Minireview

Metabolic control analysis: biological applications and insights

Mary C Wildermuth

Address: Department of Molecular Biology, Massachusetts General Hospital, 50 Blossom Street, Boston, MA 02114, USA.
E-mail: wildermu@genetics.mgh.harvard.edu

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Abstract

Metabolic control analysis provides a robust mathematical and theoretical framework for describing metabolic and signaling pathways and networks, and for quantifying the controls over these processes. Its application has already shed light on some of the principles underlying the regulation of metabolic pathways, and it is well suited to the analysis of the types of data emerging from genomic studies.

Modeling biological processes and systems

Mathematical modeling allows us to examine an event, process or system that we are unable to observe or understand directly because of its timing, magnitude, location or complexity. Models enable us to view a process or system at different organizational levels (for example, molecular or organismal) 'simultaneously', and to test responses of the system and its components to perturbations. Even incomplete or limited models can pinpoint missing or incorrect pathways or components and can help ascertain the relative importance of pathways and components in different scenarios. Models can also elucidate underlying biological design principles, sometimes challenging existing scientific dogma. They do this by extending and integrating the effects of assumptions made at one organizational level to others, and by allowing the 'visualization' of hypothetical scenarios. The term model is used broadly for the purposes of this article, and is defined as any mathematical or theoretical framework used to describe a component, process or system. This description can take many forms and make use of a variety of mathematical techniques (reviewed in [1]).

To describe biological systems, which are naturally complex and integrated, properties at different organizational levels must be related to each other in a meaningful way. Thus, the properties of a system ('systems properties') must reflect underlying molecular design principles, and equations detailing molecular components must take into account systems-level constraints and contexts. One such constraint is capacity, the maximum allowable flux. The system, as an entity, is not just an assemblage of its individual parts but has 'emergent' properties of the whole [2]. For a simple example, imagine a rubber ball that is cut into many small pieces. Much can be learned about the properties of the ball based on the individual cut pieces (for example, its elasticity), but we wouldn't know that the ball could roll.

Various modeling approaches have been used to study cellular metabolism and signaling (for reviews, see [3-7]). For the analysis of metabolism, such approaches include kinetic simulation, metabolic control analysis [8], biochemical systems theory [9,10], metabolic pathway analysis [11,12], and network analysis [6,13]. Signal transduction pathways and networks have, for the most part, been described qualitatively by the sets of expressed genes associated with the activation of a specific pathway [14,15]. Signaling has been modeled, however, for certain well-defined systems using detailed kinetics and neural-network-type approaches [2,16]. In addition, Krauss and Brand [17] have recently applied metabolic control analysis to signal transduction pathways. A description of the specific focuses and features of the above methods is not possible here. However, they can generally be grouped according to the organizational level(s) they describe and the type of data utilized. This article focuses on metabolic control analysis, a method that integrates 'local' kinetic information with systems-level information to quantitate proportions of control exerted by different components of a given pathway or system. The aim of this review is to provide the reader with the basic framework for understanding how (and why) metabolic control analysis can

be used to examine specific systems and to elucidate fundamental underlying biological design principles, which are independent of a particular system.

Metabolic control analysis

Theory

Metabolic control analysis provides a robust mathematical and theoretical framework for describing metabolic and signaling pathways and networks, and for quantifying the controls over these processes. It can deal with systems of any complexity or architecture and does not require all system components to be known *a priori*, making it a valuable postgenomic tool. It was developed in the 1970s by Kacser and Burns [18] and Heinrich and Rapoport [19]. Since then, dedicated researchers have expanded and advanced metabolic control analysis theory and applications, carefully defined the associated terms, and developed analytical and educational tools [20-25].

Metabolic control analysis uses equations based on the kinetics of enzymes (known as elasticity coefficients) to parameterize control coefficients (which describe the degree of control exerted by any given component on a particular output) resulting in response coefficients. The response to a perturbation is quantified by the summation of the response coefficients affecting the output of interest, which depends on both the control coefficients (systems properties) and elasticity coefficients (local properties). The fundamental equations are presented in Box 1 (also see Figure 1). It should be noted, explicitly, that the use of metabolic control analysis is not limited to linear pathways, but is also applicable to branching and cyclic pathways, and enzyme cascades.

Box 1

Metabolic control analysis uses control coefficients, elasticity coefficients, and response coefficients to quantify responses to perturbations. The control and elasticity coefficients are scale-less terms of the form given in Equation 1. Control coefficients (C) define the degree of control that each step in a pathway has on system variables such as flux or metabolite concentration. The control coefficient (C^A_i) of Equation 1 describes the strength of the response in variable A (e.g. flux), to a change in the steady-state rate (ν_i) of step i.

$$C^A_{\nu_i} = \frac{\delta A}{\delta \nu_i} \cdot \frac{\nu_i}{A} = \frac{\delta \ln A}{\delta \ln \nu_i} \qquad \text{where } \sum C^A_{\nu} = 1 \tag{1}$$

As illustrated in Figure 1, the flux control coefficient may range from 1 (complete control over a pathway) to 0 (no control). For a given flux J_n, the sum of the control coefficients for all enzymes affecting J_n must equal one. Thus control coefficients are systems properties and are defined in the context and constraints of the system.

Elasticity coefficients (ε) define the sensitivity of an isolated (that is, 'local') enzyme's reaction rate (under the same conditions as the system) to changes in a reaction parameter such as substrate concentration. The elasticity coefficient is derived from the kinetics of a given enzyme and often reflects the fractional change in enzyme rate associated with a fractional change in substrate concentration.

The connectivity theorems relate the systems properties of the pathway (C) to the local properties of an individual enzyme's kinetics (ε) through a common intermediate metabolite (M). They describe how metabolic perturbations propagate through the chain of enzymes comprising a metabolic network. Equation 2 details the connectivity relationship - where A may be flux or metabolite concentration.

$$\sum_i C^A_i \varepsilon^i_{[M]} = 0 \qquad \text{where } A \neq [M] \tag{2}$$

In addition to quantifying the control each step of a pathway exerts on a system variable (for example, flux), metabolic control analysis allows us to quantify the response to an external perturbation using the partitioned response coefficient (R). As shown in Equation 3, an external effector (X) such as an inhibitor would affect the rate of some enzymes in the pathway as quantified by enzyme elasticity coefficients. However, as these rates change, so do the system variables, as quantified by the control coefficient. The partitioned response coefficient therefore quantifies this change in the system variable (A) as the sum of the effects through all the enzymes (*i*) affected by the external effector (X), as shown in Equation 3.

$$R^A_X = \sum_i C^A_i \varepsilon^i_{[X]} \tag{3}$$

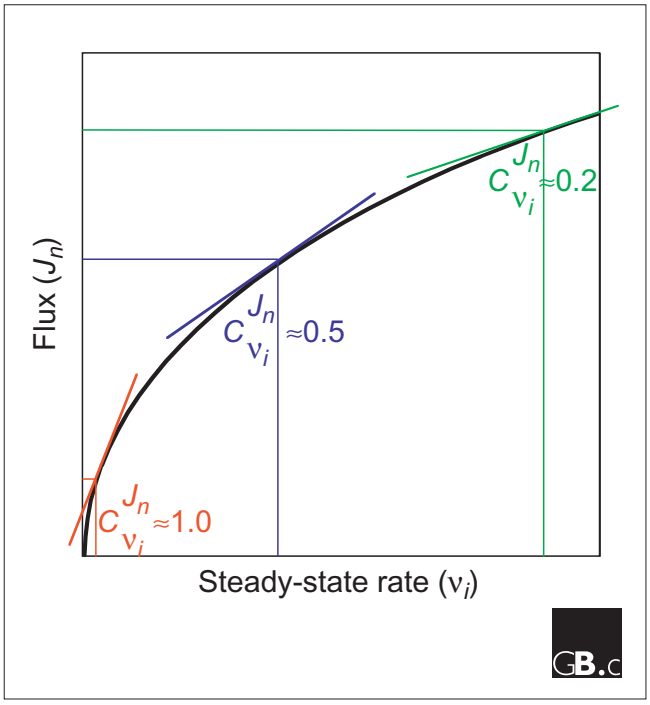

Figure 1
Flux control coefficients (C) for typical variations in pathway flux (J) measured at step n with a steady-state rate (v) at step i of a pathway. The coefficients are equal to the slope of the tangent to the curve (shown) multiplied by the scaling term v_i/J_n. This figure is adapted from [6].

Application to a specific system

Metabolic control analysis can be used to quantitate control exerted by different components of a specific system and pinpoint areas requiring further experimentation. For example, Krauss and Brand [17] recently used metabolic control analysis to quantitate the contributions of known and unknown signal transduction pathways in the early response of thymocytes to mitogen (concanavalin A, ConA) stimulation. To apply metabolic control analysis to a complex system containing both metabolic and signaling components, they gathered large parts of metabolism or signal transduction into 'black-box' groups of reactions, coupled signal transduction events to cellular variables, and limited the time frame of observation. In particular, thymocyte response to Con A stimulation was quantified by measuring steady-state respiration rates, and signaling routes (such as protein kinase C (PKC)) were grouped based on their sensitivity to specific inhibitors. The effects of these known and unknown signal transduction pathways on the mitochondrial membrane potential, a key intermediate in respiration, were also quantified. The analysis of the model system, presented in Figure 2, resulted in a quantitative topology of signaling routes involved in the early phase of mitogen stimulation of thymocytes. Novel findings, such as the significant role played by calcineurin signal transduction pathways (30% of total), highlight areas for future experimental work.

Elucidation of underlying biological design principles

The application of metabolic control analysis has altered our basic understanding of metabolic control. In particular, the belief that control over a pathway is dictated by a 'rate-limiting step' is now obsolete and is being removed from the biochemistry textbooks. Instead, it is replaced by the concept of shared control, where many - or theoretically all - enzymes in a pathway have a role in controlling the flux through the

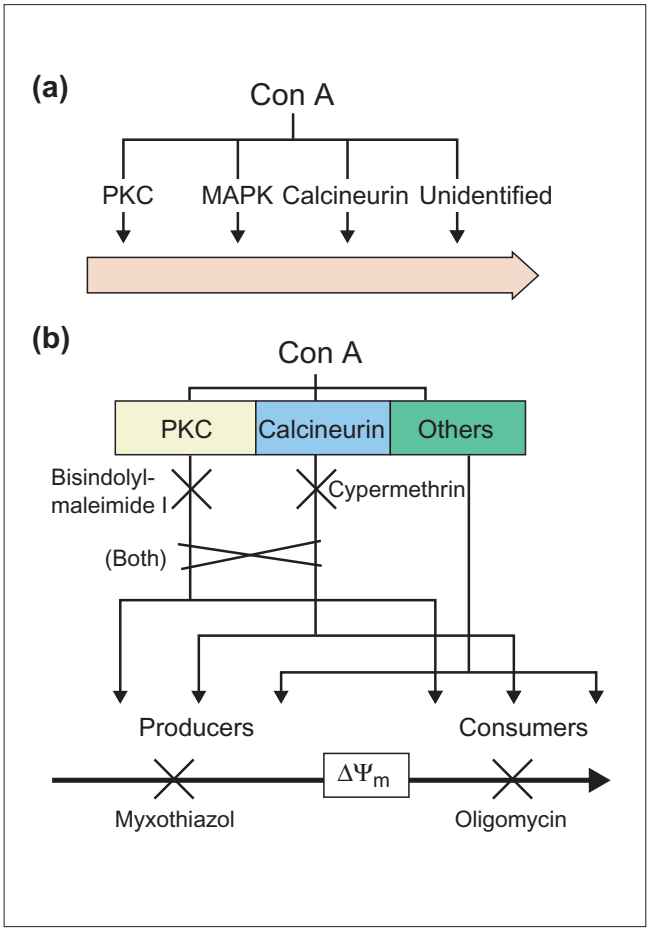

Figure 2
The model systems analyzed in [17], reproduced with permission. **(a)** Mitogen stimulation of thymocyte respiration is depicted as a single open arrow. Respiration is targeted by the mitogen Con A via a number of signal transduction pathways. Analysis of the system should allow one to establish a topology of signal routes and to weight the arrows extending through the signal transduction intermediates (PKC, MAPK, calcineurin and the unidentified pathways). **(b)** The system depicted in (a) in a modified form. Fewer signal transduction pathways are considered, but their interaction is studied with respect to two blocks of reactions that participate in respiration: the producers and the consumers of the mitochondrial membrane potential ($\Delta\Psi_m$). The responses of these target pathways to Con A via the signal transduction pathways considered can be determined using specific inhibitors of signal transduction (bisindolylmaleimide I and cypermethrin), electron transport (myxothiazol) and ATP synthesis (oligomycin).

pathway. Implicit in this is the idea that the regulation of cell metabolism requires coordinate change in the activities of many enzymes ('multisite modulation' [26]). The validity of this notion has been supported by bioengineers' lack of success in increasing a particular flux (product yield) by overexpressing the 'rate-limiting' enzyme and success by overexpressing a group of enzymes in a pathway. For example, Niederberger *et al.* [27] found that overexpression of four of the five enzymes in the yeast biosynthetic pathway leading from chorismate to tryptophan was required to significantly increase (more than eightfold) the production of tryptophan.

If the coordinated expression of enzymes in a pathway is required to significantly increase its flux, this should be an underlying design principle of organisms. In fact, the coordinated induction of enzymes to increase metabolic flux through a pathway has long been observed *in vivo*. One of many examples provided by Fell [21] is the urea synthetic pathway. The rate of urea synthesis in rats responds proportionately to the amount of protein in the diet. When rats are fed on diets that increase urea output fourfold, eight of the enzymes measured increased significantly, including all four of the urea-cycle enzymes [28]. With DNA microarrays and complete genome information, global expression data detailing the coordinated induction of pathway enzymes may be coupled with structural information on the organization of genes for pathway enzymes in operons or in clusters with common *cis*-acting elements (for example, see [29]). By performing these types of analyses on organisms responding to a variety of external effectors (such as nutrient conditions, pathogens, and so on), and on diverse organisms, this underlying design principle may be further explored.

Metabolic control analysis can also be used to explain why most mutations in diploid organisms are 'fully' recessive. Most enzymes have low-flux control coefficients; thus, a 50% reduction in enzyme concentration resulting from a null mutation in one allele of a diploid pair has little effect on the pathway flux. In addition, because pathway flux is a systems property, the influence of an alteration at one locus is measured in the whole system, minimizing the impact from any one reduction. Kacser and Burns [30] therefore posited the phenomenon of genetic dominance as the "inevitable consequence of the kinetic structure of enzyme networks" and not a result of natural selection. This conclusion was supported by Orr [31], who found the same extent of recessive mutations in artificial diploids created from the haploid organism *Chlamydomonas reinhardtii* (where the possibility of selection in the diploid was eliminated). The existence of a limited number of 'partially' recessive mutants, in which the heterozygote has an intermediate phenotype, is also consistent with metabolic control analysis. Theoretically, these enzymes (with high control coefficients) would be more likely to be a part of a very small pathway or the first enzyme of a branching pathway. Despite these studies, the 'inevitability' of dominance is still debated [32]. With the availability of complete genome information for a number of diploid organisms, genomic information on natural variants (for example, the *Arabidopsis* ecotypes Columbia and Landsberg [33]), and numerous collections of mutants, this type of question can now be addressed on a global scale.

Future directions and challenges

As illustrated above, metabolic control analysis is particularly useful for describing the theoretical aspects of regulation. This utility will continue to expand in the post-genomic era, particularly with advances in the *in vivo* imaging and quantitation of proteins and metabolites (for example, using tracer nuclear magnetic resonance). Future modeling efforts will require the integration or sampling of current mathematical approaches, including metabolic control analysis, as well as the development of new theoretical approaches and tools. As models become more complex and integrated to reflect the sheer volume of simultaneously occurring reactions in a cell, the incorporation of Monte Carlo methods (random sampling) and finite element analysis (approximations based on subdivision into smaller, more manageable elements) is likely to be necessary. In addition, the platforms and databases required to construct models of increasing complexity need to be developed in an organized and collaborative manner and to be widely accessible [34]. Access to the requisite computational resources will also become an issue. Perhaps an institute similar to the National Center for Atmospheric Research [35], which facilitates international global climate change research, could help coordinate and support biological modeling efforts.

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