Short paper

A day in the life of cell metabolism

H. Frederik Nijhout*,$, Michael C. Reed** and Cornelia M. Ulrich***

*Department of Biology, Duke University, Durham, NC 27708, USA
**Department of Mathematics, Duke University, Durham, NC 27708, USA
*** Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle WA 98109, USA

$ Corresponding author
e-mail: hfn@duke.edu

Biological systems are subject to genetic and environmental variation, much of it potentially deleterious such as a partial loss of function of a gene, an insufficient supply of nutrients, or extremes of physical factors such as temperature. Most systems have evolved mechanisms that minimize the effect of such variation on the normal function of the system. The best known of these are the many physiological homeostatic mechanisms that actively maintain a particular state and that bring the system back to that state after perturbation. More recently there has been an interest in understanding the robustness in genetic regulatory mechanisms that ensure normal temporal and spatial patterns of gene expression in the face of mutations in genes and variation in the connectivity of genetic networks. Stability and diminished sensitivity to perturbations in genetic networks can be accomplished by feedback and feed-forward loops, and by mechanisms akin to cooperativity that result in highly non-linear responses of gene transcription to regulatory inputs.

The mechanisms that confer stability or robustness are essentially of two types: passive mechanism where stability is a consequence of the particular structure and connectivity of the system, and active mechanisms where specific processes are stabilized by active and responsive changes in others. At the molecular extreme, robust gene regulatory systems are typically thought to belong mostly if not entirely to the former. At the organismal extreme, physiological homeostatic mechanisms are all examples of the latter. Metabolism and development occupy a middle ground between these two extremes. This is because metabolism and development involve molecular and genetic regulatory mechanisms that have to deal with both genetic and environmental variation. Genetic variation is a feature of populations; each individual in a population is genotypically static, and adaptation in the genetic sense is an evolutionary process. Environmental variation, by contrast, affects the individual and requires adaptive responses that vary from moment to moment. The response to varying environmental contingencies can involve changes in gene expression as well as changes in the activity of proteins by allosteric regulation or covalent modification (such as phosphorylation and methylation).

The nature of stabilizing mechanisms in development is still poorly understood. Developmental systems are in a continual state of change and it is the pattern of this change that is high stable and reproducible form individual to individual, in spite of genetic diversity and environmental variation. Metabolic systems, by contrast are typically assumed to be at some stable steady-state. The implicit assumption of most studies in metabolism, biochemistry and enzyme kinetics, is that biochemical systems reach equilibrium rapidly and are typically at steady-state. Much of enzyme kinetic and metabolic control theory is based on these assumptions.

Mechanisms that stabilize the steady-state are, by definition, mechanisms that make the system robust to perturbation. It is one of the principal aims of metabolic control analysis to describe the relative sensitivities of fluxes and metabolite concentrations to perturbations at various points in a metabolic pathway. This kind of sensitivity analysis involves letting the system relax to a steady-state and performing a small perturbation on one parameter, letting the system come to the new steady state and measuring how much the variables of the system have changed. Essentially one is taking a first partial derivative for each variable, at the original steady-state. The general properties that make metabolic systems robust to this kind of perturbation around the steady state have been extensively studied (Kacser and Burns, 1973; Heinrich and Rapoport 1974; Fell, 1992; Bagheri-Chaichian et al., 2003).

So it is natural to ask whether the steady-state represents some kind of evolved stability or robustness. If it does, then it is a stability of the first type, mentioned above. We have been studying this problem in a relatively complex metabolic network: one-carbon metabolism (Figure 1). One-carbon metabolism consists of a set of interlinked cycles. A relatively simple methionine cycle linked to a much more complex folate cycle, and the folate cycle is partially compartmentalized between the cytosol and mitochondria of a cell. One-carbon metabolism is involved in the synthesis of purines and pyrimidines, and in DNA and histone methylation, and in the synthesis of cysteine and reduced glutathione (from cystathionine). Defects in one-carbon metabolism results in defective DNA synthesis and repair, altered gene regulation, and increased sensitivity to oxidative stress. Accordingly, defects in one-carbon metabolism due to genetic mutations or insufficient dietary input are associated with a broad array of pathologies ranging form megaloblastic anemia, neural tube defects, cardiovascular and psychological disorders, and various kinds of cancer (Bailey, 1995; Choi and Mason, 2000; Lucock, 2000; Ulrich 2005). The inputs into the cycle are the amino acids methionine, serine and glycine. In addition, folate and vitamins B_6 and B_{12} act as cofactors for many of the enzymes, but their half-life in the cell is very long (3-5 months), so for our present purposes we will consider them constant. The input of amino acids varies over time with meals. It takes 4-6 hours for this system to come to a new steady state after an abrupt change in amino acid input, and this implies that under a normal dietary regime, the system is never at steady-state.

We have developed a mathematical model of one-carbon metabolism that accurately reproduces a broad diversity of experimental and clinical findings, and that has been able to make predictions that have been confirmed experimentally (Nijhout et al., 2004, 2006, 2007; Reed et al., 2004, 2006). In this model we can vary the input of amino acids in a

temporal pattern that resembles that obtained under a dietary regime of three meals a day, and calculate the time-course of fluxes and concentrations of metabolites in the network. Figure 2 shows the patterns of variation of selected fluxes and metabolite concentrations. The striking feature of Figure 2 is that some metabolites and fluxes remain constant, while others fluctuate enormously (the mitochondrial SHMT reaction even reverses direction periodically). Thus the behavior of the system in the course of a day is a curious mixture of great variation and great stability.

Interestingly, the fluxes that remain constant are exactly those that are generally thought to be critical for the various functions of one carbon metabolism such as export of one-carbon units as formate from the mitochondria, the thymidylate synthase (TS) and AICART reactions, and the DNA methylation (DNMT) reactions. This stability is achieved by dynamic changes in the velocities and concentrations of other reactions and substrates, respectively (Nijhout et al., 2006, 2007; Reed et al., 2004). Thus even though in the course of a day the system is never at steady-state, it maintains excellent stability of selected reactions, by a process that resembles physiological homeostasis.

In one-carbon metabolism there is a complex network of allosteric interaction of enzymes by substrates that occur at some distance in the pathway (e.g. SAM is an allosteric regulator of CBS and MTHFR, and 5mTHF is an allosteric regulator of GNMT). Simulations with our mathematical model have shown that these "long-range interactions" are critical for the maintenance of homeostasis (Nijhout et al., 2006, 2007; Reed et al., 2004), and we believe these allosteric interactions evolved for that function. The implication of our findings is that this system is probably never at steady-state, and many of its reactions are far from steady-state most of the time. Thus classical sensitivity analysis that relies on perturbation of the steady-state will not give useful insights into the behavior of this system. Instead, what is of interest is to know how fluctuations in input propagate though this system, and the properties that dampen such perturbations. A theoretical framework for such a fluctuation analysis has recently been developed (Anderson et al., 2007).

Acknowledgements

We would like to thank Marian Neuhouser, Jesse F. Gregory, Barry Shane and Jill James for their advice during the development of the mathematical model of folate-mediated one-carbon metabolism. This work was supported by grant DMS-0616710 from the National Science Foundation, and grant RO1 CA 105437 from the National Institutes of Health.

References

Anderson, D.A., Mattingly, J.C., Nijhout, H.F. and Reed, M.C. (2007). Propagation of fluctuations in biochemical systems, I: linear SSC networks. *Bulletin of Mathematical Biology* (in press).

Bagheri-Chaichian H., Hermisson J., Vaisnys J.R. and Wagner G.P. (2003). Effects of epistasis on phenotypic robustness in metabolic pathway. *Mathematical Biosciences* 184: 27-51.

Bailey, L.B. (1995). *Folate in Health and Disease* New York, NY: Marcel Dekker.

Choi, S.W. and Mason, J.B. (2000). Folate and carcinogenesis: an integrated scheme. *Journal of Nutrition* 130: 129-132.

Fell, D.A. (1992) Metabolic control analysis: a survey of its theoretical and experimental background. *Biochemical Journal* 286: 313–330.

Heinrich, R. and Rapoport, T. (1974). A linear steady-state treatment of enzymatic chains. General properties, control and effector strength. *European Journal of Biochemistry* 42: 89–95.

Kacser, H. and Burns, J.A. (1973). The control of flux. *Symposia of the Society for Experimental Biology* 27: 65–104.

Lucock, M. (2000). Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Molecular Genetics and Metabolism* 71: 121-138.

Nijhout, H.F., Reed, M.C., Anderson, D.F., Mattingly, J.C., James, S.J. and Ulrich, C.M. (2006). Long-range allosteric interactions between the folate and methionine cycles stabilize DNA methylation reaction rate. *Epigenetics* 1: 81-87.

Nijhout, H.F., Reed, M.C., Budu, P. and Ulrich, C.M. (2004). A mathematical model of the folate cycle. *Journal of Biological Chemistry* 279: 55008-55016.

Nijhout, H.F., Reed, M.C., Lam, S.-L., Shane, B., Jesse F Gregory, J.F. and Ulrich, C.M. (2007). In silico experimentation with a model of hepatic mitochondrial folate metabolism. *Theoretical Biology and Medical Modelling* 3:40.

Reed, M.C., Nijhout, H.F., Neuhouser, M.L., Gregory, J.F., Shane, B., James, S.J., Boynton, A. and Ulrich, C.M. (2006). A mathematical model gives insights into nutritional and genetic aspects of folate-mediated one-carbon metabolism. *Journal of Nutrition* 136: 2653-2661.

Reed, M.C., Nijhout, H.F., Sparks, R. and Ulrich, C.M. (2004). A mathematical model of the methionine cycle. *Journal of Theoretical Biology* 226: 33-43.

Ulrich, C.M. (2005). Nutrigenetics in cancer research – Folate metabolism and colorectal cancer. *Journal of Nutrition* 135: 2698-2702.

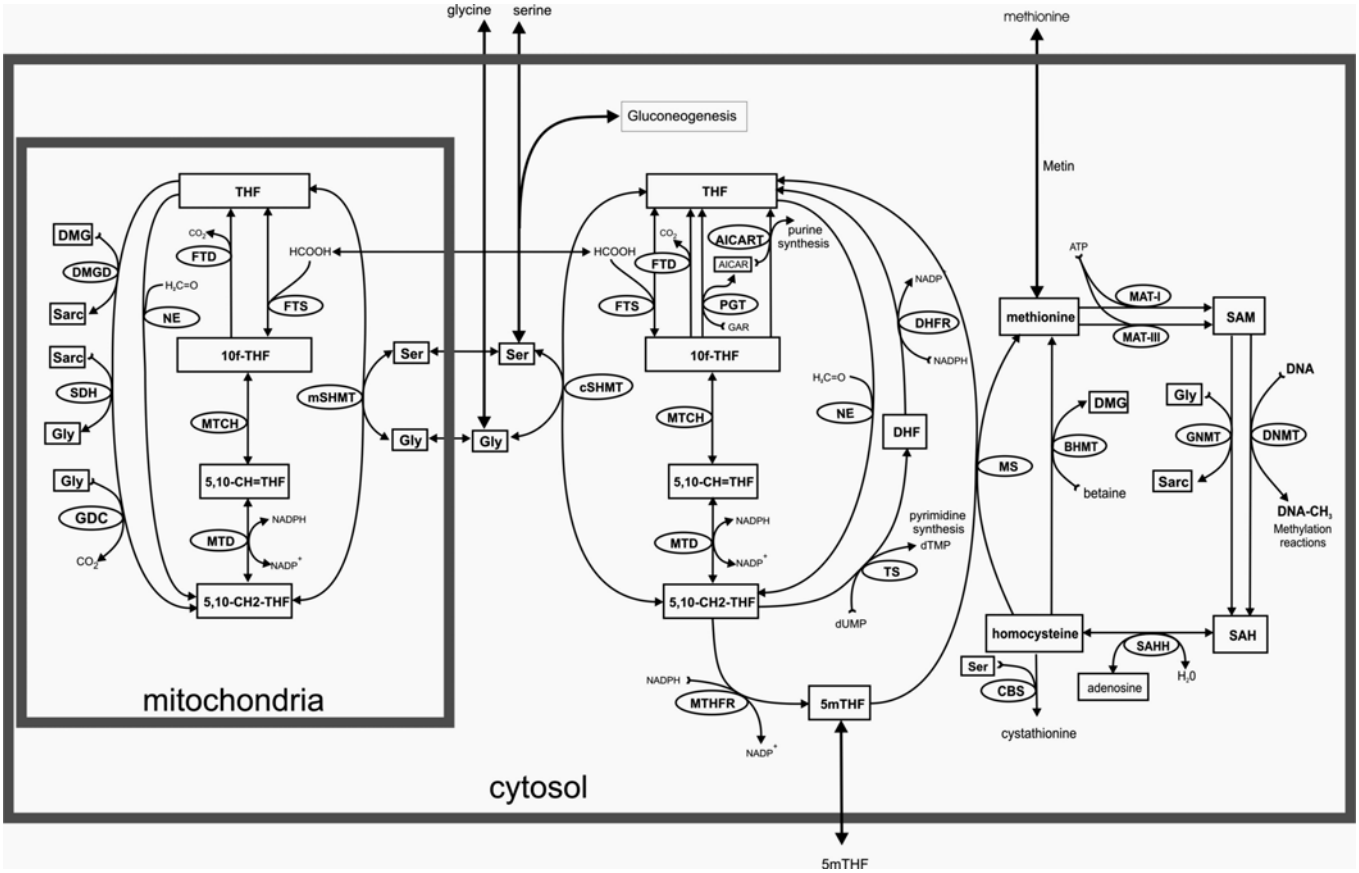

Figure 1. The reactions of one-carbon metabolism. The mathematical model of this system that was used to create the data in Figure 2 is described in Nijhout et al. (2007).

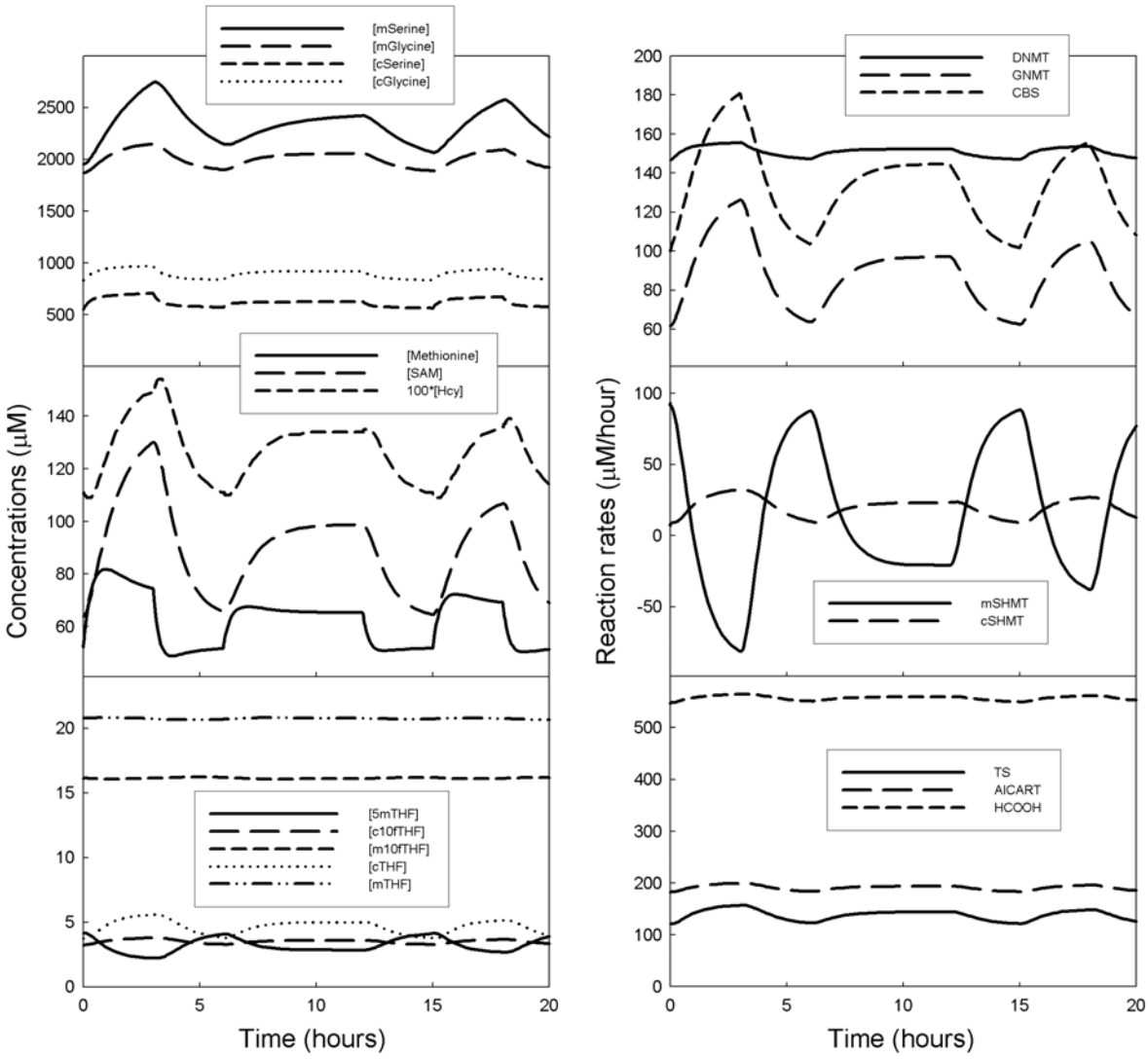

Figure 2. Fluctuations in the substrate concentrations (left column) and reaction velocities (right column) of one-carbon metabolism over a 20 hour period under simulated periodic input of glycine, serine and methionine (mimicking 3 meals). Reaction rates of selected enzymes are indicated by their acronyms from Figure 1. Mitochondrial and cytosolic SHMT reactions are indicted by the m and c prefix, respectively. The rate of formate export from the mitochondria is given by HCOOH in the lower panel of the right column.