Methylenetetrahydrofolate Synthetase Regulates Folate Turnover and Accumulation*

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Cellular folate deficiency impairs one-carbon metabolism, resulting in decreased fidelity of DNA synthesis and inhibition of numerous S-adenosylmethionine-dependent methylation reactions including protein and DNA methylation. Cellular folate concentrations are influenced by folate availability, cellular folate transport efficiency, folate polyglutamylation, and folate turnover specifically through degradation. Folate cofactors are highly susceptible to oxidative degradation in vitro with the exception of 5-formyltetrahydrofolate, which may be a storage form of folate. In this study, we determined the effects of depleting cytoplasmic 5-formyltetrahydrofolate on cellular folate concentrations and folate turnover rates in cell cultures by expressing the human methylenetetrahydrofolate synthetase cDNA in human MCF-7 cells and SH-SY5Y neuroblastoma. Cells with increased methylenetetrahydrofolate synthetase activity exhibited: 1) increased rates of folate turnover, 2) elevated generation of p-aminobenzoylegutamate in culture medium, 3) depressed cellular folate concentrations independent of medium folic acid concentrations, and 4) increased average polyglutamate chain lengths of folate cofactors. These data indicate that folate catabolism and folate polyglutamylation are competitive reactions that influence cellular folate concentrations, and that increased methylenetetrahydrofolate synthetase activity accelerates folate turnover rates, depletes cellular folate concentrations, and may account in part for tissue-specific differences in folate accumulation.

Folate is a generic term that refers to a family of structurally related compounds that contain 2-amino-4-hydroxypteridine linked to p-aminobenzoylglutamate though a methylene group. The reduced tetrahydrofolates (THF) serve as cofactors that carry one-carbon moieties for the de novo synthesis of purines (supplies the number 2 and number 8 carbon of the purine ring) and thymidylate (for the methylation of dUMP to dTMP), and for the remethylation of homocysteine to methionine (1, 2).

Methionine can be adenylylated to form S-adenosylmethionine, which is a cofactor and one-carbon donor for numerous cellular methylation reactions (3–5). Impairments in folate metabolism can result from depressed cellular folate concentrations, single nucleotide polymorphisms in genes that encode folate-dependent enzymes, or secondary nutrient deficiencies, and these impairments modify the risk for pathologies including neural tube defects, epithelial cancers, and cardiovascular disease (6). Biomarkers for impaired folate metabolism include elevated uracil content in DNA (7), DNA hypomethylation (8–10), chromosomal instability (9), and elevated serum homocysteine (3, 9, 11, 12). These biomarkers are associated with risk for folate-associated diseases and developmental anomalies, but definitive mechanisms that underlie these pathologies have not been established (12–14).

The regulation of total cellular folate concentrations is complex because it is influenced by uptake, polyglutamylation, export, and turnover (15). Carrier proteins (16) and folate receptors (17) transport folate monoglutamates into cells, but folate monoglutamates do not accumulate unless they are converted to folate polyglutamates in the cytoplasm or mitochondria, a reaction catalyzed by the enzyme folypolyglutamate synthetase (4, 18–21). Although the expression of transport proteins may adapt to extracellular folate concentrations, there is no evidence that transport fully determines intracellular folate concentrations (22–24). Folate turnover occurs by three different mechanisms: 1) newly transported folate monoglutamates can efflux out of the cell before undergoing polyglutamylation, 2) folate polyglutamates can be hydrolyzed to folate monoglutamates by the enzyme γ-glutamyl hydrolyase, then efflux out of the cell, and 3) folate can undergo catabolism, generating inactive degradation products (15). Folate catabolism is thought to be the predominant mechanism of folate turnover in both rat and human models (25) and directly affects intracellular folate concentrations (15).

Cellular folate derivatives differ in the location and oxidation state of the one-carbon moieties they carry, and also by the length of their polyglutamate peptide chain (1 to 9 glutamate residues) (19). The cellular forms of folate also differ in their susceptibility to oxidative degradation, with 5-substituted forms of reduced folate being the most stable (26, 27). 5-Formyl-THF, which is used clinically (Leucovorin™) to elevate cellular folate levels, is the most stable naturally occurring form of reduced folate. Folic acid, a synthetic form of folate found in fortified food and vitamin supplements, is also a stable form of folate. Unsubstituted forms of folate, which include THF and dihydrofolate, and the 10-substituted folates, are chemically labile and susceptible to rapid oxidative degradation in vitro (26, 27). There are two pathways for folate oxidation: one resulting in the formation of dihydro forms of the...
cofactor (dihydrofolate, 5-methyltetrahydrofolate); the other re- sulting in the oxidative cleavage of the C9–N10 bond of dihy- drofolates and tetrahydrofolates resulting in the formation of the pterin derivative and para-aminobenzoyl(poly)glutamate (pABG) (15).

Increased rates of folate catabolism and turnover can deplete cellular folate concentrations (27), and may account for differences in folate turnover rates and total folate content that exist among tissues (28–30). Localized cellular folate deficiency can occur in the absence of whole body folate deficiency, and this phenomenon may result from accelerated rates of folate catabolism (9, 15, 31, 32). This observation has been made in cancer patients, but may also occur during pregnancy (15, 31, 33, 34). Increased rates of folate catabolism during pregnancy are estab- lished in rodent models, but results from human studies have been conflicting (34–36). Elucidating the mechanisms that regulate systemic folate concentrations as well as the folate content of individual tissues is critical to understanding the complex relationships among folate status, folate metabo- lism, and disease.

**EXPERIMENTAL PROCEDURES**

**Materials**—(6S)·3′,5′,7′,9′-H5-Formyl-THF (40 Ci/mmole) and (3′,5′,7′,9′-H2-formyl (25.5 Ci/mmole) were obtained from Moravek Biochemicals, Inc. (6R, 6S)-5-Formyl-THF was from SAPEC, pABG was obtained from Sigma, and (6S)-5-methyl-THF was from Eprova. Fetal bovine serum, α-minimal essential medium (αMEM), and α-modification lacking sodium bicarbonate, folate, ribosides, ribotides, deoxyribo- sides, and deoxyribonucleotides (defined αMEM) were obtained from Hyclone Laboratories.

**Cell Lines and Medium**—SH-SY5Y human neuroblastoma cells, and cell lines expressing human methenyltetrahydrofolate synthetase (MTHFS) cDNA (SH-SY5YMTHFS1, SH-SY5YMTHFS2, and SH-SY5YMTHFSS), have been described previously (37). The human MCF-7 mammary adenocarcinoma cells (ATCC catalog number HTB22) and cell lines expressing human MTHFS DNA (CMFMTHFS1) have been reported (37). For folate turnover studies, fetal bovine serum was dialyzed against 10 volumes of phosphate-buffered saline (PBS) at 4 °C for 24 h with buffer changes every 4 h to deplete serum of folate and other small molecules. The serum was then charcoal-treated to remove any remaining folate.

**Determination of Folate Turnover in Cultured Cells**—Cell monolay- ers at 78% confluence were washed with PBS, then labeled for 12 h in αMEM lacking folate and glycine but supplemented with 25 μM (6S)-[H5-formyl-THF or 120 μM [H4]folate. For the chase, cell monolay- ers were washed with 10 ml of PBS, trypsinized, and pelleted by centrifugation. The cells were seeded in triplicate (0.3·10^6) into 100-mm culture plates containing 10 ml of αMEM supplemented with 2 μM folate acid. Cells were harvested at defined time points, the medium was removed, and the tritium in the medium was quantified. The cell monolayers were washed with PBS and lysed with 0.2 μM ammonium hydroxide. Tritium remaining in the cells was quantified using a Beckman LS 8100 liquid scintillation counter.

**Determination of Total Folate Content and Polyglutamate Chain Lengths**—Total cellular folate and folate polyglutamate chain lengths were determined by affinity/reverse phase HPLC as described elsewhere (38). This two-column HPLC method first purifies folate deriva- tives from the extract using a folate-binding protein resin, and the second phenyl column separates the individual folate derivatives (38). Folate monomers are identified with a four-channel coulometric electrochemical detector. Measurements were made from cells cultured to 70% conflu- ence in αMEM that contains 2 μM folate.

**Determination of pABG and 5-Formyl-THF Levels in Culture Me- dium**—Cells were seeded (1.45·10^6) into 100-mm Primaria culture dishes (Falcon) containing 10 ml of αMEM. Following a 2-h incubation to allow the cells to adhere to the plate, the medium was replaced with defined αMEM containing 25 μM (6S)-[H5-formyl-THF. Aliquots of medium were taken at various time points, clarified by centrifugation, then transferred to a new tube and stored at −80 °C until HPLC analyses. Reverse phase HPLC was used to separate folate degradation products from labeled folates present in the culture medium. Medium samples were spiked with a mixture of pABG, (6R,6S)-5- formyl-THF, and (6S)-5-methyl-THF, then analyzed on a Shimadzu HPLC equipped with a diode array UV spectrophotometric detector and a Luna 5 μm 250×4.6-mm C18 column (Phenomenex). A binary buffer gradient was used to separate degradation products from intact folate, with Buffer A consisting of 0.1 M sodium acetate, pH 6.0, 5 μM Pic A (folic acid) (Waters), and 1% (v/v) Buffer B (ethanol and Buffer C (methanol)). The flow rate was 1.0 ml/min. The HPLC method consisted of 100% Buffer A for the first 30 min, 75% Buffer A from 30 to 31 min, then 45% Buffer A from 31 to 42 min. With this method, pABG elutes at 6 min, 5-formyl-THF elutes at 20 min, and 5-methyl-THF elutes at 37 min. For each analysis, 1.0-ml fractions were collected into scintillation tubes, and the tritium was quantified using a Beckman LS 6500 liquid scintillation counter.

**Determination of Folate Uptake in Cultured Cells**—Cell monolay- ers at 70% confluence in 6-well plates were washed with PBS, then labeled for a total of 10 h in αMEM lacking folate and glycine but supplemented with 25 μM (6S)-[H5-formyl-THF. Cells were harvested in triplicate at the defined time points by washing with 5 ml of PBS, then trypsinizing, and pelleting by centrifugation. Viable cells (determined by their ability to exclude trypan blue) were quantified. The cell pellets were lysed with 0.2 μM ammonium hydroxide and the intracellular tritium was quantified using a Beckman LS 8100 liquid scintillation counter.

**Northern Blot Analyses**—The tissue distribution of mouse MTHFS mRNA was determined by Northern analysis using a “Multiple Tissue Northern blot” (Ambion). Each tissue sample contains 2 μg of mRNA. [3P]ATP-labeled probes were generated by asymmetric PCR amplification, using mouse MTHFS cDNA as the template and primers that amplified the first 416 bp of the sequence. The PCR amplification conditions were 25 cycles for probe construction, 94 °C for 40 s, 94 °C for 10 s, and 72 °C for 1 min for a total of 30 cycles. The β-actin control probes were generated by linear PCR amplification, using human β-actin cDNA as the template and an antisense primer. PCR cycling conditions were supplied by the manufacturer (Clontech). Hybridization of the blot was performed as directed by the manufacturer (Ambion). The tissue dis- tribution of human MTHFS mRNA was determined by Northern anal- ysis using a Multiple Tissue Northern blot (Clontech). Random [3P]ATP-labeled probes specific for MTHFS and β-actin message were generated from the corresponding human cDNAs following the manu- facturer’s protocols.

**RESULTS**

The metabolic role of 5-formyl-THF is not known. This folate derivative is unique because it does not directly participate as a cofactor in folate-dependant reactions but rather acts as an inhibitor of various folate-dependant enzymes (39). It is syn-
FIG. 1. Western blot analyses of MCF-7 and SH-SY5Y cell lines expressing human MTHFS cDNA. Human MTHFS levels were determined in crude cell extracts (60 μg/lane) from nontransfected MCF-7 and SH-SY5Y cells, and cell lines expressing human MTHFS cDNA. Human MTHFS corresponds to the immunoreactive band at 28 kDa. The identity of the upper band at 35 kDa that cross-reacts with these antibodies is not known. Recombinant mouse MTHFS (containing a 6-histidine tag) was used as a standard. Bands were quantified by densitometry as described under “Experimental Procedures.” MTHFS protein is enriched 1.8-fold in MTHFS1 cells compared with MCF-7 cells; MTHFS protein is 2.7-fold more abundant in MCF-7 cells compared with SH-SY5Y cells.

Effect of MTHFS Expression on Folate Accumulation and Turnover—The effect of increased MTHFS expression on folate accumulation was determined in MCF-7 and SH-SY5Y cells. Cells were incubated with 25.0 nM (65)-[3H]5-formyl-THF for 12 h, then viable cells were quantified using trypan blue staining. Total [3H]folate compounds were quantified in cell lysates by liquid scintillation. All values represent quadruplicate measures, and error bars are S.D. (‡, p = 0.0002, one-tailed Student’s t test; †, p < 0.0001, one-tailed Student’s t test).

FIG. 2. Effect of MTHFS on folate accumulation in MCF-7 and SH-SY5Y cells. Cells were incubated with 25.0 nM (65)-[3H]5-formyl-THF for 12 h, then viable cells were quantified using trypan blue staining. Total [3H]folate compounds were quantified in cell lysates by liquid scintillation. All values represent quadruplicate measures, and error bars are S.D. (‡, p = 0.0002, one-tailed Student’s t test; †, p < 0.0001, one-tailed Student’s t test).

TABLE I

Effect of MTHFS expression on cellular folate concentrations and folate polyglutamate chain lengths

| Cell line    | Total folate | Mean polyglutamate length µmol/mg protein | µmol/mg protein |
|--------------|--------------|------------------------------------------|-----------------|
| MCF-7        | 27.2 ± 2.9 (100%) | 3.2 ± 0.06                                |                 |
| MCFMTHFS1    | 18.6 ± 5.0 (60%)   | 4.4 ± 0.35                                |                 |
| SH-SY5Y      | 39.1 ± 5.1 (100%) | 5.7 ± 0.12                                |                 |
| SH-SY5YMHFS  | 27.9 ± 1.8 (72%)   | 6.1 ± 0.10†                               |                 |

* Value significantly different from nontransfected cells, p < 0.05 one-tailed Student’s t-test.
* Value significantly different from nontransfected cells, p < 0.01 one-tailed Student’s t-test.
* Value significantly different from nontransfected cells, p < 0.003 one-tailed Student’s t-test.

Previously, we reported the development of stable MCF-7 and SH-SY5Y cell lines that express the human MTHFS cDNA and display increased MTHFS activity (37). To determine whether depletion of 5-formyl-THF impairs folate accumulation, the effects of MTHFS activity on intracellular folate concentrations and folate turnover was determined in these cell lines. Increased expression of human MTHFS protein was verified in these cell lines by Western blot analyses (Fig. 1). Densitometry analysis indicated that MCF-7 cells that express the MTHFS cDNA contained 1.8-fold more MTHFS protein compared with nontransfected cells (Fig. 1). The specific activity of MTHFS in MCF-7 cells is 6.0 ± 0.4 pmol of 5,10-methenyl-THF formed/min/mg cell extract, whereas the specific activity of MTHFS in the MCFMTHFS1 cell line is 11.9 ± 0.8 mol of 5,10-methenyl-THF formed/min/mg cell extract, consistent with densitometry results from Fig. 1. The SH-SY5Y6 cell line that expresses the MTHFS cDNA exhibited a 100-fold elevation in MTHFS enzyme activity levels compared with nontransfected SH-SY5Y cells as described previously (Fig. 1) (37).

Effect of MTHFS Expression on Folate Accumulation and Turnover—The effect of increased MTHFS expression on folate accumulation was determined in MCF-7 and SH-SY5Y cells. Cells were cultured with [3H]5-formyl-THF for 12 h and the accumulation of radiolabeled folate was determined. [3H]5-Formyl-THF does not accumulate in cells because it equilibrates rapidly into the folate cofactor pool (37). Both MCF-7 and SH-SY5Y cell lines that express the human MTHFS cDNA contained 40% less [3H]folate compared with nontransfected cells (Fig. 2) when cultured in medium containing 25 nM [3H]5-formyl-THF. These results indicate that increased MTHFS activity decreases the capacity of two different cell lines to accumulate folate. Because folate accumulates in cells at levels that approximate the folate binding capacity of the cell (15), the inhibition of labeled folate accumulation observed above may result from: 1) inhibition of folate uptake, and/or 2) accelerated turnover of newly imported folate, and/or 3) slower rates of folate polyglutamate turnover and thereby decreased concentrations of unliganded or available binding sites for newly imported folate monoglutamates. Mechanism 3 would not be expected to alter total cellular folate concentrations, whereas mechanisms 1 and 2 would be expected to deplete cellular folate concentrations. To distinguish between these mechanisms, the concentration of total cellular folate was determined under the same culture conditions that were used to determine...
Folate accumulation (Fig. 2). The total cellular folate content was decreased by 40 and 28%, respectively, in MCF-7 and SH-SY5Y cell lines expressing the MTHFS cDNA relative to nontransfected cells cultured in medium with high folic acid concentrations (2 $\mu$M) (Table I). Therefore, increased MTHFS expression depresses intracellular folate concentrations independent of extracellular folate availability.

To determine whether depletion of cellular 5-formyl-THF concentrations influences the stability of total cellular folate, the turnover of newly imported folate was determined by pulse-chase analyses (Fig. 3). Cells that express the MTHFS cDNA displayed accelerated rates of folate turnover in both human MCF-7 and SH-SY5Y cells. Folate turnover in SH-SY5Y cells labeled with [3H]folic acid displayed biphasic rates of folate turnover (Fig. 3A). The expression of the human MTHFS cDNA in SH-SY5Y cells accelerated the rate of the initial rapid phase of turnover, but had little or no effect on the rate of the slower phase (Fig. 3A). SH-SY5Y cells labeled with [3H]5-formyl-THF (Fig. 3B) did not exhibit a biphasic rate of folate turnover, but
SH-SY5Y cells with increased MTHFS activity did display accelerated and biphasic rates of folate turnover relative to nontransfected cells. Unlike the SH-SY5Y cells (Fig. 3B), the rate of folate turnover in MCF-7 cells labeled with [1H]5-formyl-THF was biphasic (Fig. 3C), with an initial rapid phase of turnover followed by a slower phase. This difference is consistent with the greater level of endogenous MTHFS activity in MCF-7 cells compared with SH-SY5Y cells (Fig. 1). Increased MTHFS expression in MCF-7 cells accelerated the rapid phase of folate turnover, but did not influence the slower phase (Fig. 3C). During the chase following [1H]5-formyl-THF labeling, cells expressing the MTHFS cDNA lost rapidly about 50% of labeled cellular folate compared with nontransfected cells (Fig. 3, B and C). The remaining 50% of labeled cellular folate was protected from MTHFS-mediated turnover and may have entered a stable pool. The only radioactive compound identified by HPLC in the chase culture medium was pABG; no intact radioactive folates were present (data not shown).

Because MTHFS is a cytoplasmic enzyme, and cells expressing the MTHFS cDNA do not have increased MTHFS specific activity in mitochondria (37), the remaining 50% of labeled folate following the chase may represent folate that entered mitochondria. Consistent with this notion, less folate entered into this stable pool when cells were labeled with [3H]folinic acid (Fig. 3A), a folate derivative that cannot traverse the mitochondrial membrane until it is reduced to tetrahydrofolate in the cytoplasm (4, 20, 43). Alternatively, the stable pool may represent folate molecules that have been processed to their polyglutamate form by the enzyme folypolyglutamate synthetase (44). This modification increases the affinity of folate for folate-binding proteins (45). Intracellular folate polyglutamates that are protein bound are more stable than free folate derivatives because they are protected from random intracellular oxidative degradation and potentially from enzyme-mediated oxidation (15).

Increased MTHFS Expression Increases pABG Production without Affecting Folate Uptake in Vivo—To determine whether the MTHFS-induced elevations in folate turnover resulted from increased rates of folate catabolism, the accumulation of the folate degradation products (consisting primarily of pABG) was determined in culture medium during continuous exposure of cells to [3H]5-formyl-THF (Fig. 4A). MCF-7 cells expressing the human MTHFS cDNA displayed a 2-fold increased rate of pABG production compared with nontransfected cells. The primary folate catabolite observed in culture medium by HPLC was pABG, which eluted from the column at 6 min; another unidentified degradation product eluted between 6 and 15 min. With this method, intact folates do not elute until after 20 min. Initial rates of labeled 5-formyl-THF uptake were similar between both cell lines (Fig. 4B). This provides direct evidence that MTHFS activity accelerates the rate of folate catabolism without changing the rate of folate uptake. These data also indicate that MTHFS regulates intracellular folate concentrations by accelerating folate catabolism.

Effect of MTHFS on Folate Polyglutamate Chain Lengths—The MTHFS protein displays increased binding affinity for the polyglutamate forms of 5-formyl-THF compared with the monoglutamate forms (42, 46). To determine whether MTHFS-mediated folate catabolism displays preference for newly imported folate (which is primarily folate monoglutamates that have low affinity for folate-binding proteins) or folate polyglutamate species, the effect of MTHFS expression on cellular folate polyglutamate chain lengths was investigated (Table 1). Expression of MTHFS cDNA in both MCF-7 and SH-SY5Y cells increased the average polyglutamate chain length from 3.2 to 4.4, and from 5.7 to 6.1, respectively. Because shorter chain polyglutamates are underrepresented in cells with increased MTHFS activity, this indicates that MTHFS may target folate derivatives with shorter polyglutamate chain lengths for catabolism. Thus, folate that has been imported recently into the cell is most vulnerable to catabolism.

Tissue-specific Expression of Human MTHFS—Previous studies indicate that the rates of folate turnover vary among tissues (28–30). To determine whether rates of folate turnover correlate with MTHFS tissue expression profiles, commercially prepared mouse and human mRNA blots (Figs. 5, A and C, Table II) were hybridized with antisense MTHFS probes and human β-actin probes (Fig. 5B). MTHFS mRNA levels displayed tissue-specific differences in both the mouse and human mRNA blots, and the β-actin message was abundant in all tissues (human β-actin blot previously shown (47)). Both human and mouse MTHFS messages were highest in liver and kidney, with the brain containing very low levels of MTHFS message (in mouse brain tissue the MTHFS message level is 3% of the message level in liver). Differences between species were seen in the heart and lung; mouse heart tissue has the lowest level of MTHFS mRNA, whereas human heart tissue has an abundance of the MTHFS message compared with other tissues. MTHFS mRNA is not detected in human lung tissue, yet it is detectable in mouse lung tissue. The variations in mouse MTHFS mRNA levels among tissues correlate with kinetics of folate turnover among different tissues in rodents (28, 30).
The low levels of MTHFS message present in brain is noteworthy because brain folate concentrations are not affected by long term folate deficiency (29, 30).

**DISCUSSION**

The results presented in this study demonstrate that MTHFS activity regulates intracellular folate concentrations by accelerating rates of folate catabolism, and that newly imported folate, which is in the monoglutamate form and therefore not protein-bound, is vulnerable to turnover resulting from increased MTHFS activity. Furthermore, the correlation among MTHFS message levels and rates of folate catabolism in tissues indicates that MTHFS may play a key role in determining cellular folate concentrations in a tissue-specific manner.

Our current model depicting the role of MTHFS in the regulation of intracellular folate concentrations is illustrated in Fig. 6. In our system, folate monoglutamates (either folic acid or 5-formyl-THF) enter the cell and are rapidly incorporated into the folate cofactor pool as monoglutamate derivatives. Neither exogenous folic acid nor 5-formyl-THF accumulate in the cells (37). When folic acid is used as the exogenous source of folate, it is rapidly converted to dihydrofolate, then tetrahydrofolate by the enzyme dihydrofolate reductase when it enters the cell. When 5-formyl-THF is the source of exogenous folate, it is rapidly converted to 5,10-methenyl-THF by the enzyme MTHFS in the cytoplasm. Monoglutamate folates can be converted either to folate polyglutamates in the cytoplasm by the enzyme folylpolyglutamate synthetase, or folate monoglutamates enter the mitochondria where they are converted to polyglutamates and sequestered in that compartment. Folic acid and 5-substituted folate derivatives are not effective substrates for folylpolyglutamate synthetase and therefore these newly imported folate derivatives are unlikely to receive a polyglutamate tail prior to equilibration in the folate pool (48). Pulse-chase analyses demonstrate that only 50% of folate is accessible for MTHFS-mediated catabolism, and therefore stable pool(s) must exist. Mitochondria represent one potential stable pool because it is a physical compartment that lacks MTHFS activity (Fig. 6). Previous studies have shown that cytoplasmic and mitochondrial folates are not in equilibrium (4, 18, 20), although folate polyglutamates can slowly escape from this compartment (4, 19). It should be noted that some studies have found MTHFS activity in mitochondria isolated from human cells (49), but it is not found in mitochondria isolated from rabbit liver, nor have we found MTHFS activity in these cell lines (37, 50). Polyglutamate forms of folate are a second potential stable pool. Although MTHFS displays increased affinity for polyglutamate forms of folate, the data in Table I clearly indicate that monoglutamate folates are the primary or preferred targets for catabolism. However, these data do not exclude the possibility that folate polyglutamates undergo MTHFS-mediated catabolism as well, only that shorter chain folate polyglutamate derivatives are catabolized at a faster rate. Therefore, newly imported folate monoglutamates are most susceptible to catabolism because folate monoglutamates have a low affinity for folate-binding proteins. Folate polyglutamates display increased affinity for folate-binding proteins, and therefore may not be as accessible to MTHFS for catabolism. These data also indicate that folate polyglutamylation and folate degradation are competitive processes, and that catabolism may be more determinant in regulating cellular folate concentrations than folate transport.

![Folate Catabolism](https://example.com/folate_catabolism.png)

**Fig. 6. Model for the interaction of HCF and MTHFS in folate catabolism.** In this model, cytoplasmic HCF and MTHFS catabolize non-protein bound folate monoglutamates that do not escape into either stable pool 1 (cytoplasmic polyglutamate pool) or stable pool 2 (mitochondria). Exogenous 5-formyl-THF enters the folate cofactor pool through the activity of MTHFS; folic acid enters the folate cofactor pool through the activity of dihydrofolate reductase. The model predicts that MTHFS preferentially targets folate monoglutamates for catabolism. MTHFS may directly catalyze the catabolism of folate in the cytoplasm, or may increase rates of folate catabolism by altering the relative distribution of one-carbon forms of folate and thereby accelerate HCF-mediated folate catabolism. This figure does not illustrate all metabolic reactions that generate methylene-THF from THF including those catalyzed by serine hydroxymethyltransferase and the glycine cleavage system.
erates folate catabolism. First, MTHFS may have a second catalytic activity and directly catalyze the oxidation of reduced folate coenzymes. MTHFS has been demonstrated to have high affinity for several folate derivatives including 5-formyl-THF, 5-methyl-THF, THF, and dihydrofolate (46), and therefore these folate derivatives may be potential candidate substrates for MTHFS-mediated catabolism. In view of the existence of several multifunctional enzymes of folate metabolism, the possibility that MTHFS catalyzes an oxidative reaction in addition to the synthesis of methenyl-THF is worth consideration. Alternatively, MTHFS may accelerate folate catabolism by altering the distribution of folate one-carbon forms, resulting in the accumulation of more labile folate derivatives. Previously, we have demonstrated that increased MTHFS expression decreases the cellular concentrations of 5-methyl-THF and 5-formyl-THF (37), two of the most stable forms of folate, and elevates levels of 10-formyl-THF and THF, folate cofactors that are most susceptible to nonenzymatic oxidative degradation. Several studies have indicated that cellular folate concentrations are saturable and that the accumulation of cellular folate is limited by the folate-binding capacity of the cell (45) and that cellular folate-binding proteins tend to display specificity for certain one-carbon forms of folate (2). Therefore, alterations in the relative distribution of the folate one-carbon forms may influence total folate accumulation if an individual folate one-carbon derivative accumulates beyond the capacity of cellular proteins to sequester it. Recently, we identified heavy chain ferritin (HCF) as a protein that catalyzes folate catabolism in vitro and in cell cultures (27). By altering the distribution of folate cofactors, MTHFS may make certain folate derivatives more susceptible to undergo degradation by HCF-mediated catabolism. Future experimentation will be required to distinguish between these two mechanisms.

There may be advantages in maintaining low cellular folate concentrations through catabolism because it permits: 1) metabolic channeling of folate cofactors among folate-dependent enzymes, 2) competitive regulation of folate metabolic pathways, and 3) translational control of thymidylate synthetase expression (15). Channeling of folate cofactors has been shown to increase the activity of folate-dependent enzymes by decreasing $K_{m}$ and increasing $k_{cat}$ values relative to transfer by diffusion (45, 51). In vitro reconstituted folate-dependent metabolic pathways function optimally when folate levels are present in subsaturating concentrations such that the receiving enzyme active site is open and unliganded (51). Subsaturating concentrations of folate also creates competition among folate metabolic pathways for folate cofactors, and this competition may serve a regulatory function as proposed by Scott and Weir (52), that is, DNA precursor synthesis and methionine synthesis are competitive pathways. Finally, thymidylate synthetase has been shown to bind to its mRNA and repress the translation of its mRNA (53). Because mRNA and folate substrates bind to thymidylate synthetase competitively, this regulatory mechanism is operative only if thymidylate synthetase is not saturated with folate cofactors. These biochemical advantages(s) may be realized during physiological states of rapid cell division that are associated with increased rates of folate catabolism including cancer and pregnancy. Therefore, MTHFS-mediated catabolism may modulate tissue or cellular folate concentrations independent of whole body folate status, and thereby regulate folate metabolic pathways.
