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# Glycolytic Gene Expression in *Saccharomyces cerevisiae*: Nucleotide Sequence of *GCR1*, Null Mutants, and Evidence for Expression

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**In *Saccharomyces cerevisiae*, the *gcr* mutation is known to have a profound effect on the levels of most glycolytic enzymes, reducing them to 5% of normal or less in growth on noncarbohydrates. Here I report the preparation of chromosomal *gcr* insertion and deletion mutations. The null mutations were recessive, were not lethal, and caused a pattern of glycolytic enzyme deficiency similar to that seen earlier for the *gcr1-1* allele, including the partial inducibility by glucose of the residual enzyme activities. DNA sequence analysis showed that *GCR1* encoded a protein of molecular weight 94,414, with a very low codon bias index, characteristic of several *S. cerevisiae* regulatory genes; adjacent 5′ and 3′ sequences contained elements suggesting that it was transcribed, polyadenylated, and translated. RNA gel transfer hybridization experiments with purified polyadenylated RNA and a probe complementary to the 5′ portion of the open reading frame showed that *Gcr* was expressed as a polyadenylated transcript. Together with previous work, the present results suggest that the Gcr product may be a transcriptional factor necessary specifically for the high-level transcription of a limited set of genes whose products, the enzymes of glycolysis, constitute a substantial fraction of cell proteins and are responsible for the primary metabolic flux in many cells.**

In many cells the primary metabolic flux is sugar metabolism through the glycolytic pathway. The enzymes of this pathway constitute as much as 30 to 60% of the total soluble proteins in *Saccharomyces cerevisiae* (8, 10). These values represent high levels of expression of the individual genes; most of the reactions depend on single genes specifying single isozymes, and even for reactions with more than one isozyme, a single one may predominate in growth on glucose.

In *S. cerevisiae*, the various genes encoding glycolytic function, as far as is known, are unlinked. It is not clear whether there are mechanisms governing their expression as a group, but one indication for such control is the properties of *gcr1* mutants (5, 6). The *gcr1* mutation causes three significant effects. (i) Growth on glucose is quite defective, but growth on noncarbohydrates is adequate. (ii) As assessed in the latter condition, levels of most of the enzymes of glycolysis are 5% of normal or less. (iii) In the presence of sugars, levels of several of the enzymes are substantially induced, to as much as 25% of normal. Evidence suggests that the *gcr1* mutation causes a reduction in mRNA for the affected enzymes (5). A DNA clone encoding the putative *GCR1* allele has been reported (13). This clone was obtained by complementing the growth defect on glucose; in strains harboring the plasmid, glycolysis enzymes were restored to normal levels (13).

In this paper *gcr1*::*LEU2* insertion and *gcr1* deletion-*LEU2* substitution mutations were prepared in vitro and introduced into the *S. cerevisiae* genome. The profile of the glycolytic enzymes in chromosomal null mutants is presented, and in addition I show, through DNA sequencing, that *GCR1* probably codes for a large regulatory protein.

## MATERIALS AND METHODS

**Nucleic acid manipulations.** Standard techniques used throughout the course of this study are described in Maniatis et al. (18). *S. cerevisiae* DNA was prepared by the method of Sherman et al. (25). Plasmid DNA isolated from *Escherichia coli* was prepared by the sodium dodecyl sulfate (SDS)-NaOH lysis procedure described by Silhavy et al. (26).

Total *S. cerevisiae* RNA was prepared by the method of Struhl and Davis (28). Hybond-mAP (Amersham Corp.) was used to purify polyadenylated [poly(A)$^+$] by the specifications of the manufacturer.

**Plasmid constructions.** Plasmid pHB3 was prepared by deleting a 4.2-kilobase-pair (kbp) *Nco*I fragment from the right side of the insert of plasmid pGCR1. In plasmid pHB4 the leftward 2.7-kbp portion of the insert was deleted as an *Hin*dIII dropout by using the *Hin*dIII site on plasmid Yep13 (4), which lies 346 base pairs (bp) upstream from the *Bam*HI-*Sau*3A junction in plasmid pGCR1. Plasmids pHB9 and pHB12 are derivatives of plasmids Yep13 and pBR322, respectively, into which the 4-kbp *Bcl*I fragment from the central portion of the insert in plasmid pGCR1 was cloned at their *Bam*HI sites. Plasmid pHB14 is a derivative of plasmid pHB12 into which a 3-kbp DNA fragment encoding *LEU2* was cloned into the *Nco*I site of plasmid pHB12. Plasmid pHB25 was constructed in two steps: first, a 7.4-kbp *Sal*I fragment was cloned from plasmid pGCR1 into plasmid pBR322, and then the 4-kbp *Bcl*I fragment was replaced with a 3-kbp *Bgl*II fragment encoding *LEU2*.

**Transformation.** *E. coli* strains were transformed with plasmid DNA by the method of Enea et al. (7). The method of Ito et al. (11) was used to transform *S. cerevisiae* strains.

**Gel-transfer hybridization.** Gel-transfer hybridization experiments (28, 29) were carried out with cationated nylon membranes (GeneScreen; Du Pont). DNA and RNA transfer and hybridization procedures followed the protocols supplied with the membrane, except that an additional wash in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% SDS at 65°C for 30 min was included immediately prior to prehybridization. Probe DNA was generated

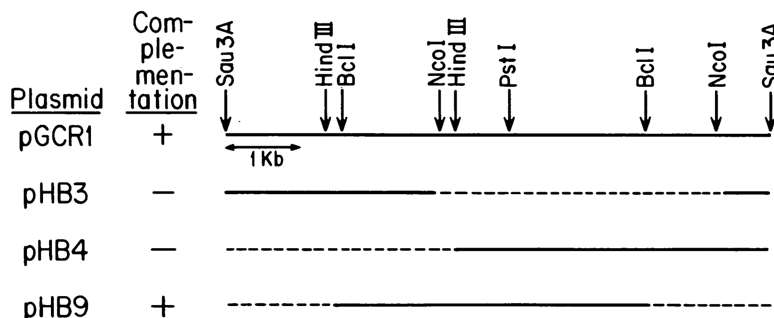


FIG. 1. Partial restriction maps of cloned plasmid DNAs used in this study and results of complementation analysis. Solid line indicates DNA carried by the clone. Plasmid pGCR1 is a derivitive of plasmid Yep13 isolated from an *S. cerevisiae* DNA gene bank prepared by partially digesting chromosomal DNA with *Sau*3A and ligating it into the *Bam*HI site of plasmid Yep13. Plasmid pGCR1 carries a 7.7-kbp insert; the *Bam*HI site was preserved at the left vector-insert junction. Plasmids pHB3 and pHB4 are "dropout" subclones of plasmid pGCR1 (see Materials and Methods for details). Plasmid pHB9 was prepared by subcloning the 3.7-kbp *Bcl*I fragment from plasmid pGCR1 into the *Bam*HI site of plasmid Yep13.

by primer extension of an appropriate M13 clone in the presence of [$^{32}$P]ATP.

**Enzyme assays.** Cultures were grown in YP medium (25) supplemented with 2% lactate and 1% glycerol. Four hours prior to harvest, half of each culture was shifted to glycolytic medium by the addition of glucose (final concentration of 2%) to the growth medium. The cultures (mid- to late-logarithmic growth phase) were harvested by centrifugation, washed, and suspended in extract buffer (3 ml/g of cell pellet), and then the extracts were prepared by passage through a French pressure cell as described previously (6). Cell debris was removed by centrifugation.

Enzyme assays were carried out as described by Clifton et al. (6), with the exception of aldolase, which was assayed by the method of Richards and Rutter (22). Protein concentration was determined by the method of Bradford (3).

**DNA sequencing.** The dideoxy chain termination method of Sanger et al. (24) was carried out as described in a protocol obtained from Amersham. Initially, the 4-kilobase (kb) *Bcl*I fragment was cloned in both orientations into the *Bam*HI site of the M13 phages M13mp18 and M13mp19 (30). To generate many of the clones used for sequencing, dropout subcloning experiments were used between sites within the fragment and sites in the polycloning region proximal to the universal priming site. Other clones were generated by cloning particular fragments into the M13 phages.

## RESULTS

**Restriction mapping and subcloning.** *gcr1* mutant strains form very small colonies on enriched medium containing glucose (e.g., colony size of 0.2 mm compared with ca. 2.5 mm for wild-type [*GCR*] strains [5]). The plasmid provisionally named pGCR1 was originally isolated from an *S. cerevisiae* DNA gene bank (chromosomal fragments from a partial *Sau*3A digest ligated into the unique *Bam*HI site of plasmid Yep13) on the basis of its complementation of this growth defect (13). Its insert proved to be 7.7 kbp in size (Fig. 1). The results of three subcloning experiments carried out to further define the region encoding Gcr$^-$-complementing activity are shown in Fig. 1. Transformation of the *leu2 gcr1* recipient, strain DFY407, with selection for Leu$^+$ and scoring of growth on glucose showed that the Gcr$^-$-complementing activity was carried by plasmid pHB9, which carries the central ca. 4-kbp portion of the insert. Transformants of strain DFY407 harboring plasmid pHB9 or pGCR1 segregated small colonies on YPD plates which proved to be Leu$^-$ Gcr$^-$, as expected. Acquisition of plasmid DNA by the yeast transformants was confirmed by reisolation and mapping of the plasmids.

Although plasmid pHB4 was unable to complement the nonreverting *gcr1-1* mutation, the plasmid was able to rescue it. Leu$^+$ transformants of *gcr1-1* mutant strains harboring plasmid pHB4 reverted to Gcr$^+$ at a frequency of $1.5 \times 10^{-5}$. DNA gel-transfer (27) hybridization experiments showed that the transformant and revertants each carried plasmid pHB4 integrated in the chromosome (data not shown). The gene conversion of *gcr1-1* to Gcr$^+$ by plasmid pHB4 shows allelism of the cloned gene with *gcr1* and further maps the mutation specified by *gcr1-1* to the right of the second *Hin*dIII site of plasmid pGCR1, as shown in Fig. 1.

**Isolation of *gcr1*::*LEU2* insertion and *gcr1* deletion-*LEU2* substitution mutations.** The inability of both plasmids pHB3 and pHB4 to complement *gcr1* suggested that the unique *Nco*I site of plasmid pHB9 (Fig. 1) was within the gene. To test this assertion, a gene disruption experiment (23) was carried out, in which foreign DNA encoding *LEU2* was cloned into the *Nco*I site. First, the 4-kb *Bcl*I fragment was cloned from plasmid pGCR1 into plasmid pBR322, yielding plasmid pHB12. Then, a 3-kbp fragment encoding *LEU2* was cloned into the *Nco*I site of plasmid pHB12, giving rise to plasmid pHB14. A transplacement experiment (23) was then carried out to determine the effect of the disruption. Plasmid pHB14 DNA was digested with *Pst*I and used to transform diploid strain DFY535 (*GCR1*/*GCR1 leu2*/*leu2*) to leucine prototrophy. Scoring of transformants on YPD medium showed them all to be Gcr$^+$, but tetrad analysis showed that Gcr$^-$ Leu$^+$ segregated 2:2 with Gcr$^+$ Leu$^-$. The same transformation done with haploid strain DFY510 (*GCR1 leu2*) gave rise to a Leu$^+$ transformant, strain DFY512, which was phenotypically Gcr$^-$. Transplacement of strain DFY512 back to Gcr$^+$ (with DNA from the nonreplicating plasmid pHB12 [*GCR1*]) restored leucine auxotrophy. The disruption experiment confirmed that the *Nco*I site is within *GCR1* or its surrounding control region and that the insertion causes a Gcr$^-$ phenotype.

The fact that *gcr1*::*LEU2*, like earlier *gcr1* mutations, conferred a recessive and nonlethal phenotype suggests that *GCR1* is not essential. However, since its function might not have been completely abolished by the insertion, a second

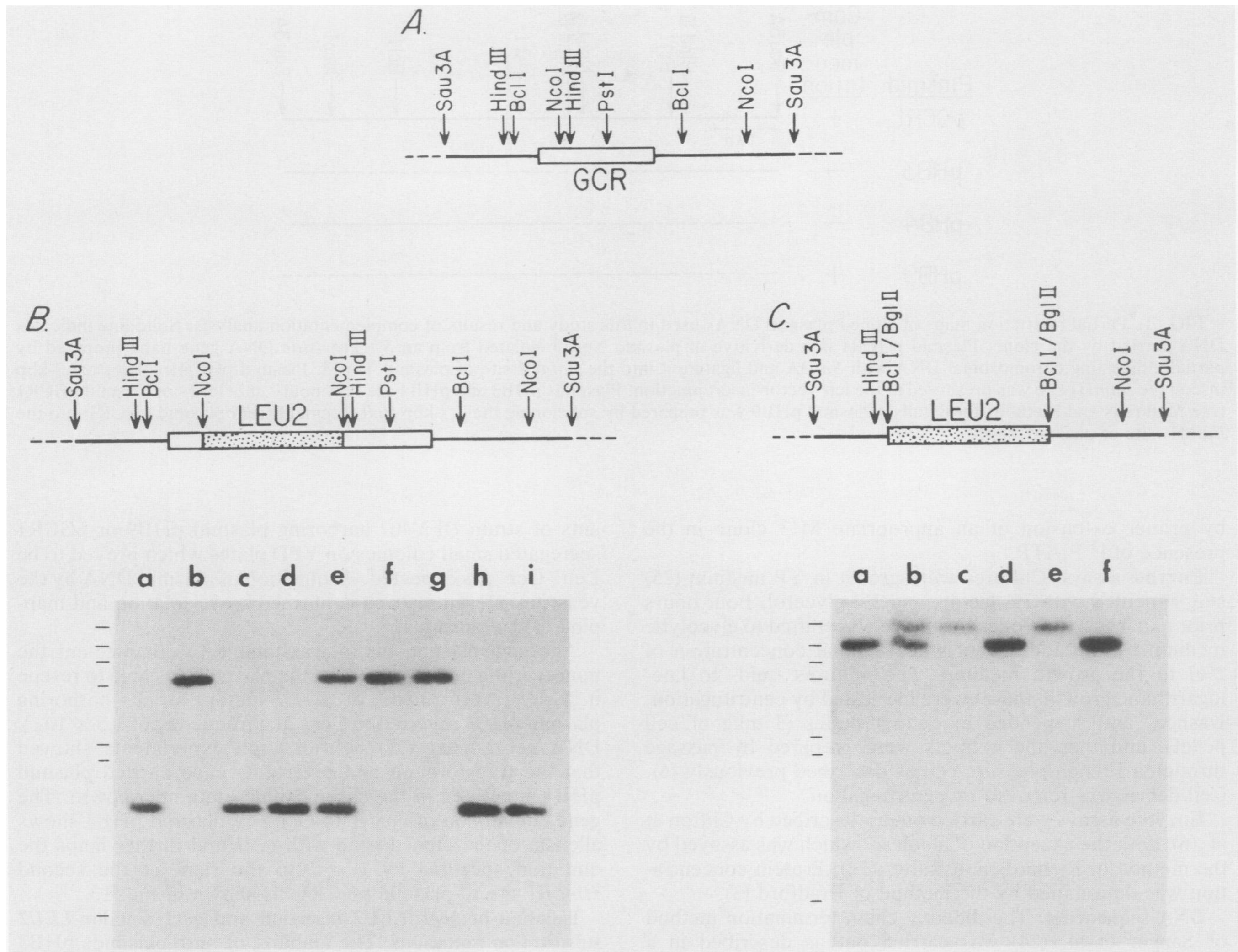


FIG. 2. Genomic structure of the *GCR1* region in wild-type *GCR1* and *gcr1* mutant strains. (A) Diagram of the genomic region encoding *GCR1* (open box) in wild-type strains; (B) diagram of the *GCR1* genomic region in strains with *gcr1*::*LEU2* insertion mutations and the results of a gel-transfer hybridization experiment with radiolabeled probe DNA homologous to the 1.2-kb *Bcl*I-*Hin*dIII fragment encoding the *Nco*I site used in the disruption experiment (see text for details). *Hin*dIII-digested chromosomal DNA (8 μg) was used for each lane. The DNAs were prepared from (a) strain DFY510 (*GCR1*), (b) strain DFY512 (*gcr1*::*LEU2*), (c) strain DFY513 (*GCR1*- repaired *gcr1*::*LEU2*), (d) strain DFY535 (*GCR1*/*GCR1*), (e) strain DFY514 (*GCR1*/*gcr1*::*LEU2*), and (f–i) strains DFY515, DFY516, DFY517, and DFY518 (spores of one complete tetrad of strain DFY514), respectively. Lines at the left denote positions of unlabeled *Hin*dIII-digested λ DNA. (C) Diagram of the *GCR1* genomic region in strains with *gcr* deletion-*LEU2* substitution mutations and the results of a gel-transfer hybridization experiment with radiolabeled probe homologous to the *Sau*3A-*Bcl*I 1.3-kb fragment (see text for details). *Bam*HI-*Pst*I-digested chromosomal DNA (8 μg) was used for each lane. The DNAs were prepared from (a) strain DFY535 (*GCR1*/*GCR1*) (b) strain DFY519 (*GCR1*/*gcr1* deletion-*LEU2* substitution mutant), (c–f) strains DYF520, DFY521, DFY522, and DFY523 (spores of one complete tetrad of strain DFY519), respectively. Lines at the left denote positions of unlabeled *Hin*dIII-digested λ DNA.

mutation was prepared in which a 3-kbp DNA fragment encoding *LEU2* was substituted for the entire 4-kbp *Bcl*I fragment (Fig. 1) (plasmid pHB25—see Materials and Methods). The new mutation was likewise introduced by transplacement into the diploid strain DFY535 (*GCR1*/*GCR1* *leu2*/*leu2*), and as with the *gcr1*::*LEU2* insertion mutation, transformants (e.g., DFY519) were $Gcr^+$ but segregated $Gcr^-$::$Leu^-$ 2:2. Thus, like the insertion mutation, the *gcr1* deletion-*LEU2* substitution mutation was not lethal.

The genomic structure of the various strains was determined by DNA gel-transfer (27) hybridization experiments. For the series of strains including the *gcr1*::*LEU2* insertion mutation (whose putative structure is indicated in Fig. 2B), chromosomal DNA was digested with *Hin*dIII and probed, after electrophoresis and transfer, with a radioactive *Bcl*I-*Hin*dIII 1.2-kbp fragment from the original clone (Fig. 2A) spanning the *Nco*I site. As expected, in $Gcr^+$ strains the probe hybridized to a band of 1.4 kbp. A second band of 4.4 kbp (i.e., larger by the size of the *LEU2* insertion) was seen in the heterozygote (*GCR1*/*gcr1*::*LEU2*, lane e), and this was the only band observed in the haploid $Gcr^-$ strains: i.e., the two $Gcr^-$ segregants of the diploid (lanes f and g) and the $Gcr^-$ transplacement of the haploid (lane b). Only the wild-type band was found in the $Gcr^+$ transplacement of the latter mutant (lane c).

For the series of strains including the *gcr1* deletion-*LEU2*

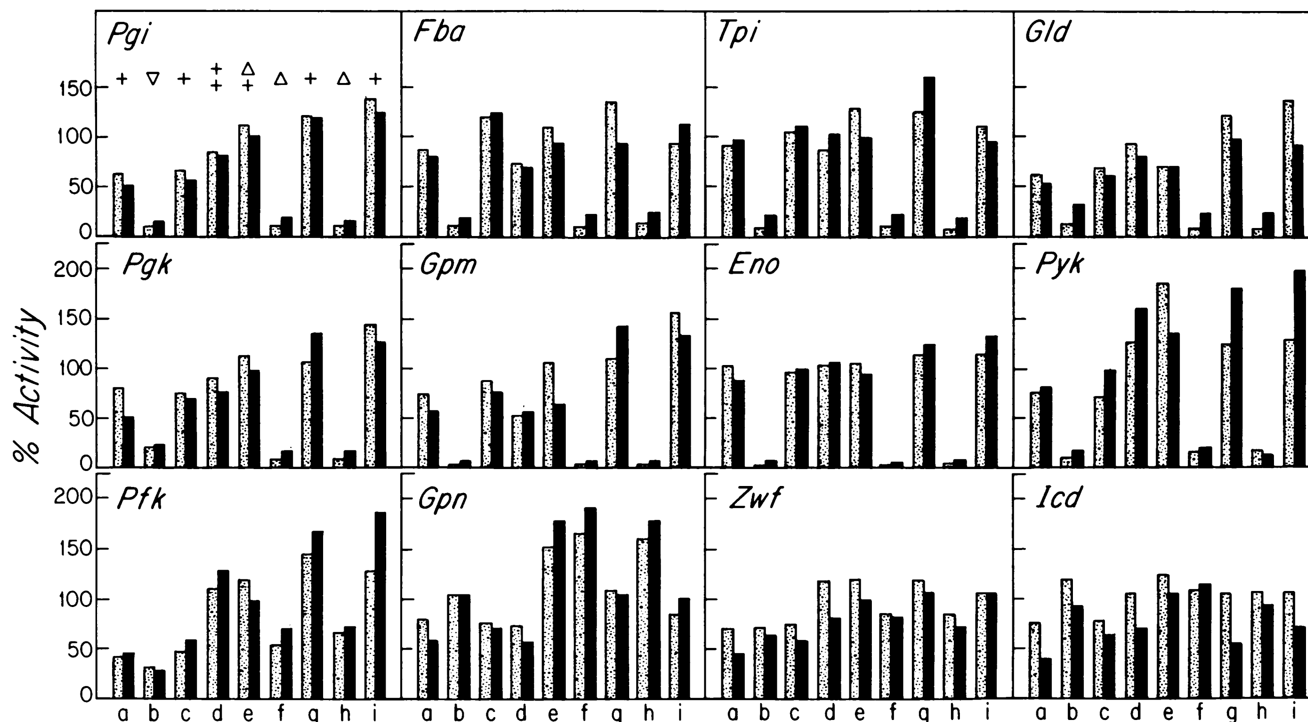


FIG. 3. Glycolytic enzyme profiles in wild-type *GCR1* and *gcr1* mutant strains. Each panel shows the relative specific activity in the cultures normalized to the average value obtained with haploid *GCR1* strains grown in gluconeogenic medium (YP enriched with 2% lactate and 1% glycerol). Enzymes assayed (wild-type activity): Pgi, phosphoglucose isomerase (1.854 U/mg); Fba, fructose bisphosphate aldolase (1.176 U/mg); Tpi, triose-phosphate isomerase (5.942 U/mg); Gld, glyceraldehyde-3-phosphate dehydrogenase (3.743 U/mg); Pgk, phosphoglycerate kinase (2.739 U/mg); Gpm, phosphoglycerate mutase (0.685 U/mg); Eno, enolase (0.422 U/mg); Pyk, pyruvate kinase (1.520 U/mg); Pfk, phosphofructokinase (0.269 U/mg); Gpm, glucose phosphorylation (0.890 U/mg); Zwf, glucose-6-phosphate dehydrogenase (0.075 U/mg); Icd, isocitrate dehydrogenase (0.056 U/mg). Stippled bars, Cultures grown in gluconeogenic medium; solid bars, cultures shifted for 4 h to glycolytic medium by the addition of glucose (to a final concentration of 2%) to gluconeogenic medium. The symbols in the first panel represent the nature of the *GCR1* allele in the strain of a given culture. a, Strain DFY510 (*GCR1*), +; b, strain DFY512 (*gcr1*::*LEU2* insertion), ▽; c, strain DFY513 (*GCR1* repaired *gcr1*::*LEU2*), +; d, strain DFY535 (*GCR1*/*GCR1*), ‡; e, strain DFY519 (*GCR1*/*gcr1*::*LEU2* deletion), △‡; f–i, strains DFY520, DFY521, DFY522, and DFY523, arising from the spores of one complete tetrad of strain DFY519 (△, +, △, +, respectively).

substitution mutation (whose putative structure is indicated in Fig. 2C), chromosomal DNA was digested with enzymes *Pst*I and *Bam*HI, and a *Sau*3A-*Bcl*I 1.3-kbp fragment (Fig. 2A) was used as the probe. The *gcr1* deletion-*LEU2* substitution mutation should cause loss of a *Pst*I site; thus, with DNA from the mutant a band larger than the one observed with DNA from wild-type strains should be present. In this experiment a 9.4-kbp fragment was found in Gcr$^+$ strains (lanes a, b, d, and f). The heterozygote (lane b) showed an additional band at ca. 18.8 kbp, and only the latter band was found in the Gcr$^-$ segregants (lanes c and e). Thus, for both series of strains the results confirmed the genetic data.

**Effect of the *gcr1*::*LEU2* insertion and *gcr1* deletion-*LEU2* substitution mutations on levels of the glycolytic enzymes.** Figure 3 shows the results of an assay for 12 enzyme activities for the same set of nine strains, a to i. Their *gcr* genotypes, as briefly indicated in the first panel, are a, *GCR1* haploid; b, *gcr1*::*LEU2* (insertion); and c, *GCR1* reformed from strain b; d, diploid *GCR1*/*GCR1*; e, diploid *GCR1*/*gcr1*::*LEU2* (substitution); and f through i, a tetrad from strain e. It was previously reported (5) for the *gcr1* mutant that the levels of most of the glycolytic enzymes were low for the eight enzyme activities shown in the first two rows of Fig. 3. The main conclusion to be drawn from the present assays is that the same pattern was also shown by the insertion (strain b) and *gcr1* deletion-*LEU2* substitution (strains f and h) mutants.

It was also reported earlier (5) that in the *gcr1-1* mutant, most of the glycolysis enzyme levels were higher in cells harvested after prolonged slow growth from a medium containing a sugar than from medium without sugar (i.e., levels 25% of normal instead of 5% of normal) and that the inducibility could be observed within a few hours of the addition of sugar. By contrast, in the wild-type strain, the levels of most of the enzymes were similar in the two conditions. In the present experiments, therefore, each strain was assayed after growth in gluconeogenic medium as well as at 4 h after glucose addition (Fig. 3). In the new *gcr1* mutants a modest induction was seen with most of the affected enzymes, whereas as before, Gcr$^+$ strains, including the heterozygote, had similar high enzyme levels in the two conditions.

Four other enzyme activities are shown in the bottom row of Fig. 3. Glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase levels were apparently unaffected by *gcr1*. The level of fructose-6-phosphate 1-kinase was less affected than that of other glycolytic enzymes, while the total level of glucose phosphorylation activity was somewhat higher in the *gcr1* strains.

**DNA sequence analysis.** Since the 4-kbp *Bcl*I restriction

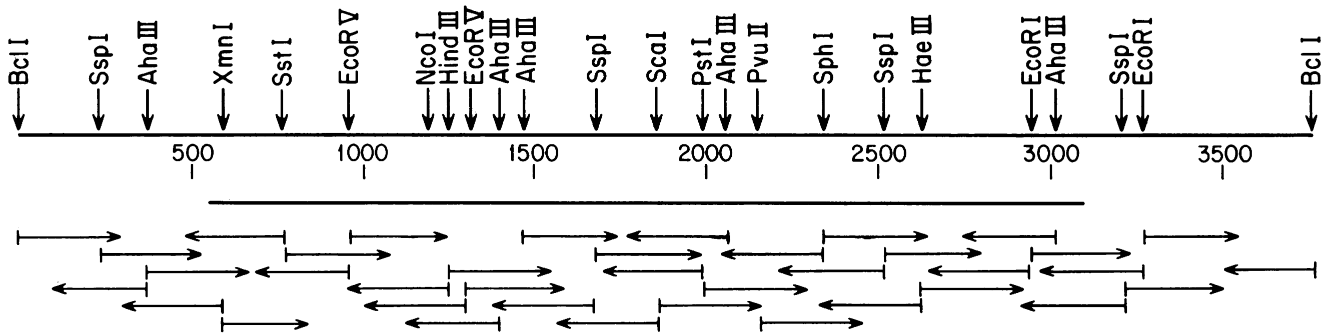


FIG. 4. DNA sequencing strategy. Except for the *NcoI* site, only restriction endonuclease sites used to generate the M13 clones used in sequencing the 4-kbp *BclI* fragment are shown. The solid bar below the map denotes the long open reading frame proposed to encode the *GCR1* gene product. The arrows represent the direction and extent of sequences obtained from a given M13 clone.

fragment carried *GCR1*, it was sequenced. Figure 4 shows the sequencing strategy used. Except for the terminal 94 bases on the left and 492 bases on the right side of the fragment, the entire *BclI* fragment was sequenced on both strands. In addition, to ensure that small restriction fragments were not missed, each restriction endonuclease site used to generate clones for sequencing was also sequenced from another clone. Figure 5 shows the entire nucleotide sequence of the 3,757 nucleotides that constitute the *BclI* restriction fragment.

The *NcoI* site used in the gene disruption experiment proved to be in an open reading frame of 857 codons which is capable of encoding a protein, the likely *GCR1* gene product, of 844 amino acids (starting with the first in-frame methionine codon). The sequence presented contains 561 nucleotides before the first base of the first Met codon and 662 nucleotides after the last base of the first stop codon in this reading frame. A promoterlike sequence (TATAAGA, underlined in Fig. 5) lies 103 bases before the putative start codon of *GCR1*. Likewise downstream from the putative *GCR1* stop codon, sequences suggestive of an *S. cerevisiae* transcription termination-polyadenylation site (31) were found. The sequence suggests the expression of a transcript of ca. 2.7 kb.

Direct evidence for the expression of this reading frame came from RNA gel-transfer (29) hybridization experiments with a probe spanning −190 nucleotides to +838 nucleotides (Fig. 5). Since preliminary experiments with total RNA were unsuccessful, presumably due to low-level expression of the transcript, poly(A)$^+$ purified RNA was prepared as an enrichment. Figure 6 shows a transcript of ca. 3.1 kb (based on single-stranded DNA molecular weight standards) in a lane loaded with poly(A)$^+$ purified RNA isolated from a 50-ml exponentially growing YPD culture of strain DFY510.

The *BclI* fragment may also include a portion of another gene. A second reading frame capable of encoding a polypeptide of at least 87 amino acids was found truncated at the right end of the fragment. The first methionine codon in this reading frame occurred at nucleotide 2936 (as indicated in Fig. 4), 401 nucleotides downstream from the termination codon of the putative *GCR1* reading frame, with a promotorlike sequence (TATATA) at nucleotide 2736. The *BclI* fragment contained no other reading frames capable of encoding polypeptides of statistically significant length in either direction. It is not known whether this second gene has any relationship to Gcr function. A larger portion of it would be carried on the non-*gcr*-complementing plasmid pHB4 than on plasmid pHB9 itself, and its partial removal in the mutant with the *gcr1* deletion-*LEU2* substitution mutation did not confer a special phenotype.

## DISCUSSION

In this paper I have shown that (i) *gcr1*-complementing activity, originally obtained as a 7.7-kbp insert in a hybrid plasmid, encodes *GCR1*; (ii) the gene is fully contained within a 3.7-kbp fragment; (iii) chromosomal insertion and *gcr1* deletion-*LEU2* substitution mutations give the same phenotype as the original *gcr1* mutation; and (iv) the fragment contains an open reading frame which is expressed as a poly(A)$^+$ transcript capable of encoding a protein of 844 amino acids, the putative *GCR1* gene product.

The first methionine codon in the *GCR1* reading frame occurs at codon position 14. As with many yeast initiation codons, it is preceded by an A at nucleotide position −3 (14) and succeeded by a T at nucleotide position +6 (1). There is a promoterlike sequence, TATAAGA, 103 bases before the putative start codon. In addition, the sequence TCAA, which is proposed to specify the precise transcription start point (9), is in an appropriate position downstream from the TATA box. The first in-frame stop codon occurs 845 codons after the ATG start codon. This stop codon is followed by another stop codon three codons later. Sequences in good agreement with the consensus sequences proposed (31) for transcription termination-polyadenylation in *S. cerevisiae* are found in the region 3′ to the stop codon.

Earlier work showed that mRNA levels for the affected genes were lower in the original *gcr1* mutant (5); thus, Gcr may function in transcription. The mechanism by which the *GCR* gene product mediates its effect remains to be elucidated. However, my working hypothesis is that the *GCR* gene product is a positive activator of glycolytic gene expression which is needed for high-level expression. The deduced amino acid sequence of the putative *GCR1* gene product indicates that it would have a molecular weight of 94,414.

The codon usage of *GCR1* (Table 1) allowed calculation (2) of its codon bias index as −0.00086. This scale indicates codon usage with respect to a preferred set of 22 codons found in highly expressed genes. Values for several glycolytic proteins are in the range 0.93 to 0.99 (2), whereas six known regulatory proteins ranged between 0.048 and −0.034 (12, 15). Thus, the value for the Gcr1 protein might accord with its being regulatory and expressed at a low level.

```
      -552          -542          -532          -522          -512          -502          -492
ATCATAACAC GCATTCTGAA AATGTTATCT GGGAGGTTTT CGATGGGTAT GGAGTTTTCC TTGATTCTCA

      -482          -472          -462          -452          -442          -432          -422
CTTATTATCC CTTGTATTGT AATTGATCCT TCAGTAATAT TTGCAGCCTT TCACAACTAT CCTTTTTTTC

      -422          -402          -392          -382          -372          -362          -352
ATTGCTTATT ACTATTGAAC CTTTTTTAGG AGTTGCCTGC TTATGCAATA TAATTTGCTG ACAAGTAGTA

      -342          -332          -322          -312          -302          -292          -282
AATTACCAGC ACAATATTAA GATTAAAAAA GAAATTAGCC AAGAGCTTGA TATATTATCT TATACACAAA

      -272          -262          -252          -242          -232          -222          -212
CCTTTCCGAC CTACTTGATA AAGCCACATA CCTCTACCTC TTCTATTAGA AATAGAAAAG TACAAAAATA

      -202          -192          -182          -172          -162          -152          -142
GCAAAAGGAA ATAATTTCTT TAAAATAACA TTGTGTGAGG TTCCAACTAT GGATTATTAA TAGAGTAACG

      -132          -122          -112          -102           -92           -82           -72
CAAACTTAAG GAAAGGAAGT GCTTTACAAT TAAGTATTTA TAAGAACGAA TTTATCCCCC AAAAAAAAGC

       -62           -52           -42           -32           -22           -12            -2
ACCTATACTT AATAAAAGGA GGGGAATAGC TATCAATTGA GTGTTGTCTG CGTCTGTCTG CGTACAAGAG

 1                15                  30                  45
G ATG AAT TTT CTG ACT CAG GCT ATG TCA GAA ACT TTT CAA GGG ACA AAT AAC
  MET Asn Phe Leu Thr Gln Ala MET Ser Glu Thr Phe Gln Gly Thr Asn Asn

        60                  75                  90                  105
AGG ATA AAA CGT AAT GTC AGG ACA CAA AGT GTG CCA TCA ACT TCC TAT AAT AAT
Arg Ile Lys Arg Asn Val Arg Thr Gln Ser Val Pro Ser Thr Ser Tyr Asn Asn

                120                 135                 150
GGC AAA GAA TCA TAT GGA CCA AAT ACT AAC CAA TTA AAT GCC CTA CTT TCT CAA
Gly Lys Glu Ser Tyr Gly Pro Asn Thr Asn Gln Leu Asn Ala Leu Leu Ser Gln

    165                 180                 195                 210
TTG GAA CAG CAA ACA AGT GTT GAT AGT ACC AGC ACG AGC TCA AAC TTT TAT TCC
Leu Glu Gln Gln Thr Ser Val Asp Ser Thr Ser Thr Ser Ser Asn Phe Tyr Ser

            225                 240                 255
ATT GCA CAA TAT ATT TTA CAA TCA TAC TTC AAG GTC AAT GTA GAT TCT CTA AAC
Ile Ala Gln Tyr Ile Leu Gln Ser Tyr Phe Lys Val Asn Val Asp Ser Leu Asn

270                 285                 300                 315
TCT CTG AAA TTG GTG GAT TTG ATA GTG GAC CAA ACT TAC CCT GAT TCT TTG ACG
Ser Leu Lys Leu Val Asp Leu Ile Val Asp Gln Thr Tyr Pro Asp Ser Leu Thr

        330                 345                 360                 375
CTG CGA AAG CTG AAT GAA GGA GCA ACG GGA CAA CCA TAC GAT TAT TTC AAT ACA
Leu Arg Lys Leu Asn Glu Gly Ala Thr Gly Gln Pro Tyr Asp Tyr Phe Asn Thr

                390                 405                 420
GTT TCT CGT GAT GCT GAT ATC TCC AAG TGT CCA ATT TTT GCG TTG ACC ATA TTT
Val Ser Arg Asp Ala Asp Ile Ser Lys Cys Pro Ile Phe Ala Leu Thr Ile Phe

    435                 450                 465                 480
TTT GTT ATA CGA TGG AGC CAC CCA AAC CCT CCA ATT TCA ATT GAG AAT TTT ACT
Phe Val Ile Arg Trp Ser His Pro Asn Pro Pro Ile Ser Ile Glu Asn Phe Thr
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FIG. 5. Nucleotide sequence of *GCR1* and the predicted amino acid sequence of the *GCR1* gene product. The DNA sequence of the 3,757-bp *Bcl*I fragment is shown. The putative TATA box and possible polyadenylation and transcription termination signals of *GCR1* are underlined. Also denoted by underlining (positions 2355–2415) is an amino acid sequence suggestive of a DNA-binding domain. The methionine of a second open reading frame (see text for details) and its putative TATA box are also indicated.

```
              495                 510                 525
ACA GTA CCG TTG CTA GAT TCA AAC TTT ATT TCT CTA AAT TCC AAT CCT TTA CTA
Thr Val Pro Leu Leu Asp Ser Asn Phe Ile Ser Leu Asn Ser Asn Pro Leu Leu

540                 555                 570                 585
TAT ATT CAA AAT CAA AAC CCA AAC AGC AAT TCA AGT GTT AAA GTT TCA AGG TCA
Tyr Ile Gln Asn Gln Asn Pro Asn Ser Asn Ser Ser Val Lys Val Ser Arg Ser

        600                 615                 630                 645
CAA ACG TTT GAA CCT TCT AAA GAG TTG ATC GAT TTG GTA TTT CCA TGG CTG TCT
Gln Thr Phe Glu Pro Ser Lys Glu Leu Ile Asp Leu Val Phe Pro Trp Leu Ser

                660                 675                 690
TAT TTG AAG CAG GAT ATG CTT CTT ATT GAT AGG ACG AAT TAC AAG CTT TAT TCT
Tyr Leu Lys Gln Asp MET Leu Leu Ile Asp Arg Thr Asn Tyr Lys Leu Tyr Ser

    705                 720                 735                 750
CTC TGT GAA CTA TTT GAA TTT ATG GGC AGG GTT GCC ATT CAG GAT CTC CGA TAT
Leu Cys Glu Leu Phe Glu Phe MET Gly Arg Val Ala Ile Gln Asp Leu Arg Tyr

            765                 780                 795
CTG AGT CAA CAT CCC TTA TTA CTA CCC AAT ATC GTA ACA TTC ATT TCA AAA TTT
Leu Ser Gln His Pro Leu Leu Leu Pro Asn Ile Val Thr Phe Ile Ser Lys Phe

810                 825                 840                 855
ATT CCT GAG TTA TTC CAA AAC GAA GAG TTT AAA GGA ATC GGT TCA ATT AAA AAT
Ile Pro Glu Leu Phe Gln Asn Glu Glu Phe Lys Gly Ile Gly Ser Ile Lys Asn

        870                 885                 900                 915
TCA AAC AAT AAT GCC CTG AAC AAT GTT ACA GGA ATA GAA ACC CAA TTT TTA AAT
Ser Asn Asn Asn Ala Leu Asn Asn Val Thr Gly Ile Glu Thr Gln Phe Leu Asn

                930                 945                 960
CCA TCT ACC GAG GAA GTG AGT CAA AAA GTT GAT TCT TAC TTT ATG GAA TTA TCA
Pro Ser Thr Glu Glu Val Ser Gln Lys Val Asp Ser Tyr Phe MET Glu Leu Ser

    975                 990                 1005                1020
AAA AAA TTA ACT ACA GAA AAT ATC AGG TTA AGT CAA GAA ATA ACA CAA CTA AAA
Lys Lys Leu Thr Thr Glu Asn Ile Arg Leu Ser Gln Glu Ile Thr Gln Leu Lys

            1035                1050                1065
GCA GAT ATG AAC TCC GTA GGC AAT GTT TGT AAC CAA ATT TTG CTG TTG CAG AGA
Ala Asp MET Asn Ser Val Gly Asn Val Cys Asn Gln Ile Leu Leu Leu Gln Arg

1080                1095                1110                1125
CAA TTG CTT TCA GGA AAT CAG GCG ATC GGA TCA AAG TCC GAA AAT ATT GTG TCT
Gln Leu Leu Ser Gly Asn Gln Ala Ile Gly Ser Lys Ser Glu Asn Ile Val Ser

        1140                1155                1170                1185
TCC ACA GGT GGG GGG ATA TTA ATA CTA GAT AAA AAT AGC ATC AAT TCG AAC GTA
Ser Thr Gly Gly Gly Ile Leu Ile Leu Asp Lys Asn Ser Ile Asn Ser Asn Val

                1200                1215                1230
CTG AGT AAT TTG GTT CAG TCG ATA GAT CCT AAT CAC TCC AAG CCC AAC GGA CAA
Leu Ser Asn Leu Val Gln Ser Ile Asp Pro Asn His Ser Lys Pro Asn Gly Gln

    1245                1260                1275                1290
GCC CAA ACA CAT CAA AGG GGT CCG AAA GGA CAA TCA CAT GCA CAG GTT CAA AGT
Ala Gln Thr His Gln Arg Gly Pro Lys Gly Gln Ser His Ala Gln Val Gln Ser

            1305                1320                1335
ACT AAT AGC CCT GCG CTA GCG CCA ATT AAC ATG TTC CCG AGC TTA AGT AAT TCT
Thr Asn Ser Pro Ala Leu Ala Pro Ile Asn MET Phe Pro Ser Leu Ser Asn Ser

1350                1365                1380                1395
ATA CAG CCG ATG CTT GGC ACC TTG CGT CCG CAA CCG CAA GAT ATA GTA CAG AAG
Ile Gln Pro MET Leu Gly Thr Leu Arg Pro Gln Pro Gln Asp Ile Val Gln Lys
```

FIG. 5—*(Continued)*

```
           1410                          1425                          1440                          1455
AGG AAG CTA CCG TTA CCA GGT TCA ATA GCC TCT GCA GCA ACA GGC AGT CCT TTT
Arg Lys Leu Pro Leu Pro Gly Ser Ile Ala Ser Ala Ala Thr Gly Ser Pro Phe

                   1470                          1485                          1500
TCT CCA TCA CCC GTT GGT GAG TCT CCC TAT AGC AAA CGC TTT AAA CTA GAC GAT
Ser Pro Ser Pro Val Gly Glu Ser Pro Tyr Ser Lys Arg Phe Lys Leu Asp Asp

  1515                          1530                          1545                          1560
AAA CCA ACT CCG TCT CAG ACG GCT CTT GAT TCC TTA CTT ACA AAA TCC ATT TCA
Lys Pro Thr Pro Ser Gln Thr Ala Leu Asp Ser Leu Leu Thr Lys Ser Ile Ser

           1575                          1590                          1605
AGC CCT AGA TTA CCC CTT TCG ACG TTG GCT AAC ACA GCT GTC ACG GAA TCT TTT
Ser Pro Arg Leu Pro Leu Ser Thr Leu Ala Asn Thr Ala Val Thr Glu Ser Phe

1620                          1635                          1650                          1665
CGC TCA CCT CAG CAG TTT CAG CAT TCT CCA GAT TTT GTA GTT GGT GGT AGC TCA
Arg Ser Pro Gln Gln Phe Gln His Ser Pro Asp Phe Val Val Gly Gly Ser Ser

        1680                          1695                          1710                          1725
AGT TCA ACA ACG GAA AAT AAC TCT AAG AAG GTA AAT GAA GAT TCT CCA TCA TCT
Ser Ser Thr Thr Glu Asn Asn Ser Lys Lys Val Asn Glu Asp Ser Pro Ser Ser

                   1740                          1755                          1770
TCT TCA AAA CTA GCT GAA CGA CCT CGT CTT CCA AAC AAC GAC TCC ACT ACT AGC
Ser Ser Lys Leu Ala Glu Arg Pro Arg Leu Pro Asn Asn Asp Ser Thr Thr Ser

  1785                          1800                          1815                          1830
ATG CCT GAA AGT CCC ACC GAG GTA GCT GGT GAT GAT GTT GAT AGG GAG AAA CCG
MET Pro Glu Ser Pro Thr Glu Val Ala Gly Asp Asp Val Asp Arg Glu Lys Pro

           1845                          1860                          1875
CCA GAG TCA AGT AAG TCG GAG CCC AAT GAT AAC AGC CCA GAA TCG AAA GAT CCT
Pro Glu Ser Ser Lys Ser Glu Pro Asn Asp Asn Ser Pro Glu Ser Lys Asp Pro

1890                          1905                          1920                          1935
GAG AAA AAT GGT AAA AAC AGT AAT CCG CTT GGT ACG GAT GCT GAC AAA CCA GTA
Glu Lys Asn Gly Lys Asn Ser Asn Pro Leu Gly Thr Asp Ala Asp Lys Pro Val

        1950                          1965                          1980                          1995
CCA ATT TCT AAT ATT CAT AAT TCT ACT GAG GCT GCA AAT TCA AGT GGT ACA GTG
Pro Ile Ser Asn Ile His Asn Ser Thr Glu Ala Ala Asn Ser Ser Gly Thr Val

                   2010                          2025                          2040
ACA AAG ACA GCT CCA TCA TTT CCG CAG AGT TCT TCT AAG TTT GAA ATT ATA AAT
Thr Lys Thr Ala Pro Ser Phe Pro Gln Ser Ser Ser Lys Phe Glu Ile Ile Asn

  2055                          2070                          2085                          2100
AAA AAG GAT ACG AAG GCG GGG CCA AAC GAG GCA ATC AAA TAC AAG CTG TCC AGA
Lys Lys Asp Thr Lys Ala Gly Pro Asn Glu Ala Ile Lys Tyr Lys Leu Ser Arg

           2115                          2130                          2145
GAA AAT AAA ACA ATA TGG GAC CTA TAT GCG GAG TGG TAT ATT GGT CTG AAC GGT
Glu Asn Lys Thr Ile Trp Asp Leu Tyr Ala Glu Trp Tyr Ile Gly Leu Asn Gly

2160                          2175                          2190                          2205
AAA TCT TCA ATA AAA AAA TTG ATT GAA AAT TAT GGC TGG CGA AGG TGG AAG GTT
Lys Ser Ser Ile Lys Lys Leu Ile Glu Asn Tyr Gly Trp Arg Arg Trp Lys Val

        2220                          2235                          2250                          2265
AGC GAA GAT TCA CAT TTT TTT CCT ACT AGA AGA ATT ATT ATG GAT TAT ATT GAA
Ser Glu Asp Ser His Phe Phe Pro Thr Arg Arg Ile Ile MET Asp Tyr Ile Glu
```

FIG. 5—(*Continued*)

```
              2280                2295                2310
ACG GAA TGT GAT CGT GGC ATA AAA CTC GGC AGG TTT ACT AAT CCT CAA CAA CCG
Thr Glu Cys Asp Arg Gly Ile Lys Leu Gly Arg Phe Thr Asn Pro Gln Gln Pro

  2325                2340                2355                2370
AGG GAG GAT ATA CGG AAG ATT TTA GTA GGG GAC CTA GAA AAG TTC AGG ATA AAT
Arg Glu Asp Ile Arg Lys Ile Leu Val Gly Asp Leu Glu Lys Phe Arg Ile Asn

          2385                2400                2415
AAC GGT CTG ACT CTG AAT TCT CTA TCA TTG TAC TTT AGA AAT TTA ACG AAA AAT
Asn Gly Leu Thr Leu Asn Ser Leu Ser Leu Tyr Phe Arg Asn Leu Thr Lys Asn

2430               2445                2460                2475
AAC AAG GAA ATT TGT ATT TTT GAA AAC TTT AAA AAT TGG AAC GTT AGA TCA ATG
Asn Lys Glu Ile Cys Ile Phe Glu Asn Phe Lys Asn Trp Asn Val Arg Ser MET

    2490                2505                2520                2535
ACA GAA GAA GAG AAA TTA AAG TAT TGC AAA AGG CGA CAT AAT ACA CCA TCT TAA
Thr Glu Glu Glu Lys Leu Lys Tyr Cys Lys Arg Arg His Asn Thr Pro Ser

      2545       2555       2565       2575       2585       2595       2605
GTTTATTGAG GTTGTCCGCG ACAATAGTTC CTTCAACAAA ATAACGAAGC GACGATAACG AGAAATGTCA

      2615       2525       2635       2645       2655       2665       2675
TTAGGTTATT ACTACCTTTA TAGAAAATAT ATATACTTAT TTAATATTTA TATAGATTTA TGTGTGTACA

      2685       2695       2705       2715       2725       2735       2745
TACCTATGCG GATGTATGCC TATGTGGGAA TTCCTAAATG TCTTTCCATC AACGACTAAA TATATATTCT

      2755       2765       2775       2785       2795       2805       2815
CATGACTGAC TATATGGGTT ACGAAAACTT AATTTTTTTG TCAAAGACCC TGGCGAATTG AGAAAACCCG

      2825       2835       2845       2855       2865       2875       2885
CTGGATAGAT GGGCTATCCG AAATTTTGAA AGATGGAAAA AACATTAATC TCATTAGCAA AAGAGGTAAG

      2895       2905       2915       2925       2935       2945       2955
ACCCTGGGTG AAGAAAAGTC CGAGGAGGGA ACACAAAAAA GTCTAGGATA ATGGCACATG AAAAGTTTAG
                                                       MET

      2965       2975       2985       2995       3005       3015       3025
TATACCTGAG AACTTCACAT TAGCGCAGTC TTTGCAATTG CAATTGCTTT ACTCTGTTGT CAAAAATCAA

      3035       3045       3055       3065       3075       3085       3095
TATAAAAACC TAGCAGACCT AATAATCAAT AGCAAAGGTA ATAAGGACAC AGTAACGTAT GGGAAAATCC

      3105       3115       3135       3145       3155       3165       3175
ACAAAAACTT AGACACTTTA CTGGTATACG TCAATGAAGG TCTACGAAAA ATTGAAAAGA CTTATACCTT

      3185       3195       3205
AAAGAAAGGG TTAGGAAATC TTGTAGTGAT C
```

FIG. 5—(*Continued*)

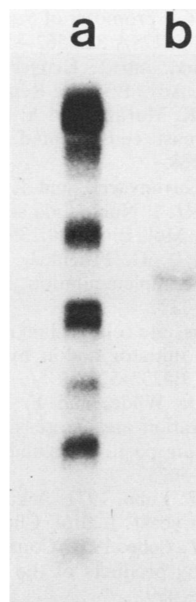


FIG. 6. RNA gel-transfer hybridization experiment. Lane A, Single-stranded end-labeled *Hin*dIII-digested λ DNA. (Upon denaturing, a phantom band of unknown origin appears at ca. 1 kb.) Lane B, Poly(A)$^+$ RNA prepared from a 50-ml exponentially growing YPD culture of strain DFY510. After electrophoresis through a 0.8% agarose–37% formaldehyde gel and transfer, the nucleic acid was hybridized with a probe complementary to the 5′ portion of the *GCR1* transcript (see text for details).

Since it is not unreasonable to expect that Gcrl protein might have DNA-binding activity, the deduced amino acid sequence was scanned for sequences suggestive of an alpha-turn-alpha motif characteristic of many DNA-binding proteins (21). One such sequence was found near the carboxy terminus of the polypeptide (the amino acids in the sequence are underlined in Fig. 5). Perhaps this sequence allows the *GCR1* gene product to interact with the control regions on the DNA of affected genes. Comparison of the deduced amino acid sequence of Gcrl with other sequences in the National Institutes of Health data base showed no significant homology with any currently catalogued sequence. The fact that the *gcr1*::*LEU2* insertion mutation gave the same phenotype as the null *gcr1* deletion-*LEU2* substitution mutation implies that if a truncated Gcr protein is formed in the insertion mutant, it is without Gcr function as presently assessed.

With respect to the mechanism of Gcr action, one approach would be a study of transcriptional starts for the affected genes in wild-type and *gcr1* mutant strains. Such information might also clarify the question of the inducibility of glycolytic enzymes in *S. cerevisiae*. The results in this and earlier (5) papers show that the levels of these enzymes are relatively constitutive, comparing growth on glucose with growth on noncarbohydrate carbon sources; a similar conclusion was reached in a recent study of the three glyceraldehyde-3-phosphate dehydrogenase genes (20). However, there is also definite knowledge of inducibility of particular enzymes or isozymes (e.g., enolase 2 [19]) and even indications for general inducibility of the pathway (16, 17). The degree to which these somewhat disparate results reflect differences in experimental conditions or strains is not known. Nonetheless, the present results strongly suggest

TABLE 1. Codon usage pattern of the *GCR1* structural gene

| Codon[a] | Amino acid | No. |
|---|---|---|
| TTT | Phe | 29 |
| TTC* | Phe | 6 |
| TTA | Leu | 18 |
| TTG* | Leu | 17 |
| CTT | Leu | 11 |
| CTC | Leu | 3 |
| CTA | Leu | 16 |
| CTG | Leu | 13 |
| ATT* | Ile | 27 |
| ATC* | Ile | 8 |
| ATA | Ile | 18 |
| ATG | MET | 11 |
| GTT* | Val | 16 |
| GTC* | Val | 3 |
| GTA | Val | 12 |
| GTG | Val | 6 |
| TCT* | Ser | 30 |
| TCC* | Ser | 12 |
| TCA | Ser | 32 |
| TCG | Ser | 5 |
| CCT | Pro | 15 |
| CCC | Pro | 8 |
| CCA* | Pro | 23 |
| CCG | Pro | 12 |
| ACT* | Thr | 15 |
| ACC* | Thr | 6 |
| ACA | Thr | 21 |
| ACG | Thr | 13 |
| GCT* | Ala | 10 |
| GCC* | Ala | 5 |
| GCA | Ala | 8 |
| GCG | Ala | 6 |
| TAT | Tyr | 15 |
| TAC* | Tyr | 7 |
| TAA | —[b] | 1 |
| TAG | — | 0 |
| CAT | His | 7 |
| CAC* | His | 2 |
| CAA | Gln | 29 |
| CAG | Gln | 15 |
| AAT | Asn | 50 |
| AAC* | Asn | 28 |
| AAA | Lys | 35 |
| AAG* | Lys | 22 |
| GAT | Asp | 32 |
| GAC* | Asp | 6 |
| GAA* | Glu | 31 |
| GAG | Glu | 16 |
| TGT* | Cys | 5 |
| TGC | Cys | 1 |
| TGA | — | 0 |
| TGG | Trp | 7 |
| CGT | Arg | 5 |
| CGC | Arg | 2 |
| CGA | Arg | 6 |
| CGG | Arg | 1 |
| AGT | Ser | 17 |
| AGC | Ser | 13 |
| AGA* | Arg | 7 |
| AGG | Arg | 14 |
| GGT* | Gly | 14 |
| GGC | Gly | 8 |
| GGA | Gly | 9 |
| GGG | Gly | 5 |

[a] Preferred codons, according to Bennetzen and Hall (2), are denoted by *.
[b] —, Stop.

that Gcr function is needed for high-level expression of the glycolytic genes in *S. cerevisiae*.

A different question concerns the extent of Gcr action. The number of genes affected by Gcr might be as few as those already recognized—eight or so glycolytic genes. The growth effect of *gcr1* mutations more or less fits with the described enzyme profile. And, as shown for *gcr1-1*, the protein pattern in SDS-polyacrylamide gel electrophoresis was generally normal except for the altered amounts of some prominent bands that are probably glycolytic proteins (5). However, there may be a variety of affected genes which are as yet unrecognized. It should also be emphasized that the levels of the various glycolytic enzymes are not equally affected by *gcr*. In the null mutants, as in *gcr1-1* (5), the largest relative effects were on phosphoglycerate mutase and enolase. And in a few cases residual enzyme levels might reflect minor isozymes whose expression is unaffected or even stimulated in the mutants.

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