

Research Paper

Long-Range Allosteric Interactions between the Folate and Methionine Cycles Stabilize DNA Methylation Reaction Rate

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ABSTRACT

Several metabolites in the folate and methionine cycles influence the activities of distant enzymes involved in one-carbon metabolism. Many hypotheses have been advanced about the functional impact of these long-range interactions. Using both steady-state and fluctuation analyses of a mathematical model of methionine metabolism, we investigate the biochemical basis for several of these hypotheses. We show that the long-range interactions provide remarkable stabilization of the DNA methylation rate in the face of large fluctuations in methionine input. In particular, they enable the system to maintain methylation in the face of low and extremely low protein input. These interactions may therefore have evolved primarily to stabilize DNA methylation under conditions of methionine starvation. In silico experimentation allows us to evaluate the independent effects of various combinations of the long-range interactions, and thereby propose a plausible evolutionary scenario.

INTRODUCTION

S-Adenosylmethionine (SAM) is the universal methyl donor for a broad range of methyltransferase reactions. Among the most important of these is the methyltransferase reaction in which cytosines at CpG sites on DNA become methylated. DNA methylation is a critical factor in the control of gene expression and both hyper- and hypo-methylation have been implicated in the inappropriate regulation of proto-oncogenes and tumor-suppressor genes, and have been associated with the development of various cancers.^{1,2} Therefore, understanding the regulatory mechanisms that control DNA methylation is important for understanding normal cell function, gene expression, and neoplastic transformation. DNA methylation depends on the availability of methyl groups provided by the folate and methionine cycles, the control of the DNA methyltransferase (DNMT) reaction, and the availability of cytosine substrates that is controlled by histones and other DNA-binding proteins. In the present paper we are solely concerned with the first two mechanisms, and so assume that the availability of methylation sites is constant.

The level of SAM and the velocity of the DNA methyltransferase reaction depend on the properties of both the methionine and folate cycles. These metabolic cycles in turn depend on the dietary intake of certain nutrients (methionine, choline, betaine) and vitamins (B₁₂, folate, B₆) and are affected by polymorphisms in the genes for enzymes of the methionine and folate cycles.³⁻⁶ Because defects in folate and methionine metabolism are associated with a large number of serious disorders (several types of cancer,⁷⁻¹⁰ cardiovascular disease,¹¹⁻¹³ neural tube defects¹⁴ and neurodegenerative diseases^{15,16}), the genes and enzymes of these cycles have been well studied.

One of the most interesting aspects of the biochemistry of these cycles is that some substrates have excitatory or inhibitory allosteric effects on distant enzymes in the reaction network: SAM inhibits MTHFR and BHMT and activates CBS and 5mTHF inhibits GNMT (acronyms for enzymes and substrates are defined in the caption of Fig. 1). It is believed that these "long-range interactions" play an important role in regulating the dynamic properties of the system.^{3,4} Using mathematical models based on established enzyme kinetics, we show that both the long-range interactions and the existence of the GNMT reaction play important and independent roles in stabilizing the methylation rate in the face of large fluctuations in methionine input. We show that this regulation is particularly important under conditions of methionine starvation. Because we can quantify the relative improvement in the stabilization of methylation that results from various combinations of these long-range interactions, we are able to propose an evolutionary scenario.

METHODS

The mathematical model used for the present study is described in the Appendix.

RESULTS

Since our purpose is to study the effects of the long-range interactions we begin by examining their effects on the steady-state concentrations of the metabolites. Figure 2 shows the steady-state values of [MET], [SAM], [SAH] and [HCY] as functions of the rate of methionine input with all four long-range interactions present (regulated) and absent (unregulated). It is evident that in the regulated cycle the metabolite levels are relatively insensitive to methionine input. By contrast, in the unregulated cycle the metabolites, in particular methionine and SAM, vary substantially as methionine input changes. Thus, we are able to establish that one important effect of the long-range interactions is to stabilize metabolite levels.

It has long been thought that the ratio [SAM]/[SAH] has important regulatory consequences⁴⁰ because SAM is the substrate for the methylation reactions, which are all inhibited by the product, SAH.²⁰ Figure 3 shows that in the fully regulated case, the [SAM]/[SAH] ratio varies only 2-fold as methionine input varies 13-fold while, in the unregulated case, the ratio varies 50-fold. The fact that the relationship between methionine input and the [SAM]/[SAH] ratio is nearly linear in the regulated case suggests that [SAM]/[SAH] is a good biomarker for methionine input. The [SAM]/[SAH] ratio is often taken as an indicator of "methylation capacity." Since the [SAM]/[SAH] ratio (Fig. 3) and the methylation rate (Fig. 4) are both monotone increasing approximately linear functions of methionine input in the regulated case, they are certainly well correlated.

Figure 2 shows that even in the fully regulated case, the steady-state value of SAM varies dramatically with methionine input (the methionine concentration, by contrast, is relatively stable). Since SAM supplies the methyl groups for the methylation reactions, one might expect that the DNA methylation reaction would be sensitive to methionine input. Contrary to this expectation, in the fully regulated case, the steady-state flux through the DNMT reaction is strikingly stable as methionine input decreases (Fig. 4, green curve). If, however, all the long-range interactions are removed, the flux through the DNMT reaction becomes extremely sensitive to methionine input (Fig. 4, red curve) and drops precipitously as methionine input declines. Thus, the long-range interactions effectively stabilize the methylation rate, particularly under conditions of methionine starvation. The underlying reason that the effect is largest at low METin is that the regulations of CBS, MTHFR, and GNMT have their strongest influence at low SAM concentrations (see the formulas in Appendix) that correspond to low methionine inputs. To see how strong this effect is, note that as methionine input drops from 100 $\mu\text{M/hr}$ to 10 $\mu\text{M/hr}$, [SAM] drops from 66 μM to 13 μM but the methylation rate drops only from 150 $\mu\text{M/hr}$ to 127 $\mu\text{M/hr}$. By contrast, at moderate and high methionine inputs the long-range

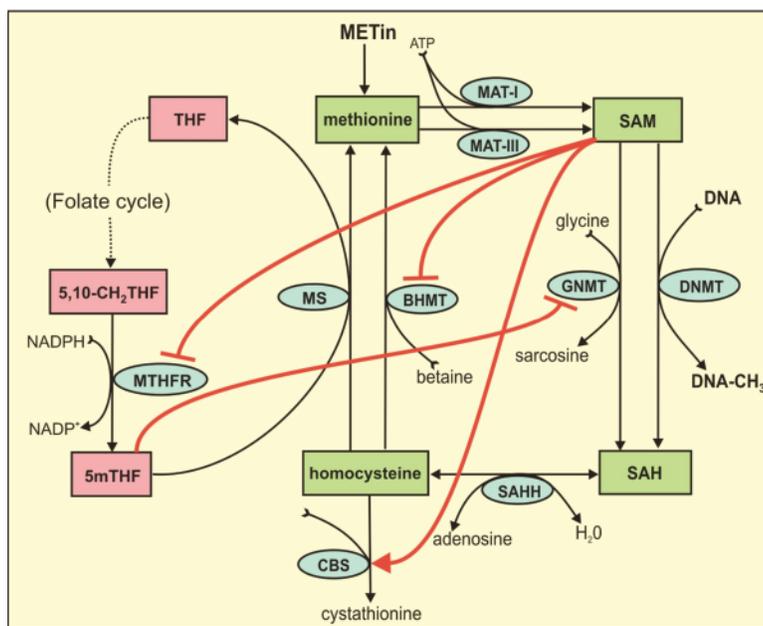


Figure 1. Long-range interactions of the methionine cycle. Substrates of the methionine cycle are in green boxes and those of the folate cycle in pink. Enzymes are in blue ellipses. Long-range interactions are shown in red; arrow indicates excitation and bars inhibition. The abbreviations used are: THF, tetrahydrofolate; SAM, S-adenosylmethionine; SAH, S-Adenosylhomocysteine; 5mTHF, 5-methyltetrahydrofolate; 5,10-CH₂-THF, 5,10-methylenetetrahydrofolate; SAHH, S-Adenosylhomocysteine hydrolase; BMT, Betaine:homocysteine methyltransferase; CBS, Cystathionine β -synthase; DNMT, DNA-methyltransferase; GNMT, Glycine N-methyltransferase; MAT, Methionine adenosyltransferase; METin, rate of methionine input; MS, Methionine synthase; MTHFR, 5,10-Methylenetetrahydrofolate reductase.

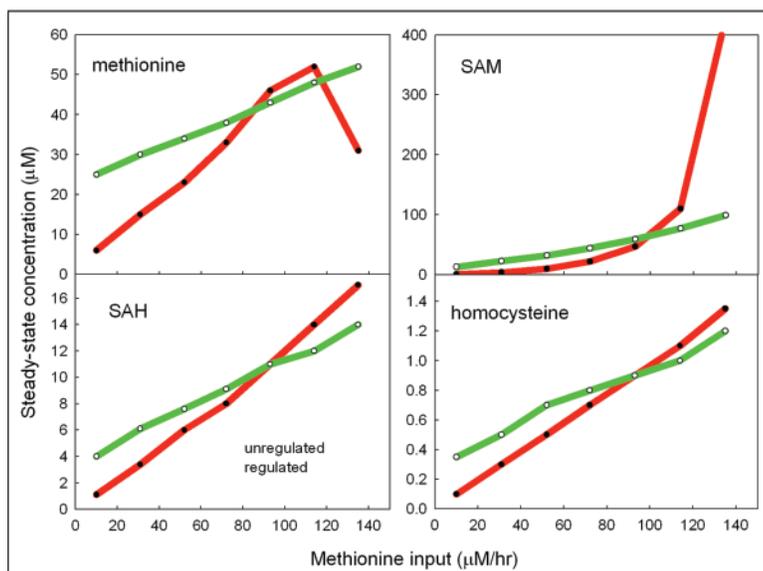


Figure 2. The steady-state concentrations MET, SAM, SAH and HCY as METin varies in the presence (green curves) and absence (red curves) of long-range interactions. The long-range interactions stabilize metabolite concentrations.

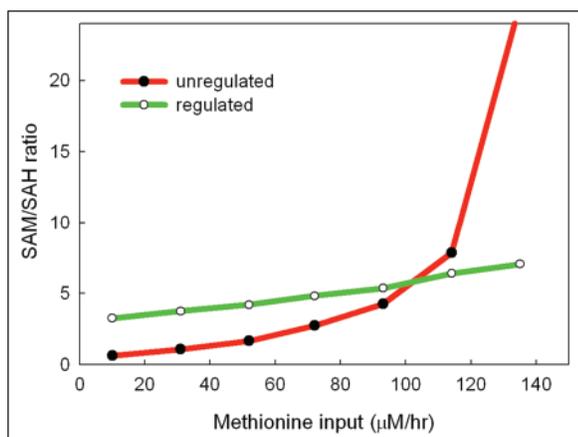


Figure 3. The [SAM]/[SAH] ratio as methionine input varies in the presence (green curve) and absence (red curve) of long-range interactions. The long-range interactions reduce the sensitivity of [SAM] to high methionine inputs and stabilize the [SAM]/[SAH] ratio.

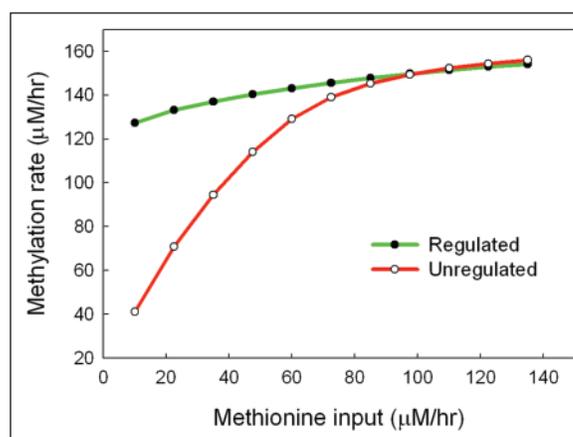


Figure 4. The rate of DNA methylation as a function of methionine input in the presence (green curve) and absence (red curve) of long-range interactions. The long-range interactions prevent the decline of methylation rate as methionine input falls.

Table 1 **Values for r for the regulated and unregulated methionine cycle, and in the presence and absence of the GNMT reaction**

	Regulated	Unregulated
GNMT	0.0072	0.088
No GNMT	0.057	0.15

interactions have little effect on methylation rate. This is because the K_m of the *DNMT* reaction is low and at moderate methionine input the reaction is already running at near saturation.

The results in Figure 4 are steady-state results; that is, for each fixed methionine input the system was allowed to relax to equilibrium and then methylation reaction velocity was calculated. In reality, methionine input never holds steady and, indeed, undergoes large fluctuations. So, another way to assess the stability of the DNA methylation rate is to determine its variation in response to such large fluctuations. Instead of using a constant methionine input, we used a continually varying random input with mean 100 $\mu\text{M/hr}$ and variance 900 (see Appendix), and calculated

$$r = \frac{\text{variance of DNA methylation rate}}{\text{variance of methionine input}}$$

The value of r tells us how much the methylation rate varies when methionine input varies. By calculating r under different circumstances, we can assess the contribution of different mechanisms to the stabilization of DNA methylation rate. The top row of Table 1 shows that when all four long-range regulations are in place, $r = 0.0072$, i.e., the rate of methylation is remarkably stable. Without any long-range regulations, $r = 0.088$. It has been proposed by Wagner et al.^{4,44} that the purpose of the GNMT reaction (in parallel to DNA methylation) is to buffer the DNA methylation rate against large swings in methionine input and [SAM]. We tested this experimentally by turning off the GNMT reaction in our model and recalculating. The bottom row of Table 1 shows that when all four long-range regulations are in place, $r = 0.057$, and without

long-range regulations, $r = 0.15$. The results presented in Table 1 demonstrate conclusively that both the long-range regulations, and the existence of the GNMT reaction, independently provide substantial stabilization of DNA methylation and both together improve the stability approximately 21-fold.

It is reasonable to assume that these long-range interactions did not all evolve simultaneously, but were added sequentially in such a way that each new interaction substantially improved fitness, which in this case we take to be correlated with the stabilization of the methylation rate. Within the mathematical model we can add the long-range interactions one at a time, and in any combination. For each case we can recompute the dependence of methylation rate on methionine input. We found that the regulation of BHMT has only a small effect on methylation rate. The reasons are twofold. First, the inhibition of BHMT only occurs at very high [SAM] (see the formula for V_{BHMT} in the Appendix) and therefore has little effect on methylation rate for low or moderate METin. Second, because the K_m of the DNMT reaction is low, the reaction runs near saturation at moderate to high levels of SAM and METin. Thus, the regulation of BHMT probably did not evolve for stabilization of methylation. The primary role of BHMT regulation appears to be the control of the fraction of homocysteine that is transsulfurated as proposed by Finkelstein and Martin,^{18,30} and as seen in reference 17.

We also found that the binding of 5mTHF to GNMT has only a small stabilizing effect on the DNMT reaction, so it is unlikely that the sole function of this long-range interaction is methylation stabilization. Since GNMT is exceedingly abundant in liver,⁴ the binding has been thought to be a storage mechanism for folate^{41,42} and a recent theoretical study³⁴ has shown that this kind of allosteric binding stabilizes the velocities in the folate cycle against fluctuations in total folate. Thus, although the presence of the GNMT reaction is important for stabilizing the DNMT reaction (see Table 1), the long-range interaction of 5mTHF with GNMT appears to be primarily concerned with the regulation of the folate cycle.

We found that both stimulation of CBS by SAM and the inhibition of MTHFR by SAM had major stabilizing effects on the DNMT rate. Figure 5 shows the effect of adding individually the long-range regulations of CBS (pink curve) and of MTHFR (blue curve) and adding both (green curve), with the regulations of

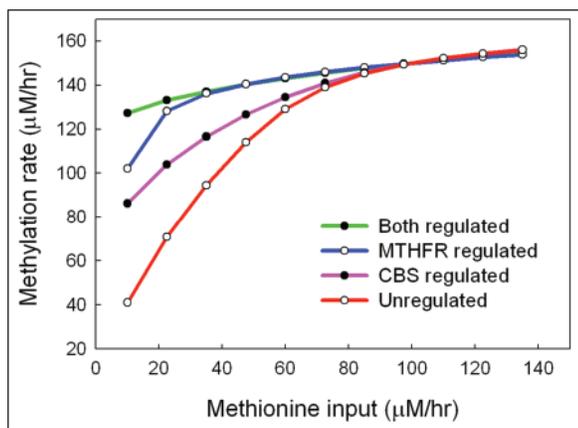


Figure 5. The rate of DNA methylation as a function of methionine input in the presence of various combinations of the long-range interactions. Red curve: the response in the absence of interactions. Pink curve: the effects of regulating CBS by SAM. Blue curve: the effect of regulating MTHFR by SAM. Green curve: the response in the presence of both CBS and MTHFR regulation. MTHFR regulation adds a substantial improvement in stabilizing the methylation rate if CBS regulation is already present, whereas CBS regulation adds only a marginal improvement if MTHFR regulation is already present. If the primary function of these regulatory interactions by SAM is to stabilize the methylation rate, then it is likely that CBS regulation evolved first.

GNMT and BHMT present in all three cases. Notice that if the CBS regulation had evolved first and was then followed by the MTHFR regulation, then each would have added substantially to stabilization. On the other hand, if the MTHFR regulation had evolved first and was then followed by the CBS regulation, then the second step would have provided only a minor benefit except for very low methionine input. Thus, in our view, it is likely that the CBS regulation evolved first followed by the regulation of MTHFR.

It has been thought that the methylation rate is also sensitive to the level of folate⁴³ because the concentration of 5mTHF affects the rate of remethylation of homocysteine to methionine via the MS reaction (Fig. 1). We used our model to test this idea. The results in Figure 6 show that high folate status is necessary to maintain the methylation rate at low and very low methionine inputs, but has only a minor effect when methionine input is moderate to high.

DISCUSSION

In this paper we have used a mathematical model to investigate the functions of four long range allosteric interactions that have been hypothesized to control the properties of the methionine cycle. We found that the inhibition of BHMT by SAM has little effect on methylation rate but controls of the fraction of homocysteine that is transsulfurated as proposed by Finkelstein and Martin.^{17,18,30} The binding of GNMT and 5mTHF also has only a small stabilizing effect on methylation rate. The primary role of this binding is probably to store folate^{41,42} and to stabilize the velocities in the folate cycle against fluctuations in total folate.³⁴ We found that both the stimulation of CBS by SAM and the inhibition of MTHFR by SAM dramatically stabilize the methylation rate against fluctuations in methionine input and [SAM]. We also found that, although regulation of GNMT activity by 5mTHF has only a small effect on methylation rate, the presence of the GNMT reaction strongly buffers the DNA methylation rate against large swings in methionine input and [SAM].

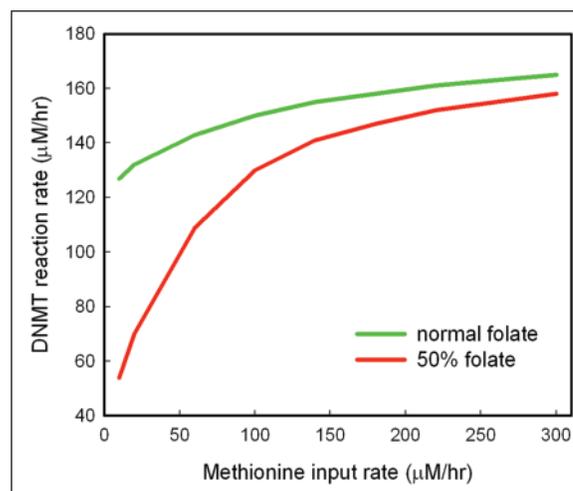


Figure 6. The effect of folate status on methylation rate. The normal and 50% levels of [5mTHF] are calculated by using formula (5) in the Appendix. The long-range interactions buffer methylation rate against declines in folate at low METin.

We recognize that no mathematical model can capture the full complexity of a biological system. First, there are substantial uncertainties and variations in measurements of kinetics because experimental data come from a diversity of tissues, organisms, and experimental procedures. Second, many of the substrates and enzymes participate in other reactions that are not in this system. Nevertheless, the model has allowed us to verify the effects of mechanisms proposed in the experimental literature and to quantify the relative magnitudes of the effects. Perhaps the greatest advantage of an explicit mathematical model is that it allows us to perform *in silico* experiments in which one or more regulations are removed, experiments that would be difficult or impossible to do *in vivo*.

The stabilizing effect of the long-range interactions on the DNA methylation rate can be explained as follows. As methionine input falls, SAM concentration declines, which has two effects. First, the decline in SAM reduces the activity of CBS so a larger fraction of homocysteine is remethylated, which tends to maintain the flux around the methionine cycle. Second, the decline in SAM releases the inhibition of MTHFR. This causes the concentration of 5mTHF to rise, which increases the inhibition of GNMT. Thus, even though the flux from SAM to SAH is lower, the inhibition of GNMT causes a larger fraction of the flux to be carried by DNMT. This second mechanism was originally hypothesized by Wagner, Briggs and Cook.⁴⁴ The mathematical model shows that each step in this relatively complicated causal chain is, indeed, correct, and that the combination of long-range interactions and the presence of the GNMT reaction are responsible for the stabilization of the DNA methylation rate.

DNA methylation, of course, depends not only on the DNMT kinetics and the availability of methyl groups, but also on the mechanisms that expose certain CpG islands to methylation. When kinetic information about these latter processes becomes available, the model can be extended to include the differential methylation of different genes.

It is interesting to note that the stabilizing effects of the long-range interactions on the methylation rate are particularly important at low methionine inputs and relatively unimportant at moderate and

high methionine inputs. Correspondingly, we found that folate status significantly affects methylation rate only at low methionine input (Fig. 6). Thus, the long-range regulatory mechanisms that we have described, as well as the connection between the folate cycle and the methionine cycle may well have evolved to protect methylation rates against periods of low and very low methionine input. Periods of protein starvation are common for some human populations today and must have been common for paleolithic humans, who are believed to have been primarily hunters and meat eaters.⁴⁵ These mechanisms have obvious benefits to any organism that is subject to repeated and prolonged periods of protein starvation and it would therefore be of great interest to discover when in the course of evolution each of these long-range biochemical interactions first arose.

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APPENDIX

The mathematical model consists of a set of four differential equations for the time dependence of the concentrations of MET, SAM, SAH and HCY, that express the rate of change of the concentrations in terms of the incoming and outgoing fluxes indicated in Figure 1. We denote each flux by V_X where X is the acronym for the corresponding enzyme and denote the methionine input, which may depend on time, by METin. Each term has units $\mu\text{M}/\text{hr}$.

$$\frac{d[\text{MET}]}{dt} = V_{\text{MS}} + V_{\text{BHMT}} + \text{METin} - V_{\text{MATI}} - V_{\text{MATIII}}, \quad (1)$$

$$\frac{d[\text{SAM}]}{dt} = V_{\text{MATI}} + V_{\text{MATIII}} - V_{\text{DNMT}} - V_{\text{GNMT}}, \quad (2)$$

$$\frac{d[\text{SAH}]}{dt} = V_{\text{DNMT}} + V_{\text{GNMT}} - V_{\text{SAHI}}, \quad (3)$$

$$\frac{d[\text{HCY}]}{dt} = V_{\text{SAHI}} - V_{\text{CBS}} - V_{\text{MS}} - V_{\text{BHMT}}, \quad (4)$$

The fluxes depend, of course, on the current values of one or more of the variables. This model is an extension of our previous model¹⁷ for the hepatic methionine cycle. That model reproduced and provided explanations for many well-known experimental and

Table 2 Values of the kinetic parameters used in the mathematical model

Enzyme	Parameter	Value [†]	Reference
MAT-I	V_{max}	260	[23]
	K_m	41	[23]
MAT-III	V_{max}	220	[24] [†]
	K_m	300	[24] [†]
	K_a	360	[23] [†]
DNMT	V_{max}	180	[25]
	K_m	1.4	[25]
	K_i	1.4	[25]
GNMT	V_{max}	160	[26] [†]
	K_m	63	[26] [†]
	K_i	18	[17]
SAHH	V_{max}	5,000	§
	K_m (forward)	10	§
	K_m (backward)	1	§
CBS	V_{max}	100,000	[19]
	K_m	1,000	[19]
MS	V_{max}	350	[17]
	K_m (5mTHF)	25	[17]
	K_m (Hcy)	0.1	[17]
BHMT	V_{max}	720	[17]
	K_m	12	[17]

[†]Units: V_{max} = $\mu\text{M/hr}$; K_m , K_a , K_i = μM ; [†]Obtained by nonlinear regression on the data in the reference.
[§]See text.

clinical observations, for example, the positive correlation of homocysteine levels with methionine input and its negative correlation with the level of 5mTHF. The model also confirmed the hypotheses of Finkelstein^{3,18,19} that SAM regulates the fraction of homocysteine that is transsulfurated to cystathionine. The current model builds on the previous one but incorporates more recent biochemical findings and includes the long-range inhibition of MTHFR by SAM and the inhibition of GNMT by 5mTHF. SAM is the methyl donor for most methyltransferases.²⁰ Other mathematical models of the methionine cycle have been developed in^{21,22} where the focus is primarily on the regulation of substrate concentrations.

Here we focus on DNA methyltransferase (DNMT). There are a large number of other methyltransferase reactions²⁰ that run in parallel to the DNMT reaction. Most methyltransferases have low K_m s for SAM and low K_i s for SAH and therefore the reactions they catalyze will behave similarly to what we describe here for DNMT. Had we included them all, the sum of their V_{max} s would add up to the V_{max} we use here for DNMT.

Kinetic equations and parameter values. For each enzyme, the K_m s are in μM and the V_{max} s and the velocities of the reactions, V , are in $\mu\text{M/hr}$. The values of the kinetic parameters used in the present model are given in Table 2.

The MAT-I kinetics are from,²³ and we take $V_{max} = 260$ and $K_m = 41$. The inhibition by SAM was derived by nonlinear regression on the data from Figure 5 in reference 23.

$$V_{\text{MAT-I}} = \left(\frac{V_{\text{max}}[\text{MET}]}{K_m + [\text{MET}]} \right) (0.23 + (0.8)e^{-0.0026[\text{SAM}]})$$

The methionine dependence of the MAT-III kinetics is from Figure 5 in reference 24, fitted to a Hill equation with $V_{max} = 220$, $K_m = 300$. The activation by SAM is from Figure 5 in reference 23, fitted to a Hill equation with $K_a = 360$.

$$V_{\text{MAT-III}} = \left(\frac{V_{\text{max}}[\text{MET}]^{1.21}}{K_m + [\text{MET}]^{1.21}} \right) \left(1 + \frac{7.2[\text{SAM}]^2}{(K_a)^2 + [\text{SAM}]^2} \right)$$

The DNA methylation reaction is given as a uni-reactant scheme with SAM as substrate. That is, the substrates for methylation are assumed constant. Their variation can be modeled by varying the V_{max} . The kinetic constants, $V_{max} = 180$, $K_m = 1.4$, and $K_i = 1.4$ are from reference 25.

$$V_{\text{DNMT}} = \frac{V_{\text{max}}[\text{SAM}]}{K_m(1 + \frac{[\text{SAH}]}{K_i}) + [\text{SAM}]}$$

The first term of the GNMT reaction is standard with $V_{max} = 160$, and $K_m = 63$ estimated from Figure 8 in reference 26. The second term is product inhibition by SAH from¹⁷ with $K_i = 18$. The third term, the long-range inhibition of GNMT by 5mTHF, was derived by nonlinear regression on the data of Figure 3 in reference 27, and scaled so that it equals 1 when the methionine input rate is 100 $\mu\text{M/hr}$.

$$V_{\text{GNMT}} = \left(\frac{V_{\text{max}}[\text{SAM}]}{K_m + [\text{SAM}]} \right) \left(\frac{1}{1 + \frac{[\text{SAH}]}{K_i}} \right) \left(\frac{4.38}{0.35 + [5\text{mTHF}]} \right)$$

The SAHH reaction is fast and reversible and we model it using standard Michaelis-Menten kinetics. The factor 5000 was chosen to make the reactions equilibrate rapidly and the $K_{m,\text{SAH}} = 10$ for SAH and $K_{m,\text{Hcy}} = 1$ for HCY were chosen so that the ratio of $[\text{SAH}]$ to $[\text{HCY}]$ is about 10:1, as observed experimentally.

$$V_{\text{SAHH}} = \left(\frac{5000[\text{SAH}]}{K_{m,\text{SAH}} + [\text{SAH}]} \right) - \left(\frac{5000[\text{HCY}]}{K_{m,\text{Hcy}} + [\text{HCY}]} \right)$$

The kinetics of CBS are standard Michaelis-Menten with $K_m = 1000$ taken from reference 19 and $V_{max} = 100000$. The form of the activation of CBS by SAM was derived by nonlinear regression on the data in references 28 and 29 and scaled so that it equals 1 when the methionine input rate is 100 $\mu\text{M/hr}$.

$$V_{\text{CBS}} = \left(\frac{V_{\text{max}}[\text{Hcy}]}{K_m + [\text{Hcy}]} \right) \left(\frac{1.15([\text{SAM}] + [\text{SAH}])^2}{30^2 + ([\text{SAM}] + [\text{SAH}])^2} \right)$$

The kinetics of MS, with $V_{max} = 350$, $K_{m,5\text{mTHF}} = 25$, $K_{m,\text{Hcy}} = 0.1$, and references are as in reference 17.

$$V_{\text{MS}} = V_{\text{max}} \left(\frac{[\text{Hcy}]}{K_{m,\text{Hcy}} + [\text{Hcy}]} \right) \left(\frac{[5\text{mTHF}]}{K_{m,5\text{mTHF}} + [5\text{mTHF}]} \right)$$

The kinetics of BHMT are taken from reference 17 with the parameters $K_m = 12$ and $V_{max} = 720$. The form of the inhibition of BHMT by SAM was derived by nonlinear regression on the data of reference 30 and scaled so that it equals 1 when the methionine input rate is 100 $\mu\text{M/hr}$.

$$V_{\text{BHMT}} = e^{-0.0021([\text{SAM}] + [\text{SAH}])} \frac{V_{\text{max}}[\text{Hcy}]}{K_m + [\text{Hcy}]}$$

Scaling of the long-range interactions. Note that each of the long-range interactions appears in a kinetic formula as a multiplicative factor. In order to compare the effect of the long-range interactions as methionine input changes we scale the factors so that they equal

one when methionine input is 100 $\mu\text{M/hr}$, which we take to be normal average hourly intake.³¹ This gives us a baseline that enables us to compare the effects of each long-range interaction as methionine input varies above and below its normal value.

Derivation of the dependence of [5mTHF] on [SAM]. The inhibition of MTHFR by SAM was derived by non-linear regression on the data of references 32 and 33 and has the form $10/(10+[SAM])$. In addition, SAH competes with SAM for binding to the regulatory domain of MTHFR. It neither activates nor inhibits the enzyme³³ but prevents inhibition by SAM; thus, we take our inhibitory factor to be

$$I = \frac{10}{10+[SAM]-[SAH]},$$

except when $[SAH] > [SAM]$, in which case we take $I = 1$. The formula for the dependence of [5mTHF] on [SAM] is derived as follows. We start with the differential equation for [5mTHF] from reference 34:

$$\frac{d[5mTHF]}{dt} = 4200 \left(\frac{[5,10\text{-CH}_2\text{-THF}]}{50+[5,10\text{-CH}_2\text{-THF}]} \right) \left(\frac{[NADPH]}{16+[NADPH]} \right) I - 350 \left(\frac{[5mTHF]}{25+[5mTHF]} \right) \left(\frac{[HCY]}{0.1+[HCY]} \right).$$

We assume typical values for kinetic constants and the concentrations of NADPH = 50 μM , 5,10- CH_2 -THF = 1 μM , and HCY = 1 μM (see ref. 34). Using these values and supposing that the reaction is at steady-state (so the right hand side equals zero), one can derive the general form for the dependence of [5mTHF] as a function of I. Scaling so that at METin = 100 $\mu\text{M/hr}$ the “normal” value of [5mTHF] is approximately 4 μM (see ref. 34), yields:

$$[5mTHF] = \frac{\frac{250}{10+[SAM]-[SAH]}}{1 - \frac{2}{10+[SAM]-[SAH]}}. \quad (5)$$

Fluctuation theory. To study the behavior of the methionine cycle under large fluctuations in methionine input we add to the “normal” input of 100 $\mu\text{M/hr}$ an Ornstein-Uhlenbeck process of mean zero and variance 900.³⁵ This means that the methionine input is continually changing in time with mean 100 $\mu\text{M/hr}$ and standard deviation 30. For simpler systems, it can be proven that after the system has run for a very long time the joint distribution of the concentrations converges to a unique distribution on \mathbb{R}^4 independent of t .³⁶ To compute this joint distribution approximately, we ran the methionine cycle with the stochastic input 80,000 times evaluating the concentrations at a fixed large time T . We found that repeating the process and/or changing T always gave the same distribution so that we were sure the distribution of the concentration vector had become stationary, i.e., independent of T . Each of the concentrations of MET, SAM, SAH and HCY has a distribution that is a marginal distribution of this joint distribution. Since we have explicit formulas for the rates of the various reactions in terms of the concentrations and because the distribution for each of the concentrations of MET, SAM, SAH and HCY can be computed from the joint distribution, we can compute the means and variances of each of the reaction rates.

It is important to note that driving the biochemical system with large-scale fluctuations is different from the methods of biochemical control theory.³⁷⁻³⁹ In that theory one takes a system at a fixed steady-state, makes a small perturbation in a parameter (perhaps an input), and allows the system to relax to a new steady-state. By

comparing the new value of a variable (a concentration or flux) to the old value, one computes the percentage change of the variable per unit percentage change in the parameter. Technically, one is computing a partial derivative. This kind of sensitivity analysis gives good information about local, linearized behavior near the initial steady-state. By contrast, we are concerned here with responses to large-scale continuous fluctuations in inputs. Technically, this means computing the variances of the distribution of each concentration or flux. This fluctuation theory gives different answers from biochemical control theory and the differences are biologically significant.