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MTHFD1 Regulates Nuclear *de novo* Thymidylate Biosynthesis and Genome Stability

Martha S. Field[1], **Elena Kamynina**[1], and **Patrick J. Stover**[1,2,#]

[1]Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853

[2]Graduate Field of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

Abstract

Disruptions in folate-mediated one-carbon metabolism (FOCM) are associated with risk for several pathologies including developmental anomalies such as neural tube defects and congenital heart defects, diseases of aging including cognitive decline, neurodegeneration and epithelial cancers, and hematopoietic disorders including megaloblastic anemia. However, the causal pathways and mechanisms that underlie these pathologies remain unresolved. Because folate-dependent anabolic pathways are tightly interconnected and best described as a metabolic network, the identification of causal pathways and associated mechanisms of pathophysiology remains a major challenge in identifying the contribution of individual pathways to disease phenotypes. Investigations of genetic mouse models and human inborn errors of metabolism enable a more precise dissection of the pathways that constitute the FOCM network and enable elucidation of causal pathways associated with NTDs. In this overview, we summarize recent evidence that the enzyme MTHFD1 plays an essential role in FOCM in humans and in mice, and that it determines the partitioning of folate-activated one carbon units between the folate-dependent *de novo* thymidylate and homocysteine remethylation pathways through its regulated nuclear localization. We demonstrate that impairments in MTHFD1 activity compromise both homocysteine remethylation and *de novo* thymidylate biosynthesis, and provide evidence that MTHFD1-associated disruptions in *de novo* thymidylate biosynthesis lead to genome instability that may underlie folate-associated immunodeficiency and birth defects.

Keywords

MTHFD1; SHMT; TYMS; DHFR; thymidylate; folate; lamin; DNA replication; multi-enzyme complex

[#]Address correspondence to: Patrick J. Stover, 127 Savage Hall, Division of Nutritional Sciences, Cornell University, Ithaca NY 14853; Tel. (607)255-9751; Fax (607)255-1033; pjs13@cornell.edu.

[1]To whom correspondence should be addressed: Patrick J. Stover, Division of Nutritional Science, 127 Savage Hall, Cornell University, Ithaca, NY USA 14853. Tel.: (607) 255-8001; Fax: (607) 255-1033; pjs13@cornell.edu

[2]Division of Nutritional Science, Cornell University, Ithaca, NY USA 14853.

I. Overview of folate-mediated one-carbon metabolism

Tetrahydrofolates (THF) are a family of cofactors that carry and chemically-activate one-carbon units for the *de novo* synthesis of guanosine, adenosine and thymidine nucleotides, and for the remethylation of homocysteine to methionine (Figure 1).[1] Folate-dependent pathways are compartmentalized in the mitochondria, cytosol and nucleus, and each compartment is associated with a particular metabolic function.[2] These folate-dependent pathways are tightly interconnected within the cell and communicate across the compartments, and thereby function as a metabolic network, as opposed to independent autonomously regulated pathways (Figure 1). Folate cofactors are also compartmentalized and do not readily exchange across compartments. Folate-dependent pathways are interconnected across compartments though the exchange of metabolic substrates, including serine, glycine and formate (Figure 1). [11]

In mitochondria, folate cofactors function in: 1) the *de novo* thymidylate synthesis for mitochondrial DNA replication, which involves the enzymes serine hydroxymethyltransferase (SHMT2), thymidylate synthase (TYMS), and dihydrofolate reductase like 1 (DHFRL1)[3]; 2) for the N-formylation of Met-tRNA for the initiation of mitochondrial protein synthesis, and 3) for the generation of formate from the catabolism of the amino acids serine, glycine, dimethylglycine and sarcosine by the enzymes SHMT2, sarcosine dehydrogenase, dimethylglycine dehydrogenase, methylenetetrahydrofolate dehydrogenase 2, methylenetetrahydrofolate dehydrogenase like-2 and methylenetetrahydrofolate dehydrogenase like-1.

In the cytosol, formate is an important source of one-carbons for FOCM. Mitochondrial-derived formate translocates to the cytoplasm where it is essential for the functioning of folate metabolism in the cytosol and the nucleus. Formate is a primary source of one-carbons for the *de novo* synthesis of purines and for the remethylation of homocysteine to methionine, catalyzed by the vitamin B12-dependent enzyme methionine synthase (MTR). Methionine can be converted to S-adenosylmethionine (AdoMet) by AdoMet synthetase. AdoMet is a cofactor for numerous methylation reactions including the methylation of DNA, RNA, proteins, neurotransmitters, phospholipids and numerous metabolites.

Nuclear folate metabolism involves the conversion of uridylate to thymidylate through reductive methylation. In this reaction, thymidylate synthase (TYMS) transfers-while simultaneously reducing- the one-carbon group from 5, 10-methylenetetrahydrofolate to deoxyuridine monophosphate, yielding thymidylate and dihydrofolate. 5, 10-methylenetetrahydrofolate can be produced either by the activity of serine hydroxymethyltransferase isozymes SHMT1 and SHMT2α[4] from serine and tetrahydrofolate or by the activity of methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) from formate and tetrahydrofolate[5,6]. Metabolic labeling studies in MCF-7 cells measured relative contribution of one-carbon group donors to thymidylate synthesis: serine contributes about 30% whereas formate contributes about 70% of one-carbon groups used by TYMS[7]. To regenerate tetrahydrofolate from dihydrofolate, cells use the activity of dihydrofolate reductase (DHFR).

In mammalian cells, the enzymes of thymidylate biosynthesis pathway are SUMOylated (covalently linked to the Small Ubiquitin-like MOdifier) during S-phase of the cell cycle and following DNA damage [5,8,9]. This enables nuclear translocation of these enzymes, where they form a physical complex with nuclear lamin proteins and other enzymes of the DNA replication machinery (Figure 2).[5] SHMT1 and SHMT2α are key enzymes in the complex, as they were shown to serve as scaffold proteins that tether the entire enzymatic complex to the nuclear lamina at sites of replication.[5] Impairments in *de novo* thymidylate synthesis result in uracil misincorporation into DNA, which leads to single- and double-strand breaks during base-excision DNA repair.[10]

II. Partitioning of folate cofactors between the *de novo* thymidylate synthesis and homocysteine remethylation pathways

Folate-dependent pathways are regulated by the availability of one-carbon activated folate cofactors. This occurs because the total cellular concentration of folate-dependent enzymes exceeds the total cellular concentration of folate (for review see[12]), thus requiring regulation of the partitioning of folate among the pathways that constitute the folate-dependent one-carbon (FOCM) network. This competition for folate cofactor is most pronounced for the partitioning of methylenetetrahydrofolate between the *de novo* thymidylate cycle and the homocysteine remethylation cycle (Figure 1).[7] Methylenetetrahydrofolate is used directly in the conversion of dUMP to dTMP by thymidylate synthase, or alternatively can be reduced to 5-methyltetrahydrofolate by the enzyme MTHFR, a reaction that commits the folate cofactor to the homocysteine remethylation pathway because this reaction is essentially irreversible *in vivo* (Figure 1). Mathematical modeling, using ordinary differential equations and the kinetic properties of methylenetetrahydrofolate reductase and thymidylate synthase, was employed to describe the competition for methylenetetrahydrofolate.[13] Under conditions of limited folate availability or folate deficiency, experimental evidence shows that *de novo* thymidylate biosynthesis has priority over homocysteine remethylation.[14]

More recent studies indicate that the competition between MTHFR and TYMS enzymes for folate cofactors is achieved not through kinetic competition but rather through partitioning in distinct cellular compartments.[6] *De novo* thymidylate synthesis occurs in the nucleus during S-phase [4], whereas MTHFR and the homocysteine remethylation cycle function in the cytosol. The competition for folate cofactors between the homocysteine remethylation and the *de novo* thymidylate synthesis pathway is explained by the partitioning of folate cofactors and the enzyme MTHFD1 between the nucleus and cytosol. Folate-deficient animals and human cells in culture were shown to enrich both folate cofactors and the MTHFD1 enzyme in the nucleus, at the expense of levels in the cytosol.[6] MTHFD1 is the primary source of methylenetetrahydrofolate generation, and therefore the nuclear enrichment of this enzyme and folate cofactors ensures the functioning of *de novo* thymidylate synthesis, but at the expense of homocysteine remethylation in the cytosol.[6]

III. Mouse models of impaired *de novo* thymidylate biosynthesis

Loss-of-function mouse models have been studied to understand the physiological consequences of impaired *de novo* thymidylate synthesis. An N-ethyl-N-nitrosourea (ENU)

mutagenesis screen yielded a T > A transversion in the TYMS codon encoding amino acid 106, causing an asparagine to lysine substitution in the folate binding site of the enzyme. This mouse model displays an early post-implantation embryonic lethal phenotype. Embryos homozygous for this mutation die due to defective gastrulation. Mice heterozygous for this mutation exhibit ~2.6 fold elevated TYMS protein in the liver compared to wild type mice.[15] This elevated TYMS expression is likely due to enhanced translation, as TYMS protein autoregulates its expression by binding its mRNA through its catalytic active site and inhibiting its translation.[16]

The *Shmt1* gene has also been disrupted in the mouse genome. Mice with a homozygous deletion of the *Shmt1* gene are viable and fertile but exhibit depressed rates of *de novo* thymidylate synthesis and elevated levels of uracil in nuclear DNA.[17] The viability of these mice is accounted for by functional redundancy with SHMT2α encoded in an alternative transcript of the *Shmt2* gene, whose primary transcript expresses the mitochondrial SHMT isozyme SHMT2.[4] The $Shmt1^{+/-}$ mice are more susceptible to intestinal tumors when crossed to the $Apc^{Min/+}$ mouse model compared to wild-type mice[18], and embryos exhibit sporadic neural tube defects when the dams are placed on a folate-deficient diet.[19,20] Interestingly, neural tube defects associated with embryonic *Shmt1* disruption can be rescued with maternal deoxyuridine supplementation, which stimulates rates of thymidylate synthesis.[21]

Homozygous disruption of *Mthfd1* in mice through a gene-trap (*gt*) is early embryonic lethal, consistent with MTHFD1 serving as a primary source of one-carbon-activated folate cofactors for purine, thymidylate and methionine synthesis.[22] Heterozygous $Mthfd1^{gt/+}$ mice exhibit lower hepatic AdoMet levels, which are expected because MTHFD1-derived methylenetetrahydrofolate is a source of one-carbons for cellular methylation reactions. Unexpectedly, $Mthfd1^{gt/+}$ mice were shown to have decreased levels of uracil in nuclear DNA, indicating enhanced rates of *de novo* thymidylate synthesis.[22] This observation was later explained by the enrichment of MTHFD1 in the nucleus at the expense of MTHFD1 levels in the cytosol when total cellular MTHFD1 is limiting, indicating that MTHFD1 preferentially supports *de novo* thymidylate biosynthesis at the expense of homocysteine remethylation.[6,23,24] During embryonic development, maternal $Mthfd1^{gt/+}$ genotype impairs fetal growth compared to wild-type dams, but neither maternal nor embryonic *Mthfd1* genotype influences risk for neural tube defects when dams are fed a folate- and choline-deficient diet.[25]

These mouse models demonstrate the multiple feedback mechanisms that protect *de novo* thymidylate biosynthesis in the nucleus. This includes the robust upregulation of TYMS expression at the level of translation, enrichment of MTHFD1 protein in the nucleus when *Mthfd1* expression is limiting, and enrichment of folate cofactors in the nucleus during folate deficiency.

IV. Human MTHFD1 mutations

A novel inborn error of metabolism was recently described resulting from loss of MTHFD1 function due to deleterious mutations in both the paternal and maternal *MTHFD1*

alleles. [23,26,27] The patient presented with severe combined immunodeficiency (SCID), megaloblastic anemia and neurologic abnormalities. Fibroblasts from the patient exhibited decreased flux of formate into methionine and dTMP by 90% and 50%, respectively, with elevated uracil in DNA, lower rates of *de novo* dTMP synthesis and increased salvage pathway dTMP generation relative to control fibroblasts. As seen in the mouse models, MTHFD1 was enriched in the nucleus in the patient fibroblasts at the expense of MTHFD1 levels in the cytosol. Patient fibroblasts exhibited increased DNA damage (double-stranded DNA breaks) compare to control fibroblasts. These results provide strong evidence for the role of MTHFD1 in nuclear *de novo* dTMP biosynthesis, and connect impaired MTHFD1 specific activity to both megaloblastic anemia and SCID.

V. Conclusions

The identification of causal pathways and associated mechanisms of folate-associated pathophysiology remains a major challenge. Because folate-dependent anabolic pathways are tightly interconnected and best described as a metabolic network, identifying the contribution of individual pathways to disease phenotypes requires relevant model systems that capture all of the system dynamics. These dynamics include metabolic subcellular compartmentation and its regulation, as well as feed back loops that maintain the functioning of the network and metabolic priority among interconnected pathways. Recent evidence suggests that the enzyme MTHFD1 plays an essential role in FOCM in humans and in mice, and that it determines the flux of folate-activated one carbon units between the folate-dependent *de novo* thymidylate and homocysteine remethylation pathways through the regulation of its nuclear localization. Furthermore, investigations of genetic mouse models and human inborn errors of metabolism are enabling a more precise dissection of the pathways that constitute the FOCM network and enable elucidation of causal pathways associated with folate pathologies including neural tube defects. Evidence to date suggests that folate-dependent *de novo* thymidylate synthesis plays an important role in folate-associated pathologies, and that the FOCM network is configured to protect this pathway at the expense of homocysteine remethylation.

Acknowledgments

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Abbreviations used are

FOCM	folate-mediated one-carbon metabolism
MTHFD1	methylenetetrahydrofolate dehydrogenase 1
SHMT1	cytoplasmic serine hydroxymethyltransferase
TYMS	thymidylate synthase
DHFR	dihydrofolate reductase
DHF	dihydrofolate

THF	tetrahydrofolate

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Highlights

- The mechanisms for folate-associated pathologies remain unresolved.
- MTHFD1 nuclear localization determines folate one-carbon unit partitioning.
- MTHFD1 may underlie folate-associated immunodeficiency and birth defects.

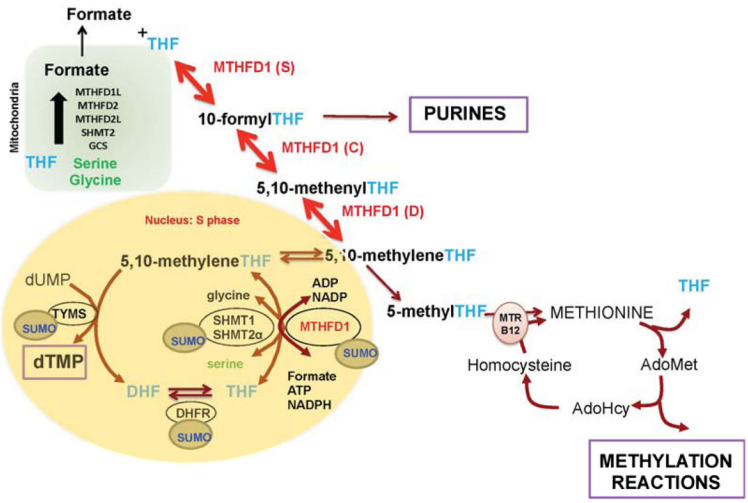

Figure 1.

Folate-Mediated One-Carbon Metabolism. One-carbon metabolism is required for the *de novo* synthesis of purines and thymidylate, and for the remethylation of homocysteine to methionine. The *de novo* thymidylate pathway is SUMOylated and translocates to the nucleus during S-phase. Mitochondria generate formate from the amino acids serine and glycine. THF, tetrahydrofolate; AdoMet, *S*-adenosylmethionine; MTHFD, Methylenetetrahydrofolate Dehydrogenase; MTR, Methionine Synthase; MTHFR, Methylenetetrahydrofolate Reductase; SHMT1, Cytoplasmic Serine Hydroxymethyltransferase; SHMT2α, Serine Hydroxymethyltransferase 2α TYMS, Thymidylate Synthase; DHFR, Dihydrofolate Reductase; GSC, Glycine Cleavage System.

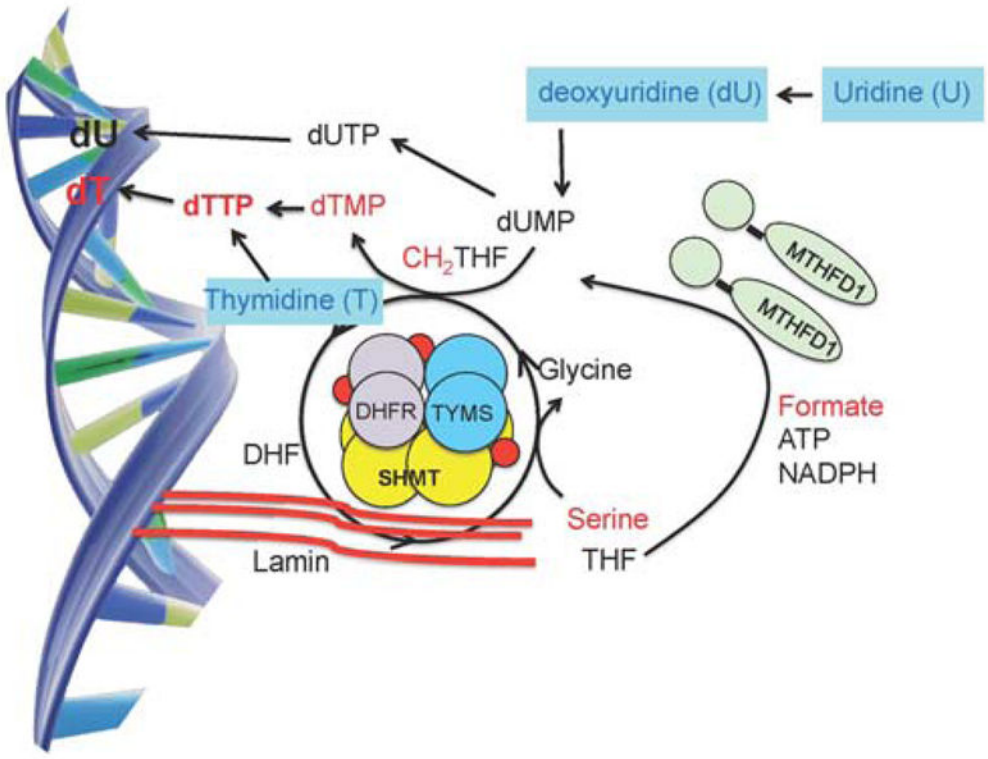

Figure 2.

The *de novo* thymidylate synthesis pathway as a nuclear multienzyme complex at sites of DNA replication. THF, tetrahydrofolate; DHF, dihydrofolate; MTHFD1, Methylenetetrahydrofolate Dehydrogenase; SHMT1, Cytoplasmic Serine Hydroxymethyltransferase; TYMS, Thymidylate Synthase; DHFR, Dihydrofolate Reductase; dUMP, deoxyuridine monophosphate; dUTP, deoxyuridine triphosphate; dTMP, thymidine monophosphate; dTTP, thymidine triphosphate.