Molecular Characterization of GCV3, the *Saccharomyces cerevisiae* Gene Coding for the Glycine Cleavage System Hydrogen Carrier Protein*

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YAL044, a gene on the left arm of *Saccharomyces cerevisiae* chromosome one, is shown to code for the H-protein subunit of the multienzyme glycine cleavage system. The gene designation has therefore been changed to GCV3, reflecting its role in the glycine cleavage system. GCV3 encodes a 177-residue protein with a putative mitochondrial targeting signal at its amino terminus. Targeted gene replacement shows that GCV3 is not required for growth on minimal medium; however, it is essential when glycine serves as the sole nitrogen source. Studies of GCV3 expression revealed that it is highly regulated. Supplementation of minimal medium with glycine, the glycine cleavage system’s substrate, induced expression at least 30-fold. In contrast, and consistent with the cleavage of glycine providing activated single carbon units, the addition of the metabolic end products that require activated single-carbon units repressed expression about 10-fold. Finally, like many amino acid biosynthetic genes, GCV3 is subject to regulation by the general amino acid control system.

The glycine cleavage system, a multienzyme complex consisting of four different subunits (P-, H-, T-, and L-proteins), catalyzes the oxidative cleavage of glycine into CO₂ and NH₃. The concomitant transfer of a methylene carbon unit to THF generates the C₁ donor 5,10-MTHF (Fig. 1). The biochemistry of glycine cleavage has been studied in organisms ranging from *Escherichia coli* (1), through plants including *Pisum sativum* (2) and *Arabidopsis thaliana* (3), to the higher eukaryotes chickens (4), cows (5), and humans (6). In contrast, very little is known about how the glycine cleavage system is regulated and its functional importance, particularly in eukaryotes. The recent identification and cloning of the yeast genes coding for the four polypeptides of the glycine cleavage system, T-protein (GenBank accession number L41522), P-protein (7), L-protein (8, 9), and H-protein (this study), facilitate the detailed molecular analysis of the glycine cleavage system and its regulation in a genetically tractable eukaryote.

In addition to the glycine cleavage system there are two other mechanisms for the synthesis of 5,10-MTHF (Fig. 1) (10); one uses glycine hydroxymethyltransferase, and the other utilizes C₁-tetrahydrofolate synthase. The multifunctional enzyme C₁-tetrahydrofolate synthase catalyzes the interconversion of 5,10-MTHF, 5,10-methenyltetrahydrofolate, 10-formyltetrahydrofolate, and THF with the concomitant production of formate (10, 11). These compounds in turn are used in a number of biosynthetic reactions that require C₁ units, including the purine nucleotides, thymidylate, and the amino acids histidine, serine, methionine, and formylmethionine (12).

Genetic analysis has shown that no single mechanism is essential for the production of 5,10-MTHF (13); however, inactivation of both glycine hydroxymethyltransferase and glycine cleavage system-dependent 5,10-MTHF synthesis renders *S. cerevisiae* growth contingent upon supplementation with formate (14). It therefore appears that the major role for formate is the shuttling of C₁ units between cellular compartments. Consistent with this role is the observation that C₁-THF synthase activity is localized to both the cytoplasmic and mitochondrial compartments (Fig. 1) (11). Indeed, the nuclear genome of *S. cerevisiae* encodes mitochondrial and cytoplasmic versions of C₁-tetrahydrofolate synthase (15). Similarly, there are two genes for glycine hydroxymethyltransferase, one for a cytoplasmic and the other for a mitochondrial version (16). In contrast, for those systems studied to date, the glycine cleavage system is localized to only the mitochondrial compartment (9, 17, 18).

That the C₁-tetrahydrofolate synthase-dependent mechanism is apparently solely responsible for the generation of formate in *S. cerevisiae* makes formate biosynthesis dependent upon 5,10-MTHF. For the two other sources of C₁ units, serine and glycine, there are at least two biosynthetic pathways (19, 20). One pathway for serine synthesis involves the conversion of 3-phosphoglycerate to serine by three enzymatic reactions. The last two of these reactions are catalyzed by the *SER1* and *SER2* gene products. A second pathway for serine synthesis is catalyzed by glycine hydroxymethyltransferase and utilizes glycine and 5,10-MTHF as its substrates. Mutants blocked in the synthesis of serine from 3-phosphoglycerate can use glycine to provide both C₁ units via the glycine cleavage system and serine via the reaction catalyzed by glycine hydroxymethyltransferase (Fig. 1). Glycine can also be synthesized in two ways. One utilizes glycine hydroxymethyltransferase to convert serine into glycine. The other pathway, which apparently derives glycine from glyoxylate, is dependent upon the *GLY1* gene (13). Since this second route is inefficient, *ser1* mutants grow very poorly on minimal media (21).

The objectives of this investigation were to establish the...
functional role of the GCV3 gene and to delineate the general features of its regulation. We show that GCV3, a gene identified by the systematic sequencing of chromosome one (22), codes for an H-protein that is essential for glycine cleavage. Studies of GCV3 expression revealed that it is induced by glycine and repressed by the metabolic products that require C1 units for their synthesis. In addition, it was established that GCV3 is subject to regulation by the general amino acid control system.

**EXPERIMENTAL PROCEDURES**

Radioactive Tracer Compounds and Oligonucleotides—[2-14C]glycine and [α-32P]dATP were purchased from ICN. Tetrahydrofolate was synthesized from folic acid using the previously described method (23). Restriction endonucleases, DNA-modifying enzymes, and ribonucleases were purchased from Bio/Can, New England Biolab, and Boehringer Mannheim. The sequences of four of the oligonucleotides used in this study were as follows: GC1, 5'-ATGGATCCCTGCTTACGGAGTTCC-3'; GC2, 5'-TCCCAAGCTTGACAGGCTAAAATGAA-3'; GC7, 5'-ATACCCGGGATCTGCTGGGAGG-3'; GC8, 5'-GATGGATCCCAAGCGAAAAATGAATTGC-3'. The sequences of the other oligonucleotides used are presented in the appropriate figures.

**Media and Culture Conditions—** S. cerevisiae-rich media (YPD) consisted of 2% Bacto-peptone, 1% yeast extract, and 2% dextrose. Two types of minimal media were used. One was standard YNB consisting of 0.175% yeast nitrogen base without amino acids and ammonium sulfate (Difeo), 0.5% ammonium sulfate, and 2% dextrose. Amino acids and uracil were supplemented as needed (24, 25). When glycine served as the sole nitrogen source, YNB was prepared with 250 mM glycine instead of ammonium sulfate. The second minimal medium is MV media. It was used for the general control-dependent GCV3 gene expression studies. As recommended, arginine (40 μg/ml) was included in this medium (26). ura3 mutants were isolated by selecting for growth on 5-fluoroorotic acid plates (27). For sporulation studies, liquid presporation and sporulation media were prepared as described previously (28). All S. cerevisiae cultures were grown at 30°C. E. coli cells were grown at 37°C.

*S. cerevisiae Strains and Strain Constructions—** The strains used in this study are described in Table I. The isogenic strains 3634, 3640, and 3646 were leu2::URA3 derivatives of the strains RH1385, RH1378, and RH1408 (28), respectively. These ura3–52 strains were converted to leu2::URA3 using the one-step gene replacement method (30). This was accomplished by transformation with the 1.6-kilobase pair ~BglII fragment from pNKY55 (31).

Strain 4049 was derived from strain 3634 by selecting for uracil auxotrophs (27). Strain 4070, a ser1::URA3 derivative of 4049, was constructed by gene replacement using the 2.0-kilobase pair ~BstE1 to ~PvuII fragment of pKM140 (20). The GCV3 gene of strains DBY745 and 4049 was replaced with URA3 to generate strains 3751 and 4404, respectively. The method used, a PCR-based modification of the gene replacement method (32), is outlined in Fig. 2. In summary, the complete URA3 gene was amplified by PCR from S288C genomic DNA using oligonucleotides GC3 and GC4. The PCR product generated is a 1.08-base pair fragment extending from 192 base pairs upstream of the URA3 translation start codon to 96 base pairs beyond the translation stop codon. In addition, the amplified fragment is flanked at both ends by 45-base pair sequences that are identical to sequences flanking the GC3 locus. The upstream end of the URA3 fragment has 45 base pairs that are identical to the sequence from nucleotide –73 to –117 relative to the GCV3 open reading frame.

**TABLE I**

| TABLE I Genotypes of S. cerevisiae strains | Strain | Genotype | Source |
|-------------------------------------------|--------|----------|--------|
| 1095–302C | a ser1 cyh4 ade2 his3 leu2 ura3 his4 thr4 gal MAL2 | YGSC™ | YGSC |
| DBY745 | a ade1–100 leu2–3 leu2–12 ura3–52 Gal+ | This study | G. Braus |
| 3751 | a ade1–100 leu2–3 leu2–12 ura3–52 gcv3Δ::URA3 Gal+ | This study | G. Braus |
| RH1385 | a ura3 | G. Braus | G. Braus |
| RH1378 | a ura3 gcd2–1 | This study | This study |
| RH1408 | a ura3–52 gcn4–103 | This study | This study |
| 3634 | a ura3 leu2::URA3 | This study | This study |
| 3640 | a ura3 gcd2–1 leu2::URA3 | This study | This study |
| 3646 | a ura3–52 gcn4–103 leu2::URA3 | This study | This study |
| 3771 | Diploid obtained by crossing DBY745 and 1095–302C | This study | This study |
| 3773 | Diploid obtained by crossing 3751 and 1095–302C | This study | This study |
| 3792A | a leu2 ade thr4 ura3 | This study | This study |
| 3792B | a ser1 leu2 his3 ade gcv3Δ::URA3 | This study | This study |
| 3792C | a ser1 leu2 his3 ade ura3 | This study | This study |
| 3792D | a leu2 ade thr4 gcv3Δ::URA3 | This study | This study |
| 4404 | a ura3 leu2 gcv3Δ::URA3 | This study | This study |
| 4049 | a ura3 leu2 | This study | This study |
| 4070 | a ura3 leu2 ser1::URA3 | This study | This study |

*a* YGSC, Yeast Genetic Stock Centre.

*b* 3792A to -D are from single tetrad.
Function and Expression of the Yeast GCV3 Gene

The 3'-end of the URA3 fragment has a 45-base pair extension that is identical to the sequence from 97 to 141 base pairs downstream of the GCV3 stop codon. Transformation of S. cerevisiae with this PCR product can result in the replacement of 665 base pairs from the GCV3 locus with a 1098-base pair fragment encoding URA3. Several Ura+ transformants of strains DBY745 and 4049 were screened using the PCR method depicted in Fig. 2 to identify Ura+ transformants that had the desired gene replacement.

Transformation, Genetic Methods, and DNA Manipulation—Standard S. cerevisiae genetic techniques such as mating, isolation of diploids, sporulation, tetrad analysis, and complementation were performed as described previously (24, 25). Transformation was performed according to the fast colony procedure (3). S. cerevisiae genomic DNA was isolated essentially as described (25). Methods for the manipulation of DNA were performed as described by Sambrook et al. (34). DNA sequencing was performed using the U.S. Biochemical Corp. Sequenase kit and the method supplied by the manufacturer. PCR amplifications (35) were performed using a Hybaid thermal reactor. For PCR-based screening using S. cerevisiae cells as the source of template, the following method was used. About one-half of a large colony was suspended in 0.5 ml of water, 5 × 10^6 to 7.5 × 10^6 cells were transferred to a microcentrifuge tube, and the volume was adjusted to 60 μl with water. The cell suspensions were boiled for 5 min, immediately frozen in liquid nitrogen, and then reboiled for another 5 min. Following vigorous vortexing for 30 s, the cell debris was removed by centrifuging at 2,200 × g for 2 min. This solution was used as the template source for a 1-hour PCR reaction.

Plasmid Construction—Two multicopy shuttle plasmids were constructed (Fig. 3). The first, a GCV3-lacZ fusion plasmid denoted as pLNGCV3-lacZ, was constructed as follows. The GCV3 promoter DNA was generated by PCR amplifying genomic DNA using primers GC1 and GC2. The PCR product was digested with HindIII and BamHI digestion. The GCV3 promoter DNA was generated by PCR amplifying genomic DNA using primers GC1 and GC2. The PCR product was digested with HindIII and BamHI digested. The plasmid clone was then used to generate the lacZ expression unit from the promoter of the GCV3 transcriptional and translational control signals from +8 to +231 relative to the GCV3 start codon. pLNGCV3 is identical to pLNGCV3-lacZ except that the GCV3-lacZ portion has been replaced by the GCV3 gene. GCV3 DNA, prepared by PCR amplification from S288C genomic DNA using oligos GC7 and GC8, was digested with BamHI and Smal and cloned into the backbone of pRS264.

Enzyme Assays—β-Galactosidase assays were performed essentially as described previously (37). All β-galactosidase activity values are averages obtained from at least three independent experiments. The values did not vary by more than ±5%.

Extracts for glycine cleavage activity assays were prepared as follows. 100-ml cultures, grown to midlog phase (A_{600} = 0.25) in YNBD medium supplemented with glycine, were harvested by centrifugation and washed twice with cold sterile water. A 100-ml volume of glass beads (acid-washed and baked) was added to the cell suspension, and the cells were disrupted by vortexing for 30 s. The cell debris was removed by centrifugation (5000 rpm for 5 min using a Beckman JA17 rotor). The supernatant was carefully recovered and subjected to three cycles of freezing in liquid nitrogen and thawing. The protein concentration was determined (38) using bovine serum albumin as the standard.

Glycine cleavage assays were performed essentially as described (39) with a few modifications. The 0.5-ml assay mixture consisted of 20 mM potassium phosphate buffer, pH 7.4, 2 mM EDTA, 10 mM benzamidine, 10 mM β-mercaptoethanol, and 1 mM phenylmethanesulfonyl fluoride). One-half volume of glass beads (acid-washed and baked) was added to the cell mixture, and the cells were disrupted using a silanil agitation (five 30-s bursts separated by 1 min on ice). After cell debris was removed by centrifugation (5000 rpm for 5 min using a Beckman JA17 rotor), the supernatant was carefully recovered and subjected to three cycles of freezing in liquid nitrogen and thawing. The protein concentration was determined (38) using bovine serum albumin as the standard.

Glycine cleavage assays were performed essentially as described (39) with a few modifications. The 0.5-ml assay mixture consisted of 20 mM potassium phosphate buffer, pH 7.4, 2 mM diethiothreitol, 1 mM tetrahydrofuran, 2.5 mM pyridoxal phosphate, 2 mM nicotinamide adenine dinucleotide, and enzyme (2 mg of crude cell extract). The glycine cleavage reaction was initiated by the addition of 66 μl of (1 μCi/μmol) [2-14C]glycine. After incubation at 30 °C for 45 min, the reaction was terminated by the sequential addition of 0.3 ml of 1 M sodium acetate, pH 4.5, 0.2 ml of 0.1 M formaldehyde, and 0.3 ml of 0.4 M dimedone in 50% ethanol. The stopped reaction was placed at 65 °C for 5 min and then placed on ice for at least 5 min. Next, 5 ml of toluene was added, followed by vigorous vortexing and then centrifugation in a clinical centrifuge for 5 min. 3 ml of the toluene layer (upper phase) was transferred to a scintillation vial containing 5 ml of EcoLite (ICN), and radioactivity was measured using an LKB RackBeta liquid scintillation spectrophotometer.
GCV3 Codes for a Glycine Cleavage H-protein and Is Essential for Glycine Cleavage Activity—A BlastX search (40) revealed that the putative protein encoded by GCV3 had significant similarity to the glycine cleavage system H-protein from organisms ranging from bacteria to mammals (Fig. 4). The presence of a lipoic acid signature suggests that Gcv3p is encoded a glycine cleavage system H-protein.

Consistent with this, a search of the S. cerevisiae genome did not identify a second H-protein gene. That glycine cleavage activity was about 1.5 times higher in the ser1::URA3 mutant (Table II) may reflect the fact that a ser1 mutation would be more dependent upon the glycine cleavage system for its supply of serine and C1 units.

GCV3 Is Required to Utilize Glycine as a Nitrogen Source but Not for Growth on Minimal Media—Once it was established that GCV3 was essential for glycine cleavage system activity in cell extracts, we wanted to study its role in vivo. Since S. cerevisiae can use glycine as a nitrogen source (7, 8) and the glycine cleavage system can generate NH3 from glycine, the effect of a gcv3::URA3 mutation on yeast's ability to use glycine as a nitrogen source was assessed. Fig. 5 shows that the utilization of glycine is dependent upon a functional GCV3 gene.

The gcv3::URA3 mutant, like its wild type parent, grows irrespective of the availability of glycine and serine (Fig. 6). Therefore, GCV3 is not essential for growth on YNB. GCV3 is, however, required for growth at wild type rates, since the gcv3::URA3 mutant (Table II) grows at the same rate as the wild type parent (2.6 h) even in the presence of glycine (data not shown).

The slow growth of a gcv3::URA3 strain was unexpected, since it should be possible to synthesize serine, glycine, and the C1 donor 5,10-MTHF without a functional glycine cleavage system (Fig. 1). One explanation for the reduced growth rate of the gcv3::URA3 strain is that 5,10-MTHF is rate-limiting for their C1 unit requirements. The amount of activity in extracts from strain 3634 was very similar to that in extracts prepared from E. coli. Further, no glycine cleavage activity was detected in the gcv3::URA3 mutant (Table II). Therefore, GCV3 encodes an essential component of the glycine cleavage system.

RESULTS AND DISCUSSION

FIG. 3. Maps of plasmids pLNGCV3-lacZ (top) and pLNGCV3 (bottom). 2μ, S. cerevisiae 2μ plasmid sequences; LacZ, coding region of E. coli lacZ gene; LEU2, S. cerevisiae LEU2 gene; ori, pBR322 origin of replication; AMP, pBR322 β-lactamase gene.

FIG. 4. Gcv3p aligned with other known glycine cleavage system H-proteins. The alignment was generated using the Clustal program (41). Asterisks represent residues that are identical in all eight H-proteins. Dots indicate residues within the alignments where only conserved amino acid replacements have occurred. Species designations are given to the right of the alignment. Also listed (right of the last set of rows) is the percentage of identity with the S. cerevisiae H-protein. Underlined residues represent a putative mitochondrial targeting signal. The solid triangle flanked by two vertical arrows indicates lysine 109, the lipoamide attachment site, and the conserved surrounding region.

The presence of a lipoic acid signature suggests that Gcv3p is mitochondrial, since the enzymatic attachment of lipoic acid occurs in the mitochondrial compartment (42). Consistent with this possibility the N-terminal 54 residues of Gcv3p contain a putative mitochondrial targeting signal (Fig. 4). The lipoic acid binding signature, significant similarity to other H-proteins, and a mitochondrial targeting signal strongly suggested that GCV3 encoded a glycine cleavage system H-protein.

To determine whether the GCV3-encoded H-protein was necessary for glycine cleavage system activity, glycine cleavage assays were done on cell extracts prepared from a wild type strain (3634) and an isogenic derivative (4404) harboring a gcv3::URA3 null mutation. The assay was also performed on extracts prepared from strain 4070, a ser1::URA3 derivative of 3634, to determine whether expression increased when cells could not use serine derived from 3-phosphoglycerate to meet
TABLE II
Glycine cleavage activity

| Strain          | Enzyme activity (pmol/min/mg protein) |
|-----------------|---------------------------------------|
| 3634 (wt)       | 106                                   |
| 4070 (ser1)     | 40                                       |
| 4044 (gcv3Δ::URA3) | 0                                    |
| DH5α            | 168                                   |

Fig. 5. **GCV3 is required for the utilization of glycine as a nitrogen source.** Growth on minimal media is shown. Left panel, minimal medium with 250 mM glycine as the nitrogen source; right panel, minimal medium with 250 mM glycine and ammonium sulfate. A, wild type strain DBY745; B, gcv3Δ::URA3 strain 3751; C, strain 3751 transformed with pLNGCV3.

TABLE III
Growth rate of wild type, ser1, and gcv3Δ::URA3 strains on minimal medium

| Strain          | No supplementation | with serine | with glycine | with formate |
|-----------------|--------------------|-------------|--------------|--------------|
| 3634 (wt)       | 2.7                | 2.6         | 2.6          | 2.5          |
| 4070 (ser1)     | >30                | 3.0         | 3.8          | 14           |
| 4044 (gcv3Δ::URA3) | 3.6               | 3.5         | 10.5         | 2.6          |

Fig. 6. **Glycine can be used in a glycine cleavage system-dependent fashion to meet cellular demand for serine.** Growth requirements of strains are shown. A, strain DBY745; B, gcv3 strain 3751; C, ser1 strain 1095–302C; D, ser1 gcv3 strain 3634 (wt) and 4070 (ser1) were used (Fig. 7A). Strain 4070 was included to test whether GCV3 expression was further elevated when demand for C1 units derived from glycine via the glycine cleavage system was increased. GCV3 expression was measured using the lacZ reporter gene on pLNGCV3-lacZ (Fig. 3). A dose response curve revealed that GCV3-lacZ expression was induced by glycine and that it plateaued at about 10 mM glycine for the SER1 strain and at 20 mM for the ser1 mutant (data not shown). Glycine induced GCV3-lacZ expression 30-fold in the SER1 strain (Fig. 7A). In the ser1 mutant, expression was the notion that the 5,10-MTHF concentration is rate-limiting in a gcv3 mutant. First, formate supplementation enables the gcv3Δ::URA3 strain to grow at the normal rate (Table III). Second, extracellular glycine severely inhibits growth of the gcv3 mutant but does not affect the wild type (Table III).

De Novo Glycine Biosynthesis Cannot Meet the Growth Requirements of a ser1 Mutant—Although a ser1 mutant (strain 4070) grows very slowly without supplementation (>30-h doubling time), it grows quite well with a 3.8-h doubling time if supplemented with glycine (Table III). Apparently, de novo glycine biosynthesis cannot meet cellular demand for glycine in a ser1 background. These results support the previous finding that supplementation with serine or glycine enables ser1 mutants to grow at near normal rates (21, 43).

C1-tetrahydrofolate Synthase, the Major and Perhaps Only Route for Formate Synthesis in S. cerevisiae—There are three main routes for the generation of activated one-carbon units. These pathways, which obtain their C1 units from serine, glycine, and formate, use glycine hydroxymethyltransferase, the glycine cleavage system, and C1-tetrahydrofolate synthase, respectively. That the double mutant (ser1 gcv3Δ::URA3) grew very slowly relative to strains harboring either one of these mutations (Fig. 2) suggested that these mutations combined to adversely influence growth on rich medium. To test whether this was due to loss of the glycine cleavage system-dependent synthesis of 5,10-MTHF, we compared the growth of wild type and ser1, gcv3Δ::URA3, and ser1 gcv3Δ::URA3 mutants on media supplemented with different combinations of glycine, serine, and formate (Fig. 6). Unlike the ser1 mutant, the double mutant could not be rescued by glycine supplementation. This suggested that glycine enabled the ser1 mutant to grow at normal rates, because it served as a precursor for both serine and 5,10-MTHF synthesis. Since formate can also serve as a precursor for 5,10-MTHF synthesis, the ability of formate supplementation to support growth was also tested. That formate and glycine enabled the ser1 gcv3Δ::URA3 strain, 3792B, to grow at normal rates (Fig. 6) indicates that the synthesis of essentially all cellular formate is dependent upon C1-tetrahydrofolate synthase. Based on the limited ability of yeast to synthesize glycine (see above) and the fact that essentially all formate is derived from 5,10-MTHF, serine derived from 3-phosphoglycerate is the source of the vast majority of cellular C1 units. In contrast, de novo synthesis of formate in E. coli can meet cellular demand for activated C1 units (44).

GCV3 Is Induced by Glycine and Repressed by the C1 Metabolic End Products—To study GCV3 regulation, strains 3634 (wt) and 4070 (ser1) were used (Fig. 7A). Strain 4070 was included to test whether GCV3 expression was further elevated when demand for C1 units derived from glycine via the glycine cleavage system was increased. GCV3 expression was measured using the lacZ reporter gene on pLNGCV3-lacZ (Fig. 3). A dose response curve revealed that GCV3-lacZ expression was induced by glycine and that it plateaued at about 10 mM glycine for the SER1 strain and at 20 mM for the ser1 mutant (data not shown). Glycine induced GCV3-lacZ expression 30-fold in the SER1 strain (Fig. 7A). In the ser1 mutant, expression was...
expression by glycine strongly suggests that the increase in glycine cleavage system activity, which occurs in response to glycine supplementation (8), is due to a transcriptional regulatory mechanism. Examination of the LDP1, GCV1, GCV2, and GCV3 genes for consensus elements that might coordinate their induction by glycine identified one potential element, with the consensus 5′-GACCTCGA-3′, that is present in the upstream regions of the four glycine cleavage system subunit genes.

Since the glycine cleavage system generates 5,10-MTHF, GCV3 expression might be repressed when cellular demand for C1 units is low. Indeed, supplementation with the C1 metabolic end products repressed GCV3-lacZ expression about 10-fold (Fig. 7B). Transcriptional control of GCV3 in response to demand for C1 units could occur via a mechanism that responds to intracellular concentrations of 5,10-MTHF, one or more of the other C1 donors, or even the C1 end products.

A second possibility is that GCV3 transcription is regulated in response to intracellular glycine levels. For example, without extracellular activated one-carbon units, essentially all C1 units must be derived from serine (see above). Two C1 units can be derived from each molecule of serine. The β-carbon of serine is added to THF during the glycine hydroxymethyltransferase-dependent conversion of serine into glycine. Glycine generated in excess of that needed for cellular metabolism (e.g., protein, purine, and heme biosynthesis) can also be used as a source of C1 units via the series of reactions catalyzed by the glycine cleavage system. Therefore, intracellular glycine levels might regulate GCV3 expression and, by extrapolation, glycine cleavage system activity to ensure the efficient use of serine and/or to prevent the potentially toxic effects of glycine accumulation.

**General Control of GCV3 Expression**—The presence of three general control response elements (Fig. 2A, GCRE1, GCRE2, and GCRE3) upstream of the GCV3 open reading frame (Fig. 2A) suggested that GCV3 is regulated by the general amino acid control system. To test this, expression of the GCV3-lacZ reporter gene was examined in strains 3634 (wt), 3640 (gcd2), and 3646 (gcn4) (Fig. 7C). If a gene is regulated by the general amino acid control system, its expression increases in response to amino acid starvation because the concentration of the transcriptional activator Gcn4p increases (45–47). Here we have used strains 3646 (gcn4), which is unable to express any Gcn4p, and 3640 (gcd2), which constitutively expresses induced levels of Gcn4p, to test whether expression is subject to general control (29). GCV3-lacZ expression in the gcd2 strain, which mimics amino acid starvation conditions, is 2.5-fold higher than in the gcn4 mutant, which mimics nonstarvation conditions. This represents a general control response that falls within the range reported for other genes that are subject to general control (48). A similar effect was obtained when histidine starvation was induced by the addition of 3-amino triazole (49, 50) (data not shown).

Perhaps, since C1 units derived via the glycine cleavage system would be available for the synthesis of amino acids like serine, methionine, and formyl methionine, GCV3 is subject to regulation by the general amino acid control system. That the upstream regions adjacent to LDP1, GCV1, and GCV2 also contain copies of the general control response element suggests that the general control system plays an important role in regulating glycine cleavage activity in yeast.

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**Fig. 7. Regulation of GCV3 expression.** A, effect of glycine supplementation on levels of β-galactosidase expressed by the isogenic strains 3634 (wild type) and 4070 (ser1) harboring pLNGCV3-lacZ. B, effect of the C1 metabolic end products on pLNGCV3-lacZ expression in strains 3634 and 4070. Cells were grown with 10 mM glycine or 10 mM glycine and the C1 metabolic end products adenine (40 µg/ml), histidine (20 µg/ml), methionine (20 µg/ml), serine (5 mM), and formate (10 mM) as indicated. C, effect of the general control system on GCV3-lacZ expression in the isogenic strains 3634 (wild type), 3646 (gcn4–103), and 3640 (gcd2–1).
REFERENCES

1. Okamura-Ikeda, K., Ohmura, Y., Fujiwara, K., and Motokawa, Y. (1993) Eur. J. Biochem 216, 539–548
2. Macerel, D., Lehrm., M., Gagnon, J., Neuburger, M., Douce, R. (1990) Biochem. J. 268, 783–789
3. Srinivasan, R., and Oliver, D. J. (1992) Plant Physiol. 98, 1518–1519
4. Yamamoto, M., Koyata, H., Matsu, C., and Hiraga, K. (1991) J. Biol. Chem. 266, 3317–3322
5. Fujiwara, K., Okamura-Ikeda, K., and Motokawa, Y. (1990) J. Biol. Chem. 265, 17463–17467
6. Fujiwara, K., Okamura-Ikeda, K., Hayasaka, K., and Motokawa, Y. (1991) Biochem. Biophys. Res. Commun. 176, 711–716
7. Sinclair, D. A., and Dawes, I. W. (1995) Genetics 140, 1213–1222
8. Sinclair, D. A., Hong, S. P., and Dawes, I. W. (1996) J. Biol. Chem. 271, 9155–9165
9. Rebeille, F., Neuburger, M., and Douce, R. (1994) J. Biochem. 115, 116–118
10. Pasternack, L. B., Laude, D. A., Jr., and A. L. (1994) Annu. Rev. Plant Physiol. Plant. Mol. Biol. 45, 325–337
11. Barlowe, C. K., and Appling, D. R. (1990) Molec. Cell. Biol. 10, 5679–5687
12. Mudd, S. H., and Cantoni, G. L (1964) in Biochemistry, 8713–8719
13. McNeil, J. B., Bognar, A. L., and Pearlman, R. E. (1996) Genetics 142, 371–381
14. McNeil, J. B., Bognar, A. L., and Pearlman, R. E. (1996) Genetics 142, 371–381
15. Srinivasan, R., and Oliver, D. J. (1992) J. Biol. Chem. 267, 7717–7725
16. Neuburger, M., Douce, R. (1994) Biochem. J. 302, 223–228
17. Melcher, K., and Entian, K. D. (1992) Curr. Genet. 21, 295–300
18. Melcher, K., and Entian, K. D. (1995) Curr. Genet. 27, 501–508
19. McKenzie, K. Q., and Jones, E. W. (1977) Nature 265, 7717–7725
20. Melcher, K., and Entian, K. D. (1992) Curr. Genet. 21, 295–300
21. McKenzie, K. Q., and Jones, E. W. (1977) Nature 265, 7717–7725
22. Bussey, H., Kaback, D. B., Zhong, W., Ye, D. T., Clark, M. W., Fortin, N., Hall, J., Dauttel, B. P. F., Keng, T., Barton, A. B., Su, Y., Davies, C. J., and Storms, R. K. (1995) FEBS Lett. 5238–5247
23. O'Dell, B. L., (1947) J. Ame. Chem. Soc. 69, 250–255
24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1989) in Current Protocols in Molecular Biology, Wiley InterScience and Green Publishing Associates, New York
26. Muzarri, G., Neiderberger, P., and Hütter, R. (1978) J. Bacteriol. 134, 48–59
27. Boeke, J., Lacroute, F., and Fink, G. (1984) Mol. Gen. Genet. 197, 345–346
28. Sherman, F. (1991) Methods Enzymol. 194, 21–37
29. Mosch, H. U., Graf, R., Schulzheim, T., and Braus, G. (1990) EMBO J. 9, 2951–2957
30. Rothstein, R. J. (1983) Methods Enzymol. 101, 202–211
31. Alani, E., Cao, L., and Klekner, N. (1987) Genetik 116, 541–545
32. Lin, C., and Fink, G. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 5238–5247
33. Arndt, K., and Fink, G. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 8516–8520
34. Harris, A. G. (1992) in The Molecular Biology of the Yeast Saccharomyces cerevisiae: Metabolism and Gene Expression, ed. M. G. D. L. (Strathern, J., Jones, E. W., and Broach, J. R., ed) pp. 319–414, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
35. Hope, I. A., Mahadevan, S., and Struhl, K. (1988) Nature 333, 635–640
36. Wek, R. C., Cannon, J. F., Deaver, T. E., and Hinnebusch, A. G. (1992) Mol. Cell. Biol. 12, 5700–5710