Regulation of the Balance of One-carbon Metabolism in Saccharomyces cerevisiae

One-carbon metabolism in yeast is an essential process that relies on at least one of three one-carbon donor molecules: serine, glycine, or formate. By a combination of genetics and biochemistry, we have shown how cells regulate the balance of one-carbon flow between the donors by regulating cytoplasmic serine hydroxymethyltransferase activity in a side reaction occurring in the presence of excess glycine. This control governs the level of 5,10-methylene tetrahydrofolate (5,10-CH₂-H₄folate) in the cytoplasm, which has a direct role in signaling transcriptional control of the expression of key genes, particularly those encoding the unique components of the glycine decarboxylase complex (GCV1, GCV2, and GCV3). Based on these and other observations, we propose a model for how cells balance the need to supplement their one-carbon pools when charged folates are limiting or when glycine is in excess. We also propose that under normal conditions, cytoplasmic 5,10-CH₂-H₄folate is mainly directed to generating methyl groups via methionine, whereas one-carbon units generated from glycine in mitochondria are more directed to purine biosynthesis. When glycine is in excess, 5,10-CH₂-H₄folate is decreased, and the regulation loop shifts the balance of generation of one-carbon units into the mitochondrial.

Tetrahydrofolate (H₄folate)‐mediated one-carbon metabolism lies at the center of a large number of essential cellular processes including methyl group biogenesis and the synthesis of nucleotides, vitamins, and some amino acids. One-carbon units are derived from catabolism of three donor molecules: serine, glycine, and formate. These are then activated and compartmentalized by attachment to H₄folate for biosynthetic processes. In most organisms, serine is the principle one-carbon donor (1) contributing to the pool of 5,10-CH₂-H₄folate by the action of serine hydroxymethyltransferase (SHMT) enzymes (see Fig. 1 and Table I, reactions 1 and 2). Glycine catabolism via the mitochondrial glycine decarboxylase multienzyme complex (GDC) (see Fig. 1 and Table I, reaction 3) also contributes to the one-carbon pool by generation of 5,10-CH₂-H₄folate (2), whereas formate activation to 10-HCO-H₄folate occurs via the synthetase activity of the Cl-tetrahydrofolate synthase trifunctional enzymes (see Fig. 1 and Table I, reactions 4a and 6c) (3). Each of the one-carbon H₄folate pools are interconverted by the cyclohydrolase (see Fig. 1 and Table I, reactions 4b and 6b) and dehydrogenase (see Fig. 1 and Table I, reactions 4a and 6a) activities of the Cl-tetrahydrofolate synthase enzymes, whereas flow between the mitochondrion and the cytoplasm is mediated by the three one-carbon donor molecules. The result is a dynamic metabolic system in which one-carbon units are interconverted between the mitochondrial and cytoplasmic compartments as well as between oxidation states. This flow has been the subject of extensive biochemical analyses (4–9) that have shown that glycine, serine, and formate can each act to supplement all of the different one-carbon pools. Much less, however, is known of how cells regulate these flows.

In contrast to the other enzyme activities for the generation and interconversion of one-carbon units, glycine catabolism via the GDC is strictly mitochondrial. This enzyme is composed of four subunits each encoded separately. These are the P-protein (encoded by GCV2), the H-protein (encoded by GCV3), the T-protein (encoded by GCV1), and the L-protein (encoded by LPD1). Each of the GCV genes is unique to the GDC and on addition of exogenous glycine has been shown to be induced transcriptionally (10–12). For GCV1 and GCV2 expression this was due to a 17-base pair sequence with the hexanucleotide 5′-CTTCTT-3′ at its core in their promoters (13), and similar sequences have been identified in the GCV3 promoter. This work also indicated that H₄folate or one of its derivatives may play a role in signaling the need for increased GCV transcription when glycine is added to the medium. This possibility led us to the hypothesis that the glycine effect is only one component of a broader one-carbon metabolism regulatory system. This has been confirmed from genome-wide transcript analysis in which the addition of glycine to yeast cells growing in minimal medium led to the induction of other genes central to one-carbon metabolism (14).

Here, we have used a combination of genetics and biochemistry to identify which derivative of H₄folate acts to signal this response, and we propose a model for 5,10-CH₂-H₄folate‐mediated regulation of metabolism through transcription. Because of the large body of work on the phenotypes of many one-carbon metabolic mutants (6, 15–17), strains were available that were specifically altered with respect to the different reduced H₄folate pools. Gene expression studies in these strains were combined with ¹³C NMR experiments to monitor one-carbon flow. These data indicated that control over cytoplasmic serine hydroxymethyltransferase activity is responsible for determining how GCV gene transcription is regulated. By monitoring
adapted from West et al. AdoMet

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vidually in appropriate strains as a single copy at the

sion have been described previously (13). These were integrated indi-

pholipid biosynthesis are as follows: PC, phosphatidyl choline; AdoHcy,

, S-adenosylhomocysteine; PE, phosphatidylethanolamine; PS, phos-

phatidylerine; and AdoMet, S-adenosylmethionine. The figure is

adapted from West et al. (9).

the levels of the 5,10-CH₂-H₄folate pool (which is normally
derived from serine), cells can up-regulate glycine catabolism
for one-carbon generation when 5,10-CH₂-H₄folate is limiting
or spare the breakdown of serine when glycine is in surplus.

EXPERIMENTAL PROCEDURES

Materials

Amino acids, 2-mercaptoethanol, [2-13C]glycine, poly(dl-Dc), and sodium
H₄folate were obtained from Sigma. 3-trimethylsilylpropionate-
2,2,3,3-d₄, sodium salt and [14C]formaldehyde were from ICN Pharma-
cuticals, and DEAE-Sephadex was from Amersham Pharmacia
Biotech. Deuterated dimethyl-d₆-sulfoxide was obtained from MSD Iso-
topes (distributed by Merck). All other materials were of highest avail-
able quality and were obtained from various commercial vendors.

Strains and Media

The yeast strains relevant to this study are listed in Table II. Yeast
were grown in minimal medium as described previously (10) with
auxotrophic requirements added at 40 mg liter⁻¹. To elicit the glycine
response, glycine was added to a final concentration of 10 mM; a 2 mM
sodium formate stock (pH 7.0) was used to supplement media where
indicated.

Gene Expression Analyses

Constructs and Yeast Transformation—Plasmids carrying the full-
length GCV2 promoter:JocZ fusion (pRH2 and pH1), full-length
GCV1 promoter:JocZ fusion, and full-length GCV3 promoter:JocZ fu-

sion have been described previously (13). These were integrated indi-

vidually in appropriate strains as a single copy at the URA3 locus using
the lithium acetate transformation method (22).

β-Galactosidase Assays—Yeast were grown to early log phase in the
media indicated and harvested for assays as described previously (10).

NMR

Cell Growth, 13C-Labeling, and Extract Preparation—NMR studies
followed the method described in Ref. 6. A 1-liter culture of cells
was grown to late log phase in yeast minimal medium with or without 100
mg liter⁻¹ [2-13C]-labeled glycine. Cells were harvested and resus-
pended in 20 ml of 0.3 N HCl and warmed over a boiling water bath for
1 h. This solution was centrifuged, the supernatant was replaced with
fresh HCl, and the process was repeated. The supernatants from the
two extractions were pooled, reduced using a Rotavapor, and dried in a
Speedivac. Samples were stored at this stage at ~80 °C until analysis.

Immediately prior to NMR, samples were resuspended in 1 ml of deu-
terated dimethyl-d₆-sulfoxide.

NMR Analysis—NMR spectra were recorded on a Bruker DMX 600
spectrometer equipped with a TXI-XYP probe at a temperature of 298
K. Chemical shifts are relative to internal 3-trimethylsilyl propionate-
2,2,3,3-d₄, sodium salt set to 0 ppm. (This results in our measured shifts
being ~1.4 ppm lower in frequency than those reported relative to
tetramethylsilicon.) Metabolites were identified by comparison with
previously published data on chemical shifts (Table III) (6). Relative
intensities of lines within “triplets” produced by mixtures of single 13C
and double 13C-13C isotopomers were extracted with line shape decon-
volving using the Bruker WINNMR program. 13C NMR spectra were
acquired using power-gated decoupling. A total of 2400 scans with an
acquisition time of 1.14 s and recycle delay of 5.0 s were acquired for
each sample, resulting in an overall time of 4 h 7 min per sample. The
spectral width was 190 ppm, and 64,000 points were collected. The
pulse lengths were 9.0 μs, with a 78-degree flip angle. Spectra were
processed with exponential multiplication and line broadening of 3 Hz.

Chemical Synthesis of 5,10-CH₂-H₄folate

Labeled 5,10-CH₂-H₄folate was prepared from H₄folate and
[13C]formaldehyde, based on a previously published method (23). Equimolar proportions of H₄folate and formaldehyde (5% [13C]formal-
dehyde) were combined in a stopped oxygen- and light-free container.
The solution was brought to pH 5 by the dropwise addition of 1 N NaOH,
and the mixture was incubated at room temperature for 15 min. The
reaction mixture was left at 4 °C for 60 min after which the pH was
adjusted with 1 N NaOH to 6.5 using 1 N NaOH. The 5,10-CH₂-H₄folate
was concentrated at 4 °C on a 10-ml DEAE-Sephadex column pre-equilibrated with 50 mM
(NH₄)₂CO₃, and washed immediately before loading with 100 ml of 50
mM 2-mercaptoethanol. After loading, the column was washed with 20
ml of 50 mM 2-mercaptoethanol and then eluted with a 100-ml gradient
of 10–500 mM (NH₄)₂CO₃ containing 50 mM 2-mercaptoethanol (pro-
gress was monitored by absorbance at 295 nm). Fractions (2 ml) were
collected, and those containing 5,10-CH₂-H₄folate were identified by
absorbance and scintillation counting. Concentration and purity were
estimated by correlating the pteridine ring concentration (ε₉₀ = 30,000
m⁻¹ cm⁻¹) with the amount of radioactivity (1 mmol = 1.3 × 10¹² dpm
in a given fraction. Maximum purity was estimated at greater than
90%.

Samples were freeze-dried and stored under argon at ~80 °C away
from light. Prior to use, the powder was resuspended in 50 mM
2-mercaptoethanol.

Gel Mobility Shift Assay

Assays were performed as described previously (24) using purified
protein extracts obtained from heparin-Sepharose chromatography
(25). Proteins were extracted from yeast strain BWG1–7A, purified, and
incubated with 40-base pair DNA fragments harboring the glycine
regulatory region of GCV2. These were prepared, separated by electro-
phoresis, and analyzed as described previously (13). The relative
amount of DNA present in the DNA/protein complexes was estimated
by PhosphorImager analysis. To reduce the effects of loading inconsis-
tencies, these values were normalized to the total DNA present in each
lane.

RESULTS

One-carbon metabolism comprises a complex set of reactions
that contribute to many central biosynthetic pathways. Several
approaches can be taken to identify components of the signal-
ning system regulating the glycine response, although direct
biochemical analyses are difficult because of the ability of the
reactants to interchangeably supplement the pools of one-car-
bon-derived compounds and the relative instability of the
H₄folate derivatives. By combining extensive genetic analysis
with biochemical approaches, several groups have provided
clear indications of the flow of metabolites and the conse-
dquences of specific metabolic blocks (6, 15–17). In particular,
Appling and co-workers have effectively combined genetics
with the use of 13C NMR to provide a wealth of information on
the biochemistry of one-carbon metabolic flow. Here, we have
combined genetic and biochemical approaches to extend our
previous finding that a folate species plays a role in signaling
the glycine response of the GCV2 genes (13). Because glycine
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Table I

| Reaction | Enzyme | Gene designation | Metabolism and phenotype of mutant strains relevant to this study |
|----------|--------|------------------|---------------------------------------------------------------|
| 1        | Cytoplasmic serine hydroxymethyltransferase (SHMT) | SHM2 | Requires adenine for optimal growth |
| 2        | Mitochondrial serine hydroxymethyltransferase (SHMT) | SHM1 | No growth requirements |
| 3        | Glycine decarboxylase multienzyme complex (GDC) | GCV1, GCV2, GCV3, and LPD1 | Cannot assimilate one-carbon units from serine in the cytosol (or generate serine from glycine and 5,10-CH2-H4folate) |
| 4a–4c    | Cytoplasmic trifunctional C1-tetrahydrofolate synthase (Ade3p) | ADE3 | Methionine auxotrophy |
| 5        | NAD-dependent 5,10-CH2-H4folate dehydrogenase (Mtd1p) | MTD1 | No growth requirement |
| 6a–6c    | Mitochondrial trifunctional C1-tetrahydrofolate synthase (Mis1p) | MIS1 | No growth requirement |
| 7        | 5,10-CH2-H4folate reductase (MTHFR) | MET13 | Metionine auxotrophy |
| 8        | 5,10-CH2-H4folate synthetase (MTHFS) | MTHFS (YER183c) | No growth requirements |

* See Fig. 1.

** Enzyme activities of trifunctional C1-tetrahydrofolate synthases: a) 5,10-CH2-H4folate dehydrogenase, b) 5,10-CH2-H4folate cyclohydrolase, and c) 10-HCO-H4folate synthetase.

Table II

| Yeast strains used |
|-------------------|
| Strain            | Genotype          | Source/reference |
| BWG1–7A           | MAT ade1–100 his4–519 leu2–3,112 ura3–52 | (18) |
| gsd1              | MAT gcv1 leu2 met1 ser1 ura3–52 | (19) |
| MET13             | MAT leu2Δ1 trp1Δ63 ura3–52 | (Mutant phenotype described in Ref. 20) |
| met13             | MAT leu2Δ1 trp1Δ63 ura3–52 kanMX: YGL125u<sup>a</sup> | |
| DAY 4             | MAT his4 leu2 ser1 trp1 ura3–52 | (21) |
| WHY 1             | MAT his4 leu2 ser1 trp1 ura3–52 AYER183c | Donated by D. Appling |
| ADE3 SHM2         | MAT his3 his4 leu2 trp1 ura3–52 | This study |
| ade3–130 SHM2     | MAT ade3–130 his3 leu2 trp1 ura3–52 | This study |
| ADE3 shm2         | MAT his3 leu2 trp1 ura3–52 shm2–LEU2 | This study |
| ade3–130 shm2     | MAT ade3–130 his3 leu2 trp1 ura3–52 shm2–LEU2 | This study |

* Requires methionine for growth.

Table III

| Yeast strains used |
|-------------------|
| Strain            | Genotype          | Chemical shifts of metabolites |
|                   |                   | Metabolite | C-1 | C-2 | C-3 | C-4 | C-5 | C-8 |
| BWG1–7A           | MAT ade1–100 his4–519 leu2–3,112 ura3–52 | Adenine | 145.5 | 155.5 | 147.1 |
| gsd1              | MAT gcv1 leu2 met1 ser1 ura3–52 | Choline | 57 | 68.8 | 55.3 |

catabolism via the glycine decarboxylase reaction directly contributes to the one-carbon pool, we first tested whether a mutant lacking GDC activity could still elicit a glycine response.

The Glycine Response Is Independent of the Ability to Metabolize Glycine—We previously proposed that changes in an intermediate or product of one-carbon metabolism (e.g., a derivative of H4folate) provide the signal for the glycine response. To test whether these changes require glycine catabolism, we transformed a strain mutant for glycine cleavage activity with a normally regulated GCV2::lacZ fusion (pRH2) and compared reporter gene expression in minimal medium with that in minimal medium containing 10 μM glycine (Fig. 2). A 3- to 4-fold increase in gene expression on the addition of glycine was observed, which is typical of the wild-type glycine response previously reported (10). Because this strain is mutant for the GCV1 gene product encoding the T-protein of the glycine decarboxylase complex and has no detectable GDC activity, it is unable to use glycine as a sole one-carbon and nitrogen source (26). Thus glycine catabolism has no direct effect on H4folate-mediated one-carbon metabolism. To reconcile this result with the data indicating that GCV transcriptional signaling involves a H4folate intermediate, we sought a one-carbon metabolic mutant unable to elicit a normal glycine response. We reasoned that this strain would reveal how glycine acts to control one-carbon metabolism and the balance of cellular H4folate molecules.

Mutation of Cytosolic Serine Hydroxymethyltransferase Disrupts the Glycine Response of the GCV Genes—The cytoplasmic SHMT reaction is normally the principle route for the synthesis of one-carbon charged H4folate derivatives using H4folate and serine as substrates (Fig. 1 and Table I, reaction 1). Fig. 3 shows the effect of a mutation eliminating cytosolic SHMT activity (shm2) on glycine induction of expression of all three GCV genes. Expression of each of the GCV genes was constitutively high in minimal medium with and without glycine. Interestingly, the elevated level of expression in this strain in minimal medium was about the same as that of the wild type (ADE3 SHM2) grown in minimal medium containing glycine. The mutation in this strain therefore elicited the same increase...
in gene expression as seen for the addition of glycine to the wild type.

To determine whether the effect of this mutation was due to a disturbance of one-carbon metabolism and not simply loss of the SHMT enzyme, we supplemented the cytosolic one-carbon pool with formate. From Fig. 1 and Table I it can be seen that catabolism of formate in the cytosol should compensate for any one-carbon imbalance of an ADE3 shm2 strain via the activity of C1-tetrahydrofolate synthase (ADE3 gene product; Ref. 7). This enzyme first activates the one-carbon units derived from formate to 10-HCO-H4folate before conversion to 5,10-CH2-H4folate. In the wild-type strain (ADE3 SHM2) addition of excess formate caused a decrease in GCV2 gene expression (Fig. 4A). This reduction was even more evident in the ADE3 shm2 strain, occurring at a lower formate concentration. This effect was eliminated by the introduction of a mutation in the ade3 gene. The lack of response to formate by the ade3–130 shm2 strain shows that assimilation of cytoplasmic one-carbon units from formate is required for the down-regulation of gene expression. Interestingly, in these experiments the effect on control of adding glycine to the wild type was similar to that of the shm2 mutation. That is, there was an ADE3-dependent reduction in GCV gene expression induced by glycine on the addition of formate (Fig. 4B). We propose, therefore, that the presence of excess glycine acts in a similar manner to the shm2 mutation by causing a reduction in the level of one of the cytoplasmic one-carbon charged H4folates and that this decrease then acts as the signal to increase GCV gene expression.

**The Principle Effect of Mutation of Cytosolic SHMT Is a Reduction in Cytosolic 5,10-CH2-H4folate—**Two principle lines of evidence support the model that the ADE3 shm2 strain is limited in its cytosolic one-carbon pool. First, when grown in minimal medium, it has a slightly slower growth rate than the wild type that can be restored by the addition of adenine to the medium (Table IV). This is also consistent with the observation made by West et al. (9) that a strain that was unable to oxidize cytoplasmic 5,10-CH2-H4folate could not generate sufficient one-carbon units in the mitochondrion for purine synthesis to support wild-type growth. This strain recovered when adenine was added to the medium.

Secondly, we used 13C NMR to follow the incorporation of one-carbon units from the mitochondrion into molecules derived from the cytoplasmic one-carbon H4folate pools. When cells are grown in the presence of exogenous glycine it is incorporated into choline and adenine both directly as an intact molecule and also as a one-carbon unit from the cytoplasmic pool. Applying and co-workers (6) have shown that monitoring the incorporation of [2,13C]glycine into choline and adenine allows an insight into the flow of mitochondrial one-carbon metabolism to the cytoplasm.

Fig. 5 shows a comparison of the relevant regions of the NMR spectra for extracts of three mutants used for the gene expression studies (ADE3 SHM2, ade3–130 SHM2, and ADE3 shm2). Fig. 5A shows that there was an approximate 4-fold reduction in the incorporation of label into choline in the ADE3 shm2 strain when compared with either the wild-type or the ade3–130 SHM2 strain. This demonstrated that synthesis of choline was principally derived from one-carbon units formed as a result of the cytoplasmic SHMT reaction. Residual labeled cho-

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**Fig. 2.** Expression of the full-length GCV2::lacZ gene fusion (pRH2) transformed into a strain mutant for glycine decarboxylase activity. Strain gsd1 is mutant in the gene encoding the GDC T-protein (gcd1) and therefore cannot catabolize glycine. Error bars represent the standard deviation of β-galactosidase assays performed in triplicate.

**Fig. 3.** The glycine response of GCV1, GCV2, and GCV2 genes is disrupted in an shm2 knockout strain. The GCV3::lacZ gene fusions were transformed as single copies into a strain mutant for the cytosolic serine hydroxymethyltransferase (ADE3 shm2) and its isogenic wild type (ADE3 SHM2). Strains are described in Table II. Error bars represent the standard deviation of β-galactosidase assays performed in triplicate. Dmin, minimal medium (10).

**Fig. 4.** Exogenous formate alters GCV gene transcription by altering the one-carbon metabolic balance. One-carbon metabolic mutant strains transformed with the full-length GCV2::lacZ gene fusion were grown in minimal medium with the addition of formate (concentration as indicated) (left) or minimal medium containing 10 mM glycine with the addition of 25 mM formate (right). β-Galactosidase assays were performed on exponentially growing cells as described under “Experimental Procedures.” Values are relative to those without the addition of formate. Error bars represent the standard deviation of β-galactosidase assays performed in triplicate. Dmin, minimal medium (10).
H4folate) could be candidates, and 10-HCO-H4folate remains a
major drain on the one-carbon pool is at the level of
methionine for growth.

The data also show that 10-HCO-H4folate levels were not as
greatly affected in the ADE3 shm2 mutant. 5,10-CH2-H4folate
is normal in a strain lacking cytoplasmic C1-tetrahydrofolate synthase activity. The
full-length GCV2::lacZ gene fusion was transformed as a single copy into a strain mutant in the gene encoding the cytoplasmic C1-tetrahydrofolate synthase (ade3–130 SHM2). Error bars represent the standard deviation of β-galactosidase assays performed in triplicate. Dmin, minimal medium (10).

### Table IV

| Strain                | Mediuma | Minimal | Minimal + adenine |
|-----------------------|---------|---------|------------------|
| ADE3 SHM2 (wild type) | 2.4 h   | 2.3 h   |                  |
| ADE3 shm2             | 3.5 h   | 2.4 h   |                  |

* Requires methionine for growth.

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**Fig. 5.** 13C NMR labeling of (A) choline and (B) adenine in extracts from cells grown on [2-13C] glycine. Spectra from strains ade3–130 SHM2, ADE3 shm2, and ADE3 SHM2 are compared; chemical shifts of relevant metabolites are listed in Table III.
is encoded by the *MET13* gene product that supplies one-carbon units for the synthesis of methionine (28). This pathway transfers the methyl moiety via S-adenosylmethionine to the C-4 position of choline (resonating at ~55.3 ppm in $^{13}$C NMR spectra; Table III). Fig. 5A shows that there was a significant reduction in labeling of this carbon in the ADE3 *shm2* strain, indicating lowered levels of 5-CH$_3$H$_4$folate in the cell. The possibility that changes in the 5-CH$_3$H$_4$folate pool mediate the glycine response was therefore analyzed in a *met13* strain lacking MTHFR activity.

The *met13* strain retained the ability to elicit the glycine response (Fig. 7). Furthermore, in contrast to the constitutively high expression in the ADE3 *shm2* mutant, the level of GCV2 expression in minimal medium was markedly lower than in the wild-type strain (less than 5%). This was not an artifact of methionine addition to the growth medium because the ADE3 *shm2* strain retained constitutively high levels of expression under the same culture conditions (data not shown). This result was not surprising because we have previously noted that excess methionine caused a slight increase in GCV2 expression (13). This experiment excludes the possibility that low 5-CH$_3$H$_4$folate is the cause of increased GCV2 expression in the ADE3 *shm2* strain. Moreover under all four conditions described above in which the cytosplasmic 5,10-CH$_2$H$_4$folate pool would be in surplus, the level of GCV gene expression was down-regulated. This effect was most dramatic in the *met13* mutant when the cytoplasmic pool of 5,10-CH$_2$H$_4$folate would be greatest because of the block to synthesis of 5-CH$_3$H$_4$folate and because one-carbon metabolism via the SHMT reaction is directed more toward the generation of methyl groups (methionine, choline, and dTMP) than of purines (see “The Principle Effect of Mutation of Cytosolic SHMT Is a Reduction in Cytosolic 5,10-CH$_2$H$_4$folate” above and “How Is 5,10-CH$_2$H$_4$folate Used in the Cell to Maintain a One-carbon Metabolic Balance”).

How Could the Addition of Glycine to Cells Affect 5,10-CH$_2$H$_4$folate Levels? The evidence above indicates that 5,10-CH$_2$H$_4$folate is the molecule mediating the regulation of the GCV genes. Because metabolism of glycine via the GDC is not essential to elicit a glycine response, we sought to explain how the addition of glycine to cells could modulate the cytoplasmic concentration of 5,10-CH$_2$H$_4$folate. In a detailed analysis of the regulation and catalytic mechanism of SHMT from rabbit liver and *Escherichia coli*, Schirch et al. (29) have shown that glycine in the range of 4–37.5 mM inhibited the conversion of serine to glycine and 5,10-CH$_2$H$_4$folate. Hence end-product inhibition of the SHMT reaction is one explanation. More significantly, however, was the discovery that 5-CH$_3$H$_4$folate or 5-HCO-H$_4$folate binds SHMT in conjunction with glycine to form a dead-end complex that dramatically inhibits the activity of the enzyme (30). The 20-fold reduction of GCV gene expression in the *met13* strain (Fig. 7) when grown in minimal medium may thus reflect the consequences of additive metabolic disturbances leading to a build up of cytoplasmic 5,10-CH$_2$H$_4$folate levels: disruption of the use of 5,10-CH$_2$H$_4$folate for 5-CH$_3$H$_4$folate synthesis and loss of 5-CH$_3$H$_4$folate inhibition of SHMT activity.

The second important control molecule, 5-HCO-H$_4$folate, is produced from 5,10-CH$_1$H$_4$folate by the SHMT from rabbit liver and *E. coli* in the presence of excess glycine (31). This reaction appears in *E. coli* to be the sole source of 5-HCO-H$_4$folate, a metabolite that is also present in yeast but whose function until now was not known. Hence both end-product inhibition and the effect of glycine in conjunction with folate derivatives in dead-end complex formation would lead to a reduction in 5,10-CH$_2$H$_4$folate synthesis on addition of glycine to the cell. This situation mimics that observed above in the ADE3 *shm2* strain.

To What Extent Does the Above Dead-end Complex Formation Contribute to the in Vivo Transcription Response to Glycine?—GCV gene expression was assayed in strain WHY1, mutant for the putative 5,10-CH$_1$H$_4$folate synthetase gene (YER183c). This mutant cannot convert 5-HCO-H$_4$folate to 5,10-CH$_1$H$_4$folate.

Blocking this reaction (Fig. 1 and Table I, reaction 8) would lead to an elevated intracellular concentration of 5-HCO-H$_4$folate and should therefore result in increased inhibition of SHMT, decreased 5,10-CH$_2$H$_4$folate, and altered control of GCV gene transcription. If this reaction were important in the control system, then in the absence of exogenous glycine the mutant should show increased GCV expression because of the very high affinity of SHMT for intracellular glycine in the presence of 5-HCO-H$_4$folate (30). The mutant should also show a further response on addition of exogenous glycine because this is the third component of the ternary complex. Both outcomes were observed as shown in Fig. 8.

5,10-CH$_2$H$_4$folate Affects the Binding to DNA of a Protein That Binds the Control Motif in the Promoter of Glycine-responsive Genes—We have previously identified by gel mobility shift analysis a protein that binds to the glycine response region mediating the transcriptional control of the GCV genes. This binding was increased by the addition of H$_4$folate in *vitro* (13). Because the above data implicated 5,10-CH$_2$H$_4$folate in the

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2 W. Holmes and D. Appling, personal communication.
control of GCV2 transcription, we synthesized the compound and used it in gel mobility shift assays. Fig. 9 shows that addition of up to 2 μM 5,10-CH2-H4folate led to a decrease in the binding of the protein to the specific DNA; this decrease, however, was only to 60% of the initial level. Our previous work using H4folate showed an increase in DNA/protein complex formation but at a higher concentration than that measured here for 5,10-CH2-H4folate (50 μM–1 mM). Other commercially available folates (folic acid, folinic acid, and 5-CH3-H4folate) had no effect on complex formation over the range up to 1 mM (data not shown).

DISCUSSION

It is understood how metabolites flow through the various one-carbon metabolic pathways in the cell (4–9), but less is known on mechanisms controlling this flow. It has been shown here that there is an inverse relationship between cytoplasmic 5,10-CH2-H4folate levels and GCV gene expression. We propose that this is a control mechanism whereby the cell regulates the balance of serine, glycine, and one-carbon metabolism to meet conflicting demands. This is achieved by altering the balance of one-carbon metabolism between the cytoplasm and the mitochondrion to ensure a constant supply of one-carbon units to the important biosynthetic pathways such as those concerned with purine and pyrimidine biosynthesis. At the center of this control loop is regulation of the levels of cytoplasmic 5,10-CH2-H4folate.

How is 5,10-CH2-H4folate Used in the Cell to Maintain a One-carbon Metabolic Balance?—Fig. 1 and Table I show the many ways in which 5,10-CH2-H4folate is involved in metabolism in the cytoplasm. Our data and those of many others show that the SHMT-catalyzed reaction serves as the principle source of this molecule, which is the primary contributor to the one-carbon pool (1). Pathways that are involved in the consumption of 5,10-CH2-H4folate include methionine synthesis via the action of the MTHFR enzyme and 5,10-CH2-H4folate synthesis via the methylene-H4folate dehydrogenase function of the C1-tetrahydrofolate synthase enzyme. When each of these functions was disturbed, alterations in the level of 5,10-CH2-H4folate signaled the need for the cell to synthesize one-carbon units from the alternate donor molecule glycine and thus shift the balance of one-carbon metabolism into the mitochondrion. From these data, we have been able to gain an insight into the regulation and flow of one-carbon metabolites in the cell under normal metabolic conditions.

Our results indicate that when cells are growing normally cytoplasmic 5,10-CH2-H4folate is used primarily for the production of methyl groups via methionine biosynthesis and secondly for the production of more oxidized C1-H4folate derivatives and metabolites such as purines. For purine synthesis there is therefore a balance between one-carbon metabolites derived from mitochondrial metabolism of glycine and the cytoplasmic conversion of serine to 5,10-CH2-H4folate. This model is supported by the fact that a ser1 allele (ade9, defective in its ability to produce serine from glycolytic intermediates) was originally identified as an adenine auxotroph when serine was limiting (32). At low serine levels, adenine limited growth of this strain, indicating that serine can be channeled preferentially into fulfilling the requirements of the cytoplasmic 5,10-CH2-H4folate pool for 5-CH3-H4folate and subsequently methionine biosynthesis at the expense of the more oxidized C1-H4folates required for purine biosynthesis. However, whereas flow from the cytoplasmic 5,10-CH2-H4folate pool for synthesis of purines may have lower priority, it does still play a role in normal cell growth.

Exogenous adenine was required for the shm2 strain to grow at an optimal rate, indicating that the mitochondrial one-carbon pathway cannot fully maintain the requirements for purine biosynthesis. However, Pasternack et al. (6) have shown that at least 25% of one-carbon units for purine synthesis are mitochondrially derived. They concluded that the normal pathway for glycine-derived one-carbon units is through the synthesis in the mitochondrion of formate, which is exported to the cytosol for purine synthesis. Thus the normal balance for flow of one-carbon units derived from serine is toward methyl group biosynthesis, whereas glycine derived from serine in the mitochondrion supplements the supply of one-carbon units for purine biosynthesis.

Under What Circumstances Is This Flow Altered?—Under physiological conditions the cell needs to adjust the synthesis and degradation of serine and glycine to fulfill the requirements for protein synthesis as well as one-carbon metabolism and hence needs to regulate the balance of one-carbon flow. We explored two situations in which the use of glycine for one-carbon metabolism was enhanced (shown by an increase in GCV gene expression): when cells were grown with exogenous glycine and when the SHM2 gene was disrupted. These two situations resulted in a similar reduction of the levels of cytoplasmic 5,10-CH2-H4folate, which forms the basis for the signal to increase GCV gene transcription. Interestingly, in a recent study on the global transcriptional effects of the DNA-damaging reagent methyl methanesulfonate, the transcription of GCV2, GCV1, and GCV3 was increased 12.5-, 7.5-, and 2.6-fold, respectively (33). According to the regulatory mechanism proposed above, a decrease in 5,10-CH2-H4folate levels due to an increased demand on cytoplasmic one-carbon metabolism for nucleotide biosynthesis would signal a need to increase GCV gene transcription. Because the principle route for one-carbon unit synthesis is through the cytoplasmic SHMT, it was not surprising that loss of its function resulted in a decrease of 5,10-CH2-H4folate levels. It was less apparent, however, how addition of glycine also affected the levels. We have shown that the side reaction of the cytoplasmic SHMT described by Schirch and co-workers (29–31), which results in its inhibition by ex-
Regulation of One-carbon Metabolism

Regulation of SHMT activity by a side reaction dictates cytoplasmic 5,10-CH₂-H₄folate levels, which mediate transcription of genes involved in generation of one-carbon units from glycine. This control loop has the effect of shifting the balance of one-carbon metabolism toward the mitochondrial reactions. Solid black arrows indicate enzyme-catalyzed reactions; gray lines indicate control of enzyme activity; and the dashed line indicates regulation of gene expression.

Inhibition of the activity of cytoplasmic SHMT alters the cytoplasmic 5,10-CH₂-H₄folate level, which modulates expression of genes involved in one-carbon metabolism, including those in the glycine cleavage complex. Transcription of the GCV genes is repressed by high levels of cytoplasmic 5,10-CH₂-H₄folate, a situation that signals that alternate sources of one-carbon units are not required. When cytoplasmic 5,10-CH₂-H₄folate levels are low, however (either through one-carbon “starvation” or the presence of excess glycine, causing production of 5-HCO-H₄folate, which inhibits SHMT activity), the cell calls upon glycine catabolism in the mitochondrion to supplement its one-carbon requirements.

These conclusions also explain the unexpected observation by Pasternack et al. (6) of an apparent interdependence of two physically compartmentalized enzyme activities. Yeast ade3–30 mutants lacking the synthetase activity of cytoplasmic C1-tetrahydrofolate synthase are unable to activate formate to 10-HCO-H₄folate for purine synthesis. In a particular genetic background, ade3–30 cells could not use glycine to replace serine to supplement their one-carbon pool. Under normal conditions, glycine metabolized by the GDC and mitochondrial SHMT produces serine. This is exported to the cytoplasm, where cytoplasmic SHMT acts to fulfill cytoplasmic one-carbon metabolic requirements. We propose that when glycine accumulates, flow through cytoplasmic SHMT is reduced (by glycine inhibition); cytoplasmic 5,10-CH₂-H₄folate levels drop, and one-carbon metabolism is channelled to the mitochondrion. If reductive synthesis of 5,10-CH₂-H₄folate is blocked by the ade3–30 mutation and glycine inhibits production in the oxidative direction, the cells are effectively starved for cytoplasmic one-carbon-loaded H₄folate molecules. Lack of mitochondrial C1-tetrahydrofolate synthase (encoded by MIS1) activity could reverse this phenotype. The mis1 mutation may act to increase production of serine in the mitochondrion (because of an inability to oxidize 5,10-CH₂-H₄folate) and thus increase serine export to the cytoplasm. Excess serine may partially overcome the SHMT block, thus supplying one-carbon-loaded H₄folate molecules to the cytoplasm.

By What Mechanism Does 5,10-CH₂-H₄folate Effect Transcriptional Change?—Our previous work demonstrated that H₄folate could directly interact with a protein binding to the DNA sequence harboring a glycine response control sequence (GRR) (13). This interaction altered its ability to bind but only occurred at H₄folate levels above those found in the cell. Here, we describe an interaction with 5,10-CH₂-H₄folate that also altered the ability of the protein to bind the GRR, but at lower and physiologically significant concentrations. Addition of high levels of 5,10-CH₂-H₄folate did not, however, completely inhibit binding of the protein to the GRR.

Because glycine induction of the GCV genes has been shown to be coordinately regulated, it is interesting to note that control of GCV2 and GCV3 transcription is regulated by repression, whereas that of GCV1 is regulated by activation (13). These data indicate that signaling may be mediated by 5,10-CH₂-H₄folate altering the activity of the DNA-binding protein rather than by affecting its ability to bind DNA and that changes in in vitro binding reactions were the results of a conformational change in the protein due to binding of the folate. In vivo, the signaling mechanism may be further complicated by a very low concentration of free folates present in cells because of substrate channeling of folates between enzymes (reviewed in Ref. 34). The most likely explanation involves the existence of a protein that can detect 5,10-CH₂-H₄folate levels, possibly by association with the cytoplasmic SHMT enzyme and dictated by the presence of the competing folates that regulate SHMT activity. By dissociation and subsequent nuclear localization, the protein may function to modulate gene transcription. Purification and identification of the transcription factor that binds the GRR and effects transcriptional activity will help answer these questions. Work on identification of the DNA-binding protein is under way in our laboratory.

Furthermore, because 5,10-CH₂-H₄folate is central to one-carbon metabolism, we predict that this control system forms the basis for a new metabolic transcriptional regulon. We are currently investigating the genome-wide transcriptional consequences of alterations to the cytoplasmic 5,10-CH₂-H₄folate pool.

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