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The *GCR1* Gene Encodes a Positive Transcriptional Regulator of the Enolase and Glyceraldehyde-3-Phosphate Dehydrogenase Gene Families in *Saccharomyces cerevisiae*

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The intracellular concentrations of the polypeptides encoded by the two enolase (*ENO1* and *ENO2*) and three glyceraldehyde-3-phosphate dehydrogenase (*TDH1*, *TDH2*, and *TDH3*) genes were coordinately reduced more than 20-fold in a *Saccharomyces cerevisiae* strain carrying the *gcr1-1* mutation. The steady-state concentration of glyceraldehyde-3-phosphate dehydrogenase mRNA was shown to be approximately 50-fold reduced in the mutant strain. Overexpression of enolase and glyceraldehyde-3-phosphate dehydrogenase in strains carrying multiple copies of either *ENO1* or *TDH3* was reduced more than 50-fold in strains carrying the *gcr1-1* mutation. These results demonstrated that the *GCR1* gene encodes a *trans*-acting factor which is required for efficient and coordinate expression of these glycolytic gene families. The *GCR1* gene and the *gcr1-1* mutant allele were cloned and sequenced. *GCR1* encodes a predicted 844-amino-acid polypeptide; the *gcr1-1* allele contains a 1-base-pair insertion mutation at codon 304. A null mutant carrying a deletion of 90% of the *GCR1* coding sequence and a *URA3* gene insertion was constructed by gene replacement. The phenotype of a strain carrying this null mutation was identical to that of the *gcr1-1* mutant strain.

Yeast glycolytic enzymes make up 25 to 60% of the soluble protein in the organism (7). In most strains of the yeast *Saccharomyces cerevisiae*, the intracellular concentrations of glycolytic enzymes remain constant in cells grown on glucose or gluconeogenic carbon sources. A notable exception is the enzyme encoded by one of the enolase genes (*ENO2*), which is induced more than 20-fold in cells grown in glucose (12). In some yeast strains, as well as under certain growth conditions, it has been reported that several other glycolytic enzyme activities are induced when cells are shifted from gluconeogenic carbon sources to glucose (11). This paradox could be explained if many glycolytic genes are capable of glucose-dependent induction only under certain defined physiological conditions or in certain defined genetic backgrounds. Both positive and negative *cis*-acting regulatory sequences have been identified within the 5′ flanking region of the enolase genes (4; R. Cohen, T. Yokoi, J. P. Holland, and M. J. Holland, submitted for publication). By analogy with other yeast genes (1, 6), regulation of glycolytic gene expression by *cis*-acting sequences is likely to be mediated by *trans*-acting regulatory proteins.

Clifton et al. (3) reported the isolation of a recessive mutation designated *gcr1-1*. The specific activities of many glycolytic enzymes, including glyceraldehyde-3-phosphate dehydrogenase and enolase, are reduced to 2 to 5% of the wild-type level in strains carrying the *gcr1-1* mutation. In vitro translational studies suggest that this mutation affects the intracellular levels of glycolytic mRNAs (2). The *GCR1* gene, therefore, appears to encode a positive transcription factor which is involved in coordinate expression of glycolytic genes.

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In this report, we describe the effects of the *gcr1-1* mutation on expression of the two enolase and three glyceraldehyde-3-phosphate dehydrogenase structural genes. We present direct evidence that the *GCR1* gene encodes a *trans*-acting regulator of enolase and glyceraldehyde-3-phosphate dehydrogenase gene expression. The primary structures of the *GCR1* gene and the *gcr1-1* mutant allele are reported. Strains carrying a *gcr1* null mutation were constructed and characterized. The implications of these results for coordinate regulation of glycolytic gene expression are discussed.

MATERIALS AND METHODS

Materials. ^{125}I-protein A was generously provided by John Hershey, University of California, Davis. Oligonucleotides for sequencing were provided by the DNA Synthesis Group, Cetus Corp., Emeryville, Calif.

Strains and growth conditions. *S. cerevisiae* S173-6B (α *leu2-3 leu2-112 his3-1 trp1-289 ura3-52*) was provided by F. Sherman, University of Rochester, Rochester, N.Y. *S. cerevisiae* S173-LA is identical to S173-6B and carries an *eno1* deletion mutation (12). *S. cerevisiae* DFY1 (wild type) and DFY67 (*gcr1-1*) were generously provided by D. G. Fraenkel, Harvard Medical School, Boston, Mass. Yeast strains were grown at 30°C and harvested in early log phase (A_{660}, 1.0). Cells were grown in YP medium (1% yeast extract, 2% peptone) or a defined medium containing 0.67% yeast nitrogen base without amino acids (Difco Laboratories, Detroit, Mich.) supplemented with 2 μg of uracil per ml where indicated. Carbon sources were 2% glucose, 2% glycerol–2% lactate, or 2% glycerol– 2% lactate–2% maltose.

Analysis of glycolytic proteins. Western blotting with anti-enolase polyclonal antibody was carried out as previously described (4). Identification of glyceraldehyde-3-phosphate

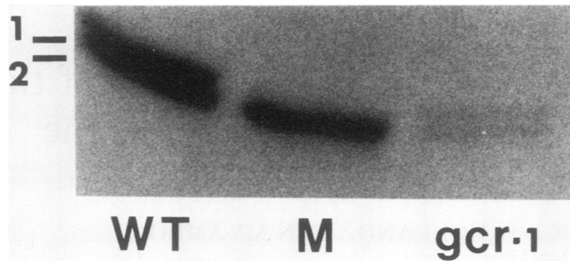

FIG. 1. The steady-state concentrations of the enolase polypeptides in a strain carrying the *gcr1-1* mutation. Western blotting assays were performed with whole-cell extracts prepared from wild-type strain S173-6B (WT), strain S173-LA carrying an *eno1* mutation (M), and strain S137-6B carrying a *gcr1-1* mutation *(gcr1-1)* as described in Materials and Methods. The locations of the enolase 1 (band 1) and enolase 2 (band 2) polypeptides are indicated. Cells were grown in YP medium containing 2% glycerol plus 2% lactate.

dehydrogenase polypeptides by two-dimensional polyacrylamide gel electrophoresis was as previously described (13). One-dimensional (SDS)-polyacrylamide gel electrophoresis of yeast whole-cell extract was as previously described (12).

Analysis of glycolytic mRNA. In vitro translation of total yeast cellular RNA was performed by using a wheat germ cell-free extract (9). Northern blotting studies used a nick-translated 2.1-kilobase (kb) *Hin*dIII fragment isolated from p*gap*491, which contains the *TDH3* structural gene (8), or nick-translated *Hin*dIII fragments from pGCR5 and pGCR6. Solution hybridization reaction mixtures contained 100 μg of total cellular RNA and a nick-translated *Hin*fI fragment (574 base pairs) (5,000 cpm per reaction mixture; specific activity, 10^7 cpm/μg) containing coding sequences from the *TDH3* structural gene. Hybridization was carried out in 0.05- to 1.0-ml reaction mixtures containing 0.3 M NaCl, 25 mM Tris (pH 7.4), 1 mM EDTA, and 0.3% SDS at 65°C for 2.5 to 250 min. Hybrid formation was monitored by hydroxyapatite chromatography.

Yeast transformation and cloning. Yeast transformations were performed by using the alkali cation procedure described by Ito et al. (10). Multicopy plasmids (CV13) carrying a 2.1-kb *Hin*dIII fragment containing the *TDH3* structural gene (8) or a 6.6-kb *Bam*HI fragment containing the *ENO2* structural gene (4) were used to transform *S. cerevisiae* S173-6B or an isogenic strain carrying the *gcr1-1* mutation. The *GCR1* structural gene was isolated by transforming *S. cerevisiae* S173-6B carrying a *gcr1-1* mutation with a centromere-based plasmid library containing genomic DNA (partially *Sau*3A digested) isolated from *S. cerevisiae* DFY1. The pBR322-based plasmid vector contained a *URA3* gene, the *TRP1* autonomous replication sequence, and a 1.1-kb fragment of DNA containing the centromere from chromosome 11. Yeast genomic DNA was ligated into a unique *Bam*HI site in the plasmids. Ura$^+$ transformants that grew at wild-type rates in medium containing 2% glucose were isolated. Two plasmids carrying the putative *GCR1* gene were isolated and identified from these transformants (pGCR1 or pGCR2). Subcloning analysis was performed by blunt-end ligating DNA fragments from pGCR1 or pGCR2 into the *Bam*HI site in the centromere plasmid vector. These plasmids (pGCR3 to pGCR8) were used to transform *S. cerevisiae* S173-6B carrying a *gcr1-1* mutation to uracil independence.

The *gcr1-1* mutant allele was isolated by transforming *S. cerevisiae* S173-6B carrying a *gcr1-1* mutation with a linearized pGCR3 plasmid in which sequences extending from *Xho*I to *Sac*I were removed. Ura$^+$ transformants containing a centromere-based plasmid in which sequences extending from the *Xho*I site to the *Sac*I site were restored by gene conversion with the *gcr1-1* mutant allele were isolated and characterized.

A *gcr1* null mutant was constructed by transformation of strain S173-6B with a linear fragment of DNA containing a deletion of approximately 90% of the *GCR1* coding sequences and a *URA3* gene insertion. *GCR1* coding sequences extending from a *Sac*I site at a codon 66 to an *Eco*RI site at codon 797 in pGCR3 were removed and replaced with a fragment of DNA containing the yeast *URA3* gene. A *Sal*I fragment containing this deletion-insertion mutation was isolated and used to transform a diploid strain (isogenic to strain S173-6B) to uracil independence. After sporulation and tetral dissection, Ura$^+$ haploid cells were identified. Genomic DNA was isolated from these cells, digested with *Sal*I, and subjected to Southern blotting analysis with a nick-translated pGCR3 probe to confirm that the wild-type *GCR1* gene was replaced with the null mutant allele.

DNA sequencing. A restriction endonuclease cleavage map of the overlapping genomic sequences in pGCR1 and pGCR2 was generated by standard procedures. The DNA sequences of both strands of *GCR1* and *gcr1-1* mutant alleles were determined by the dideoxy sequencing method (15) with synthetic DNA primers. Sequence reactions were performed as described above, with minor modifications to allow the reactions to take place in a microtiter plate. In some regions, it was necessary to substitute deoxyinosine triphosphate for deoxyguanosine triphosphate (4:1) to resolve compressions in the gel.

RESULTS

The *GCR* structural gene regulates transcription of the enolase and glyceraldehyde-3-phosphate dehydrogenase structural gene. The specific activities of enolase and glyceraldehyde-3-phosphate dehydrogenase are reduced to 2 to 5% of wild-type levels in strains carrying the *gcr1-1* mutation (2, 3). To determine whether this reduction in specific activity is accompanied by a proportional decrease in the steady-state concentration of the enzymes, the polypeptides encoded by the two enolase genes *(ENO1* and *ENO2)* and the three glyceraldehyde-3-phosphate dehydrogenase genes (*TDH1*, *TDH2*, and *TDH3)* were analyzed. The two enolase polypeptides were resolved after one-dimensional SDS-polyacrylamide gel electrophoresis (12). Western blotting analysis showed that both of the enolase polypeptides were reduced approximately 50-fold in cells carrying the *gcr1-1* mutation (Fig. 1). Glyceraldehyde-3-phosphate dehydrogenase polypeptides were resolved after two-dimensional polyacrylamide gel electrophoresis as previously described (13). Several of the most abundant cellular proteins were substantially reduced in extracts prepared from the strain carrying the *gcr1-1* mutation (Fig. 2), consistent with the fact that glycolytic enzymes are very abundant proteins in *S. cerevisiae*. All of the glyceraldehyde-3-phosphate dehydrogenase polypeptides were reduced to undetectable levels in a strain carrying the *gcr1-1* mutation. These data showed that the *GCR1* gene regulates the steady-state concentration of the polypeptide synthesized from all of the glyceraldehyde-3-phosphate dehydrogenase and enolase genes.

The steady-state concentrations of enolase and glyceraldehyde-3-phosphate dehydrogenase mRNAs in the *gcr1-1* mutant strain was determined by cell-free translation. Total RNA was isolated from strains carrying the *gcr1-1*

mutation and the wild-type parental strain grown in media containing different carbon sources. RNA isolated from the mutant strain grown in a medium containing glycerol plus lactate failed to direct the synthesis of a number of abundant polypeptides when compared with RNA isolated from the wild-type strain (Fig. 3). The amounts of enolase and glyceraldehyde-3-phosphate dehydrogenase polypeptides synthesized under the direction of RNA isolated from the *gcr1-1* mutant strain were substantially reduced. Based on these observations, we concluded that the *gcr1-1* mutation affects the intracellular concentrations of translatable enolase and glyceraldehyde-3-phosphate dehydrogenase mRNAs.

When the *gcr1-1* mutant strain is grown on a medium containing glycerol, lactate, and maltose, the specific activities of several glycolytic enzymes increase (2, 3). Under these growth conditions, the specific activity of glyceraldehyde-3-phosphate dehydrogenase is approximately 25% of wild-type levels. When total RNA isolated from the *gcr1-1* mutant strain grown in glycerol-lactate-maltose was translated in vitro, the pattern of polypeptide synthesis was qualitatively and quantitatively similar to the pattern observed for RNA isolated from mutant cells grown in glycerol plus lactate (Fig. 3). The observed increase in the specific activity of glyceraldehyde-3-phosphate dehydrogenase did not appear to be accompanied by a proportional increase in mRNA under these growth conditions.

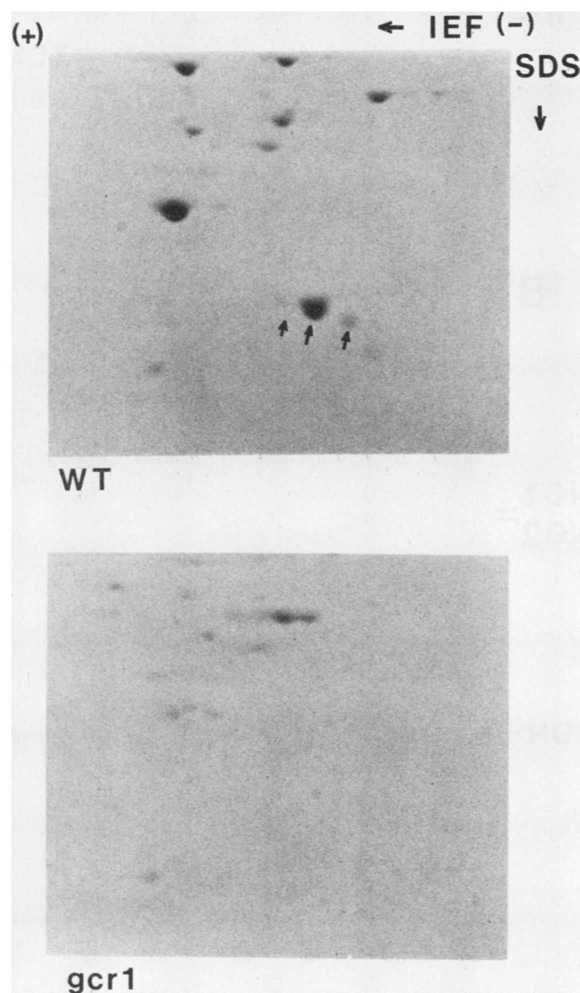

FIG. 2. Steady-state concentration of the glyceraldehyde-3-phosphate dehydrogenase polypeptides in a strain carrying the *gcr1-1* mutation. Two-dimensional polyacrylamide gel electrophoresis was performed with identical amounts of whole-cell extracts prepared from wild-type strain S173-6B (WT) and strain S173-6B carrying a *gcr1-1* mutation (gcr1) as described in Materials and Methods. The directions of isoelectric focusing (IEF) and SDS-polyacrylamide gel electrophoresis (SDS) are indicated. Arrows indicate the positions of the glyceraldehyde-3-phosphate dehydrogenase polypeptides. Cells were grown in YP medium containing 2% glycerol plus 2% lactate.

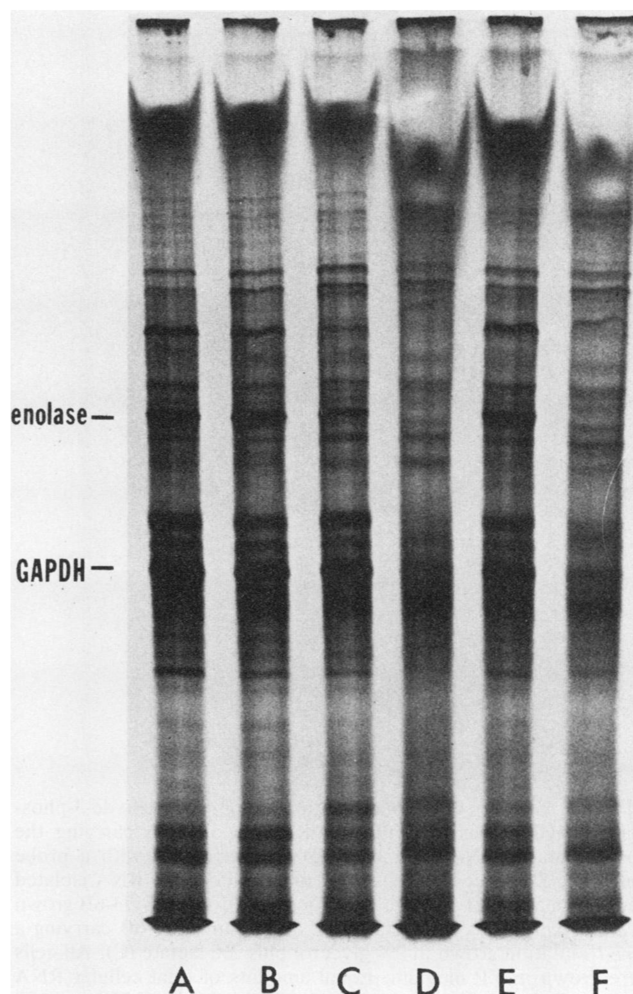

FIG. 3. In vitro translation of total cellular RNA isolated from wild-type and *gcr1-1* mutant strains. Total cellular RNAs isolated from (A) wild-type strain DFY1 grown in 2% glucose (B) wild-type strain DFY1 grown in 2% maltose; (C) wild-type strain DFY1 in 2% glycerol plus 2% lactate, (D) strain DFY67 (*gcr1-1*) grown in 2% glycerol plus 2% lactate, (E) wild-type strain DFY1 grown in 2% glycerol–2% lactate–2% maltose, and (F) strain DFY67 (*gcr1-1*) grown in 2% glycerol–2% lactate–2% maltose were translated in a wheat germ cell-free extract in the presence of [^{35}S]methionine and subjected to SDS-polyacrylamide gel electrophoresis and autoradiography. All cells were grown on YP medium. The locations of the enolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polypeptides are indicated.

The intracellular concentration of glyceraldehyde-3-phosphate dehydrogenase mRNA in cells carrying the *gcr1-1* mutation was determined by Northern blotting (Fig. 4). A 574-base-pair hybridization probe from within the coding sequences of the *TDH3* structural gene was used in this

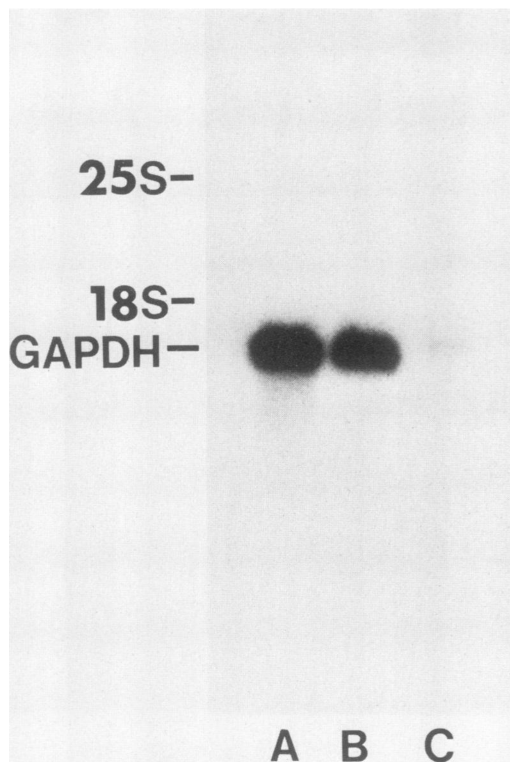

FIG. 4. Steady-state concentration of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in a strain carrying the *gcr1-1* mutation. Northern blotting was performed with a probe containing *TDH3* coding sequences and total cellular RNA isolated from strain S173-6B grown in 2% glucose (A), strain S173-6B grown in 2% glycerol plus 2% lactate (B), and strain S173-6B carrying a *gcr1-1* mutation grown in 2% glycerol plus 2% lactate (C). All cells were grown in YP medium. Equal amounts of total cellular RNA were applied to each lane of the gel. The positions of 25S and 18S rRNAs are indicated.

experiment. This probe cross-hybridizes with mRNA synthesized from each of the three glyceraldehyde-3-phosphate dehydrogenase structural genes (13). Previous studies showed that the intracellular concentration of glyceraldehyde-3-phosphate dehydrogenase synthesized from each of the three structural genes is approximately twofold higher in cells grown on medium containing glucose versus glycerol plus lactate (13, 14). As expected, the steady-state concentration of total glyceraldehyde-3-phosphate dehydrogenase mRNA was approximately two-fold higher in cells grown in glucose versus glycerol plus lactate. The steady-state concentration of total glyceraldehyde-3-phosphate dehydrogenase mRNA in the *gcr1-1* mutant strain grown on a medium containing glycerol plus lactate was less than 5% of the level observed in the wild-type strain grown under the same conditions (Fig. 4). Since the *TDH1*, *TDH2*, and *TDH3* genes account for 10, 25, and 65% of the glyceraldehyde-3-phosphate dehydrogenase activity in yeast cells (14), it is likely that the *gcr1-1* mutation affects transcription of all three genes. Similar results were obtained for enolase total mRNA levels (data not shown). These data showed that the decrease in specific activities of enolase and glyceraldehyde-3-phosphate dehydrogenase in the *gcr1-1* mutant strain resulted from a proportionate decrease in the steady-state concentrations of the respective total mRNAs.

The *GCR1* structural gene encodes a *trans*-acting regulator

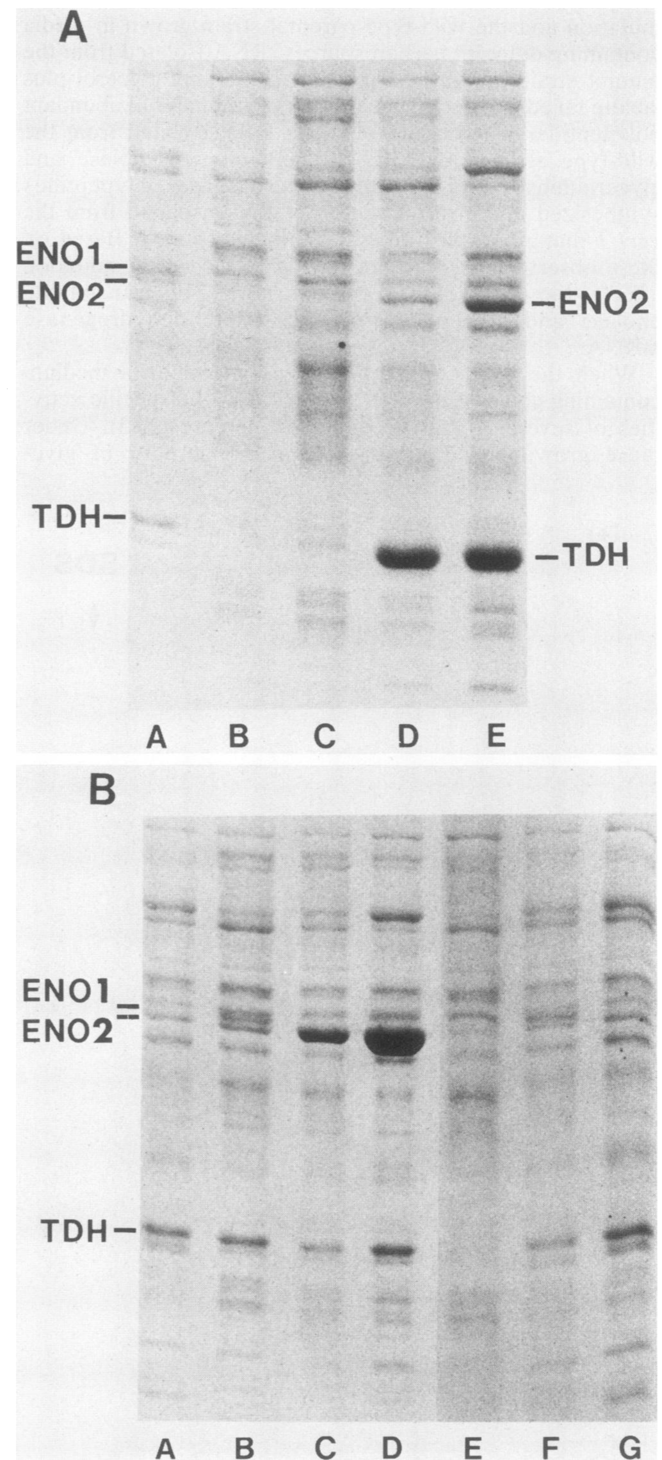

FIG. 5. Expression of cloned *TDH3* and *ENO2* genes in a strain carrying a *gcr1-1* mutation. SDS-polyacrylamide gel electrophoresis was performed with whole-cell extracts prepared from cells grown in YP medium. The positions of the enolase 1 (ENO1) and enolase 2 (ENO2) and glyceraldehyde-3-phosphate dehydrogenase (TDH) polypeptides are indicated. (Panel A) Whole-cell extracts were prepared from (lanes): A, S173-6B grown in 2% glycerol plus 2% lactate; B, S173-LA (*eno1*) grown in 2% glycerol plus 2% lactate; C, S173-6B carrying a *gcr1-1* mutation and multiple copies of the *TDH3* gene grown on 2% glycerol plus 2% lactate; D, S173-6B carrying multiple copies of the *TDH3* gene grown on 2% glycerol plus 2% lactate; and E, S173-6B carrying multiple copies of the *TDH3* gene

of enolase and glyceraldehyde-3-phosphate dehydrogenase gene expression. Expression of the *ENO2* and *TDH3* structural genes cloned into a multicopy plasmid containing a 2μm origin of replication was analyzed in wild-type and *gcr1-1* mutant strains. Wild-type cells carrying a multicopy plasmid containing a cloned segment of DNA containing the *TDH3* structural gene overproduced glyceraldehyde-3-phosphate dehydrogenase polypeptide when grown on medium containing either glucose of glycerol plus lactate as a carbon source (Fig. 5A). A *gcr1-1* mutant strain carrying the same plasmid did not overexpress glyceraldehyde-3-phosphate dehydrogenase. Similar results were obtained by using wild-type and *gcr1-1* mutant cells carrying the *ENO2* structural gene on a multicopy plasmid (Fig. 5B). Since the intracellular concentrations of glyceraldehyde-3-phosphate dehydrogenase and enolase in wild-type strains carrying multiple copies of each respective gene are 20 to 40% of the total cellular protein, failure to detect these proteins at elevated concentrations in the *gcr1-1* mutant background provided compelling evidence that this mutation leads to a 50-fold reduction in the transcription of these genes and that the *GCR1* gene product is a *trans*-acting regulator of transcription of these genes.

Isolation and characterization of the *GCR1* structural gene. The *GCR1* structural gene was isolated by genetic complementation of a *gcr1-1* mutant strain. A strain carrying *ura3* and *gcr1-1* mutations was transformed with a library of genomic DNA isolated from *S. cerevisiae* DFY1 with a centromere-based plasmid as described in Materials and Methods. Ura$^+$ transformants were selected that grew at wild-type rates on a medium containing glucose as the carbon source. Two types of transformant were identified after polyacrylamide gel electrophoresis of total cellular proteins isolated from putative transformants (Fig. 6). One type had restored the wild-type pattern of cellular proteins, whereas the other yielded a pattern of cellular protein that was indistinguishable from that of the *gcr1-1* strain. Two transformants from the former class were further analyzed. Genetic analysis performed with the second class of transformants showed them to be pseudorevertants that grew more rapidly on a medium containing glucose but did not restore wild-type levels of enolase or glyceraldehyde-3-phosphate dehydrogenase activity.

Restriction endonuclease mapping studies of the plasmids (pGCR1 and pGCR2) isolated from the two transformants revealed overlapping yeast genomic DNA sequences (Fig. 7). The ability of sequences within the overlapping region to complement the *gcr1-1* mutation was tested with subcloned restriction fragments (Fig. 7). These data revealed the approximate location of the complementing gene. The failure of pGCR5 or pGCR6 to complement the *gcr1-1* mutant strain suggested that the common *Hin*dIII site in these two plasmids lies within the coding sequences of the complementing gene. Northern blotting analysis was conducted by using the *Hin*dIII fragments in pGCR5 and pGCR6 as probes. Both probes hybridized to the same 3,000-base transcript (Fig. 8).

grown on 2% glucose. (Panel B) Whole-cell extracts were prepared from (lanes): A and G, S173-LA (*eno1*) grown on 2% glycerol plus 2% lactate; B and F, S173-6B grown on 2% glycerol plus 2% lactate; C, S173-6B carrying multiple copies of the *ENO2* gene grown on 2% glycerol plus 2% lactate; D, S173-6B carrying multiple copies of the *ENO2* gene grown on 2% glucose; and E, S173-6B carrying a *gcr1-1* mutation and multiple copies of the *ENO2* gene grown on 2% glycerol plus 2% lactate.

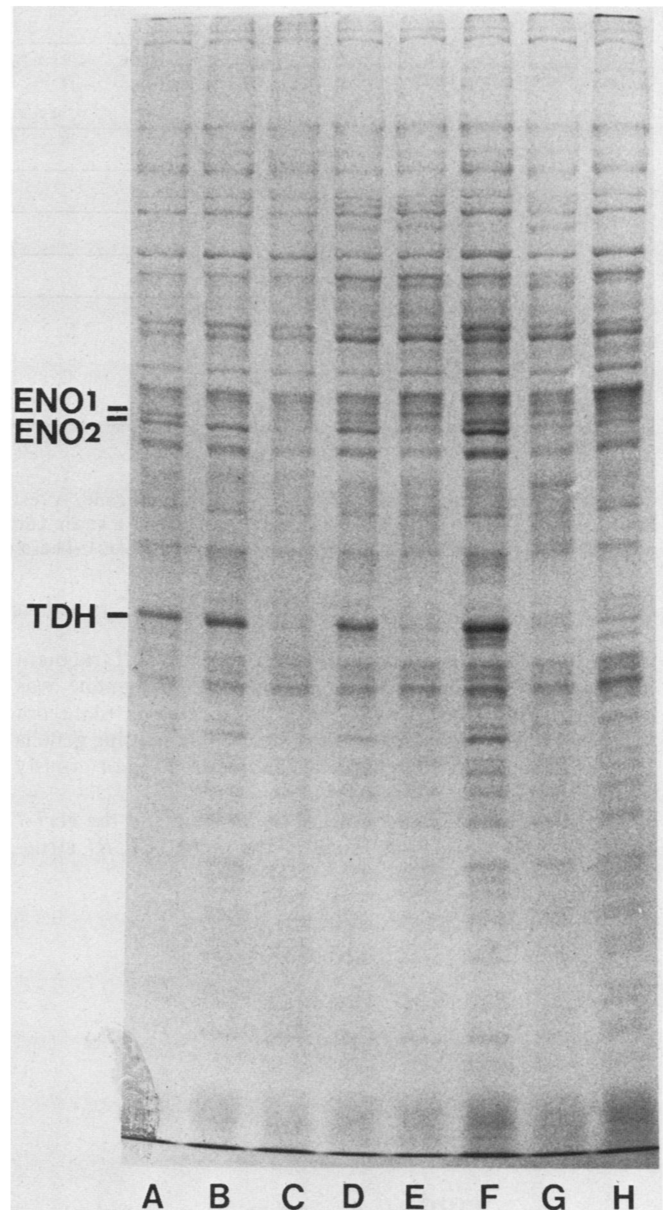

FIG. 6. Isolation of the *GCR1* gene by complementation of a strain carrying the *gcr1-1* mutation. SDS-polyacrylamide gel electrophoresis of whole-cell extracts was performed as described in Materials and Methods. Extracts were prepared from (A) strain S173-6B, (B) strain S173-LA (*eno1*), and (C to H) Ura$^+$ transformants which grew on medium containing 2% glucose obtained after transformation of strain S173-6B carrying a *gcr1-1* mutation with a plasmid library containing yeast genomic DNA. The patterns of proteins present in extracts prepared from transformants carrying plasmids pGCR1 (lane D) and pGCR2 (lane F) are similar to that of an extract prepared from strain S73-6B (lane A). Cells were grown in YP medium containing 2% glycerol plus 2% lactate. The positions of the enolase 1 (ENO1), enolase 2 (ENO2), and glyceraldehyde-3-phosphate dehydrogenase (TDH) polypeptides are indicated.

These results demonstrated that the coding sequence of the complementing gene spans the *Hin*dIII site.

Southern blotting analysis was performed with genomic DNA isolated from the wild-type strain and a strain carrying the *gcr1-1* mutation. Genomic DNA was limit digested with *Sal*I, and DNA filter blots were hybridized with a nick-

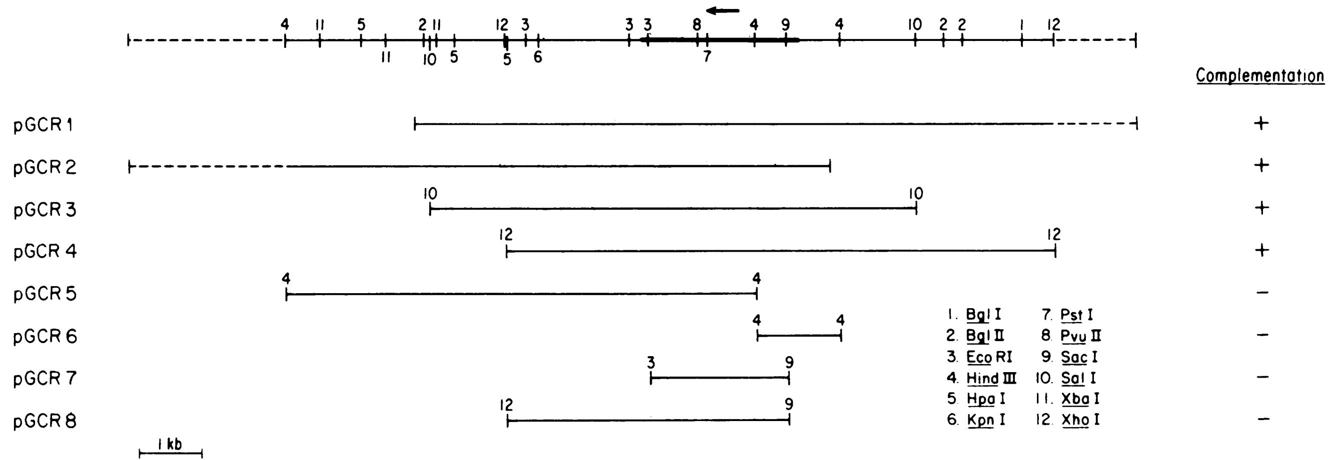

FIG. 7. Identification of the cloned *GCR1* structural gene. A restriction endonuclease map of overlapping yeast genomic DNA sequences in two plasmids (pGCR1 and pGCR2) that complement a strain carrying the *gcr1-1* mutation. Plasmids pGCR3 to pGCR8 contain segments of DNA isolated from pGCR1 or pGCR2 (indicated by bars). These plasmids were tested for their ability to complement a strain carrying the *gcr1-1* mutation.

translated probe corresponding to the genomic *Sal*I fragment in pGCR3. A single 7.7-kb genomic *Sal*I fragment was observed with DNA isolated from both strains (data not shown). These data indicated that the complementing gene is present in one copy per haploid genome and is not grossly altered in the mutant strain.

The nucleotide sequences of the *GCR1* gene and the *gcr1-1* allele. The nucleotide sequence of the cloned *GCR1* structural gene is presented in Fig. 9. The gene contains one continuous open reading frame encoding a putative 844-amino-acid polypeptide. These data, as well as the observation that the open reading frame does not contain the -TACTAAC- sequence found in the 3′ terminal sequences of all yeast mRNA intervening sequences, suggest that the *GCR1* gene does not contain any intervening sequence(s). The predicted size of the *GCR1* gene is consistent with the Northern blotting data shown in Fig. 8. Comparison of the amino acid sequence predicted from the sequence of the *GCR1* gene with known sequences in protein structure data bases revealed no significant homologies.

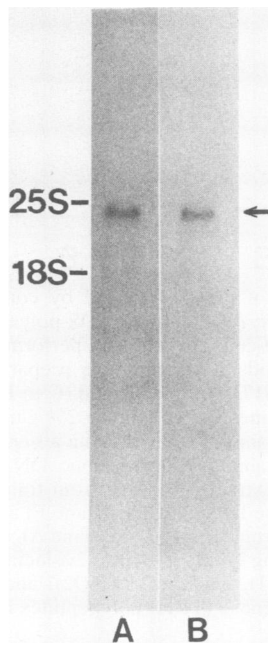

FIG. 8. Identification of the transcript encoded by the *GCR1* gene. Northern blotting analysis was performed with total cellular RNA isolated from strain S173-6B grown in YP medium containing 2% glucose. RNA blots were hybridized with nick-translated *Hin*dIII fragments isolated from pGCR5 (A) or pGCR6 (B). The positions of 25S and 18S rRNAs are indicated. The arrow indicates the position of a 3,000-base transcript.

The *gcr1-1* allele was also cloned and sequenced as described in Materials and Methods. The mutant gene contains 1-base-pair insertion (T/A) at codon 304. This frameshift mutation would lead to premature termination of translation. A strain carrying a *gcr1* null mutation was constructed by transforming *S. cerevisiae* S173-6B with a fragment of DNA containing a deletion of 90% of the *GCR1* coding sequences and a *URA3* structural gene insertion at the site of the deletion. Replacement of the *GCR1* allele with the allele containing this deletion-insertion mutation was confirmed by Southern blotting analysis (data not shown). The growth phenotype of strains carrying this null mutation is identical to that of strains carrying the *gcr1-1* mutant allele, as are the patterns of the cellular proteins observed after SDS-polyacrylamide gel electrophoresis (data not shown). Diploid strains that are heterozygous for the recessive *gcr1-1* mutation and the recessive deletion-insertion mutation display the same phenotype observed for haploid strains carrying either mutation. These observations further confirmed that the cloned complementing gene is the *GCR1* structural gene.

DISCUSSION

The experiments described in this paper provided direct evidence that the *GCR1* gene encodes a *trans*-acting protein that positively regulates expression of the two enolase and three glyceraldehyde-3-phosphate dehydrogenase genes. It is likely that the effects of the *gcr1-1* mutation on the specific

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                                                                                                                                                                    AAGCTTCAT CGGTAAACAA ACCACAGTTA   29
ATAGCGAGAA AGTCGGCAAG TGACAAACGA CGGTTATATT TAATTGGCCT TTGATATATA TTGAAAATGG TATGGTATGA TCATAACACG CATTCTGAAA ATGTTATCTG GGAGGTTTTC GATGGGTATG GAGTTTTCCT TGATTCTCAC TTATTATCCC  189
TTGTATTGTA ATTGATCCTT CAGTAATATT TGCAGCCTTT CACAACTATC CTTTTTTTCA TTGCTTATTA CTATTGAACC TTTTTTAGGA GTTGCCTGCT TATGCAATAT AATTTGCTGA CAAGTAGTAA ATTACCAGCA CAATATTAAG ATTAAAAAAG  349
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GATTATTAAT AGAGTAACGC AAACTTAAGG AAAGGAAGTG CTTTACAATT AAGTATTTAT AAGAACGAAT TTATCCCCCA AAAAAAGCCA CCTATACTTA ATAAAGGGAG GGGAATAGCT ATCAATTGAG TGTTGTCTGC GTCTGTCTGC GTACAAGAGG  669

ATG AAT TTT CTG ACT CAG GCT ATG TCA GAA ACT TTT CAA GGG ACA AAT AAC AGG ATA AAA CGT AAT GTC AGG ACA CAA AGT GTG CCA TCA ACT TCC TAT AAT AAT GGC AAA GAA TCA TAT GGA CCA AAT ACT   801
Met Asn Phe Leu Thr Gln Ala Met Ser Glu Thr Phe Gln Gly Thr Asn Asn Arg Ile Lys Arg Asn Val Arg Thr Gln Ser Val Pro Ser Thr Ser Tyr Asn Asn Gly Lys Glu Ser Tyr Gly Pro Asn Thr    44

AAC CAA TTA AAT GCC CTA CTT TCT CAA TTG GAA CAG CAA ACA AGT GTT GAT AGT ACC AGC ACG AGC TCA AAC TTT TAT TCC ATT GCA CAA TAT ATT TTA CAA TCA TAC TTC AAG GTC AAT GTA GAT TCT CTA   933
Asn Gln Leu Asn Ala Leu Leu Ser Gln Leu Glu Gln Gln Thr Ser Val Asp Ser Thr Ser Thr Ser Ser Asn Phe Tyr Ser Ile Ala Gln Tyr Ile Leu Gln Ser Tyr Phe Lys Val Asn Val Asp Ser Leu    88

AAC TCT CTG AAA TTG GTG GAT TTG ATA GTG GAC CAA ACT TAC CCT GAT TCT TTG ACG CTG CGA AAG CTG AAT GAA GGA GCA ACG GGA CAA CCA TAC GAT TAT TTC AAT ACA GTT TCT CGT GAT GCT GAT ATC  1065
Asn Ser Leu Lys Leu Val Asp Leu Ile Val Asp Gln Thr Tyr Pro Asp Ser Leu Thr Leu Arg Lys Leu Asn Glu Gly Ala Thr Gly Gln Pro Tyr Asp Tyr Phe Asn Thr Val Ser Arg Asp Ala Asp Ile   132

TCC AAG TGT CCA ATT TTT GCG TTG ACC ATA TTT TTT GTT ATA CGA TGG AGC CAC CCA AAC CCT CCA ATT TCA ATT GAG AAT TTT ACT ACA GTA CCG TTG CTA GAT TCA AAC TTT ATT TCT CTA AAT TCC AAT  1197
Ser Lys Cys Pro Ile Phe Ala Leu Thr Ile Phe Phe Val Ile Arg Trp Ser His Pro Asn Pro Pro Ile Ser Ile Glu Asn Phe Thr Thr Val Pro Leu Leu Asp Ser Asn Phe Ile Ser Leu Asn Ser Asn   176

CCT TTA CTA TAT ATT CAA AAT CAA AAC CCA AAC AGC AAT TCA AGT GTT AAA GTT TCA AGG TCA CAA ACG TTT GAA CCT TCT AAA GAG TTG ATC GAT TTG GTA TTT CCA TGG CTG TCT TAT TTG AAG CAG GAT  1329
Pro Leu Leu Tyr Ile Gln Asn Gln Asn Pro Asn Ser Asn Ser Ser Val Lys Val Ser Arg Ser Gln Thr Phe Glu Pro Ser Lys Glu Leu Ile Asp Leu Val Phe Pro Trp Leu Ser Tyr Leu Lys Gln Asp   220

ATG CTT CTT ATT GAT AGG ACG AAT TAC AAG CTT TAT TCT CTC TGT GAA CTA TTT GAA TTT ATG GGC AGG GTT GCC ATT CAG GAT CTC CGA TAT CTG AGT CAA CAT CCC TTA TTA CTA CCC AAT ATC GTA ACA  1461
Met Leu Leu Ile Asp Arg Thr Asn Tyr Lys Leu Tyr Ser Leu Cys Glu Leu Phe Glu Phe Met Gly Arg Val Ala Ile Gln Asp Leu Arg Tyr Leu Ser Gln His Pro Leu Leu Leu Pro Asn Ile Val Thr   264

TTC ATT TCA AAA TTT ATT CCT GAG TTA TTC CAA AAC GAA GAG TTT AAA GGA ATC GGT TCA ATT AAA AAT TCA AAC AAT AAT GCC CTG AAC AAT GTT ACA GGA ATA GAA ACC CAA TTT TTA AAT CCA TCT ACC  1593
Phe Ile Ser Lys Phe Ile Pro Glu Leu Phe Gln Asn Glu Glu Phe Lys Gly Ile Gly Ser Ile Lys Asn Ser Asn Asn Asn Ala Leu Asn Asn Val Thr Gly Ile Glu Thr Gln Phe Leu Asn Pro Ser Thr   308

GAG GAA GTG AGT CAA AAA GTT GAT TCT TAC TTT ATG GAA TTA TCA AAA AAA TTA ACT ACA GAA AAT ATC AGG TTA AGT CAA GAA ATA ACA CAA CTA AAA GCA GAT ATG AAC TCC GTA GGC AAT GTT TGT AAC  1725
Glu Glu Val Ser Gln Lys Val Asp Ser Tyr Phe Met Glu Leu Ser Lys Lys Leu Thr Thr Glu Asn Ile Arg Leu Ser Gln Glu Ile Thr Gln Leu Lys Ala Asp Met Asn Ser Val Gly Asn Val Cys Asn   352

CAA ATT TTG CTG TTG CAG AGA CAA TTG CTT TCA GGA AAT CAG GCG ATC GGA TCA AAG TCC GAA AAT ATT GTG TCT TCC ACA GGT GGG GGG ATA TTA ATA CTA GAT AAA AAT AGC ATC AAT TCG AAC GTA CTG  1857
Gln Ile Leu Leu Leu Gln Arg Gln Leu Leu Ser Gly Asn Gln Ala Ile Gly Ser Lys Ser Glu Asn Ile Val Ser Ser Thr Gly Gly Gly Ile Leu Ile Leu Asp Lys Asn Ser Ile Asn Ser Asn Val Leu   396

AGT AAT TTG GTT CAG TCG ATA GAT CCT AAT CAC TCC AAG CCC AAC GGA CAA GCC CAA ACA CAT CAA AGG GGT CCG AAA GGA CAA TCA CAT GCA CAG GTT CAA AGT ACT AAT AGC CCT GCG CTA GCG CCA ATT  1989
Ser Asn Leu Val Gln Ser Ile Asp Pro Asn His Ser Lys Pro Asn Gly Gln Ala Gln Thr His Gln Arg Gly Pro Lys Gly Gln Ser His Ala Gln Val Gln Ser Thr Asn Ser Pro Ala Leu Ala Pro Ile   440

AAC ATG TTC CCG AGC TTA AGT AAT TCT ATA CAG CCG ATG CTT GGC ACC TTG GCT CCG CAA CCG CAA GAT ATA GTA CAG AAG AGG AAG CTA CCG TTA CCA GGT TCA ATA GCC TCT GCA GCA ACA GGC AGT CCT  2121
Asn Met Phe Pro Ser Leu Ser Asn Ser Ile Gln Pro Met Leu Gly Thr Leu Ala Pro Gln Pro Gln Asp Ile Val Gln Lys Arg Lys Leu Pro Leu Pro Gly Ser Ile Ala Ser Ala Ala Thr Gly Ser Pro   484

TTT TCT CCA TCA CCC GTT GGT GAG TCT CCC TAT AGC AAA CGC TTT AAA CTA GAC GAT AAA CCA ACT CCG TCT CAG ACG GCT CTT GAT TCC TTA CTT ACA AAA TCC ATT TCA AGC CCT AGA TTA CCC CTT TCG  2253
Phe Ser Pro Ser Pro Val Gly Glu Ser Pro Tyr Ser Lys Arg Phe Lys Leu Asp Asp Lys Pro Thr Pro Ser Gln Thr Ala Leu Asp Ser Leu Leu Thr Lys Ser Ile Ser Ser Pro Arg Leu Pro Leu Ser   528

ACG TTG GCT AAC ACA GCT GTC ACG GAA TCT TTT CGC TCA CCT CAG CAG TTT CAG CAT TCT CCA GAT TTT GTA GTT GGT GGT AGC TCA AGT TCA ACA ACG GAA AAT AAC TCT AAG AAG GTA AAT GAA GAT TCT  2385
Thr Leu Ala Asn Thr Ala Val Thr Glu Ser Phe Arg Ser Pro Gln Gln Phe Gln His Ser Pro Asp Phe Val Val Gly Gly Ser Ser Ser Ser Thr Thr Glu Asn Asn Ser Lys Lys Val Asn Glu Asp Ser   572

CCA TCA TCT TCT TCA AAA CTA GCT GAA CGA CCT CGT CTT CCA AAC AAC GAC TCC ACT ACT AGC ATG CCT GAA AGT CCC ACC GAG GTA GCT GGT GAT GAT GTT GAT AGG GAG AAA CCG CCA GAG TCA AGT AAG  2517
Pro Ser Ser Ser Ser Lys Leu Ala Glu Arg Pro Arg Leu Pro Asn Asn Asp Ser Thr Thr Ser Met Pro Glu Ser Pro Thr Glu Val Ala Gly Asp Asp Val Asp Arg Glu Lys Pro Pro Glu Ser Ser Lys   616

TCG GAG CCC AAT GAT AAC AGC CCA GAA TCG AAA GAT CCT GAG AAA AAT GGT AAA AAC AGT AAT CCG CTT GGT ACG GAT GCT GAC AAA CCA GTA CCA ATT TCT AAT ATT CAT AAT TCT ACT GAG GCT GCA AAT  2649
Ser Glu Pro Asn Asp Asn Ser Pro Glu Ser Lys Asp Pro Glu Lys Asn Gly Lys Asn Ser Asn Pro Leu Gly Thr Asp Ala Asp Lys Pro Val Pro Ile Ser Asn Ile His Asn Ser Thr Glu Ala Ala Asn   660

TCA AGT GGT ACA GTG ACA AAG ACA GCT CCA TCA TTT CCG CAG AGT TCT TCT AAG TTT GAA ATT ATA AAT AAA AAG GAT ACG AAG GCG GGG CCA AAC GAG GCA ATC AAA TAC AAG CTG TCC AGA GAA AAT AAA  2781
Ser Ser Gly Thr Val Thr Lys Thr Ala Pro Ser Phe Pro Gln Ser Ser Ser Lys Phe Glu Ile Ile Asn Lys Lys Asp Thr Lys Ala Gly Pro Asn Glu Ala Ile Lys Tyr Lys Leu Ser Arg Glu Asn Lys   704

ACA ATA TGG GAC CTA TAT GCG GAG TGG TAT ATT GGT CTG AAC GGT AAA TCT TCA ATA AAA AAA TTG ATT GAA AAT TAT GGC TGG CGA AGG TGG AAG GTT AGC GAA GAT TCA CAT TTT TTT CCT ACT AGA AGA  2913
Thr Ile Trp Asp Leu Tyr Ala Glu Trp Tyr Ile Gly Leu Asn Gly Lys Ser Ser Ile Lys Lys Leu Ile Glu Asn Tyr Gly Trp Arg Arg Trp Lys Val Ser Glu Asp Ser His Phe Phe Pro Thr Arg Arg   748

ATT ATT ATG GAT TAT ATT GAA ACG GAA TGT GAT CGT GGC ATA AAA CTC GGC AGG TTT ACT AAT CCT CAA CAA CCG AGG GAG GAT ATA CGG AAG ATT TTA GTA GGG GAC CTA GAA AAG TTC AGG ATA AAT AAC  3045
Ile Ile Met Asp Tyr Ile Glu Thr Glu Cys Asp Arg Gly Ile Lys Leu Gly Arg Phe Thr Asn Pro Gln Gln Pro Arg Glu Asp Ile Arg Lys Ile Leu Val Gly Asp Leu Glu Lys Phe Arg Ile Asn Asn   792

GGT CTG ACT CTG AAT TCT CTA TCA TTG TAC TTT AGA AAT TTA ACG AAA AAT AAC AAG GAA ATT TGT ATT TTT GAA AAC TTT AAA AAT TGG AAC GTT AGA TCA ATG ACA GAA GAA GAG AAA TTA AAG TAT TGC  3177
Gly Leu Thr Leu Asn Ser Leu Ser Leu Tyr Phe Arg Asn Leu Thr Lys Asn Asn Lys Glu Ile Cys Ile Phe Glu Asn Phe Lys Asn Trp Asn Val Arg Ser Met Thr Glu Glu Glu Lys Leu Lys Tyr Cys   836

AAA AGG CGA CAT AAT ACA CCA TCT TAA GTTTATTGAG GTTGTCCGCG ACAATAGTTC CTTCAACAAA ATAACGAAGC GACGATAACG AGAAATGTCA TTAGGTTATT ACTACCTTTA TAGAAAATAT ATATACTTAT TTAATATTTA  3324
Lys Arg Arg His Asn Thr Pro Ser ...                                                                                                                                             844

TATAGATTTA TGTGTGTACA TACCTATGCG GATGTATGCC TATGTGGGAA TTC                                                                                                                  3377
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FIG. 9. The nucleotide sequence of the *GCR1* structural gene. The nucleotide sequence of 3,377 base pairs of genomic DNA was determined as described in Materials and Methods. The predicted amino acid sequence from an 844-codon open reading frame is indicated below the nucleotide sequence.

activities of other yeast glycolytic enzymes (2, 3) are also mediated at the level of transcription. Given the observation that the *gcr1-1* mutant allele contains a frameshift mutation, it is likely that the protein encoded by the mutant allele is nonfunctional. This suggestion is supported by the observation that strains carrying a *gcr1* null mutation have the same phenotype as *gcr1-1* strains. The *GCR1* gene is, therefore, not essential for cell viability but is required for efficient and coordinate expression of glycolytic genes.

The specific activities of several glycolytic enzymes increase when strains carrying the *gcr1-1* mutation are grown in medium supplemented with maltose (2, 3). Under these growth conditions, the specific activity of enolase did not increase appreciably; however, the specific activity of glyceraldehyde-3-phosphate dehydrogenase increased to 25% of wild-type levels. In vitro translation studies (Fig. 3) did not support a commensurate 25% increase in glyceraldehyde-3-phosphate dehydrogenase mRNA when cells were grown in the presence of maltose. We did observe a three- to fivefold increase in the doubling time of the *gcr1-1* mutant strain when 2% maltose was included in medium containing 2% glycerol plus 2% lactate. The observed increase in the specific activity of glyceraldehyde-3-phosphate dehydrogenase may have been due to accumulation of enzyme in these cells rather than a large increase in transcription of the genes.

When *ENO2* or *TDH3* genes were introduced into a *gcr1-1* mutant strain on a multicopy episomal plasmid, expression of these genes was reduced more than 50-fold over that in wild-type strains. These observations suggested that the *cis*-acting sequence which mediate the effect of the *GCR1* gene product on transcription of *ENO2* and *TDH3* are located within or adjacent to the genes. Expression of *ENO1* (Cohen et al., submitted), *ENO2* (4), and *TDH3* (unpublished data) is positively regulated by *cis*-acting sequences located within the 5′ flanking regions of these genes. It is possible that the *GCR1* gene product modulates the activity of these *cis*-acting regulatory sequences. Although the polypeptide encoded by the *GCR1* gene does not share significant homology with any other known DNA-binding proteins, it is possible that this polypeptide binds to glycolytic gene *cis*-acting regulatory sequences analogous to *GAL4* protein binding to the *cis*-acting regulatory sequences of *GAL1* (5). Alternatively, the protein encoded by the *GCR1* gene may modulate binding of another protein to these sequences. Further studies will be necessary to distinguish between these possibilities. Regardless of the mechanism of action of the *GCR1* protein, it is clear that this protein plays a significant role in coordinate regulation of transcription of many yeast glycolytic genes.

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ADDENDUM IN PROOF

Baker (H. V. Baker, Mol. Cell. Biol. 6:3774–3784, 1986) recently reported the nucleotide sequence of the *GCR1* structural gene and characterization of a *gcr1* null mutation. Our results and his are in essential agreement.

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