Identification of a Novel One-carbon Metabolism Regulon in Saccharomyces cerevisiae*§

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Cristy L. Gelling, Matthew D. W. Piper‡, Seung-Pyo Hong§, Geoffrey D. Kornfeld, and Ian W. Dawes¶

From the Ramaciotti Centre for Gene Function Analysis and School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, New South Wales 2032, Australia

Glycine specifically induces genes encoding subunits of the glycine decarboxylase complex (GCV1, GCV2, and GCV3), and this is mediated by a fall in cytoplasmic levels of 5,10-methylenetetrahydrofolate caused by inhibition of cytoplasmic serine hydroxymethyltransferase. Here it is shown that this control system extends to genes for other enzymes of one-carbon metabolism and de novo purine biosynthesis. Northern analysis of the response to glycine demonstrated that the induction of the GCV genes and the induction of other amino acid metabolism genes are temporally distinct. The genome-wide response to glycine revealed that several other genes are rapidly co-induced with the GCV genes, including SHM2, which encodes cytoplasmic serine hydroxymethyltransferase. These results were refined by examining transcript levels in an shm2Δ strain (in which cytoplasmic 5,10-methylenetetrahydrofolate levels are reduced) and a met13Δ strain, which lacks the main methylenetetrahydrofolate reductase activity of yeast and is effectively blocked at consumption of 5,10-methylene tetrahydrofolate for methionine synthesis. Glycine addition also caused a substantial transient disturbance to metabolism, including a sequence of changes in induction of amino acid biosynthesis and respiratory chain genes. Analysis of the glycine response in the shm2Δ strain demonstrated that apart from the one-carbon regulon, most of these transient responses were not contingent on a disturbance to one-carbon metabolism. The one-carbon response is distinct from the Baslp purine biosynthesis regulon and thus represents the first example of transcriptional regulation in response to activated one-carbon status.

One-carbon metabolism is a key pathway involved in providing single carbon units for the biosynthesis of purines, thymidylates, serine, methionine, and N-formylmethionyl tRNA. The importance of this pathway is highlighted by the large number of human diseases and disorders associated with folate deficiency and disturbances to folate-mediated reactions (1). Tetrahydrofolate biosynthesis is also the target of several drugs used in rheumatoid arthritis, psoriasis, and cancer treatment, whereas microbial folate biosynthesis is inhibited by folate analogs such as the sulfonamide antibacterial agents (2, 3).

One-carbon units are activated by attachment at various oxides to the carrier molecule tetrahydrofolate (H4folate). They are derived principally from the reactions catalyzed by serine hydroxymethyltransferase (SHMT), which cleaves serine to generate 5,10-methylenetetrahydrofolate (5,10-CH2-H4folate) and glycine (Fig. 1, reactions 1 and 2) (4). They can also be generated from folate, via synthesis of 10-HCO-H4folate by the synthetase activity of the C1-tetrahydrofolate synthase enzymes (Fig. 1, reactions 5c and 6c) (5) or as 5,10-CH2-H4folate from glycine cleavage by the mitochondrial glycine decarboxylase complex (GDC) (Fig. 1, reaction 3) (6).

One-carbon unit activation occurs in both the cytoplasm and the mitochondrion, but one-carbon-substituted H4folate derivatives are not directly exchanged between compartments to any significant extent (7). The intercompartmental flow of one-carbon units is instead maintained by the movement of serine, glycine, and formate, which are interconverted by the one-carbon metabolic enzyme activities duplicated in the cytosol and mitochondrion. One-carbon metabolism therefore operates as a flexible system whereby different sources and locations of one-carbon units can supplement and substitute for each other in order to maintain the supply to anabolic pathways (8–10).

GCV1, GCV2, and GCV3 of Saccharomyces cerevisiae encode the protein subunits that are unique to the glycine decarboxylase complex. The fourth subunit, lipoamide dehydrogenase, is present in several other multienzyme complexes (11). These genes are up-regulated following addition of glycine to the medium (12–14) via a glycine response element with the consensus sequence CATCN7CTTCTT (15). Previously, it was demonstrated that this glycine effect is a response to a decrease in cytosolic 5,10-CH2-H4folate levels rather than directly to glycine (16). This was proposed to reflect inhibition of SHMT through the formation of a “dead-end” complex with glycine and 5-HCO-H4folate (17, 18). In Escherichia coli and in mammalian liver tissue 5-HCO-H4folate is formed by a side reaction of SHMT in the presence of glycine (19).

These results raised the possibility that the one-carbon response of the GCV genes reflects a more general system controlling transcription of one-carbon metabolism genes. In this study, microarray analysis was used to characterize the transcriptional response of S. cerevisiae to the addition of glycine. A

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§ The on-line version of this article (available at http://www.jbc.org) contains Table VI.

¶ Present address: Dept. of Biology, University College London, Darwin Bldg., Gower St. London WC1E 6BT, UK.

† To whom correspondence should be addressed. Tel.: 61-2-9385-2089; Fax: 61-2-9385-1050; E-mail: i.dawes@unsw.edu.au.
sequence of regulatory responses was identified that indicated extensive transient physiological effects of the change, including disturbance to amino acid and mitochondrial metabolism. In addition, genes involved in one-carbon metabolism and purine biosynthesis were temporally co-regulated over the time course. Further refinement of the genes included in this regulon was obtained by microarray analysis of strains deleted for the genes encoding the key one-carbon metabolism enzymes: methylenetetrahydrofolate reductase (MTHFR, encoded by MET13) and cytoplasmic serine hydroxymethyltransferase (cytoplasmic SHMT, encoded by SHM2) (20, 21).

Because the regulon was found to include genes involved in purine nucleotide biosynthesis, the glycine response of a GCV2::lacZ fusion construct in strains deleted for the BAS1 and GCN4 genes was examined.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—The yeast strains used in this study are listed in Table I.

Glucose minimal medium (Dmin) contained 2% (w/v) d-glucose, 0.17% yeast nitrogen base without amino acids (Difco), and 0.5% ammonium sulfate. Auxotrophic requirements were added at 40 mg/μl, except for uracil, which was added at 80 mg/μl. For the microarray experiments using strains from the BY4743 series, 40 mg/liter methionine and adenine were also added to all media. Where indicated, glycine was added to a final medium concentration of 10 mM.

For microarray analysis of the response to glycine in strain BWG1-7A, yeast were grown in Dmin at 30 °C to an A600 of 1.0 and then diluted by half into either Dmin or Dmin plus glycine. Samples were subsequently harvested at eight time points over 4 h and poured onto −80 °C crushed ice prior to centrifugation. For the equivalent Northern analysis, parallel control samples were harvested, whereas for the microarray experiment a pooled zero time reference control was harvested as well as a 2-h growth control culture without added glycine.

For microarray analyses of the shm2Δ, met13Δ, and BY4743 strains, triplicate cultures were grown as above to an A600 of 0.5 before harvesting.

For the glycine response in shm2Δ and BY4743, cultures were grown as above to an A600 of 0.5, divided in two at zero time, and 20% (w/v) glycine stock solution was added to 1 aliquot such that the final glycine concentration was 10 mM. The glycine-treated and control cultures were further split into triplicate samples, which were harvested at 0.5 h. Northern blot confirmation of microarray observations was also performed in this way, except samples from control and glycine-treated cultures were harvested 18 min and 2 h after zero time. For the analysis of GCV2::lacZ expression constructs, cells were grown in Dmin to an A600 of 0.9−1.0, then diluted in an equal volume of fresh Dmin or Dmin plus glycine, and harvested after 2 h.

Total RNA was extracted by the AE-phenol procedure described in Schmitt et al. (22). For microarray analyses, this was followed by on-column DNase I digestion and clean-up using an RNeasy kit from Qiagen. RNA purity and integrity were checked by UV spectrophotometry and denaturing agarose-gel electrophoresis.

Northern Analysis—Northern blotting was performed as described in Schmitt et al. (22). Probes were generated by random primer labeling of PCR-amplified 1-kb regions of the ACT1, ADE1, ARG4, CYT1, GCV1, GCV2, GCV3, and SHM2 open reading frames with α-32P-dCTP and [α-32P]dATP (PerkinElmer Life Sciences). Hybridization and prehybridization were performed at 65 °C in RapidHyb buffer (Amersham Biosciences). Membrane-bound radioactivity was quantified using a Bio-Rad PhosphorImager.

Microarray Production—S. cerevisiae oligonucleotide microarrays were obtained from the Ramaciotti Centre for Gene Function Analysis (Sydney, Australia) and consisted of 40-mer oligonucleotide probes for 6,250 yeast open reading frames (MWG Biotec) printed in duplicate on epoxy-coated glass microarray slides (Eppendorf). Slides were processed by baking at 120 °C with divalent ethyleneglycol on the same day as hybridization according to the substrate manufacturer’s instructions.

cDNA Synthesis, Labeling, and Hybridization—cDNA synthesis and labeling were carried out according to a modification of the amino-allyl dye-coupling protocol (24). Briefly, cDNA was synthesized from 20 μg of total RNA by reverse transcriptase (Invitrogen) using a 2:1 mixture of 5-(3-aminallyl)-uridine 5′-triphosphate (Sigma) to dTTP and the RNA subsequently hydrolyzed with NaOH. cDNA was purified using QiAquick columns (Qiagen) and labeled with N-hydroxysuccinimide esters of either Cy3 or Cy5 (Amersham Biosciences).

Hybridization was performed at 37 °C for 14–16 h in DIG EasyHyb (Roche Diagnostics) with 0.5 mg/ml E. coli tRNA and 0.5 mg/ml yeast RNA. The slides were washed 3 times in 1× SSC (0.15 M sodium chloride, 0.015 μM trisodium citrate), 0.1% SDS at 50 °C, followed by rinsing in 1× SSC, dried by centrifugation, and scanned in an Applied Precision ArrayWoRx E Biochip Reader.

For time course analysis of the glycine response in strain BWG1-7A, each slide was prepared in technical duplicate, using reciprocal labeling. A culture without added glycine was treated identically as a growth control; this did not show any significant expression changes over the 4-h period. For the comparison between deletion mutant strains, each replicate sample from the BY4743, shm2Δ, and met13Δ cultures was hybridized to one microarray slide, using a pooled wild-type sample as the reference control. For the glycine response of the BY4743 and shm2Δ strains, each replicate glycine-treated sample was co-hybridized with a replicate control sample.

Data Analysis—Microarray image analysis was performed in GenePix Pro 3.0 (Axon Instruments) following by normalizing the fluorescence intensities by the LOWESS method in GeneSpring 5.0 (Silicon Genetics). Welch’s analysis of variance (with a Benjamini-Hochberg multiple testing correction) was used to determine which genes showed significantly changed expression either at any time point after glycine addition or in any sample of the experiments using mutant strains. Genes showing significant expression changes were hierarchically clustered by using the program Cluster (25) using complete linkage clustering. Correlation coefficients of a one-dimensional self-organizing map was used to determine leaf order in the dendograms. When clustering across experiments, to compensate for the differences in sample number each sample was weighted such that the combined weight of samples from a single experiment was one. Web-based FunSpec software was used to detect cluster nodes that were enriched for genes with particular functional annotations (26).

Normalized data and cluster results are available at www.genomics.unsw.edu.au/microarray/data/gelling.

Hexamer motifs (with up to two degenerate positions) that were over-represented 800 bp upstream of genes from the one-carbon regulon were detected using Yeast Motif Finder and FindExplanator (27, 28), both available at bio.cs.washington.edu. Genes with 10 or more significant motif matches were used. GCV2 Expression Consequence Analyses—The PREZ plasmid carrying the full-length GCV2 promoter::lacZ fusion described previously (15) was integrated into the indicated strains as a single copy at the URA3 locus using the lithium acetate transformation method (29). Yeast were
Table I

S. cerevisiae strains used in this study

| Strain | Genotype | Source/Ref. |
|--------|----------|-------------|
| BWG1-7A | MATa; ade1-10; his4-51;9 leu2-3, 112; ura3-52 | 54 |
| BY4743 | MATa/α; his3Δ1/3Δ1; leu2Δ0/leu2Δ0; lys2Δ0/lys2Δ0; MET15/Δ0; ura3Δ0/ura3Δ0 | 34 |
| BY4743 gcn1Δ | MATa/α; his3Δ1/3Δ1; leu2Δ0/leu2Δ0; lys2Δ0/lys2Δ0; MET15/Δ0; ura3Δ0/ura3Δ0; YDR019c/kanMX4; YDR019c/kanMX4 | 34 |
| BY4743 met13Δ | Mata/α; his3Δ1/3Δ1; leu2Δ0/leu2Δ0; lys2Δ0/lys2Δ0; MET15/Δ0; ura3Δ0/ura3Δ0; YDR019c/kanMX128; YGR115w/kanMX4 | 34 |
| BY4743 shm2Δ | Mata/α; his3Δ1/3Δ1; leu2Δ0/leu2Δ0; lys2Δ0/lys2Δ0; MET15/Δ0; ura3Δ0/ura3Δ0; YLR065c/kanMX4; YLR065c/kanMX4 | 34 |
| F113 | MATa; ino1-113, ura3-52, CAN1p | A. G. Hinnebusch |
| H112 | MATa; ino1-113, ura3-52, CAN1p; bas1Δ | This study |
| F212 | MATa; ino1-113, ura3-52, CAN1p, gcn4-103 | A. G. Hinnebusch |
| H122 | MATa; ino1-113, ura3-52, CAN1p, gcn4-103; bas1Δ | This study |

Grown as described above and then harvested and assayed for ß-galactosidase activity as described previously (14).

bas1 Deletion Mutant Generation—The bas1 mutants were derived from GCN4 wild-type (F113) or gcn4 mutant (F212) strains by the pool/pop-out gene replacement technique described in Rothstein (90) using the yEp5-based BAS1 deletion plasmid pB1559 (deleted for the SspI Xhol fragment: -179–520 bp from start codon) (31).

RESULTS

The cellular response to glycine addition includes induction of the GCV genes, which encode the specific subunits of the glycine decarboxylase complex (14, 15). Piper et al. (16) demonstrated that this response is mediated by a decrease in cytoplasmic levels of 5,10-CH2-H4folate. Given the central role of H4folate intermediates in one-carbon metabolism, it was proposed that this novel “glycine response” might represent a more general one-carbon metabolic transcriptional control system (regulon). To test this hypothesis it was necessary to identify all genes whose transcription is altered by the addition of glycine coordinately with the induction of the GCV genes.

Kinetik of the Transcriptional Response to Glycine Addition in GCV1, GCV2, and GCV3—In order to establish the kinetics of induction of the GCV genes, temporal changes in the transcript levels of GCV1, GCV2, and GCV3 following glycine addition were examined by Northern analysis. Glycine (to 10 mM) was added to cultures of S. cerevisiae strain BWG1-7A growing in minimal medium, and samples were taken at intervals. The results in Fig. 2 show that induction of each of the GCV genes occurred with similar kinetics. Glycine induction began within 30 min, reached a maximal level within an hour, and remained at an elevated level with a slight decline from maximal induction after 4 h.

Glycine has several different metabolic fates; it can act as a nitrogen and/or a one-carbon unit source, or it can be used in protein synthesis and other biosynthetic processes (purine, glutathione, and heme biosynthesis). Induction of the one-carbon regulon is therefore probably not the only transcriptional response to glycine addition. Indeed, addition of glycine also resulted in induction of the ARG4 gene, which is a member of the Gcn4p-mediated response to changes in amino acid availability (32). However, the timing of ARG4 induction was distinct from the response of the GCV genes (Fig. 2), indicating that a time course analysis can distinguish the one-carbon regulon from other changes mediated by glycine addition.

The Genome-wide Transcriptional Response to Glycine Addition—For genome-wide analysis of the response to glycine, cells were grown to mid-exponential phase; glycine was added at zero time, and samples were harvested at intervals up to 240 min. The zero time sample was used as one slide as a reference. 949 genes showed a significant change in expression at some time after addition of glycine. Hierarchical clustering (Fig. 3A) identified groups of genes that were temporally co-regulated. Genes that showed expression changes greater than 2-fold in a 2-h growth control sample are provided as Supplemental Ma-

![Figure 2](https://example.com/image2.png)

**Fig. 2.** Northern analysis of GCV1, GCV2, GCV3, and ARG4 expression after addition of glycine. RNA was harvested from cells in Dmin and Dmin + 10 mM glycine at the time points indicated and probed for GCV1, GCV2, GCV3, and ARG4 transcripts. Each membrane was also probed for ACT1 as a RNA loading control; the example shown is from the GCV1 membrane. Data were quantified on a Phospho-Imager, normalized to ACT1, and expressed as fold induction from zero time expression in the control samples.
Table II). Amino acid biosynthesis genes, particularly those from the arginine pathway, were transiently induced after 1 h (Fig. 3A, node a; Table II), consistent with the timing of ARG4 expression obtained from the Northern analysis (Fig. 2). Although this temporal pattern was not evident for all known amino acid biosynthetic genes (data not shown), it was apparent when those genes shown by Natarajan et al. (33) to be induced during mild leucine/histidine starvation were considered. These genes presumably represent those most highly induced by amino acid starvation.

Glycine addition also led to a transient down-regulation of ribosome biogenesis (Fig. 3A, nodes e and f; Table II). Genes involved in transcription and processing of rRNA were repressed 30 min after glycine addition and remained so for 2 h. Ribosomal
protein genes showed a similar pattern, with a 30-min delay from the initial repression of rRNA-processing genes.

**Rapid Induction of One-carbon Metabolism and Purine Biosynthesis Genes following Glycine Addition**—The most rapid response identified was induction of genes involved in one-carbon metabolism and purine biosynthesis 10 min after glycine addition. Only a small number of genes showed strong expression changes in the first 10 min. Of the eight genes that showed significant change and greater than 2-fold induction at this time point, five have known functions in one-carbon metabolism or a one-carbon unit-dependent process.

In addition to being rapidly induced, the one-carbon metabolism genes were also co-regulated over the rest of the time course together with purine biosynthesis genes. These were in a set that showed rapid induction followed by maintenance of the new transcript levels over 4 h (Fig. 3A, node d). Glycine induction of ADE1 and SHM2 was confirmed by Northern analysis (Fig. 5).

Temporal co-regulation of this set of genes (Fig. 3A, node d) with GCV1, GCV2, and GCV3 strongly implies that they represent at least part of a one-carbon regulon. However, some of these genes are of unknown function or have no obvious connection to one-carbon metabolism. Additional evidence to support this, including a gene in the one-carbon regulon, could be obtained by observing its expression across a number of different disturbances affecting the balance of one-carbon metabolism.

GCV2 expression is decreased, but still glycine-responsive, in a met13 strain, which lacks the main methylenetetrahydrofolate reductase activity of the cell (16). The effect of this mutation is thus to reduce synthesis of 5-CH3-H4folate from 5,10-CH2-H4folate. Conversely, expression of GCV2 was constitutively high and showed no further induction upon glycine addition in an shm2 strain, which had reduced cytosolic levels of 5,10-CH2-H4folate. If the members of the one-carbon regulon are indeed
regulated by levels of cytoplasmic 5,10-CH2-H4folate, they should show similar responses in these mutants.

Co-regulation of One-carbon Metabolism and Purine Biosynthesis Genes under Conditions of Altered 5,10-CH2-H4folate—Transcript levels in cells grown in minimal medium of strains deleted for the SHM2 and MET13 genes were therefore compared with those in the isogenic wild-type in the homozogous diploid BY4743 background. Microarray analyses of the glycine response in both the BY4743 wild-type and the otherwise isogenic shm2Δ strain were also performed. Northern analysis in this wild-type strain demonstrated that transcription of both ADE1 and SHM2 was induced 2 h after glycine addition (Fig. 6), and hence cultures were harvested at this time. Because the met13Δ strain is a methionine auxotroph, and the shm2Δ strain is a partial adenine auxotroph (20, 35), it was necessary to provide both nutrients to all three strains.

The complete set of glycine-responsive genes was hierarchically clustered across all three experiments. These data included the temporal expression profile following glycine addition to strain BWG1-7A and expression 2 h after glycine addition to strains BY4743 and shm2Δ, as well as expression in shm2Δ and met13Δ strains with respect to wild type. From Fig. 3B a group of genes that showed the expected behavior is apparent, including GCV1, GCV2, and GCV3. These results confirmed that at least ADE1, ADE2, ADE5,7, ADE8, ADE13, ADE17, GCV1, GCV2, GCV3, MTD1, and SHM2 are co-regulated during perturbations to one-carbon metabolism (Table III). The glycine response was not completely abolished in the shm2Δ strain; however, for all putative members of the regulon the induction ratio was reduced, most markedly for SHM2, ADE17, GCV1, GCV2, and GCV3 that were the most responsive to glycine.

In order to detect any potential regulatory elements involved in this co-regulation, the promoters of genes in the node indicated in Fig. 3A in blue were analyzed. These include all but one of the known one-carbon metabolism genes from the entire “one-carbon metabolism node,” plus SHM2 (see below). Of these genes, only ADE13 did not contain a match (allowing two mismatches) within its promoter region to the CATCNCTTCTT glycine-response element (15). The promoters were searched for over-represented hexamer motifs (Table IV). The most significantly over-represented motif was TGACTC, which corresponds to the binding consensus of both the Gen4p (general amino acid control) and Bas1p (purine regulatory) transcription factors. The next most significant motif was TCYTCY, which partially matches the CTTCTT motif found in the glycine regulatory element of GCV2.

The use of the shm2 and met13 strains enabled a refinement of the composition of the “one-carbon” regulon relative to that obtained from timing of the glycine response in BWG1-7A. Interestingly, BDH1, which encodes a (2R,3R)-2,3-butanediol dehydrogenase showed a similar expression pattern to those genes in the core one-carbon metabolism node, but it has no known role in one-carbon metabolism.

Fig. 3C shows genes of one-carbon metabolism and purine biosynthesis that were not clustered into the one-carbon node or were excluded from analysis because of the lack of significant expression changes. Clearly SHM2 is also a member of the regulon and does not cluster with the others only because of the lack of SHM2 transcript in the shm2Δ strain. SHM2 showed very similar expression to the GCV genes over the compendium of publicly available genome-wide expression studies clustered by Hughes and co-workers (36). ADE4 demonstrated behavior clearly consistent with membership of the one-carbon regulon, whereas ADE6, ADE12, and ADE3 are also possible candidates. MET6, MET13, LPD1, and SER1 also showed features making them potential candidates for inclusion in the one-carbon regulon. Most of the remaining one-carbon metabolism genes do not appear to be members of the regulon (or at least not to be very highly regulated members). In particular, two of the most highly induced genes, SHM2 and ADE17, have homologs that are clearly not regulated by 5,10-CH2-H4folate status, SHM1 and ADE16. SHM1 encodes the mitochondrial SHMT, which under normal conditions contributes ~5% of total cellular SHMT activity (8). ADE16 encodes a minor isoform of Ade17p that is not transcriptionally co-regulated with ADE17 (37–39). The extent of the main components of the one-carbon regulon is indicated in Fig. 7.

Additionally, two genes (SNZ1 and SNO1) showed similar expression patterns to those of the one-carbon regulon, including decreased glycine induction in the shm2Δ strain. However, there was a delay of about an hour in the timing of their glycine induction in BWG1-7A. These adjacent and divergent genes are both involved in pyridoxine biosynthesis.

Genome-wide Transcriptional Responses to Conditions of Altered 5,10-Methylenen-Hfolate—Fig. 3B revealed that the induction of amino acid biosynthesis, stress, and respiratory chain genes following glycine addition also occurred in strain BY4743 (although often to a smaller magnitude than in strain BWG1-7A) but that the repression of ribosomal protein genes was only slight, and repression of rRNA processing genes was absent (Table V).

Characterization of the glycine response of the shm2Δ strain provided an opportunity to gauge what proportion of the glycine responses might ultimately be linked to SHMT function. Of the major transcriptional responses previously identified from temporal analysis of the glycine response, only the one-carbon regulon showed consistent differences in glycine induction between BY4743 and shm2Δ. Several other genes were induced more than 2-fold in BY4743 relative to shm2Δ, but this set was not detectably enriched for any other functional class (data not shown). From this it was concluded that none of the major secondary responses to glycine identified in the time course are totally dependent on function of the cytoplasmic SHMT encoded by SHM2.

The Glycine Response of GCV2 Is Independent of Both BAS1 and GCV4—Purine biosynthetic genes form a regulon controlled by the Bas1p/Pho2p transcription factors (39, 40). Because many genes of the one-carbon regulon overlap with those of the Bas1p/Pho2p purine biosynthesis regulon, and the GCV2 glycine-response element contains a potential Bas1p-binding
motif (15), a bas1Δ strain was used to investigate the possible role of Bas1p/Pho2p in the “one-carbon response.”

The bas1Δ was generated in both wild-type and gcn4Δ backgrounds, in order to account for general amino acid control, which might be achieved by glycine addition in the absence of a functional one-carbon response. Expression of a \( \text{GCV2:}\lambda\text{lacZ} \) fusion construct was thus examined in the wild-type F113, F113 gcn4Δ, and F113 bas1Δ, and F113 gcn4Δ/bas1Δ strains (Fig. 8). The glycine response was retained in all mutants tested, although both appear to play some role in maintaining a basal level of \( \text{GCV2} \) expression in minimal medium.

**DISCUSSION**

In combination with the evidence that \( \text{GCV2} \) expression is regulated by cytoplasmic 5,10-\( \text{CH}_2\)-\( \text{H}_4 \)folate (16), these results indicate that there is a set of genes co-regulated in response to changes in cellular one-carbon levels, and these form a previously unknown one-carbon metabolism regulon.

The composition of this one-carbon regulon is consistent with a response to 5,10-\( \text{CH}_2\)-\( \text{H}_4 \)folate limitation. Induction of the \( \text{GCV} \) genes and \( \text{SHM2} \) would increase generation of 5,10-\( \text{CH}_2\)-\( \text{H}_4 \)folate from both serine and glycine cleavage. The inclusion of \( \text{SHM2} \) is consistent with a response to one-carbon unit levels rather than glycine directly, because in yeast the reaction catalyzed by cytoplasmic SHMT is thought to operate predominantly in the direction of glycine synthesis (8). The transcriptional induction of \( \text{SHM2} \) is consistent with the observation of Botsford and Parks (41) that SHMT activity in \( S. \text{cerevisiae} \) was induced 4-fold by 10 mM glycine.

Up-regulation of purine biosynthetic genes may increase the efficiency of this pathway when cytoplasmic one-carbon units are depleted. The enzymes of the purine biosynthetic pathway and cytosolic one-carbon metabolism may physically interact to facilitate substrate channeling, although not as a classical multienzyme complex (42, 43). Coupling expression of purine pathway genes to those of one-carbon metabolism under these conditions may be required to maintain optimal functioning of the system as a whole. Additionally, induction of the one-carbon regulon would result in an increased contribution from mitochondrial one-carbon metabolism, which is metabolically closely linked with purine biosynthesis, because at least 25% of one-carbon units incorporated into purines are derived from mitochondrially derived formate, irrespective of the availability of cytoplasmic one-carbon units (44).

The \( \text{SNZ1} \) and \( \text{SNO1} \) genes play a role in pyridoxine biosynthesis (45) and are induced in response to amino acid and nucleotide starvation (33, 46). Because both cytoplasmic SHMT and the glycine decarboxylase complex utilize a pyridoxal 5’-phosphate co-enzyme, it is possible that expression of \( \text{SNZ1/SNO1} \) responds to the increased production of these two enzymes. The 1-h delay between induction of the one-carbon regulon and \( \text{SNZ1/SNO1} \) indicates that the two responses are probably distinct. Interestingly, \( \text{SNZ1} \) and \( \text{SNO1} \) were found to be among the genes most highly induced by Gcn4p in response to amino acid starvation (33), which is consistent with the requirement for many different pyridoxal 5’-phosphate-dependent aminotransferases in amino acid metabolism. The alteration in \( \text{SNZ1} \) and \( \text{SNO1} \) expression in the \( \text{shm2} \Delta \) and \( \text{met13} \Delta \) mutants, which do not show induction or repression of the rest of the Gcn4p regulon, also implies that \( \text{SNZ1/SNO1} \) may be regulated in response to demand for pyridoxine.

Many members of the minimal one-carbon regulon are regulated by adenine levels via the Bas1p/Pho2p purine biosynthesis transcription factors (39, 47). In addition, \( \text{GCV1} \) (47) and \( \text{GCV2} \) show decreased basal expression in \( \text{bas1} \) strains, and the glycine regulatory region of \( \text{GCV2} \) includes a TGA motif that matches the consensus binding motif of Bas1p. However, the one-carbon regulon is distinct from the Bas1p/Pho2p
purine response for several reasons. First, the presence of adenine in the growth medium, and the fact that strain BWG1-7A has ade1 and his4 mutations, makes it unlikely that the observed one-carbon regulon can be attributed to secondary purine nucleotide de-repression caused by one-carbon unit limitation. Second, the regulon includes GCVI, which has been

![Fig. 7. Main genes of the one-carbon regulon.](image)

**TABLE V**

Over-represented functional categories amongst genes co-regulated under conditions of altered one-carbon metabolism (nodes of dendrogram in Fig. 3B)

| Node (Fig. 3B) | p value | Genes in node from functional category | % node | % genome |
|---------------|---------|--------------------------------------|--------|---------|
| g GO biological process<sup>a</sup> | 1.98 × 10<sup>-11</sup> | HIS4 ARO3 TRP2 LEU1 TRP5 BAT1 CPA2 ILV5 LEU4 | 29.4 | 1.4 |
| i GO biological process<sup>a</sup> | 1.18 × 10<sup>-5</sup> | PYC2 BNA1 SNO1 SNZ1 ZWF1 | 14.7 | 0.9 |
| j GO biological process<sup>a</sup> | 8.66 × 10<sup>-6</sup> | TPS1 NTH1 UBC5 HSP12 CTT1 YGP1 PEP4 | 28 | 3.2 |
| k MIPS functional classification | 2.90 × 10<sup>-14</sup> | RPY1 CYC7 COX13 QCR9 QCR10 CYC1 COX12 CB2 | 50 | 1.4 |
| lRibosome biogenesis<sup>b</sup> | <1.00 × 10<sup>-14</sup> | RPL11B RPL16A RPL17B RPS1B RPL16B RPS3 | 23.1 | 2.2 |

<sup>a</sup> Represents the approximate probability that the cluster would contain the observed number of genes from a particular functional annotation by chance.

<sup>b</sup> Functional categories from the Gene Ontology™ Consortium (GO) (www.geneontology.org).
demonstrated not to be regulated by purine levels (47). Third, although the glycyine regulatory region is required for the glycyne response, the TGACT motif contained within it is not a primary response to glycine addition, in particular the functioning of other enzymes involved in amino acid metabolism. For example, glycine acts as a serine analog in weak competitive inhibition of tryptophan synthase (48, 49) and in non-competitive inhibition of 3-phosphoglycerate dehydrogenase, the first step of serine synthesis (50).

As a consequence of the diversity of cellular processes in which one-carbon end products are involved, including DNA synthesis and methylation reactions, perturbations to this system have far-reaching consequences. This is particularly evident in humans, where the availability of folate for one-carbon metabolism is known to be essential for normal embryonic development, as well having impacts on neural function, cardiovascular disease, alcoholic liver disease, and carcinogenesis (51–53). It is possible that factors influencing one-carbon activation of H4folate, as opposed to H2folate availability, may also have clinical relevance.

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One-carbon Metabolism Regulon

7081
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Cristy L. Gelling, Matthew D. W. Piper, Seung-Pyo Hong, Geoffrey D. Kornfeld and Ian W. Dawes

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