamp, and R. Najarian.** 1983. The isolation, characterization, and sequence of the pyruvate kinase gene of Saccharomyces cerevisiae. J. Biol. Chem. **258:**2193–2201.
10. **Cai, M., and R. W. Davis.** 1990. Yeast centromere binding protein CBF1, of the helix-loop-helix protein family, is required for chromosome stability and methionine prototrophy. Cell **61:**437–446.
11. **Carmen, A., and M. J. Holland.** 1994. The upstream repression sequence from the yeast enolase gene ENO1 is a complex regulatory element that binds multiple *trans*-acting factors including REB1. J. Biol. Chem. **269:**9790–9797.
12. **Casadaban, M. J., A. A. Martinez, S. K. Shapira, and J. Chou.** 1983. Beta-galactosidase gene fusions for analyzing gene expression in Escherichia coli and yeast. Methods Enzymol. **100:**293–308.
13. **Chambers, A., C. Stanway, J. S. Tsang, Y. Henry, A. J. Kingsman, and S. M. Kingsman.** 1990. ARS binding factor 1 binds adjacent to RAP1 at the UASs of the yeast glycolytic genes PGK and PYK1. Nucleic Acids Res. **18:**5393–5399.
14. **Clifton, D., and D. G. Fraenkel.** 1981. The *gcr* (glycolysis regulation) mutation of *Saccharomyces cerevisiae*. J. Biol. Chem. **256:**13074–13078.
15. **Cohen, R., J. P. Holland, T. Yokoi, and M. J. Holland.** 1986. Identification of a regulatory region that mediates glucose-dependent induction of the *Saccharomyces cerevisiae* enolase gene *ENO2*. Mol. Cell. Biol. **6:**2287–2297.
16. **Cohen, R., T. Yokoi, J. P. Holland, A. E. Pepper, and M. J. Holland.** 1987. Transcription of the constitutively expressed yeast enolase gene *ENO1* is mediated by positive and negative *cis*-acting regulatory sequences. Mol. Cell. Biol. **7:**2753–2761.
17. **Ferre, D. A., G. C. Prendergast, E. B. Ziff, and S. K. Burley.** 1993. Recognition by Max of its cognate DNA through a dimeric b/HLH/Z domain. Nature (London) **363:**38–45.
18. **Fisher, F., and C. R. Goding.** 1992. Single amino acid substitutions alter helix-loop-helix protein specificity for bases flanking the core CANNTG motif. EMBO J. **11:**4103–4109.
19. **Gallwitz, D., and R. Seidel.** 1980. Molecular cloning of the actin gene from yeast Saccharomyces cerevisiae. Nucleic Acids Res. **8:**1043–1059.
20. **Hitzeman, R. A., F. E. Hagie, J. S. Hayflick, C. Y. Chen, P. H. Seeburg, and R. Derynck.** 1982. The primary structure of the Saccharomyces cerevisiae gene for 3-phosphoglycerate kinase. Nucleic Acids Res. **10:**7791–7808.
21. **Holland, J. P., and M. J. Holland.** 1979. The primary structure of a glyceraldehyde-3-phosphate dehydrogenase gene from Saccharomyces cerevisiae. J. Biol. Chem. **254:**9839–9845.
22. **Holland, J. P., L. Labieniec, C. Swimmer, and M. J. Holland.** 1983. Homologous nucleotide sequences at the 5′ termini of messenger RNAs synthesized from the yeast enolase and glyceraldehyde-3-phosphate dehydrogenase gene. J. Biol. Chem. **258:**5291–5299.
23. **Holland, M. J., T. Yokoi, J. P. Holland, K. Myambo, and M. A. Innis.** 1987. The *GCR1* gene encodes a positive transcriptional regulator of the enolase and glyceraldehyde-3-phosphate dehydrogenase gene families in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**813–820.
24. **Huie, M. A., E. W. Scott, C. M. Drazinic, M. C. Lopez, I. K. Hornstra, T. P. Yang, and H. V. Baker.** 1992. Characterization of the DNA-binding activity of GCR1: in vivo evidence for two GCR1-binding sites in the upstream activating sequence of *TPI* of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**2690–2700.
25. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
26. **Kohalmi, S. E., and B. A. Kunz.** 1988. Role of neighbouring bases and assessment of strand specificity in ethylmethanesulphonate and N-methyl-N′-nitro-N-nitrosoguanidine mutagenesis in the SUP4-o gene of Saccharomyces cerevisiae. J. Mol. Biol. **204:**561–568.
27. **McAlister, L., and M. J. Holland.** 1985. Differential expression of the three yeast glyceraldehyde-3-phosphate dehydrogenase genes. J. Biol. Chem. **260:**15019–15027.
28. **Nikoloff, D. M., P. McGraw, and S. A. Henry.** 1992. The INO2 gene of Saccharomyces cerevisiae encodes a helix-loop-helix protein that is required for activation of phospholipid synthesis. Nucleic Acids Res. **20:**3253.
29. **Pepper, A. E.** 1990. Genetic analysis of *cis*- and *trans*-acting elements that mediate negative regulation of the yeast enolase gene *ENO1*. Ph.D. dissertation. University of California, Davis.
30. **Rodicio, R., J. J. Heinisch, and C. P. Hollenberg.** 1993. Transcriptional control of yeast phosphoglycerate mutase-encoding gene. Gene **125:**125–133.
31. **Scott, E. W., and H. V. Baker.** 1993. Concerted action of the transcriptional activators REB1, RAP1, and GCR1 in the high-level expression of the glycolytic gene *TPI*. Mol. Cell. Biol. **13:**543–550.
32. **Slater, M. R., and E. A. Craig.** 1987. Transcriptional regulation of an *hsp70* heat shock gene in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**1906–1916.
33. **Uemura, H., and D. G. Fraenkel.** 1990. *gcr2*, a new mutation affecting glycolytic gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**6389–6396.
34. **Uemura, H., and Y. Jigami.** 1992. Role of GCR2 in transcriptional activation of yeast glycolytic genes. Mol. Cell. Biol. **12:**3834–3842.
35. **Voronova, A., and D. Baltimore.** 1990. Mutations that disrupt DNA binding and dimer formation in the E47 helix-loop-helix protein map to distinct domains. Proc. Natl. Acad. Sci. USA **87:**4722–4726.
36. **Willett, C. E., C. M. Gelfman, and M. J. Holland.** 1993. A complex regulatory element from the yeast gene *ENO2* modulates GCR1-dependent transcriptional activation. Mol. Cell. Biol. **13:**2623–2633.
37. **Yokoi, T.** Unpublished data.
38. **Yoshida, K., N. Ogawa, and Y. Oshima.** 1989. Function of the PHO regulatory genes for repressible acid phosphatase synthesis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **217:**40–46.