Supplementary information

Rat liver folate metabolism can provide an independent functioning of associated metabolic pathways

Aleksandr V. Zaitsev[1], Michael V. Martinov[2], Victor M. Vitvitsky[2], Fazoil I. Ataullakhanov[1,2,3].

[1]Department of Physics, Moscow State University, Moscow 119991, Russia, [2]Center for Theoretical Problems of Physico-Chemical Pharmacology, Russian Academy of Sciences, Moscow 119991, Russia, [3]Dmitry Rogachev National Medical Research Center for Pediatric Hematology, Oncology, and Immunology, Moscow 117997, Russia.

Supplementary text S1. Constant metabolite concentrations in the model.

Table S1.1. Constant metabolite concentrations in the model.

Metabolite	Model (μM)	Experiment (μmol/kg liver)	References
ATP	1600	1500 - 2000	[1–4]
ADP	1000	600 - 1300	[1,5,6]
dUMP	200	170 - 240	[7]
Gly	1800	1400 – 1900	[8–10]
NADP	15	4-54	[11–13]
NADPH	320	180 - 370	[11–13]
Pi	2300	2100 - 2700	[1]
Ser	590	400 - 700	[8–10]

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Supplementary text S2. Reaction rate equations.

AT (AICAR Transformylase, EC 2.1.2.3). AT catalyzes irreversible reaction of formyl group transfer from 10-THF to AICAR via a random sequential bi-bi mechanism [1] providing the second of the two folate-requiring reactions in purine *de novo* biosynthesis. The reaction rate in the model is described by the corresponding equation:

$$V^{AT} = V_{max}^{AT} \frac{[AICAR]}{[AICAR]+K_{m,AICAR}^{AT}} \cdot \frac{[10-THF]}{[10-THF]+K_{m,10-THF}^{AT}} \quad \text{(S2.1)}$$

DHFR (Dihydrofolate reductase, EC 1.5.1.3). DHFR catalyzes irreversible reduction of DHF to THF using NADPH as a cofactor. The reaction mechanism is random sequential bi-bi [2]. Under physiological conditions the enzyme is saturated with NADPH (Supplementary texts S1, S4). Thus, the reaction rate can be described by Michaelis-Menten equation with one substrate:

$$V^{DHFR} = V_{max}^{DHFR} \frac{[DHF]}{K_m^{DHFR}+[DHF]} \quad \text{(S2.2)}$$

FTHFD (Formyltetrahydrofolate dehydrogenase, EC 1.5.1.6). FTHFD catalyzes irreversible reaction of NADP - dependent oxidation of 10-THF to THF and CO_2 [3,4]. Under intracellular conditions the enzyme is supposed to be saturated with NADP, since the corresponding Michaelis constant value is substantially lower than the intracellular NADP concentration (Supplementary texts S1, S4). Also, the enzyme is strongly inhibited by THF [5–7]. In the model the FTHFD reaction rate is described by the equation taken from [7]:

$$V^{FTHFD} = \frac{V_{\max}^{FTHFD}[10-THF]}{K_{m,10-THF}^{FTHFD}\left(1+\frac{[THF]}{K_{i,THF}^{FTHFD}}\right)+[10-THF]} \quad \text{(S2.3)}$$

FTHFS (Formyltetrahydrofolate synthetase, EC 6.3.4.3). FTHFS catalyzes the reversible ATP-dependent synthesis of 10-formyltetrahydrofolate from formate and tetrahydrofolate. While a ping-pong mechanism was proposed for the reaction based on the enzyme crystal structure analysis [8] there are no strong evidences supporting this mechanism. The reaction rate in the model is described by equation for random-order sequential bi-bi mechanism [9,10] with assumption that enzyme is saturated with ATP and ADP (Supplementary texts S1, S4).

$$V^{FTHFS} = \frac{\frac{V_{max}^{FTHFS}}{K_{m,THF}K_{m,HCOOH}}\left([THF][HCOOH] - \frac{[10-THF][P_i]}{K_{eq}^{FTHFS}\frac{[ATP]}{[ADP]}}\right)}{1 + \frac{[THF]}{K_{m,THF}^{FTHFS}} + \frac{[HCOOH]}{K_{m,HCOOH}^{FTHFS}} + \frac{[10-THF]}{K_{m,10-THF}^{FTHFS}} + \frac{[P_i]}{K_{m,Pi}^{FTHFS}} + \frac{[THF][HCOOH]}{K_{m,THF}^{FTHFS}K_{m,HCOOH}^{FTHFS}} + \frac{[10-THF][P_i]}{K_{m,10-THF}^{FTHFS}K_{m,Pi}^{FTHFS}}}$$

(S2.4)

GFIT/FITCD (Glutamate formimidoyltransferase/formimidoyltetrahydrofolate cyclodeaminase, EC 2.1.2.5/EC 4.3.1.4) GFIT/FITCD is a bifunctional enzyme that catalyzes two consecutive reactions: transfer of formimino group from formiminoglutamate to tetrahydrofolate, and deamination of formiminotetrahydrofolate with formation of CH-THF. Transferase reaction follows the random sequential bi-bi mechanism [11]. The overall reaction is considered to be irreversible [12,13]. For the polyglutamate forms of folates, almost 100% channeling between the catalytic sites of the enzyme was shown [14]. Therefore, we did not include in the model formiminotetrahydrofolate and describe the rate of the overall reaction using a single equation for random sequential bi-bi mechanism:

$$V^{GFIT/FITCD} = V_{max}^{GFIT/FITCD}\frac{[FIGlu]}{[FIGlu]+K_{m,FIGlu}^{GFIT/FITCD}} \cdot \frac{[THF]}{[THF]+K_{m,THF}^{GFIT/FITCD}} \quad \text{(S2.5)}$$

GT (GAR Transformylase, EC 2.1.2.2). This enzyme catalyzes the irreversible transfer of the formyl group from 10-formyltetrahydrofolate to the amino nitrogen of GAR [15], providing the first of the two folate-requiring reactions in purine *de novo* biosynthesis. According to the literature data [16] the reaction rate is described by equation for ordered sequential (bi-bi) mechanism with 10-THF binding first:

$$V^{GT} = V_{max}^{GT}\frac{[GAR][10-THF]}{K_{i,10-THF}^{GT}K_{m,GAR}^{GT} + K_{m,GAR}^{GT}[10-THF] + K_{m,10-THF}^{GT}[GAR] + [10-THF][GAR]}$$

(S2.6)

MS (Methionine synthase, EC 2.1.1.13). This enzyme catalyzes an irreversible methyl group transfer from CH3-THF to homocysteine via an ordered bi-bi mechanism with CH3-THF as the first substrate and methionine as the first product [17,18]. The equation for MS reaction rate was taken from [19]:

$$V^{MS} = \frac{V_{max}^{MS}}{1+\frac{K_{m,CH3-THF}^{MS}}{[CH3-THF]}+\frac{K_{m,Hcy}^{MS}}{[Hcy]}\left(1+\frac{K_{d,CH3-THF}^{MS}}{[CH3-THF]}\right)} \quad \text{(S2.7)}$$

We have changed MS activity in the model from 6.6 [19] to 3 mmol/h kg liver for better description of experimental dependence of CH3-THF concentration on folate pool.

MTHFD/MTHFC (Methylentetrahydrofolate dehydrogenase / Methylenetetrahydrofolate cyclohydrolase, EC 1.5.1.5 / EC 3.5.4.9). MTHFD and MTHFC reactions are catalyzed by the same trifunctional cytoplasmic enzyme – C1-tetrahydrofolate-synthase (C1-THFS). MTHFD activity provides the reversible NADP/NADPH dependent transformation between CH2-THF and CH-THF. MTHFC provides the reversible transformation between 10-THF and CH-THF. Also, the enzyme demonstrates effective channeling between MTHFD and MTHFC. Equations for corresponding reaction rates were constructed in this work and described in Supplementary text S3 together with the enzyme kinetic parameters.

MTHFR (Methylenetetrahydrofolate reductase, EC 1.5.1.20). This enzyme catalyzes irreversible reduction of CH2-THF to CH3-THF using NADPH as a cofactor. The reaction mechanism is ping-pong with NADPH as the first substrate and CH3-THF as the second product. MTHFR is allosterically inhibited by AdoMet. The equation for MTHFR reaction rate was taken from [19].

$$V^{MTHFR} = V_{max}^{MTHFR} \frac{\frac{1}{1+\frac{[AdoMet]}{K_{i,AdoMet}^{MTHFR}\left(1+\frac{[AdoHcy]}{K_{i,AdoHcy}^{MTHFR}}\right)}}+\frac{0.046}{1+\frac{K_{i,AdoMet}^{MTHFR}\left(1+\frac{[AdoHcy]}{K_{i,AdoHcy}^{MTHFR}}\right)}{[AdoMet]}}}{1+\frac{K_{m,NADPH}^{MTHFR}}{[NADPH]}\left(1+\frac{[CH_3-THF]}{K_{i,CH3-THF}^{MTHFR}}\right)+\frac{K_{m,CH2-THF}^{MTHFR}}{[CH_2-THF]}} \quad \text{(S2.8)}$$

Activity of MTHFR in the model was changed from 4 [19] to 0.9 mmol/h kg liver for better description of experimental dependence of CH3-THF concentration on folate pool.

MTHFS (Methenyltetrahydrofolate synthase, EC 6.3.3.2). This enzyme catalyzes irreversible ATP-dependent conversion of 5-THF to CH-THF [20,21]. Reaction mechanism is random sequential bi-bi [22]. In cells the enzyme is saturated with ATP (Supplementary texts S1, S4), so the reaction rate is described by the Michaelis-Menten equation with only one substrate:

$$V^{MTHFS} = V_{max}^{MTHFS} \frac{[5-THF]}{K_m^{MTHFS}+[5-THF]} \quad \text{(S2.9)}$$

SHMT (Serine hydroxymethyl transferase, EC 2.1.2.1). SHMT catalyzes reversible conversion between THF and serine and CH2-THF and glycine. Reaction mechanism is random sequential bi-bi [23]. The enzyme is inhibited by 5-THF and CH3-THF [24]. The inhibition constant value for 5-THF monoglutamate is equal to 130 μM [24]. Even after a 10 fold decrease that can be expected for polyglutamate this value remains high compared to 5-THF concentration in cells (Table 3). That is why we neglected this inhibition in the model. Inhibition of SHMT by CH3-THF is competitive with THF and CH2-THF [25]. The rate of SHMT reaction in the model is described by the following equation:

$$V^{SHMT} = \frac{\frac{V_{max}^{SHMT}}{K_{m,THF}K_{m,Ser}}\left([THF][Ser]-\frac{[CH_2-THF][Gly]}{K_{eq}^{SHMT}}\right)}{1+\frac{[THF]}{K_{m,THF}^{SHMT}}+\frac{[Ser]}{K_{m,Ser}^{SHMT}}+\frac{[CH_2-THF]}{K_{m,CH_2-THF}^{SHMT}}+\frac{[Gly]}{K_{m,Gly}^{SHMT}}+\frac{[THF][Ser]}{K_{m,THF}^{SHMT}K_{m,Ser}^{SHMT}}+\frac{[CH_2-THF][Gly]}{K_{m,CH_2-THF}^{SHMT}K_{m,Gly}^{SHMT}}} \quad \text{(S2.10)}$$

where

$$K_{m,THF}^{SHMT} = K_{m0,THF}^{SHMT}\left(1 + \frac{[CH3-THF]}{K_{i1,CH3-THF}^{SHMT}}\right)$$

$$K_{m,CH2-THF}^{SHMT} = K_{m0,CH2-THF}^{SHMT}\left(1 + \frac{[CH3-THF]}{K_{i2,CH3-THF}^{SHMT}}\right)$$

TS (Thymidilate synthase, EC 2.1.1.45). TS catalyzes conversion of dUMP to dTMP via reductive methylation with CH2-THF. The reaction mechanism is ordered bi-bi with dUMP as the first substrate and DHF as the first product [26]. The enzyme is inhibited competitively by 10-THF [27]. According to the literature data the TS Michaelis constant value for dUMP lies in the range of 2.5 – 6.8 μM [26,28] that is several fold lower compared with physiological concentration of dUMP (Supplementary text S1). Thus, in cells the enzyme is saturated with dUMP and we used the Michaelis-Menten equation with one substrate including competitive inhibition with 10-THF to describe the TS reaction rate in the model:

$$V^{TS} = V_{max}^{TS} \frac{[CH2-THF]}{K_{m,CH_2-THF}^{TS}\left(1+\frac{[10-THF]}{K_{i,10-THF}^{TS}}\right)+[CH_2-THF]} \quad \text{(S1.11)}$$

Synthesis of 5-THF. In cells 5-THF is produced from 10-THF or CH-THF in non-enzymatic reactions, as well as in side reactions catalyzed by MTHFC/D, SHMT, and, probably, by some

other enzymes [29,30]. In the model the rates of 5-THF production from 10-THF (V^{5THFS1}) and from CH-THF (V^{5THFS2}) are described by Michaelis-Menten equations with parameters presented in Supplementary text S4:

$$V^{5THFS1} = V_{max}^{5THFS1} \frac{[10-THF]}{K_m^{5THFS1}+[10-THF]} \quad \text{(S1.12)}$$

$$V^{5THFS2} = V_{max}^{5THFS2} \frac{[CH-THF]}{K_m^{5THFS2}+[CH-THF]} \quad \text{(S1.13)}$$

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Supplementary text S3. Methylenetetrahydrofolate dehydrogenase/Methylenetetrahydrofolate cyclohydrolase (EC1.5.1.5 / EC 3.5.4.9)

Methylenetetrahydrofolate dehydrogenase and methylenetetrahydrofolate cyclohydrolase reactions are catalyzed by the same cytoplasmic protein that also possesses formyltetrahydrofolate synthetase activity. Both, methylenetetrahydrofolate dehydrogenase and methylenetetrahydrofolate cyclohydrolase activities associate with one (N-terminal) domain of the enzyme while formyltetrahydrofolate synthase activity associates with another (C-terminal) domain [1]. That is why we consider the formyltetrahydrofolate synthetase reaction as an independent activity.

Methylenetetrahydrofolate dehydrogenase activity (MTHFD - EC 1.5.1.5) provides the reversible NADP/NADPH dependent transformation between CH2-THF and CH-THF. Methylenetetrahydrofolate cyclohydrolase activity (MTHFC – EC 3.5.4.9) provides reversible transformation between 10-THF and CH-THF. Interestingly, the methylenetetrahydrofolate cyclohydrolase activity is detected mostly in the absence of NADP/NADPH cofactors in vitro [2,3]. In the presence of the cofactors (under physiological conditions) almost all consumed 10-THF is converted to CH2-THF without releasing CH-THF that demonstrates channeling between MTHFC and MTHFD. Also, in MTHFD reaction only 50% of CH2-THF is converted to CH-THF while the rest is channeled to 10-THF [4].

Based on the literature data [5] we suggested the scheme of reactions provided by MTHFD and MTHFC which is shown in Fig. S3.1. We assumed random sequential binding of all folates and cofactors to the enzyme in both MTHFD and channeling (MTHFD/C) reactions. According to the scheme the enzyme can be involved only in one reaction: MTHFD, MTHFC, or MTHFD/C. In other words, the reactions cannot be catalyzed independently. MTHFC reaction can proceed only between corresponding enzyme-folate complexes lacking bound NADP/NADPH cofactors. The channeling between 10-THF and CH2-THF in the scheme is provided due to the absence of direct conversion of E•NADPH•10-THF complex to E•NADPH•CH-THF complex and vise-versa (Fig. S3.1). To provide 50% channeling of CH2-THF to 10-THF in the scheme we set the rate constant of forward MTHFD reaction (k_{Df}) equal to the rate constant of forward channeling reaction (k_{Cf}) (Fig. S3.1).

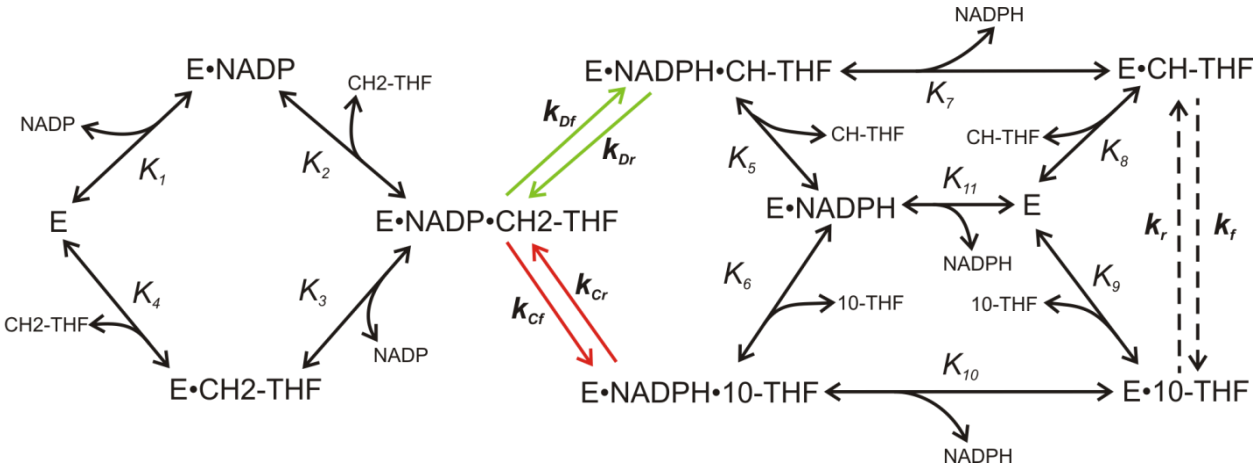

Fig. S3.1. Scheme of reactions catalyzed by methylenetetrahydrofolate dehydrogenase and methylenetetrahydrofolate cyclohydrolase. E denotes enzyme. Solid black arrows show association-dissociation of corresponding enzyme-substrate complexes. K_1-K_{11} denote corresponding dissociation constants. Black dashed arrows show forward and reverse MTHFC reactions with forward (k_f) and reverse (k_r) rate constants. Green and red arrows show MTHFD and channeling reactions respectively with forward (k_{Df} and k_{Cf}) and reverse (k_{Dr} and k_{Cr}) rate constants.

Using the scheme in Fig. S3.1, one can obtain equations for the reaction rates.
To make the equations less bulky let us introduce the following designations:

CH2-THF → CH2
CH-THF → CH
10-THF → CHO
NADPH→NPH
NADP→NP

V^{MTHFC} - the rate of methyltetrahydrofolate cyclohydrolase reaction without NADPH
V^{MTHFD} - the rate of methyl tetrahydrofolate dehydrogenase reaction
$V^{MTHFD/C}$ - the channeling rate between CH_2-THF and 10-THF

The positive direction of the V^{MTHFC}, V^{MTHFD}, and $V^{MTHFD/C}$ rates correspond to the reaction rate constants with index *f* in Fig. S3.1.
The rates of MTHFC, MTHFD, and MTHFC/D reactions are described by the following equations:

$$V^{MTHFC} = [E.CH] * k_f - [E.CHO] * k_r$$
$$V^{MTHFD} = [E.NP.CH2] * k_{Df} - [E.NPH.CH] * k_{Dr} \quad \text{(S3.1)}$$
$$V^{MTHFD/C} = [E.NP.CH2] * k_{Cf} - [E.NPH.CHO] * k_{Cr}$$

Here expressions in square brackets denote concentrations of corresponding enzyme-substrate complexes. Assuming a rapid equilibrium in all stages of the scheme in Fig. S3.1 except the catalytic stages, we obtain the following system of equations:

$$K_1 = \frac{[NP]*[E]}{[E.NP]}$$
$$K_2 = \frac{[E.NP]*[CH2]}{[E.NP.CH2]}$$
$$K_4 = \frac{[E]*[CH2]}{[E.CH2\,]}$$
$$K_5 = \frac{[E.NPH]*[CH]}{[E.NPH.CH]}$$
$$K_6 = \frac{[E.NPH]*[CHO]}{[E.NPH.CHO]} \quad \text{(S3.2)}$$

$$K_8 = \frac{[E]*[CH]}{[E.CH]}$$

$$K_9 = \frac{[E]*[CHO]}{[E.CHO]}$$

$$K_{11} = \frac{[E]*[NPH]}{[E.NPH]}$$

$$[\mathrm{E}_0] = [E] + [E.NP] + [E.NPH] + [E.NP.CH2] + [E.NPH.CH] + [E.NPH.CHO] + [E.CH] + [E.CHO] + [E.CH2]$$

Using the system of equations (S3.2), we obtain equations for the concentrations of enzyme-substrate complexes:

$$[E.NP.CH2] = \frac{[E_0][NP][CH2]}{[NP][CH2]+K_1K_2\left(1+\frac{[NP]}{K_1}+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}+\frac{[CHO]}{K_6}\right)+\frac{[CHO]}{K_9}+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)}$$

$$[E.NPH.CH] = \frac{[E_0][NPH][CH]}{[NPH][CH]+K_5K_{11}\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CHO]}{K_6}\right)+\frac{[CHO]}{K_9}+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)}$$

$$[E.CH] = \frac{[E_0][CH]}{[CH]+K_8\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}+\frac{[CHO]}{K_6}\right)+\frac{[CHO]}{K_9}+\frac{[CH2]}{K_4}\right)} \quad \text{(S3.3)}$$

$$[E.CHO] = \frac{[E_0][CHO]}{[CHO]+K_9\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}+\frac{[CHO]}{K_6}\right)+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)}$$

$$[E.NPH.CHO] = \frac{[E_0][NPH][CHO]}{[NPH][CHO]+K_6K_{11}\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}\right)+\frac{[CHO]}{K_9}+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)}$$

And finally, using equations (S3.3) and (S3.1) we obtain the equations for the rates of MTHFC, MTHFD and MTHFD/C reactions:

$$V^{MTHFC} = \frac{[E_0][CH]k_f}{[CH]+K_8\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}+\frac{[CHO]}{K_6}\right)+\frac{[CHO]}{K_9}+\frac{[CH2]}{K_4}\right)} - - \frac{[E_0][CHO]k_r}{[CHO]+K_9\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}+\frac{[CHO]}{K_6}\right)+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)} \quad \text{(S3.4)}$$

$$V^{MTHFD} = \frac{[E_0][NP][CH2]k_{Df}}{[NP][CH2]+K_1K_2\left(1+\frac{[NP]}{K_1}+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}+\frac{[CHO]}{K_6}\right)+\frac{[CHO]}{K_9}+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)} - \\ - \frac{[E_0][NPH][CH]k_{Dr}}{[NPH][CH]+K_5K_{11}\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CHO]}{K_6}\right)+\frac{[CHO]}{K_9}+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)} \quad \text{(S3.5)}$$

$$V^{MTHFD/C} = \frac{[E_0][NP][CH2]k_{Cf}}{[NP][CH2]+K_1K_2\left(1+\frac{[NP]}{K_1}+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}+\frac{[CHO]}{K_6}\right)+\frac{[CHO]}{K_9}+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)} - \\ - \frac{[E_0][NPH][CHO]k_{Cr}}{[NPH][CHO]+K_6K_{11}\left(1+\frac{[NP]}{K_1}\left(1+\frac{[CH2]}{K_2}\right)+\frac{[NPH]}{K_{11}}\left(1+\frac{[CH]}{K_5}\right)+\frac{[CHO]}{K_9}+\frac{[CH]}{K_8}+\frac{[CH2]}{K_4}\right)} \quad \text{(S3.6)}$$

The equation parameter values were taken from the literature or calculated from the literature data. We choose parameter values consistent with Haldane relationships for the enzymatic reactions.

$$K_{eq1} = \frac{[CHO][NPH]}{[CH2\,][NP]} = \frac{K_{Cf}K_6K_{11}}{K_{Cr}K_1K_2} \quad \text{(S3.7)}$$

$$K_{eq2} = \frac{[CHO]}{[CH]} = \frac{K_fK_9}{K_rK_8} \quad \text{(S3.8)}$$

$$K_{eq3} = \frac{[CH][NPH]}{[CH2][NP]} = \frac{K_{Df}K_5K_{11}}{K_{Dr}K_1K_2} \quad \text{(S3.9)}$$

Here K_{eq1}, K_{eq2}, *and* K_{eq3} denote equilibrium constants for MTHFD/C, MTHFC, and MTHFD reactions respectively.

The equation parameter values and corresponding comments are presented in Table S3.1.

Table S3.1. **Kinetic parameters of MTHFD and MTHFC (EC 1.5.1.5/EC 3.5.4.9).**

Values for all catalytic constants obtained at temperature other than 37 ^{o}C were recalculated to values at 37 ^{o}C using temperature dependence coefficient of 2 per 10 ^{o}C. In several cases literature data for Michaelis constant values were used to evaluate values of corresponding dissociation constants. Similar to all other enzyme kinetic parameters (Supplementary text S4) for MTHFD/C we used Michaelis, inhibition, and dissociation constant values for folates obtained with polyglutamate forms of folates, if available. If data obtained with polyglutamate forms of folates were not available we used data obtained with monoglutamates decreased by factor of about ten. The exact parameters values in these cases were chosen to satisfy the Haldane equations (S3.7-S3.9) and detailed balance equations.

Parameter (Units)	Model	Experiment	Comments and references
E_0 (μM)	3.3	3.3	Pig liver [6].
k_{Df} (1/s)	48	18	Recombinant human liver cytoplasmic enzyme [7].
		50	Pig liver [2].
		38[a]	Rat liver, calculated from [6,8].
k_{Dr} (1/s)	63	60	Recombinant human liver cytoplasmic enzyme [7].
k_f (1/s)	189	250	Recombinant human liver cytoplasmic enzyme [7].
		320	Pig liver [2].
		134[b]	Rat liver, calculated from [6,8].

k_r (1/s)	24	25	Recombinant human liver cytoplasmic enzyme [7].
k_{Cf} (1/s)	48	-	$k_{Cf} = k_{Df}$ according to 50% channeling in forward MTHFD reaction [4]
k_{Cr} (1/s)	24	25	Recombinant human liver cytoplasmic enzyme [7].
K_1 (μM)	9.6	-	Calculated from the detailed balance equation: $K_1 \cdot K_2 = K_3 \cdot K_4$.
K_2 (μM)	3	3.0	Pig liver, *Km* for CH2-THF pentaglutamate for MTHFD [9].
		2.4 - 4.2	Pig liver, *Km* for CH2-THF pentaglutamate for MTHFD [10].
K_3 (μM)	8	9-13	Pig liver, *Km* for NADP for MTHFD with CH2-THF pentaglutamate [10].
K_4 (μM)	3.6	39	Recombinant human liver cytoplasmic enzyme, *Kd* for CH2-THF monoglutamate in the absence of NADP/NADPH [5].
K_5 (μM)	0.1	-	Calculated from equation (S3.9).
K_6 (μM)	0.8	7	Recombinant human liver cytoplasmic enzyme, *Kd* for 10-THF monoglutamate in the presence of NADP/NADPH [5].
K_8 (μM)	6	55	Mouse liver, *Km* for CH-THF monoglutamate for MTHFC [11].
		43	Recombinant human liver cytoplasmic enzyme, *Km* for CH-THF monoglutamate for MTHFC [7].
		16.4	Recombinant human liver cytoplasmic enzyme, *Km* for CH-THF monoglutamate for MTHFC [12].
K_9 (μM)	16	190	Recombinant human liver cytoplasmic enzyme, *Kd* for 10-THF monoglutamate for MTHFC [5].

		51	Recombinant human liver cytoplasmic enzyme, *Km* for 10-THF monoglutamate for MTHFC [7].
K_{10} (μM)	14.4	13	Recombinant human liver cytoplasmic enzyme, *Km* for NADPH for overall reverse MTHFD/C reaction with 10-THF monoglutamate [7].
K_{11} (μM)	288	-	Calculated from the detailed balance equation: $K_{11} \cdot K_6 = K_9 \cdot K_{10}$
K_{eq1}	16	16	Recombinant human liver cytoplasmic enzyme [5]
K_{eq2}	21	21	Calculated from the nonenzymatic first order reaction rate constants [7]
K_{eq3}	0.76	0.76[c]	Calculated from [5,7]

[a] Using MTHFD activity in rat liver V_{max}^{MTHFD} = 456 mmol/h kg tissue [8] and liver enzyme concentration of 3.3 μM [6], one can get the value for k_{Df} = 456/(3.3*3.6) ≈ 38 1/s.

[b] Using MTHFC activity in rat liver V_{max}^{MTHFC} = 1596 mmol/h kg tissue [8] and liver enzyme concentration of 3.3 μM [6], one can get the value for k_f = 1596/(3.3*3.6) ≈ 134 1/s.

[c] Taking values for K_{eq1} = 16 [5] and for K_{eq2} = 21 [7] and using equations S3.7-S3.9 one can get the value for $K_{eq3} = K_{eq1}/K_{eq2}$ = 0.76.

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Supplementary text S4. Enzyme kinetic parameters.

In the model we tried to used enzyme kinetic parameters obtained for rodent liver, if available, making preference for rat liver. All enzyme activities used in the model were obtained for rat liver and presented as mmol per hour per kg of wet liver, that is a very close estimation for mmol per hour per liter. In case if enzyme activity in the literature was normalized to protein we recalculated the activity as per kg using protein concentration of 200 g per kg of wet liver [1]. If enzyme activity or catalytic constant in the literature was measured at temperature other than 37 ^{o}C, its value was recalculated for 37 ^{o}C using temperature coefficient of 2 per 10 ^{o}C. In cells folates exist mainly in the polyglutamate forms containing 5-6 glutamate residues per folate molecule. Such polyglutamate forms have 2 - 100 times higher affinity to enzymes compared with monoglutamate forms [2–4]. While folate Michaelis, inhibition, and dissociation constant values obtained at different conditions are presented in Table S4.1, only those, obtained for the polyglutamate forms of folates were used in the model. In a few cases when constant for polyglutamate was not available in the literature we used value of constant obtained for monoglutamate divided by ten. Commetnts in the Table S4.1 describe enzyme source and, glutamination status of folates. If nothing is mentioned about glutamination satatus it means that data were obtained with monoglutamate form of the corresponding folate.

Table S4.1. Enzyme kinetic parameters.

Parameter (Units)	**Model**	**Experiment**	**Comments and References**
AT (AICAR transformylase, EC 2.1.2.3)			
V_{max}^{AT} (mmol/h kg liver)	8.8	8.8	Rat liver [5].
$K_{m,10-THF}^{AT}$ (μM)	5.9	5.9	Human breast cancer cells MCF-7, 10-THF pentaglutamate [6].
$K_{m,AICAR}^{AT}$ (μM)	10	10	Human recombinant enzyme [7].
DHFR (Dihydrofolate reductase, EC 1.5.1.3)			
V_{max}^{DHFR}	4.9	109	Rat liver [8].
		57	Rat liver [9].

(mmol/h kg liver)		4.9	Rat liver [10].
$K_{m,DHF}^{DHFR}$ (μM)	0.034	0.1	Rat liver [11].
		0.74	Pig liver [12].
		0.17	Rat liver [13].
$K_{m,NADPH}^{DHFR}$ (μM)	The enzyme is saturated with NADPH	3.2	Pig liver [12].
		0.72	Rat liver [13].
		0.2	Rabbit liver [14].
FTHFD (Formyltetrahydrofolate dehydrogenase, EC 1.5.1.6).			
V_{max}^{FTHFD} (mmol/h kg liver)	25	45	Rat liver [15].
		14	Rat liver [16].
		29	Rat liver [17].
		11.2	Rat liver [18].
		23.1	Rat liver [19].
		26.1	Rat liver [20].
$K_{m,NADP}^{FTHFD}$ (μM)	The enzyme is saturated with NADP	0.9	Rat liver [17].
		1	Rat liver [21].
		3.5	Pig liver [22].
$K_{i,THF}^{FTHFD}$ (μM)	0.015	0.015	Rabbit liver, THF pentaglutamate [23].
		1	Pig liver [22].
$K_{m,10-THF}^{FTHFD}$ (μM)	0.9	0.9	Rabbit liver, 10-THF pentaglutamate [23].
FTHFS (Formyltetrahydrofolate synthetase, EC 6.3.4.3)			
V_{max}^{FTHFS} (mmol/h kg liver)	432	162	Rat liver [9].
		384	Rat liver [24].
		504	Rat liver [25].
$K_{eq}^{FTHFS} = = \frac{[ADP][P_i][10-THF]}{[ATP][HCOOH][THF]}$	53	115	[26]
		20-60	[27]

$K_{m,THF}^{FTHFS}$ (µM)	1	239	Mouse liver [28].
		15	Rabbit liver [29].
		<1	Rabbit liver, THF pentaglutamate. [30].
$K_{m,ATP}^{FTHFS}$ (µM)	The enzyme is saturated with ATP	47	Mouse liver [28].
		67	Rabbit liver [29].
		40	Rabbit liver, with THF pentaglutamate [30].
$K_{m,HCOOH}^{FTHFS}$ (µM)	4	18700	Mouse liver [28].
		166	Rabbit liver [29].
		4	Rabbit liver, with THF pentaglutamate [30].
$K_{m,ADP}^{FTHFS}$ (µM)	The enzyme is saturated with ADP	130	C. cylindrosporum [31]
$K_{m,H2PO4}^{FTHFS}$ (µM)	5000	5000	C. cylindrosporum [32].
$K_{m,10-THF}^{FTHFS}$ (µM)	120	10000	C. cylindrosporum [31].
		120	C. cylindrosporum, 10-THF triglutamate [31].
GFIT/FITCD (Glutamate formimidoyltransferase/formimidoyltetrahydrofolate cyclodeaminase, EC 2.1.2.5/EC 4.3.1.4)			
$V_{max}^{GFIT/FITCD}$ (mmol/h kg liver)	216	215	Rat liver [19].
		213	Rat liver [20].
$K_{m,THF}^{GFIT/FITCD}$ (µM)	2	3.4	Pig liver, THF pentaglutamate.[33].
		0.7	Pig liver, THF pentaglutamate [34].
		100	Pig liver [35].
$K_{m,FIGlu}^{GFIT/FITCD}$ (µM)	400	12000	Pig liver [36].
		11000	Pig liver [35].
		5800	Pig liver [37]
		400	Pig liver, with THF pentaglutamate [38].
GT (GAR transformylase, EC 2.1.2.2)			
V_{max}^{GT} (mmol/h kg liver)	0.8	1.6	Rat liver [5].
		0.7	Rat liver [39].

$K_{m,10-THF}^{GT}$ (µM)	0.8	15	Mouse leukemia cells L1210 [39].
		0.8	Mouse lymphoma cells L5178Y [40].
$K_{m,GAR}^{GT}$ (µM)	10	17	Mouse leukemia cells L1210 [39].
		2.5	Mouse lymphoma cells L5178Y [41].
$K_{i,10-THF}^{GT}$ (µM)	0.36	3.6	Mouse lymphoma cells L5178Y [41].
MS (Methionine synthase, EC 2.1.1.13)			
V_{max}^{MS} (mmol/h kg liver)	0.59	3.1	Rat liver [42].
		0.5	Rat liver [18].
		1.2	Rat liver [24].
		0.9	Rat liver [43].
$K_{m,CH3-THF}^{MS}$ (µM)	4	4	Rat liver, CH3-THF pentaglutamate [44].
		89	Recombinant rat enzyme [45].
		12.8	Pig liver [46].
		0.5-8.4	Pig liver, THF hexaglutamate [47].
$K_{m,Hcy}^{MS}$ (µM)	2.5	2.6	Recombinant rat enzyme [45].
		2.2	Pig liver [46].
$K_{d,CH3-THF}^{MS}$ (µM)	14.2	142	Pig liver [46].
MTHFD/MTHFC (Methylenetetrahydrofolate dehydrogenase/methylenetetrahydrofolate cyclohydrolase (EC1.5.1.5 / EC 3.5.4.9) Reaction rate equations and kinetic parameters for MTHFD and MTHFC are presented in Supplementary text S3.			
MTHFR (Methylenetetrahydrofolate reductase, EC 1.5.1.20)			
V_{max}^{MTHFR} (mmol/h kg liver)	0.9	3.4	Rat liver [48].
		0.7	Rat liver [18].
		0.34	Rat liver [19].
		1.9	Rat liver [49].
$K_{m,CH2-THF}^{MTHFR}$ (µM)	2	88	Pig liver, [50].

		19	Pig liver, [51].
		0.1-1.7	Pig liver, CH2-THF from triglutamates to hexaglutamates [52].
$K_{m,NADPH}^{MTHFR}$ (µM)	120	16	Pig liver [51].
		15-185	Pig liver, with CH2-THF from triglutamates to hexaglutamates [52].
$K_{i,CH3-THF}^{MTHFR}$ (µM)	2	20	Pig liver [53].
$K_{i,AdoMet}^{MTHFR}$ (µM)	3	0.9	Pig liver [54].
		3	Pig liver [55].
$K_{i,AdoHcy}^{MTHFR}$ (µM)	3	3	Pig liver [56].
MTHFS (Methenyltetrahydrofolate synthase, EC 6.3.3.2)			
V_{max}^{MTHFS} (mmol/h kg liver)	12.6	14	Rat liver [57].
		8	Human liver [58].
$K_{m,5-THF}^{MTHFS}$ (µM)	0.5	0.6	Human liver, 5-THF-pentaglutamate [59].
		0.5	Rabbit liver [60].
		5	Recombinant mouse enzyme [61].
		8	Rabbit liver [62].
		0.2	Rabbit liver, 5-THF-pentaglutamate [14].
$K_{m,ATP}^{MTHFS}$ (µM)	The enzyme is saturated with ATP	300	Rabbit liver [60].
		20	Human liver [59]
		300	Rabbit liver [14].
		769	Recombinant mouse enzyme [61].
SHMT (Serine hydroxymethyl transferase, EC 2.1.2.1)			
V_{max}^{SHMT} (mmol/h kg liver)	500	400	Rat liver [9].
		758	Rat liver [8].
		216	Rat liver [63].

$K_{eq}^{SHMT} = = \frac{[CH2-THF][Gly]}{[THF][Ser]}$	8	8 - 12	[64]
$K_{m,Ser}^{SHMT}$ (µM)	1000	510	Rat liver [65].
		210	Pig liver, with THF-triglutamate [66].
		1000	Rabbit liver, with THF hexaglutamate [3].
		700	Monkey liver [67].
		360	Rabbit liver [64].
$K_{m,Gly}^{SHMT}$ (µM)	5000	5000	Rabbit liver, with CH2-THF hexaglutamate [3].
		1700	Rabbit liver [64].
$K_{m0,THF}^{SHMT}$ (µM)	0.5	1.7	Pig liver, THF-triglutamate [66].
		0.1-0.5	Rabbit liver, THF-hexaglutamate [3].
		4.1	Human recombinant enzyme [68].
		10	Rabbit recombinant enzyme [68].
		40-60	Rabbit liver [69].
$K_{m0,CH2-THF}^{SHMT}$ (µM)	1.7	17	Rabbit liver [64].
$K_{i1,CH3-THF}^{SHMT}$ (µM)	3	30	Pig liver. Calculated for monoglutamate using data from [64].[b]
$K_{i2,CH3-THF}^{SHMT}$ (µM)	14	140	Pig liver. Calculated for monoglutamate using data from [64].[b]
TS (Thymidylate synthase EC 2.1.1.45)			
V_{max}^{TS} (mmol/h kg liver)	0.09	0.04	Rat liver [70].
		0.12	Rat liver [71].
		0.14	Rat liver [1].
$K_{m,dUMP}^{TS}$ (µM)	The enzyme is saturated with dUMP	6.8	Rat liver [72].
		2.5	Rat liver [73].
$K_{m,CH2-THF}^{TS}$ (µM)	0.7	65	Rat liver [72].
		2	Rat liver [73].
		0.7	Rat hepatoma cells H35, CH2-THF

			heptaglutamate [74].
$K_{i,10-THF}^{TS}$ (µM)	0.1	0.1	Rat hepatoma cells H35, 10-THF heptaglutamate [74].
Synthesis of 5-THF			
V_{max}^{5THFS1} (mmol/h/l liver)	0.015	0.015	Rat liver [57].
$K_{m,10-THF}^{5THFS1}$ (µM)	0.9	9	Rat liver [57].
V_{max}^{5THFS2} (mmol/h/l liver)	6.5	6.5	Rabbit liver, calculated from [3,75].
$K_{m,CH-THF}^{5THFS2}$ (µM)	4	40	Rabbit liver [75].

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Supplementary text S5. Turnover of folate pool in hepatocytes.

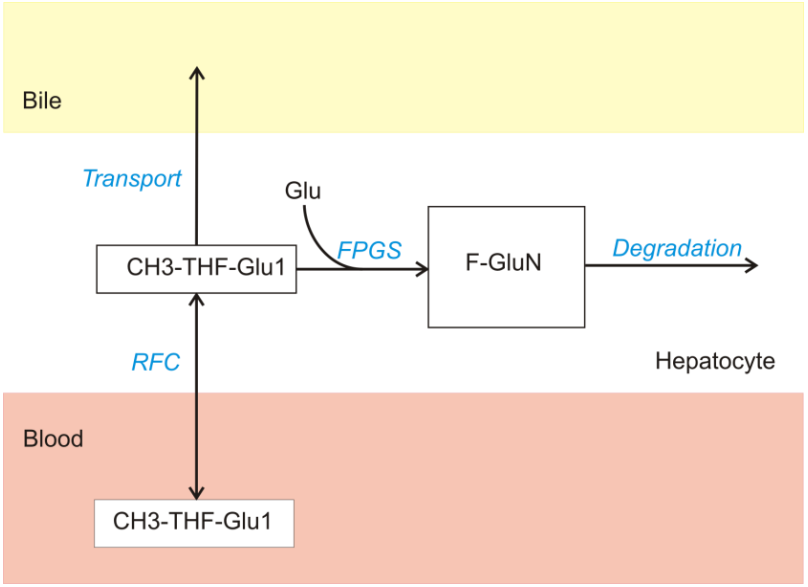

Fig. S5.1. Scheme of folate pool turnover in hepatocytes. Designations: CH3-THF-Glu1 – monoglutamate of methyl-tetrahydrofolate; F-GluN – polyglutamate forms of folates forming intracellular folate pool; Glu – glutamate; RFC – reduced folate carrier transporting monoglutamate forms of folates, FPGS – folylpolyglutamate synthase.

The main circulating form of folates is monoglutamate of methyl-tetrahydrofolate (CH3-THF-Glu1). Under physiological conditions it enters hepatocytes primarily via the anion exchange transporter - reduced folate carrier (RFC) [1] (Fig. S5.1). Once in the hepatocyte, CH3-THF-Glu1 can be transported from the hepatocyte to the bile ducts, or it can be converted to polyglutamate forms by the enzyme folylpolyglutamate synthase (FPGS) [2]. Intracellular folate pool consists of the polyglutamate forms containing 5-6 glutamate residues per folate molecule. The polyglutamate forms do not pass through cell membrane and are much more efficient substrates for enzymes of folate metabolism than free folates or monoglutamate forms [2]. There are no known specific mechanisms of intracellular folates degradation and most likely intracellular folates degrade via slow non-enzymatic oxidation. While there is a lysosomal enzyme gamma-glutamyl hydrolase [3] which cleaves glutamate residues from folates, it probably helps to degrade already damaged oxidized folates. Folates that get into the bile are transported to the intestine where they are re-absorbed to blood and again enter the liver through the portal vein. Thus, folates are involved in entero-hepatic circulation [4,5].

The kinetics of folate pool can be described by the following equation:

$$\frac{dF}{dt} = V^{FPGS} - V^{degr} \quad \text{(S5.1)}$$

where F is folate pool (sum of concentrations of polyglutamate forms of folates), V^{FPGS} is the rate of folylpolyglutamate synthase, V^{degr} is the rate of non-enzymatic folate pool degradation. The rate for non-enzymatic folate degradation can be described as follows:

$$V^{degr} = k^{degr} \cdot F \quad \text{(S5.2)}$$

where k^{degr} is reaction rate constant for the non-enzymatic folate pool degradation. The estimation for the rate constant k^{degr} of folate pool degradation at physiological conditions can be obtained from experiments with prolonged feeding of rats with folate-free diet. [6,7]. The initial drop in folate concentration in rat liver gives a rate of decrease in folate pool (*Vdegr*) equal to 0.06 μmol/h kg liver. It gives us a value for the rate constant $k^{degr} = V^{degr}/F = 0.06/20 = 3*10^{-3}$ h^{-1}. Under steady-state conditions the rate of folate pool degradation is equal to the rate of its accumulation:

$$V^{FPGS} = k^{degr} \cdot F \quad \text{(S5.3)}$$

That gives us an estimation for the characteristic time of folate pool turnover that is equal to $1/k^{degr}$ = 333 h. This time is several orders of magnitude bigger than characteristic times of folate metabolites and folate dependent processes (Table 2, 5). Thus for all metabolic processes associated with folate metabolism in our model we can consider folate pool as a constant model parameter.

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