^{13}C NMR Detection of Folate-Mediated Serine and Glycine Synthesis in Vivo in *Saccharomyces cerevisiae*†

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Received May 13, 1992

ABSTRACT: *Saccharomyces cerevisiae* has both cytoplasmic and mitochondrial C_1-tetrahydrofolate (THF) synthases. These trifunctional isozymes are central to single-carbon metabolism and are responsible for interconversion of the THF derivatives in the respective compartments. In the present work, we have used ^{13}C NMR to study folate-mediated single-carbon metabolism in these two compartments, using glycine and serine synthesis as metabolic endpoints. The availability of yeast strains carrying deletions of cytoplasmic and/or mitochondrial C_1-THF synthase allows a dissection of the role each compartment plays in this metabolism. When yeast are incubated with [^{13}C]formate, ^{13}C NMR spectra establish that production of [3-^{13}C]serine is dependent on C_1-THF synthase and occurs primarily in the cytosol. However, in a strain lacking cytoplasmic C_1-THF synthase but possessing the mitochondrial isozyme, [^{13}C]formate can be metabolized to [2-^{13}C]glycine and [3-^{13}C]serine. This provides in vivo evidence for the mitochondrial assimilation of formate, activation and conversion to [^{13}C]CH_2-THF via mitochondrial C_1-THF synthase, and subsequent glycine synthesis via reversal of the glycine cleavage system. Additional supporting evidence of reversibility of GCV in vivo is the production of [2-^{13}C]glycine and [2,3-^{13}C]serine in yeast strains grown with [3-^{13}C]serine. This metabolism is independent of C_1-THF synthase since these products were observed in strains lacking both the cytoplasmic and mitochondrial isozymes. These results suggest that when formate is the one-carbon donor, assimilation is primarily cytoplasmic, whereas when serine serves as one-carbon donor, considerable metabolism occurs via mitochondrial pathways.

Tetrahydrofolate (THF)[1]-mediated one-carbon metabolism is essential in many cellular processes including nucleic acid biosynthesis, mitochondrial and chloroplast protein biosynthesis, amino acid metabolism, and methyl group biogenesis (Blakley, 1969). In most organisms, the major source of one-carbon units is the 3-carbon of serine, derived from glycolytic intermediates (Schirch, 1984). The one-carbon unit is transferred to THF in a reaction catalyzed by serine hydroxymethyltransferase (SHMT) (Figure 1, reaction 4) to generate 5,10-methylene-THF (CH_2-THF) and glycine. This form of the coenzyme is then distributed between several pathways, depending on the needs of the cell. In eukaryotes, reactions 1–3 are catalyzed by a trifunctional enzyme termed C_1-THF synthase. Figure 1 depicts the central role that C_1-THF synthase plays in interconverting these folate derivatives between the various states of oxidation required by these many pathways. In the yeast *Saccharomyces cerevisiae*, two isozymes of C_1-THF synthase exist, both encoded by nuclear genes. The *ADE3* gene encodes a cytoplasmic C_1-THF synthase (Jones, 1972; Staben & Rabinowitz, 1986), and the *MIS1* gene encodes a mitochondrial isozyme (Shannon & Rabinowitz, 1986, 1988). It is not known whether higher eukaryotes also possess a trifunctional mitochondrial isozyme, but all three activities of C_1-THF synthase have been demonstrated in rat liver mitochondria (Barlowe & Appling, 1988).

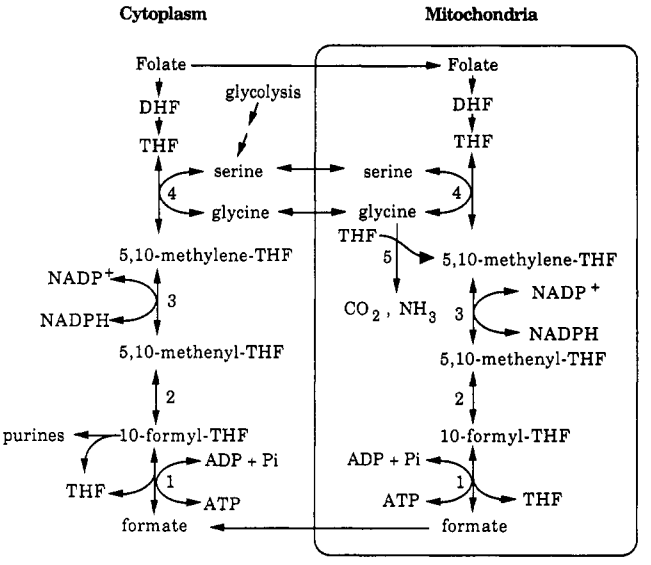

FIGURE 1: Proposed organization of the enzymes of single-carbon metabolism. Reactions 1, 2, and 3, 10-formyl-THF synthetase (EC 6.3.4.3), 5,10-methenyl-THF cyclohydrolase (EC 3.5.4.9), and 5,-10-methylene-THF dehydrogenase (EC 1.5.1.5), respectively, are catalyzed by C_1-THF synthase. Reaction 4 is SHMT (EC 2.1.2.1). Reaction 5 is GCV (EC 2.1.2.10) and is found only in the mitochondria.

We are interested in the role of these two isozymes in the coordination of folate-mediated one-carbon metabolism between the two compartments. We have previously proposed an intercompartmental pathway in which mitochondrial one-carbon units are derived from cytoplasmic serine after its transport into mitochondria (Barlowe & Appling, 1988; Appling, 1991). Cleavage of serine to glycine and CH_2-THF via mitochondrial SHMT donates a one-carbon unit into the mitochondrial pool. CH_2-THF can be oxidized to 10-formyl-

† This work is supported by the Foundation for Research, by NIH Grant DK36913 to D.R.A., and by NSF CHE 9013384 to D.A.L.

* Recipient of a Research Career Development Award from the National Institutes of Health. To whom correspondence should be addressed: 512-471-5842.

[1] Abbreviations: CH_2-THF, 5,10-methylene-THF; CHO-THF, 10-formyl-THF; CH^+-THF, 5,10-methenyl-THF; GCV, glycine cleavage enzyme system; NMR, nuclear magnetic resonance; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

0006-2960/92/0431-8713$03.00/0

THF by the sequential reactions CH_2-THF dehydrogenase and 5,10-methenyl-THF (CH^+-THF) cyclohydrolase (Figure 1, reactions 3 and 2). This 10-formyl-THF can be utilized in mitochondrial protein synthesis (Staben & Rabinowitz, 1984) or converted to formate via 10-formyl-THF synthetase (Figure 1, reaction 1) for transport out of mitochondria. Although these enzymes are all reversible, mitochondrial synthesis of serine from formate via this pathway has not been demonstrated and cellular serine requirements are considered to be fulfilled via serine synthesis from glycolytic intermediates. In fact, mutation of phosphoserine aminotransferase (*ser1*) in yeast creates a strain requiring supplemental serine for growth (Jones & Fink, 1982). The serine requirement can also be satisfied with formate plus glycine (McKenzie & Jones, 1977). It is proposed that cytoplasmic C_1-THF synthase converts the formate to CH_2-THF (Figure 1, reactions 1–3), which then condenses with glycine in a reaction catalyzed by cytoplasmic SHMT (Figure 1, reaction 4) to produce serine. *ser1*$^-$ yeast strains lacking one or more of the activities of cytoplasmic C_1-THF synthase fail to grow on formate plus glycine, thus confirming the critical role of this enzyme for utilization of formate (McKenzie & Jones, 1977; Barlowe & Appling, 1990). Furthermore, mutations in the *MIS1* gene that encodes the mitochondrial isozyme do not affect growth on glycine plus formate (Shannon & Rabinowitz, 1988; Barlowe & Appling, 1990). Although these results again point to the cytoplasmic pathway as the major route for formate assimilation, they do not rule out a contribution by the mitochondrial pathway. Growth studies also indicate the ability of high glycine alone to substitute for the serine requirement of a *ser1*$^-$ strain (McKenzie & Jones, 1977). It is proposed that under these conditions the glycine cleavage enzyme system (GCV) (Figure 1, reaction 5) cleaves some of the glycine to CO_2 and CH_2-THF. High glycine thus provides both the one-carbon unit (CH_2-THF) and the two-carbon unit (glycine) required for serine synthesis by SHMT. Since GCV is a mitochondrial enzyme (Motokawa & Kikuchi, 1971) and folate derivatives have not been shown to cross the mitochondrial membrane to any significant extent (Cybulski & Fisher, 1981; Barlowe & Appling, 1988; Horne et al., 1989), it is reasonable that serine synthesis from glycine occurs via mitochondrial SHMT. Although in vitro studies of GCV isolated from chicken (Okamura-Ikeda et al., 1987) and rat (Motokawa & Kikuchi, 1969) liver mitochondria indicate that this enzyme system is reversible, to our knowledge, in vivo evidence for glycine synthesis via GCV has not been reported.

In the present work, we have used in vivo ^{13}C metabolic labeling of various yeast mutants and ^{13}C NMR analysis of metabolic end products to investigate the respective in vivo roles of the cytoplasmic and mitochondrial isozymes of C_1-THF synthase and SHMT in the metabolism of one-carbon units donated by formate or serine. These pathways have been difficult to study by traditional ^{14}C metabolic labeling strategies due to the potential for tracer carbons to become incorporated at multiple positions in several end products and the presence of parallel pathways in the two compartments. The ability of ^{13}C NMR to distinguish which carbon(s) in a particular metabolite is (are) labeled, used in combination with various yeast strains harboring specific metabolic blocks, enables the enzymatic route and compartment of biosynthesis of a metabolite under investigation to be determined. In the present work we develop the application of this approach for the monitoring of flux thru C_1-THF synthase, SHMT, and GCV by label incorporation into glycine and serine. These experiments provide new insights into our current understanding of single-carbon metabolism including demonstrations of (i) the ability of both the cytoplasmic and mitochondrial pathway to metabolize formate to the 3-carbon of serine, implying mitochondrial assimilation of formate; (ii) glycine synthesis in the mitochondria by reversal of GCV using CH_2-THF derived from either formate or the 3-carbon of serine; and (iii) serine resynthesis (synthesis of serine incorporating carbons derived from serine) involving mitochondrially produced glycine.

EXPERIMENTAL PROCEDURES

Materials and Strains. Haploid strains of *Saccharomyces cerevisiae* were used in all studies. Yeast strains DAY4 (**a** *ADE3 MIS1 ser1 ura3-52 trp1 leu2 his*$^-$), KSY9 (**a** *ADE3 ser1 ura3-52 mis1*::*URA3*), DAY3 (**a** *ade3-130 MIS1 ura3-52 trp1 leu2 his4 ser1*), and KSY8 (α *ser1 ade3-130 ura3-52 mis1*::*URA3*) have been described previously (Shannon & Rabinowitz, 1988; Barlowe et al, 1989). *ade3-130* is a chromosomal deletion (Jones, 1977), and *mis1*::*URA3* is a gene disruption (Shannon & Rabinowitz, 1988); these mutations result in complete absence of the respective enzymes.

Cell Growth, ^{13}C Labeling, and Extract Preparation. Yeast cultures of 750 mL, grown aerobically at 30 °C in rich media (1% yeast extract, 2% bactopeptone, 2% glucose), were harvested at mid-log phase by centrifugation at 4000 rpm for 4 min and resuspended in 25 mL of yeast minimal media (yeast nitrogen base) containing 2% glucose, L-leucine (30 mg/L), L-histidine (20 mg/L), L-tryptophan (20 mg/L), uracil (20 mg/L), and adenine (20 mg/L). Cultures were incubated aerobically (except as indicated, Figure 6) at 30 °C and provided with labeled and/or unlabeled substrates as indicated in the figure legends. After 3 h of incubation extracts were prepared for NMR analysis. Cultures were centrifuged at 4000 rpm for 4 min, washed with 25 mL of 1.8 mM KH_2PO_4/118 mM NaCl (pH 7.4), and then resuspended in 1.8 mL of the same buffer containing 4 M 2-mercaptoethanol (in order to maintain reduction state of folate derivatives). Cells were disrupted by vortexing for 4 min with glass beads, boiled for 5 min, and centrifuged for 25 min at 25000*g*. A portion of the resulting supernatant was transferred to a 5-mm NMR tube and overlaid with argon prior to capping.

Just prior to incubation with labeled substrates, an aliquot of growing cells was removed from each culture and spread on drop out plates to test for contamination and strain verification.

NMR Analysis. ^{13}C NMR spectra were obtained on a Nicolet Analytical Instruments NT 360 equipped with a 5-mm probe at 90 MHz. A pulse width corresponding to a 90° flip angle was used, and data were collected with a 200-ms delay and continuous broad-band decoupling. A total of 3600 scans were acquired over a sweep width of 20 000 Hz. A total of 64K data points were collected and zero filled prior to Fourier transform. Characteristic chemical shifts of appropriate metabolites were established by natural abundance spectra in a DAY4 extract background produced as described above with unlabeled compounds provided during incubation.

RESULTS

Metabolism of [^{13}C]Formate and [2-^{13}C]Glycine to Serine. Figure 2 demonstrates the utility of ^{13}C NMR to follow serine synthesis from glycine and a one-carbon unit derived from formate. An extract of DAY4 (*ADE3*$^+$ *MIS1*$^+$), which is wild type for both cytoplasmic and mitochondrial C_1-THF synthase, was incubated with unlabeled glycine and formate

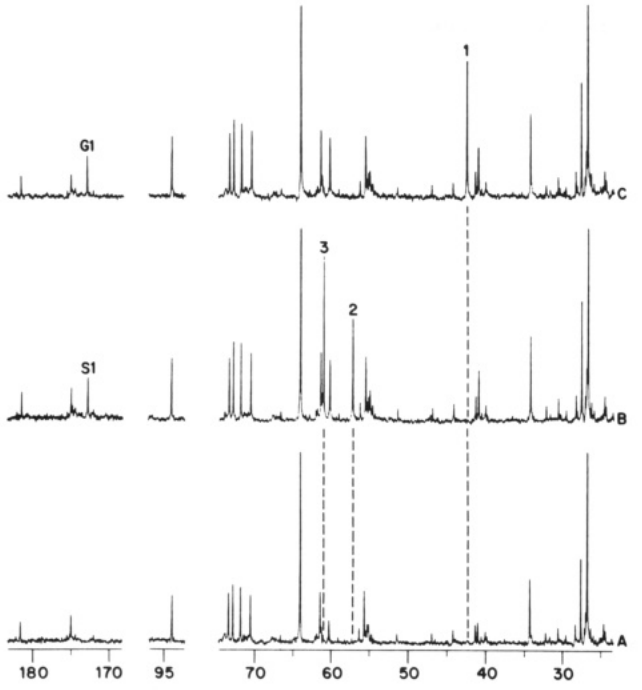

FIGURE 2: Full-scale ^{13}C NMR spectra of cell extract of DAY4 (*ADE3*$^+$ *MIS1*$^+$) spiked with (A) nothing, (B) 16 mg/mL unlabeled serine, or (C) 16 mg/mL unlabeled glycine. Resonances: (1) 2-glycine; (2) 2-serine; (3) 3-serine.

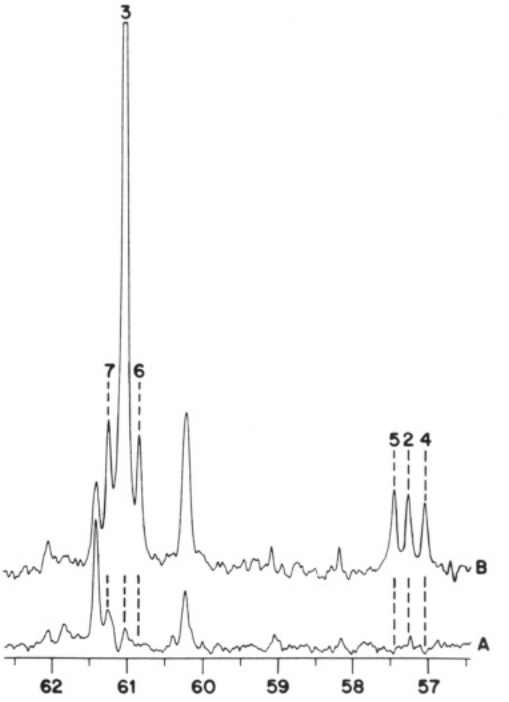

FIGURE 3: ^{13}C NMR spectra of cell extract of DAY4 (*ADE3*$^+$ *MIS1*$^+$) incubated for 3 h with (A) 0.25 mg/mL unlabeled formate and 20 mg/mL unlabeled glycine or (B) 0.25 mg/mL [^{13}C]formate and 20 mg/mL [2-^{13}C]glycine. Resonances: (2) 2-serine; (3) 3-serine; (4–7) 2,3-serine.

and prepared as described above. The spectrum obtained (Figure 2A) serves as a control and establishes that no significant peaks exist at the resonance frequencies associated with 2-serine, 3-serine, or 2-glycine. Peaks present in this spectrum result from the 1.1% naturally occurring ^{13}C of cellular metabolites normally present at high concentrations in the cell. These resonances do not interfere with analysis of spectra acquired from experiments involving incorporation of ^{13}C. Direct addition of unlabeled serine (1.1% natural abundance ^{13}C) to this extract allows characterization of the resonance frequencies associated with the carbons of serine for this system to be 172.8 ppm for C1 (Figure 2B, resonance S1), 57.2 ppm for C2 (Figure 2B, resonance 2), and 60.97 ppm for C3 (Figure 2B, resonance 3). Direct addition of glycine (1.1% natural abundance ^{13}C) to a second aliquot of the DAY4 extract allows characterization of the resonance frequencies associated with the carbons of glycine to be 173.2 ppm for C1 (Figure 2C, resonance G1) and 42.3 ppm for C2 (Figure 2C, resonance 1). The carbons at position 1 in serine and glycine do not derive from the folate-mediated one-carbon; subsequent figures will display only the regions of the spectra encompassing C2 and C3 of serine and C2 of glycine.

Extracts prepared from DAY4 (*ADE3*$^+$ *MIS1*$^+$) metabolizing [^{13}C]formate and [2-^{13}C]glycine (Figure 3B) reveal the presence of three labeled species of serine in the cell: [3-^{13}C]-serine (resonance 3), [2-^{13}C]serine (resonance 2), and [2,3-^{13}C]serine (resonances 4–7). [2,3-^{13}C]Serine yields four peaks due to carbon–carbon coupling, i.e., the splitting of the resonance frequency associated with each ^{13}C carbon by a neighboring ^{13}C into a doublet. The J_{23} coupling constant of these doublets is typical of adjacent ^{13}C carbons. This provides a powerful tool for distinguishing doubly vs singly labeled products. Thus, the triplets seen in Figure 3B indicate the presence of both the doubly labeled and singly labeled species in the extract. Figure 3A shows that control extracts prepared from DAY4 incubated with unlabeled formate and glycine show insignificant accumulation of ^{13}C-labeled serine. Small resonances appear due to serine synthesis from the unlabeled

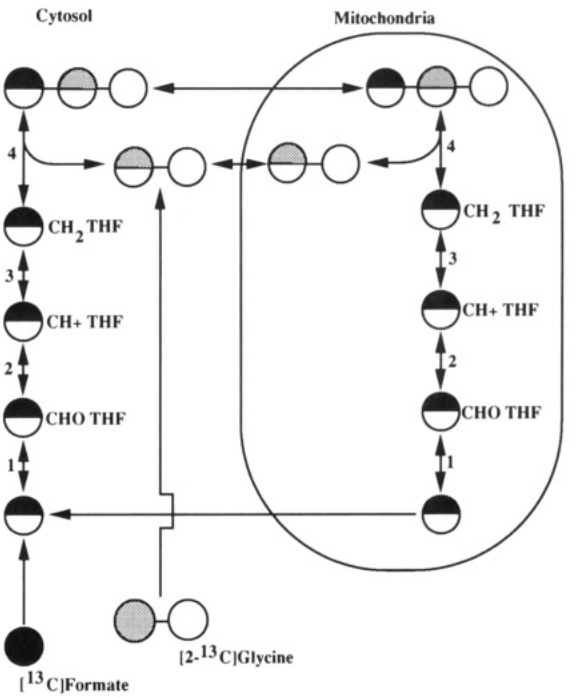

FIGURE 4: Expected distribution of carbons derived from formate and glycine into serine. Refer to Figure 1 for enzyme names. Black circles indicate ^{13}C carbons derived from [^{13}C]formate, and shaded circles indicate ^{13}C carbons derived from [2-^{13}C]glycine. Half circles indicate equilibration of the introduced label with endogenous unlabeled pools of formate and glycine.

formate and glycine (actually 1.1% natural abundance ^{13}C). The following reactions describe the previously proposed pathway to [2,3-^{13}C]serine from [^{13}C]formate plus [2-^{13}C]-glycine (Figure 4). [^{13}C]Formate is converted to [^{13}C]CH_2-THF by C_1-THF synthase (reactions 1–3, Figure 4). [^{13}C]-CH_2-THF and [2-^{13}C]glycine are used by SHMT for serine synthesis (reaction 4, Figure 4). The presence of singly labeled

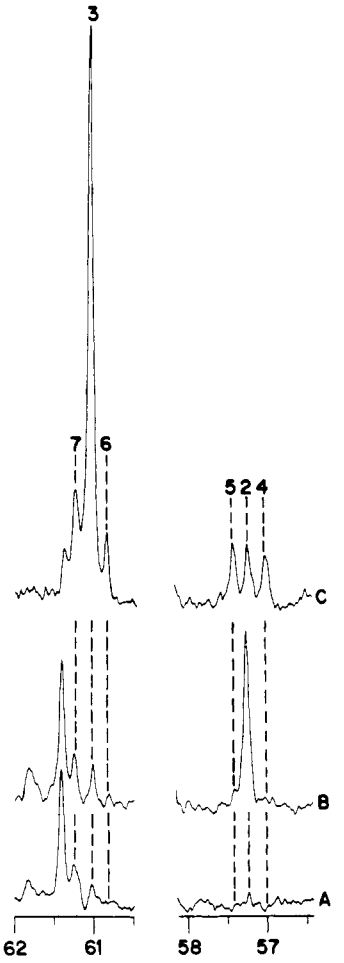

FIGURE 5: ^{13}C NMR spectra of cell extract of DAY4 (*ADE3*$^+$ *MIS1*$^+$) incubated for 3 h with (A) 0.25 mg/mL unlabeled formate and 20 mg/mL unlabeled glycine, (B) 0.25 mg/mL unlabeled formate and 20 mg/mL [2-^{13}C]glycine, or (C) 0.25 mg/mL [^{13}C]formate and 20 mg/mL unlabeled glycine. Resonances: (2) 2-serine, (3) 3-serine; (4–7) 2,3-serine.

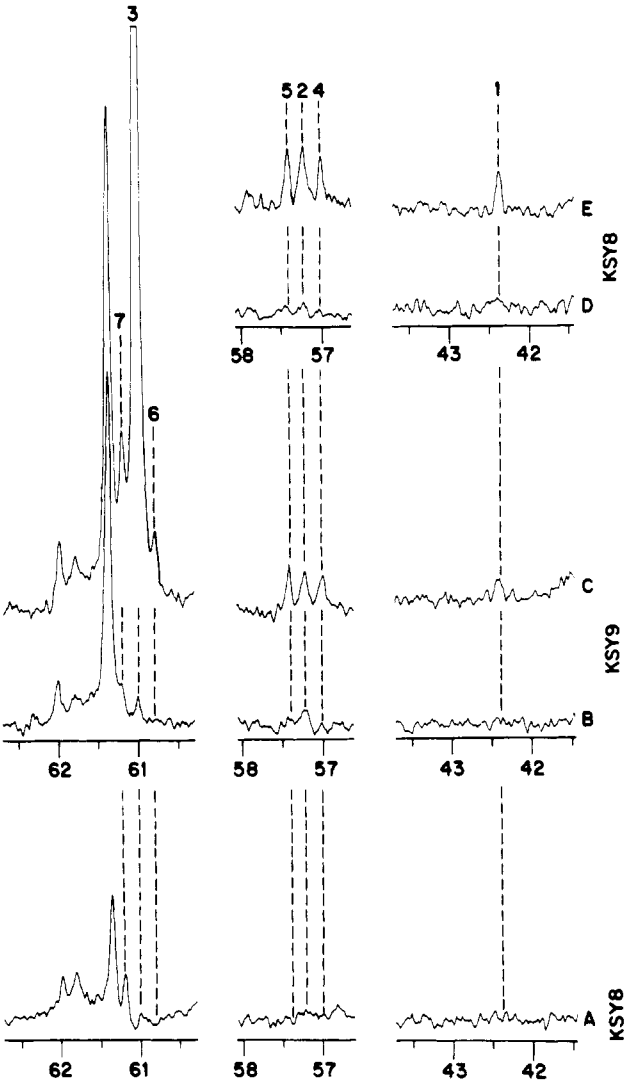

FIGURE 6: ^{13}C NMR spectra of yeast extract. (A) KSY8 (*ade3*$^-$ *mis1*$^-$) incubated with 0.25 mg/mL [^{13}C]formate and 2 mg/mL unlabeled glycine, (B) KSY9 (*ADE3*$^+$ *mis1*$^-$) incubated with 0.25 mg/mL unlabeled formate and 2 mg/mL unlabeled glycine, (C) KSY9 (*ADE3*$^+$ *mis1*$^-$) incubated with 0.25 mg/mL [^{13}C]formate and 2 mg/mL unlabeled glycine, (D) KSY8 (*ade3*$^-$ *mis1*$^-$) incubated with 1.125 mg/mL unlabeled serine, and (E) KSY8 (*ade3*$^-$ *mis1*$^-$) incubated with 1.125 mg/mL [3-^{13}C]serine. Resonances: (1) 2-glycine; (2) 2-serine; (3) 3-serine; (4–7) 2,3-serine.

serine (resonances 2 and 3) in Figure 3B indicates that endogenous, unlabeled pools of both glycine and formate are present in the cell which compete with the ^{13}C-labeled substrates provided. The relative heights of these peaks indicate either a much greater endogenous pool of glycine than CH_2-THF or a difference in the equilibration of the introduced label with the existing unlabeled pools.

The next two experiments verify the source of the label in these three species of serine. DAY4 (*ADE3*$^+$ *MIS1*$^+$) cultures were incubated with either labeled glycine or labeled formate. Figure 5B shows the spectrum of a DAY4 culture incubated with [2-^{13}C]glycine and unlabeled formate. This spectrum displays the resonance associated with [2-^{13}C]serine (resonance 2), indicating incorporation of glycine C2 into serine C2 via SHMT. A small amount of [3-^{13}C]serine (resonance 3) can also be seen in this spectrum. This is not surprising since glycine cleavage via GCV is known to contribute to the single-carbon pool, thus providing [^{13}C]CH_2-THF for mitochondrial [3-^{13}C]serine synthesis. Figure 5C is the spectrum of a DAY4 culture incubated with unlabeled glycine and [^{13}C]formate and demonstrates the appearance of the resonance frequency associated with [3-^{13}C]serine (resonance 3), indicating the incorporation of formate into serine C3 as expected. This spectrum, however, also has resonances associated with the presence of ^{13}C label at C2 of serine (resonances 2 and 4–7). Metabolism to C2 of serine would first require formate incorporation at C2 of glycine (see below), thus providing a [2-^{13}C]glycine for synthesis of serine in the SHMT reaction. Further experiments characterize the role of both the cytoplasmic and mitochondrial C_1-THF synthase in metabolism of formate to glycine and serine.

Cytosolic C_1-THF Synthase Metabolism of Formate. Strains KSY9 (*ADE3*$^+$ *mis1*$^-$), and KSY8 (*ade3*$^-$ *mis1*$^-$) were exposed to [^{13}C]formate and unlabeled glycine for 3 h and extracts prepared as described in Experimental Procedures. The spectrum obtained from strain KSY8 (*ade3*$^-$ *mis1*$^-$) shows no incorporation of label into serine or glycine (Figure 6A). Spectra from extracts of KSY8 incubated with unlabeled substrates are virtually identical (data not shown). Since this strain completely lacks both the mitochondrial and cytoplasmic isozyme, formate assimilation into glycine and serine can be considered to require these isozymes. Strain KSY9, which contains the cytoplasmic C_1-THF synthase only, shows considerable accumulation of [3-^{13}C]serine (resonance 3) and some accumulation of [2-^{13}C]serine (resonance 2), [2,3-^{13}C]-serine (resonances 4–7), and [2-^{13}C]glycine (resonance 1) (compare spectra B and C of Figure 6). Incorporation of

folate-mediated one-carbon units into the 2-position of glycine requires reversal of the mitochondrial GCV for synthesis of [2-^{13}C]glycine (Figure 1, reaction 5).

Furthermore, since it appears that reduced folate coenzymes are unable to cross the mitochondrial membrane (Cybulski & Fisher, 1981; Barlowe & Appling, 1988; Horne et al., 1989), glycine synthesis via reversal of the GCV would involve mitochondrially produced [^{13}C]CH_2-THF. Further metabolism of the resulting [2-^{13}C]glycine to [2-^{13}C]serine or [2,3-^{13}C]serine requires unlabeled or labeled CH_2-THF, respectively, in the reaction catalyzed by SHMT (Figure 1, reaction 4). Mitochondrial [^{13}C]CH_2-THF under these conditions could arise by transport of cytosolically produced [3-^{13}C]serine and donation of C3 via mitochondrial SHMT.

Metabolism of [3-^{13}C]Serine. In order to verify that label incorporation into serine C2 and glycine C2 resulted from further metabolism of [3-^{13}C]serine, strains DAY4 (*ADE3*$^+$ *MIS1*$^+$) and KSY8 (*ade3*$^-$ *mis1*$^-$) were incubated with [3-^{13}C]-serine. Figure 6 shows that KSY8 accumulates [2-^{13}C]glycine (resonance 1) (compare spectra D and E of Figure 6). Thus, mitochondrial synthesis of [2-^{13}C]glycine from a single-carbon unit derived from [3-^{13}C]serine is independent of the presence of either C_1-THF synthase isozyme. These two spectra also demonstrate incorporation of ^{13}C into [2-^{13}C]serine (resonance 2) and [2,3-^{13}C]serine (resonances 4 and 5). These products suggest that [3-^{13}C]serine enters the mitochondria, where it is cleaved to [^{13}C]CH_2-THF plus unlabeled glycine via SHMT (Figure 8A, reaction 4). This [^{13}C]CH_2-THF is then used for [2-^{13}C]glycine synthesis via reversal of GCV (Figure 8A, reaction 5). [2-^{13}C]Serine and [2,3-^{13}C]serine are then synthesized by reversal of SHMT using the [2-^{13}C]glycine and either unlabeled or labeled CH_2-THF, respectively (Figure 8A, reaction 4). The spectrum obtained from incubation of DAY4 with [3-^{13}C]serine (data not shown) is virtually identical to that in Figure 6E, indicating that this route to serine synthesis also occurs with both mitochondrial and cytosolic isozymes present.

Mitochondrial C_1-THF Synthase Metabolism of Formate. Previous growth experiments have shown that formate cannot satisfy the one-carbon requirement of *ser1*$^-$ strains lacking cytoplasmic C_1-THF synthase, even when the mitochondrial isozyme is present (Barlowe & Appling, 1990). These results suggested that formate utilization is entirely cytoplasmic, via conversion to CH_2-THF by C_1-THF synthase (Figure 1). The following set of experiments examines the ability of mitochondria to metabolize formate to glycine and serine.

While cytosolic formate incorporation occurs readily in aerated cells, incubation of DAY3 (*ade3*$^-$ *MIS1*$^+$) and KSY8 (*ade3*$^-$ *mis1*$^-$) with [^{13}C]formate revealed that mitochondrial formate incorporation into serine and glycine was enhanced in poorly aerated cells. Figure 7 shows extracts of DAY3 and KSY8 incubated with [^{13}C]formate under low aeration conditions. Strain KSY8 shows no indication of label incorporation into either serine or glycine (compare spectra A and B of Figure 7). As seen in Figure 7A, KSY8 incubation with unlabeled substrates accumulates an easily detectable pool of glycine. Any incorporation of ^{13}C-labeled formate into this pool of glycine would have dramatically increased this peak. [^{14}C]Formate uptake studies verified the ability of KSY8 to uptake formate (data not shown). Lack of incorporation of [^{13}C]formate confirms that metabolism of [^{13}C]formate to glycine and serine requires the presence of either the cytoplasmic or mitochondrial C_1-THF synthase. Figure 7D shows that strain DAY3 does metabolize [^{13}C]-formate to [2-^{13}C]glycine (resonance 1) and [3-^{13}C]serine

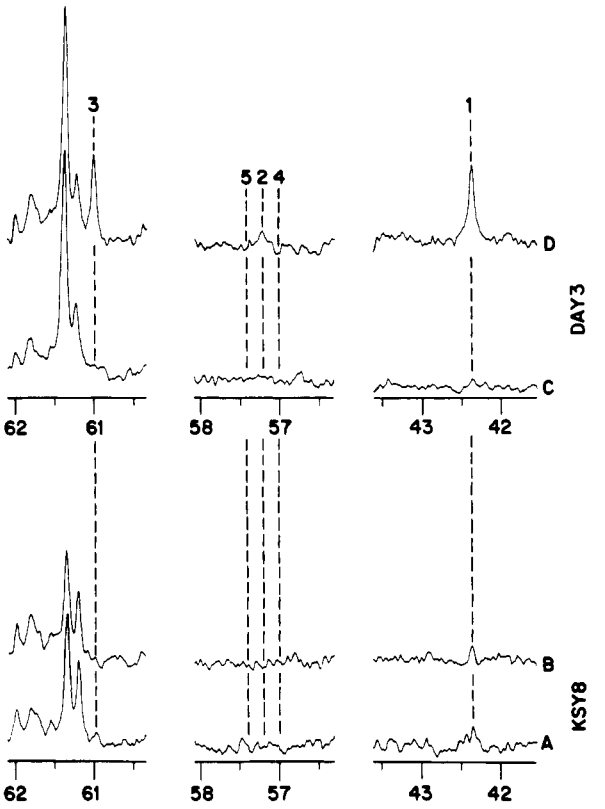

FIGURE 7: ^{13}C NMR spectra of yeast extract. (A) KSY8 (*ade3*$^-$ *mis1*$^-$) incubation with 0.25 mg/mL unlabeled formate and 2 mg/mL unlabeled glycine, (B) KSY8 (*ade3*$^-$ *mis1*$^-$) incubation with 0.25 mg/mL [^{13}C]formate and 2 mg/mL unlabeled glycine, (C) DAY3 (*ade3*$^-$ *MIS1*$^+$) incubation with 0.25 mg/mL unlabeled formate and 2 mg/mL unlabeled glycine, and (D) DAY3 (*ade3*$^-$ *MIS1*$^+$) incubation with 0.25 mg/mL [^{13}C]formate and 2 mg/mL unlabeled glycine. Resonances: (1) 2-glycine; (2) 2-serine; (3) 3-serine; (4–7) 2,3-serine.

(resonance 3). A small amount of [2-^{13}C]serine also accumulates (resonance 2). Since DAY3 lacks cytoplasmic C_1-THF synthase, assimilation of [^{13}C]formate must occur via mitochondrial C_1-THF synthase by conversion to [^{13}C]CH_2-THF and utilization by GCV (Figure 8B) to synthesize [2-^{13}C]-glycine or SHMT to synthesize [3-^{13}C]serine. Considering the inability of [^{13}C]CH_2-THF to traverse the mitochondrial membrane, synthesis of [3-^{13}C]serine must occur via mitochondrial SHMT. Furthermore, since no [2,3-^{13}C]serine is present, the mitochondrially produced [2-^{13}C]glycine appears to be unavailable for mitochondrial serine production via SHMT.

DISCUSSION

The study of folate-mediated one-carbon metabolism and its compartmentation in eukaryotes presents unique challenges that result from parallel pathways in the cytosol and mitochondria, the potential for regulatory interactions between these two pathways, and the wide variety of metabolites involved (Appling, 1991). To date, most information about these pathways has come from growth studies, ^{14}C tracer studies, or studies of isolated enzymes. ^{13}C NMR provides a powerful complement to the older methods due to its ability to distinguish ^{13}C label at specific carbons in a compound (Jeffrey et al., 1991). In the present work, we have followed the metabolism of ^{13}C-labeled one-carbon units in various wild-type and mutant strains of *Saccharomyces* to begin a dissection of the compartmentation of folate-mediated one-carbon metabolism. In combination with ^{13}C NMR, the

availability of yeast strains lacking the cytoplasmic, mitochondrial, or both isozymes of C_1-THF synthase allows the synthesis of a metabolic product to be attributed to a specific enzyme and compartment.

Studies of the metabolism of [^{13}C]formate in KSY8 (*ade3*$^-$ *mis1*$^-$) revealed no assimilation of label into cellular products during a 3-h exposure (Figure 7A,B). This is in agreement with previous studies in which KSY8 was unable to accumulate ^{14}C label following exposure to [^{14}C]formate (Barlowe & Appling, 1990). These results confirm that one or both of the C_1-THF synthase isozymes are required for formate utilization in yeast.

Although rat liver mitochondria have previously been shown to rapidly take up formate (Chappell & Haarhoff, 1967; Cybulski & Fisher, 1977), previous growth studies in yeast (McKenzie & Jones, 1977; Shannon & Rabinowitz, 1988; Barlowe & Appling, 1990) have suggested that the mitochondrial C_1-THF synthase does not play a role in formate assimilation, leading us to propose a one-way flux of formate out of the mitochondria (Figure 1) (Barlowe & Appling, 1988). Studies presented herein using strains lacking one or both isozymes of C_1-THF synthase provide in vivo evidence of conversion of [^{13}C]formate to [^{13}C]CH_2-THF by both the cytoplasmic and mitochondrial C_1-THF synthase (Figure 8). Subsequent utilization of this [^{13}C]CH_2-THF is indicated by the appearance of ^{13}C label at C3 of serine and C2 of glycine, in reactions catalyzed by SHMT (Figure 1, reaction 4) and GCV (Figure 1, reaction 5), respectively. Metabolism of [^{13}C]-formate to [2-^{13}C]glycine and [3-^{13}C]serine in DAY3 (lacking the cytoplasmic C_1-THF synthase) indicates that formate does indeed enter mitochondria in vivo to be metabolized by the mitochondrial pathway. However, the small amount of mitochondrial serine synthesis is apparently insufficient to support normal growth of a *ser1*$^-$ strain. Since the mitochondrial C_1-THF synthase represents only about 10% of the total C_1-THF synthase activity in a wild-type strain (*ADE3*$^+$ *MIS1*$^+$) (Shannon & Rabinowitz, 1986), it is likely that flux through this enzyme is rate-limiting for serine synthesis in an *ade3*$^-$ *ser1*$^-$ strain, although some other step, such as formate transport, cannot be ruled out.

The appearance of [2-^{13}C]glycine from [^{13}C]formate (Figure 7) provides direct in vivo evidence for the synthesis of glycine via reversal of GCV using [^{13}C]CH_2-THF, CO_2, and NH_3 substrates. The glycine cleavage system is a major route of both glycine and serine catabolism in vertebrates (Kikuchi & Hiraga, 1982). Genetic studies have demonstrated a role for GCV in the generation of one-carbon units in yeast as well (Ogur et al., 1977). Although this reaction is readily reversible in vitro (Kikuchi & Hiraga, 1982), glycine synthesis via GCV in vivo has not been previously reported. Data presented here clearly demonstrate that glycine synthesis from formate via the combined C_1-THF synthase/GCV reactions occurs in vivo in yeast. Both compartments can contribute to this pathway, as summarized in Figure 8. The first stage requires C_1-THF synthase for activation of [^{13}C]formate and conversion to [^{13}C]CH_2-THF. If the mitochondrial isozyme is involved (Figure 8B), the [^{13}C]CH_2-THF can be used directly by GCV, along with CO_2 and NH_3, for the synthesis of [2-^{13}C]glycine. If the cytoplasmic isozyme is involved (Figure 8A), the [^{13}C]CH_2-THF is first used to synthesize [3-^{13}C]serine via cytoplasmic SHMT, which then enters the mitochondria. Mitochondrial SHMT then removes C3 of serine, producing a mitochondrial pool of [^{13}C]CH_2-THF which can be used by GCV to synthesize [2-^{13}C]glycine. The critical role of C_1-THF synthase in these pathways is

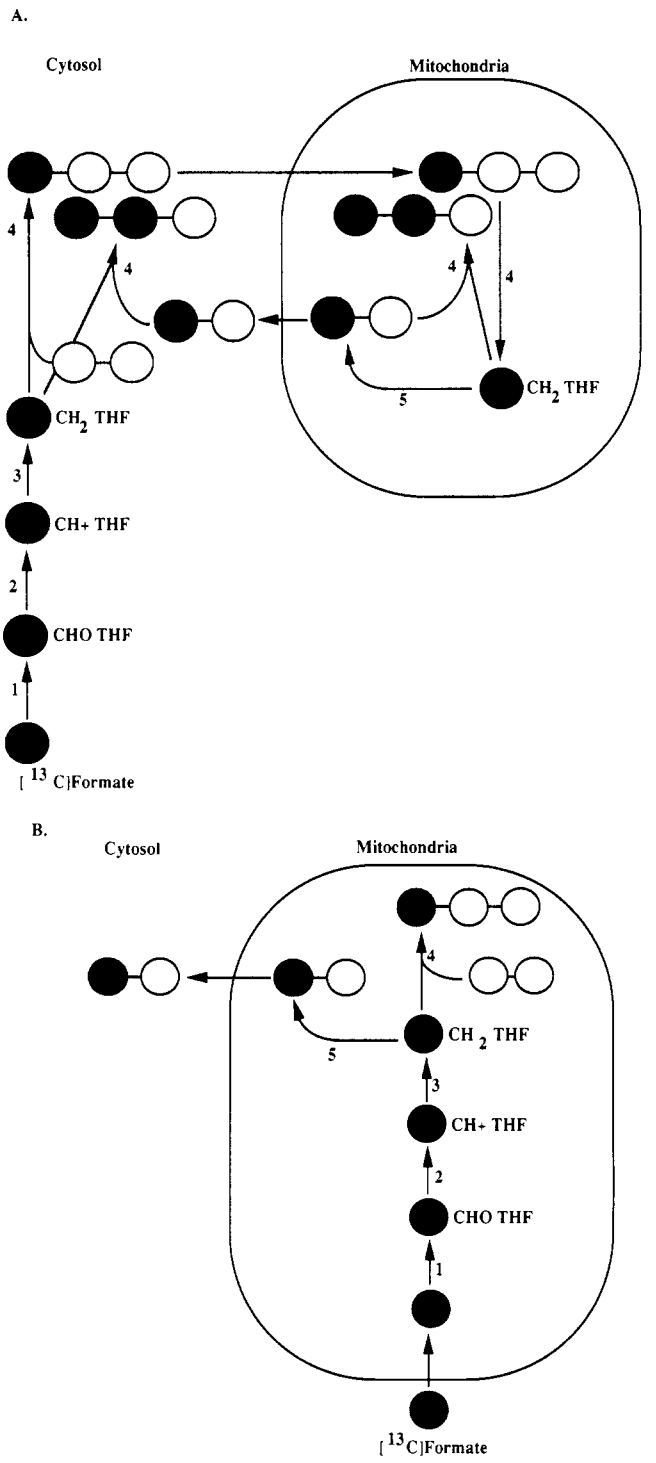

FIGURE 8: Proposed route of formate incorporation into glycine and serine via (A) cytoplasmic C_1-THF synthase and (B) mitochondrial C_1-THF synthase.

demonstrated by the inability of KSY8 (*ade3*$^-$ *mis1*$^-$) to synthesize [2-^{13}C]glycine from [^{13}C]formate (Figure 7B).

Our results also suggest the possibility of two pools of glycine in yeast. Wild-type yeast (e.g., DAY4) synthesize three labeled species of serine from labeled formate: [3-^{13}C]serine, [2-^{13}C]serine, and [2,3-^{13}C]serine, in addition to [2-^{13}C]-glycine. [2,3-^{13}C]Serine must be produced via SHMT from a glycine unit labeled at C2 and a labeled one-carbon unit, [^{13}C]CH_2-THF. While synthesis of [2-^{13}C]glycine is confined to the mitochondria (reversal of GCV), further synthesis to [2,3-^{13}C]serine could occur via the mitochondrial SHMT or the cytosolic SHMT after transport of labeled glycine to the

cytosol (Figure 8A). DAY3, on the other hand, while synthesizing [2-^{13}C]glycine, only produces one species of serine, that labeled at C3 (Figure 7D). Synthesis of [3-^{13}C]serine in DAY3 is restricted to the mitochondria since [^{13}C]CH_2-THF cannot cross the mitochondrial membrane. The lack of [2,3-^{13}C]serine in this strain indicates that only unlabeled glycine was used in the SHMT reaction, in spite of the presence of [2-^{13}C]glycine produced in the mitochondria. This result suggests two pools of glycine, only one of which is available for mitochondrial serine synthesis. One possibility is that the [2-^{13}C]glycine produced in the mitochondria is immediately transported to the cytosol (see Figure 8B). This is supported by the appearance of a small amount of [2-^{13}C]serine (Figure 7D), indicating serine synthesis from [2-^{13}C]glycine and an unlabeled single-carbon unit (available in the cytosol). Alternatively, some form of compartmentation could exist within the mitochondria. If the [2-^{13}C]glycine synthesized by GCV were not immediately transported to the cytosol, a labeled mitochondrial glycine pool would exist, somehow separated from the unlabeled pool used to synthesize serine. The present data cannot distinguish between these two possibilities.

In conclusion, this work establishes an experimental basis of directing label into the synthetase or dehydrogenase functions of either the cytoplasmic or mitochondrial C_1-THF synthase selectively and establishes a means of tracing C_1-THF synthase-independent folate-mediated single-carbon metabolism. These should prove useful tools in future experiments concerning additional information about these pathways and their compartmentation.

ACKNOWLEDGMENT

We thank the Nuclear Magnetic Resonance Laboratory at The University of Texas at Austin for their assistance and A. D. Sherry for critical reading of the manuscript.

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