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Phytochemistry 68 (2007) 2139–2151
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Computational approaches to the topology, stability and dynamics of metabolic networks

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Received 5 March 2007; received in revised form 15 April 2007
Available online 14 June 2007

Abstract

Cellular metabolism is characterized by an intricate network of interactions between biochemical fluxes, metabolic compounds and regulatory interactions. To investigate and eventually understand the emergent global behavior arising from such networks of interaction is not possible by intuitive reasoning alone. This contribution seeks to describe recent computational approaches that aim to asses the topological and functional properties of metabolic networks. In particular, based on a recently proposed method, it is shown that it is possible to acquire a quantitative picture of the possible dynamics of metabolic systems, without assuming detailed knowledge of the underlying enzyme-kinetic rate equations and parameters. Rather, the method builds upon a statistical exploration of the comprehensive parameter space to evaluate the dynamic capabilities of a metabolic system, thus providing a first step towards the transition from topology to function of metabolic pathways. Utilizing this approach, the role of feedback mechanisms in the maintenance of stability is discussed using minimal models of cellular pathways.

Keywords: Kinetic modeling; Metabolomics; Systems biology; Complex networks; Nonlinear dynamics; Bifurcations

1. Introduction

Cellular metabolism and its regulation represents a large-scale dynamical system and complex dynamic behavior has been observed for a wide variety of metabolic pathways. Among the most classical and well-known examples are temporal variations of metabolic intermediates in the photosynthetic Calvin cycle (Laisk and Walker, 1986; Giersch, 1986; Ryde-Petterson, 1991), and the yeast glycolytic pathway (Selkov, 1975; Danø et al., 1999), along with many other descriptions of complex behavior in biochemical systems (Heinrich and Schuster, 1996; Goldbeter, 1997; Morgan and Rhodes, 2002; Tyson et al., 2003). More general, dynamic properties of cellular regulatory systems, such as multi-stability, sustained oscillations or irreversible switching, are considered to be essential for cellular regulation and constitute the conceptual basis for many, if not most, physiological properties of living cells, such as time-keeping by circadian clocks (Goldbeter, 2002), regulation of cell division (Tyson, 1991; Tyson et al., 2001), cellular signaling (Bhalla and Iyengar, 1999, 2001, 2003), or cell differentiation (Laurent and Kellershohn, 1999; Freeman, 2000).

However, also within a seemingly much simpler scenario, such as a metabolic system at a steady state, the dynamic properties play a crucial role to ensure and maintain the function and stability of the system. Utilizing an intricate network of regulatory interactions and feedback loops, the evolution of cellular metabolism has developed a variety of biochemical and metabolic strategies to ensure metabolic homeostasis, prevent depletion of metabolic intermediates and allow for an optimal response to changing environmental conditions (Sweetlove and Fernie, 2005).

This contribution seeks to summarize and describe recent computational approaches to elucidate the structure, topology and dynamics of metabolic pathways. While not aiming at a comprehensive review of the field, the contribution will specifically focus on computational and mathematical approaches to elucidate the transition from structure to dynamics of metabolic systems. Key questions relate to the dynamic stability of metabolic networks, the role of

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doi:10.1016/j.phytochem.2007.04.041

feedback regulation in the maintenance of stability, the relevance of dynamic properties for metabolic regulation, as well as the implications of dynamic properties with respect to biotechnological modifications of metabolic systems.

The paper is organized as follow: Section 2 gives a brief overview over current approaches to describe cellular metabolism, ranging from topological and stoichiometric analysis to detailed kinetic models of metabolic pathways. Subsequently, a recent approach is described that aims to contribute to a transition from structure to function of metabolic networks and allows to infer a variety of dynamic properties from structural knowledge. In Section 3, a minimal model of the TCA cycle in plant leaves is investigated and the role of feedback mechanisms with respect to dynamic properties is discussed. Section 4 gives a summary of the results in the context of stability and robustness of metabolic networks.

2. Computational approaches to metabolism

Cellular metabolism is characterized by interwoven networks of metabolic fluxes and regulatory interactions, involving multiple and interacting hierarchies of regulation and timescales. The experimentally observable global behavior of such systems, often involving antagonistic relationships between several components, cannot be predicted or understood by intuitive reasoning alone. In this respect, mathematical modeling of cellular metabolism provides an appropriate framework to represent and investigate the principles of metabolic organization (Csete and Doyle, 2002; Tyson et al., 2003; Wolkenhauer and Mesarovic, 2005): at its core, mathematical modeling aims to translate the current knowledge and assumptions about cellular components and their interactions into a mathematical representation – thus providing a precise and well-defined vocabulary to study the integrated behavior of metabolic networks on a systems level.

In this respect, mathematical modeling has many facettes. As yet, the current assumptions and beliefs about cellular metabolism can, of course, not be given in terms of a single model. Rather, computational approaches utilize a hierarchy of descriptions, involving different levels of detail and complexity. A schematic overview is given in Fig. 1 and discussed in the following sections.

2.1. Kinetic models of cellular pathways

Tracing back to the beginning of the last century, mathematical modeling of metabolic processes traditionally has a strong emphasis on the development of detailed kinetic models of cellular pathways, based on explicit enzyme-kinetic rate equations. Over the past decades, the mathematical and numerical analysis of detailed kinetic core models has made numerous significant contributions to elucidate and understand the general principles of metabolic regulation and control – culminating in the formulation of metabolic control analysis (MCA), a mathematical theory to describe the control and regulatory properties of metabolic systems (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; Heinrich and Schuster, 1996; Fell, 1997). A brief synopsis of the mathematical nomenclature of explicit kinetic models is given in Box 1.

More recently, several initiatives have been made to extend this 'bottom-up' approach towards more comprehensive large-scale dynamic models of cellular metabolism (Tomita et al., 1999; Kitano, 2001; Loew and Schaff, 2001; Slepchenko et al., 2003; Ishii et al., 2004; Kitano, 2005). However, while such 'whole cell models' are certainly a desirable goal, their construction is as yet hampered by a number of fundamental difficulties – most importantly the

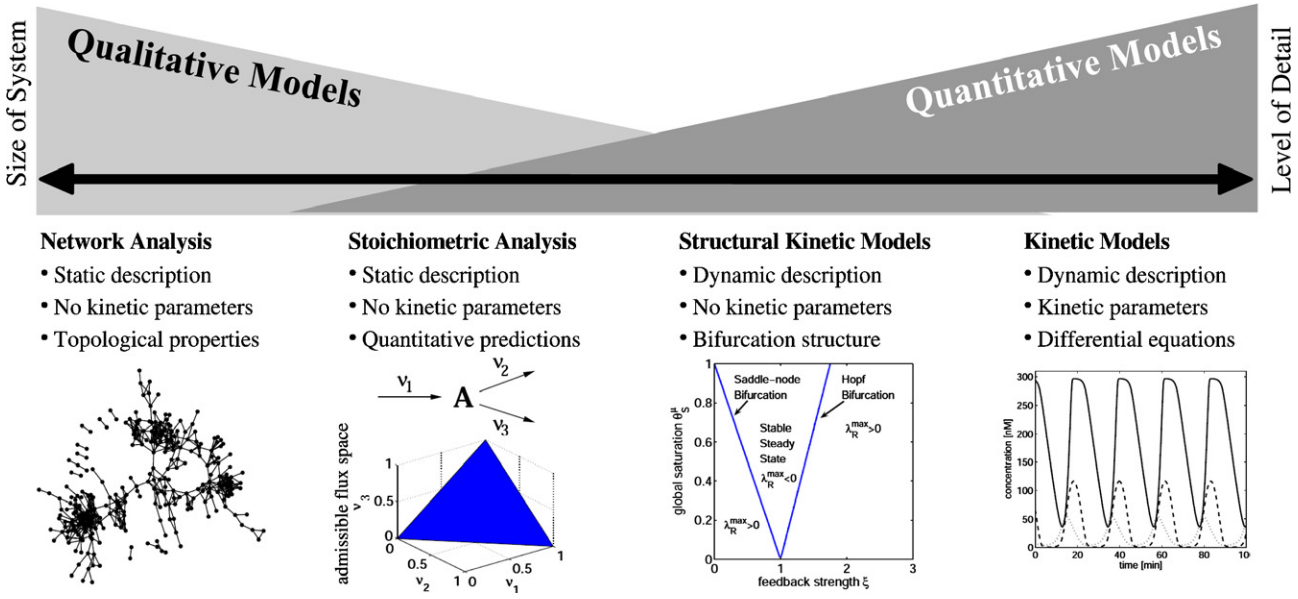


Fig. 1. Current mathematical representations of metabolic networks utilize a hierarchy of descriptions, ranging from topological and stoichiometric descriptions to detailed kinetic models of cellular pathways.

inevitable computational complexity of such models in combination with a notorious lack of reliable quantitative information about the kinetic parameters. Moreover, even when the construction of large kinetic models becomes feasible, there remains dissension about what the benefits of such large models would be. While, historically, mathematical modeling often deliberately sacrificed aspects of biological realism for sake of interpretability, the quest for genome-scale dynamic models of metabolism also entails a shift in paradigm towards comprehensive and predictive, but not necessarily intelligible, '*in silico*' models.

Nonetheless, explicit kinetic models still allow for the most detailed and quantitative evaluation of the dynamics and function of metabolic systems. In particular, a detailed understanding and prediction of functional properties, such as oscillations or irreversible switching, is not possible based on topological considerations alone, but as yet often necessitates the construction of explicit kinetic models. Recent examples of detailed kinetic models are given in Morgan and Rhodes (2002), the JWS Online Repository (Olivier and Snoep, 2004; Snoep, 2005), the BioModels Database (http://www.ebi.ac.uk/biomodels/) or the CellML Model Repository (http://www.cellml.org/models/).

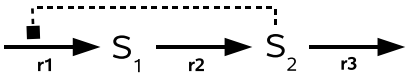
Box 1

Assuming spatial homogeneity, the dynamics of a metabolic system can be described by a set of ordinary differential equations

$$\frac{d\mathbf{S}(t)}{dt} = \mathbf{N} \cdot \boldsymbol{v}(\mathbf{S}, \mathbf{k}),$$

where $\mathbf{S}(t)$ denotes the time-dependent vector of metabolite concentrations and $\mathbf{N}$ the stoichiometric matrix. The vector of enzyme-kinetic rate equations $\boldsymbol{v}$ consists of nonlinear functions, which depend on the substrate concentrations $\mathbf{S}$ as well as on a set of parameters $\mathbf{k}$ – usually a set of Michaelis constants $\boldsymbol{K}_\mathbf{m}$, maximal reaction velocities $\boldsymbol{v}_\mathbf{m}$ and equilibrium constant $\boldsymbol{K}_\mathbf{eq}$. Given the functional form of the rate equations, a set of parameters $\mathbf{k}$, and an initial condition $\mathbf{S}(0)$, the differential equations can be solved numerically to obtain the time-dependent behavior of all metabolites under consideration.

Example. For the simple pathway,



incorporating inhibition of the first reaction by S_2, the corresponding set of equations reads

$$\frac{d}{dt}\begin{bmatrix} S_1(t) \\ S_2(t) \end{bmatrix} = \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \cdot \begin{bmatrix} v_1(\mathbf{S}, \mathbf{k}) \\ v_2(\mathbf{S}, \mathbf{k}) \\ v_3(\mathbf{S}, \mathbf{k}) \end{bmatrix}.$$

Given the explicit functional form of the rate equations, e.g. $v_2(S_1) = v_{m2}S_1/(K_1 + S_1)$, $v_3(S_2) = v_{m3}\ S_2/(K_2 + S_2)$, and $v_1(S_2) = v_{m1}/(1 + (S_2/K_i)^n)$, the numerical values of the seven kinetic parameters, , and an initial condition $S(0)$, the system is fully specified.

2.2. Topological approaches and network analysis

In the view of the limits of large-scale kinetic modeling it is not surprising that structure-oriented and graph-theoretic approaches to investigate metabolic networks have attracted substantial interest recently. In particular, present-day advances in genome sequencing and annotation, and thus the possibility to reconstruct large (up to 'genome-scale') metabolic networks for several microbial organisms, have triggered a number of substantial contributions to the computational and mathematical analysis of large metabolic networks (Jeong et al., 2000; Edwards and Palsson, 2000; Fell and Wagner, 2000; Förster et al., 2003; Papin et al., 2003; Duarte et al., 2007).

At the most basic level, a metabolic network can be interpreted as a bipartite graph, consisting of a set of nodes that represent the metabolic substrates, a second set of nodes representing the biochemical interconversions, as well as a set of directed or undirected links between them. As proposed by several authors, the bipartite graph can be collapsed into either a substrate graph (Jeong et al., 2000) or a reaction graph (Wagner and Fell, 2001), constituting the objects of interest for most applications of complex network theory (Barabási and Oltvai, 2004). Examples of both representations are given in Fig. 2.

For the substrate graph, a vast number of studies consistently showed that the degree distribution of the graph is highly heterogeneous ('scale-free'), i.e., most metabolic substrates participate in only a few reactions, whereas some metabolites ('hubs') participate in a much larger number of reactions (Jeong et al., 2000; Wagner and Fell, 2001; Ma and Zeng, 2003). The list of highly connected metabolites is dominated by the ubiquitous co-factors, such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), and nicotinamide adenine dinucleotide (NAD) in its various forms, as well as by metabolites relating to central metabolism, namely glycolysis and the tricarboxylic acid (TCA) cycle. As argued by Wagner and Fell (2001), this possibly reflects the evolutionary origin of cellular metabolism, as highly connected metabolites should be among the phylogenetically oldest – in accordance with basic growth models of scale-free networks (Barabasi and Albert, 1999).

While the heterogeneous degree distribution itself is certainly no news to most biochemists (Sweetlove and Fernie, 2005), a result of more general importance is that the degree distribution has some consequences for the robustness and error tolerance of complex networks (Albert et al., 2000), here identified with the persistence of topological network indices upon removal of nodes or links. Various other

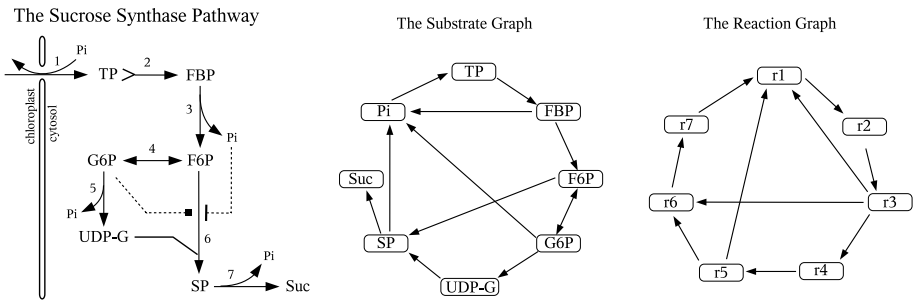


Fig. 2. A simplified model of the sucrose synthase pathway, adapted and redrawn from Heldt et al. (1986) and Morgenthal et al. (2006). *Center and Right:* Shown is the projection as a substrate and reaction graph: Taking directionality into account, two substrates are connected if they participate in a common reaction as substrate and product, respectively. Similar for the reaction graph. A deduction of the possible dynamics is not possible from the graph-theoretic representation alone. *Abbreviations*: TP, triosephosphates; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; SP, sucrose phosphate; Suc, sucrose; Pi, free cytosolic inorganic phosphate.

topological characteristics of complex networks have been studied for metabolic systems. Examples include the clustering coefficient, as well as the remarkably short average path-length between metabolites ('small-world property') – an indicator of the time required to spread information or perturbations within the network (Wagner and Fell, 2001). Further work has dealt with the topological damage generated by the deletion of enzymes (Lemke et al., 2004), the comparison of topological structure between various organisms (Ma and Zeng, 2003), as well as hierarchies and modularity in metabolic networks (Holme et al., 2003; Ravasz et al., 2002; Gagneur et al., 2003).

However, while complex network analysis has certainly contributed significantly to the understanding of metabolic network architecture, the graph-based analysis of metabolic networks also holds several drawbacks. Apart from as yet incomplete knowledge of network structure and ambiguities in the representation, see Arita (2004) and Osterman (2006) for more detailed discussions, the topological approach does not straightforwardly allow to incorporate dynamic properties into the description of the system. Despite considerable efforts, the precise relationship between topological structure on the one hand, and dynamic and functional properties on the other hand, remains largely unclear (Ronen et al., 2002; Klipp et al., 2004; Prill et al., 2005; Ingram et al., 2006). Furthermore, despite the superficial similarity of the substrate graph representation to other examples of complex networks, many aspects of metabolic networks differ fundamentally from those of other networks of cellular interactions. Metabolic networks are actually hypergraphs, i.e, networks where edges (reactions) connect to several nodes (metabolites), necessitating more advanced methods than simple graph theory for their analysis. Indeed, due to this inherent hypergraph structure and dynamics dominated by the interconversion of metabolic intermediates, the interpretation and analysis of experimentally observed metabolomic data is fundamentally different from, e.g. co-expression of transcripts (Steuer et al., 2003; Camacho et al., 2005; Morgenthal et al., 2006; Steuer, 2006). To allow for an investigation of the structure and dynamics of metabolic networks, the picture has thus to be augmented by more specific structural and kinetic aspects.

2.3. Stoichiometric analysis

Among the most profound deficiencies of the simple graph-based description of metabolism is that it does not account for the specific structural properties of metabolic networks. In contrast to this shortcoming, stoichiometric analysis seeks to explicitly make use of the structural nature of metabolic systems (Varma and Palsson, 1994; Schuster et al., 2000): The stoichiometric approach amounts to an analysis of the stoichiometric matrix $\mathbf{N}$ whose elements indicate how many molecules of each substrate are consumed and produced in each reaction. Knowledge of the stoichiometry puts constraints on the feasible flux distributions, thus providing information that can be utilized to predict and explore the functional capabilities of metabolic networks (Varma and Palsson, 1994; Edwards and Palsson, 2000; Stelling et al., 2002; Price et al., 2003; Famili et al., 2003).

Assuming the system operates at a steady state or, equivalently, considering the time-averaged behavior for stationary metabolite concentrations $\langle \Delta \mathbf{S} \rangle = \mathbf{0}$, the differential equations given in Box 1 can be interpreted as a linear equation for the flux vector $v(\mathbf{S},\mathbf{k})$. Reflecting the law of mass conservation, any feasible flux vector v^0 has to obey the equation $\mathbf{N}v^0 = \mathbf{0}$, independent of the parameter values $\mathbf{k}$. As the number of rows of the stoichiometric matrix (number of metabolites) is usually smaller than the number of columns (number of reactions or fluxes), the equation is under-determined and has no unique solution. However, the feasible flux distributions of a metabolic network consisting of r reactions are restricted to the null-space of the stoichiometric matrix and thus described by only r – rank ($\mathbf{N}$) free parameters – instead of full set of r unknown reaction rates (Heinrich and Schuster, 1996; Klamt et al., 2002).

This reduction of the admissible flux space is exploited by a number of computational approaches, most notably *Flux balance analysis* (FBA) (Varma and Palsson, 1994; Schilling et al., 1999; Palsson, 2000). Setting up an objective function for the steady state flux vector v^0, a flux distribution that minimizes or maximizes the objective function can be obtained. Possible objective functions include maximal biomass yield, maximal energy (ATP) production, or maximal nutrient uptake, among various others. Flux balance

analysis has resulted in a vast number of application (Palsson, 2002; Papin et al., 2003; Almaas et al., 2004; Holzhütter, 2004; Stephanopoulos et al., 2004), including recent approaches to incorporate the regulation of gene expression (Covert et al., 2001). Of particular interest are recent efforts to augment the stoichiometric balance equations with thermodynamic constraints – providing a link between concentration and flux in metabolic networks, and allowing to infer putative metabolic regulation (Beard et al., 2002; Holzhütter, 2004; Kümmel et al., 2006).

A closely related concept to FBA, but based on the exhaustive enumeration of all feasible flux vectors, was proposed by Schuster et al. (2000). The metabolic network is decomposed into distinct, but possibly overlapping, pathways: The *elementary flux modes* (EFMs) of the system. An EFM is defined as a minimal set of reactions capable of working together in a steady state. The set of EFMs is unique for a given metabolic network and all feasible flux vectors can be described as linear combinations of EFMs. Interestingly, EFMs are often found to correspond to distinct modes of behavior of the system, i.e. though an observed flux distribution can be a combination of all possible flux modes, many biologically realized flux distributions closely relate to one (or few) single flux modes only (Klamt and Stelling, 2003; Papin et al., 2004). An example of the decomposition into elementary flux modes is given in Fig. 3.

Similar to flux balance analysis, the concept of elementary flux modes has resulted in a vast number of applications to analyze and predict metabolic network functionality (Stelling et al., 2002; Schuster et al., 2002; Klamt et al., 2002; Poolman et al., 2003; Klamt and Gilles, 2004; Klamt, 2006). Several software packages are available that allow for the computation of elementary flux modes for medium-sized metabolic networks (Hoops et al., 2006; Klamt et al., 2007).

Within the hierarchy of current computational approaches to metabolism, in particular considering a tradeoff between required knowledge and predictive power, the stoichiometric analysis of metabolic networks must be considered the most successful computational approach to date. It is far more predictive than simple graph-based schemes, computationally feasible even for large networks, and does not require the extensive knowledge of kinetic parameters and rate equations as detailed kinetic modelling.

2.4. Structural kinetic modeling

Similar to topological approaches, stoichiometric analysis does not straightforwardly allow to incorporate dynamic properties into the description of the system. In particular, the stoichiometric balance equation $\mathbf{N}v(\mathbf{S^0}) = \mathbf{0}$ allows no conclusions about the dynamic features or possible dynamic behavior of a metabolic state, characterized by a flux distribution $v(\mathbf{S^0})$ and metabolite concentrations $\mathbf{S^0}$.

To this end, aiming at a transition from structure to dynamics of metabolic networks, a recently proposed approach augments the stoichiometric analysis with kinetic properties, thus providing a bridge between structural analysis and explicit dynamic simulations (Steuer et al., 2006, 2007). Without requiring knowledge about the explicit functional form of the enzyme-kinetic rate equations and parameters, the method describes the possible dynamics of metabolic systems, as well as the stability and robustness of metabolic states, and concomitantly identifies the relevant interactions and parameters governing the dynamic properties of the system.

The analysis is based on a simple observation: In most application, an explicit kinetic model is not required. Rather, a large variety of dynamical properties are readily accessible using only a local linear approximation of the system at its respective state. In particular, the dynamic response to perturbations, the stability of a metabolic state, as well as the transition to oscillatory behavior, is fully determined using a local approximations of the system.

Formally, the local linear approximation is obtained from a Taylor series expansion of the metabolic system at a (not necessarily unique or stable) steady state. The linear term of the expansion, the *Jacobian matrix*, governs the dynamic response of the system in the vicinity of the metabolic state (Kaplan and Glass, 1995). Unfortunately, the estimation of the Jacobian matrix, defined as the partial

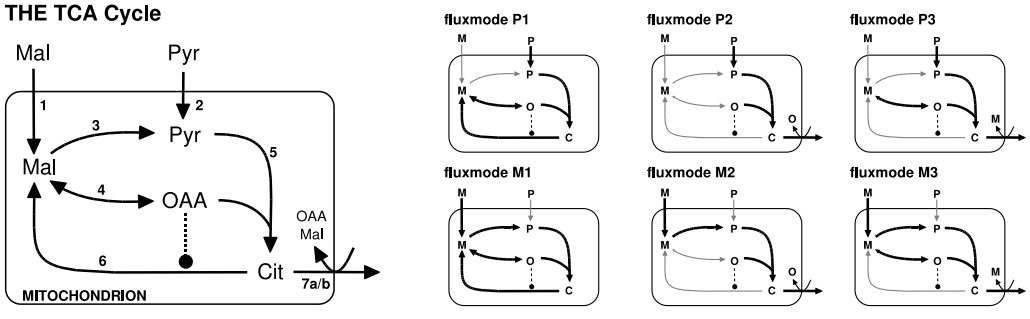


Fig. 3. A highly simplified model of the plant mitochondrial TCA cycle, adapted from a more detailed model described elsewhere (Steuer et al., 2007). The TCA cycle either exports metabolic intermediates for biosynthesis (reactions v_{7a} and v_{7b}) or contributes to ATP production (reaction v_6). The pathway gives rise to *6* elementary flux modes (EFMs), i.e. minimal sets of reactions capable of working together in a steady state. The EFMs can be roughly identified with distinct modes of operation of the TCA cycle: The synthesis of ATP via v_6 (EFMs P1 and M1) and the export of intermediates for biosynthesis (EFMs P2, P3, M2, M3). Furthermore, the flux modes distinguish between import of malate (EFMs M1, M2, M3) and pyruvate (EFMs P1, P2, P3) into the mitochondrion. *Abbreviations.* Mal, malate; Pyr, pyruvate; OAA, oxaloacetate; Cit, citrate.

derivative of the rate equations with respect to the metabolic variables $\mathbf{S}(t)$ at the state $\mathbf{S^0}$, usually requires explicit knowledge of the enzyme-kinetic rate equations. However, even in the face of lacking enzyme-kinetic information, it is still possible to specify the structure of the Jacobian matrix, such that each element is restricted to a well-defined and biochemically interpretable interval. Then, just as the stoichiometric balance equation puts constraint on the feasible flux distributions, the structure of the Jacobian matrix puts constraints on the possible dynamic behavior of the metabolic network and defines the *dynamic capabilities* at the metabolic state (Heinrich and Schuster, 1996; Steuer et al., 2006).

Structural kinetic modeling (SKM) thus amounts to construct a parametric representation of the Jacobian matrix of a metabolic system, such that each element can be assigned to a well-defined interval even when detailed knowledge about the functional form of the rate equations is not available. As detailed elsewhere (Steuer et al., 2006, 2007), the Jacobian matrix of a metabolic system can always be written as a product of two matrices $\mathbf{\Lambda}$ and $\boldsymbol{\theta}^{\boldsymbol{\mu}}_{\boldsymbol{x}}$:

$$\mathbf{J_s} = \mathbf{\Lambda} \cdot \boldsymbol{\theta}^{\boldsymbol{\mu}}_{\boldsymbol{x}} \tag{1}$$

A brief synopsis of the mathematical definitions is given in Box 2. The first matrix $\mathbf{\Lambda}$ reflects the stoichiometry and the metabolic state of the system, as characterized by a flux distribution v^0 and metabolite concentrations $\mathbf{S^0}$. In addition, the (usually unknown) elements of the matrix $\boldsymbol{\theta}^{\boldsymbol{\mu}}_{\boldsymbol{x}}$ correspond to the '*effective kinetic order*' or '*normalized saturation*' of each reaction with respect to its substrates, products and allosteric effectors. Each nonzero element of the matrix $\boldsymbol{\theta}^{\boldsymbol{\mu}}_{\boldsymbol{x}}$ can be assigned to a well-defined interval, even when the specific functional form of the rate equation is not known. See Box 2 for examples (Steuer et al., 2006).

Both matrices span the associated *parameter space* of the metabolic system. At each metabolic state, defined by $v(\mathbf{S^0})$ and $\mathbf{S^0}$, the unknown elements of $\boldsymbol{\theta}^{\boldsymbol{\mu}}_{\boldsymbol{x}}$ define the range of possible dynamics at the metabolic state. In particular, the balance equation $\mathbf{N}v^0 = \mathbf{0}$ does not imply actual dynamic stability of the metabolic state. Rather, the real parts of the eigenvalues of the Jacobian determine whether a metabolic state is stable or whether a small perturbation is exponentially amplified. Given a small perturbation, the dynamic response can be classified according to four distinct scenarios: (i) The system returns to its original state, the perturbation is damped out. (ii) The return to the original state is oscillatory, i.e., the system overshoots, resulting in damped transient oscillations. (iii) The initial perturbation is amplified, i.e., the metabolic state is unstable. The system does not return to the original state. (iv) The metabolic state is unstable and the divergence from the metabolic state is oscillatory. Formally, the distinct scenarios can be deduced from the spectrum of eigenvalues of the Jacobian matrix at a metabolic state, see Fig. 4 for a schematic overview. Of particular interest are also transitions between the different cases (bifurcations), indicating qualitative changes in the dynamics of the system. The parametric representation this allows for a quantitative statistical exploration of the possible dynamics at each metabolic state.

A simple example. Prior to an application to a more complex scenario, the method of structural kinetic modeling (SKM) is illustrated using the simple pathway already depicted in Box 1. Within the conventional approach, based on explicit differential equations, the Jacobian of the system is given by the partial derivatives of the rate functions at the steady state $\mathbf{S^0}$, with the numerical values depending nontrivially (as mediated by the steady state $\mathbf{S^0}$) on all parameter values $\mathbf{k}$. Assuming, for illustrative purposes only, simple mass-action kinetics $v_2 = k_2 S_1$ and $v_3 = k_3 S_2$, but retaining the nonlinear inhibition of S_2 on v_1, a possible system of explicit differential equations is

$$\begin{aligned} \frac{\mathrm{d}S_1}{\mathrm{d}t} &= \frac{v_\mathrm{m}}{1 + \left[\frac{S_2}{K_i}\right]^2} - k_2 S_1 \\ \frac{\mathrm{d}S_2}{\mathrm{d}t} &= k_2 S_1 - k_3 S_2 \end{aligned} \tag{2}$$

Within SKM, the explicit differential equations are replaced by a parametric representation of the pathway in

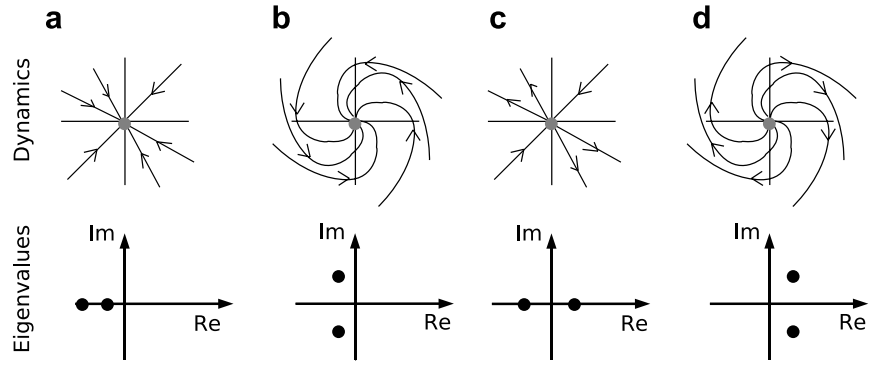


Fig. 4. A metabolic state that satisfies the steady state condition $\mathbf{N}\boldsymbol{v}^{\mathbf{0}} = \mathbf{0}$ must not necessarily be stable. The local dynamics in the vicinity of the steady state can be classified according to the eigenvalues of the Jacobian: (a) Stable node: All real parts of the eigenvalues are negative, the largest real value $\lambda_\mathrm{R}^{\max}$ determines the slowest timescale of the system. (b) Stable focus: The eigenvalues determining the slowest timescale have complex conjugate imaginary parts, corresponding to oscillatory dynamics. (c) Saddle: The real part $\lambda_\mathrm{R}^{\max}$ is positive. Any small perturbation is exponentially amplified. (d) Unstable focus: The dynamic behavior has an oscillatory component, corresponding to the (complex conjugate) imaginary parts. Transitions between the different scenarios occur via a Hopf bifurcation (b → d) or a bifurcation of the saddle-node type (a → c).

terms of the generalized parameter matrices $\mathbf{\Lambda}$ and $\boldsymbol{\theta}_x^{\boldsymbol{\mu}}$. The metabolic state is defined by the flux distribution v^0, constraint by $v^0 := v_1^0 = v_2^0 = v_3^0$, and metabolite concentrations $\mathbf{S^0} = \{S_1^0, S_2^0\}$. The elements of the matrix $\boldsymbol{\theta}_x^{\boldsymbol{\mu}}$ are unknown and restricted to well-defined intervals (see Box 2 for the choice of intervals).

Using similar assumptions as for the explicit differential equations (mass-action kinetics for v_2 and v_3, thus $\theta_x^{\mu} = 1$ for the respective reactions), the Jacobian is thus fully specified by the metabolic state $\{v^0, S_1^0, S_2^0\}$ and the (normalized) strength ξ of the feedback inhibition of $v_1(S_2)$ by S_2:

$$\mathbf{J_s} = \mathbf{\Lambda}\boldsymbol{\theta}_x^{\boldsymbol{\mu}} = \begin{pmatrix} -\frac{v^0}{S_1^0} & -\frac{v^0}{S_1^0}\xi \\ \frac{v^0}{S_2^0} & -\frac{v^0}{S_2^0} \end{pmatrix} \tag{3}$$

Similar to the four kinetic parameters in the explicit equations, these four parameters represent a genuine, but alternative, parameterization of the system. Both sets of parameters can be interconverted. However, the alternative parametrization holds a number of advantages, specifically: (i) The parameters do not depend on any explicit choice of the rate equations. In particular, the parametrization of the inhibition ξ is not restricted to the explicit functional form given in Eq. (2), but holds for a large class of possible rate functions. (ii) The parameters directly specify the Jacobian matrix, and thus the linear response at the metabolic state, requiring no further computational effort.

Aiming at the *dynamic behavior* of the system, the Jacobian is now evaluated in terms of the generalized parameters. Without loss of generality, the units of concentration and time are arbitrary, hence we set $S_1^0 := 1$ and $v^0 := 1$. Using the abbreviation $\beta := S_1^0/S_2^0$, the Jacobian matrix depends on the two parameters $\{\beta,\xi\}$ only. The dynamics of the system can be categorized according to the eigenvalues of the Jacobian: The simple pathway is at a stable metabolic state for all possible parameter values $\{\beta,\xi\}$, i.e., the largest positive real part of the eigenvalues is always negative $\lambda_{\mathrm{R}}^{\max} < 0$. The slowest timescale of the system, as determined by the magnitude of $\lambda_{\mathrm{R}}^{\max}$, depends mainly on the ratio $\beta = S_1^0/S_2^0$ of metabolite concentrations.

Fig. 5 summarizes the dynamic behavior of the simple pathway over the parameter space $\{\beta,\xi\}$, confirming several well-known properties of negative feedback regulation: The negative feedback speeds up the response time of the system and can induce damped (transient) oscillations (Case *b* in Fig. 4), which are most pronounced for a ratio of concentrations close to unity $\beta \approx 1$. For strongly differing substrate concentrations $\beta \gg 1$ or $\beta \ll 1$, the damped oscillations vanish. Importantly, for fixed feedback strength, the largest real part of the eigenvalues exhibits a well-defined minimum – corresponding to an optimally fast response to perturbations (Rosenfeld et al., 2002). It is emphasized, that the analysis presented in Fig. 5 is not based on explicit knowledge of the functional form of the feedback inhibition. Rather, all results hold for an arbitrary choice of rate equations, as long as they conform to basic assumptions about biochemical rate functions (Steuer et al., 2006).

Box 2:

Structural kinetic modeling is based on a decomposition of the Jacobian matrix of a metabolic system into a product of two matrices. The system of differential equations $\dot{S}_i = \sum_j N_{ij} v_j(\mathbf{S}, \mathbf{k})$ can be rewritten as

$$\frac{\mathrm{d}}{\mathrm{d}t}\frac{S_i}{S_i^0} = \sum_j \frac{v_j^0}{S_i^0} N_{ij} \frac{v_j(\mathbf{S})}{v_j^0},$$

where $\mathbf{S^0}$ denotes a (not necessarily unique or stable) steady state and $v^0 = v(\mathbf{S^0},\mathbf{k})$ the associated flux distribution. Using the definitions

$$\Lambda_{ij} := \frac{v_j^0}{S_i^0} N_{ij} \quad \text{and} \quad \mu_j(\mathbf{S}) := \frac{v_j(\mathbf{S})}{v_j^0},$$

and the variable transformation $x_i := S_i(t)/S_i^0$, the Jacobian with respect to the new variables $\mathbf{x}$ is

$$\mathbf{J_s} = \mathbf{\Lambda}\boldsymbol{\theta}_x^{\boldsymbol{\mu}} \text{ with } \boldsymbol{\theta}_x^{\boldsymbol{\mu}} := \left.\frac{\partial \boldsymbol{\mu}(\mathbf{x})}{\partial \mathbf{x}}\right|_{\mathbf{x^0}=\mathbf{1}}$$

The elements of the matrix $\mathbf{\Lambda}$ contain the structure and the timescales of the system, as defined by the vectors of flux values v^0 and metabolite concentrations $\mathbf{S^0}$, respectively. The elements of the matrix $\boldsymbol{\theta}_x^{\boldsymbol{\mu}}$ specify the normalized degree of saturation of each reaction with respect to each of its substrates, defined in close analogy to the (scaled) elasticity coefficients of metabolic control analysis. In particular, $\theta_x^{\mu} \in [0,1]$ for all substrates and $\theta_x^{\mu} \in [0,-1]$ for all products of a reaction. For allosteric regulation $\theta_x^{\mu} \in [0,n]$ and $\theta_x^{\mu} \in [0,-n]$ for activation and inhibition, respectively. The eigenvalues of the Jacobian matrix then determine the stability of the steady state $\mathbf{S^0}$, as well as qualitative changes in the dynamics of the system, most notably the existence of bifurcation of the Hopf and Saddle-node type.

Example.

For the pathway specified in Box 1, the corresponding matrices are given as

$$\mathbf{\Lambda} = \begin{bmatrix} \frac{v^0}{S_1^0} & -\frac{v^0}{S_1^0} & 0 \\ 0 & \frac{v^0}{S_2^0} & -\frac{v^0}{S_2^0} \end{bmatrix} \quad \boldsymbol{\theta}_x^{\boldsymbol{\mu}} = \begin{bmatrix} 0 & -\xi \\ \theta_{S_1}^2 & 0 \\ 0 & \theta_{S_2}^3 \end{bmatrix},$$

with entries determined by the steady state concentrations S_i^0, the flux v^0, the (normalized) saturation or effective kinetic order $\theta_{S_1}^2 \in [0,1]$ of the reaction v_2 to its substrate S_1 and, likewise, the (normalized) saturation $\theta_{S_2}^3 \in [0,1]$ of the reaction v_3 to its substrate S_2, as well as the (normalized) strength of the feedback inhibition $\xi \in [0,n]$ of S_2 on v_1.

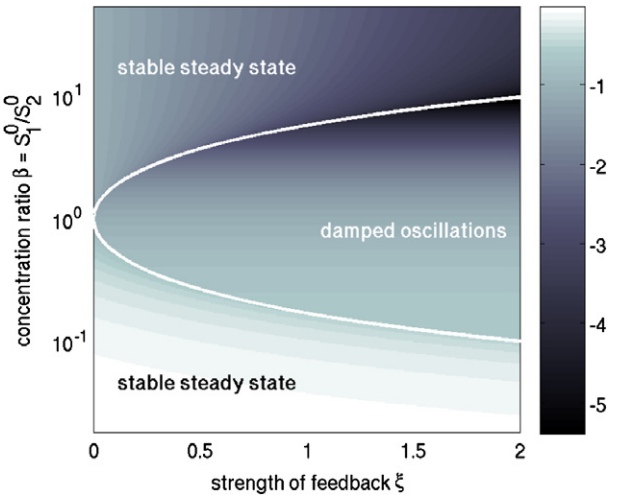


Fig. 5. The response–time of the simple example pathway to perturbations of the metabolic state as a function of the parameters $\{\beta,\xi\}$. Shown is the largest real part λ_R^{max} of the eigenvalues of the Jacobian. Darker colors correspond to large negative values (see colorbar), indicating a faster return to the steady state. Within the region marked by the white line, the eigenvalues have nonzero imaginary parts, corresponding to transient damped oscillations. Increasing the negative feedback ξ speeds up the response time of the system, with a well-defined minimum at the (upper) transition to transient oscillatory dynamics.

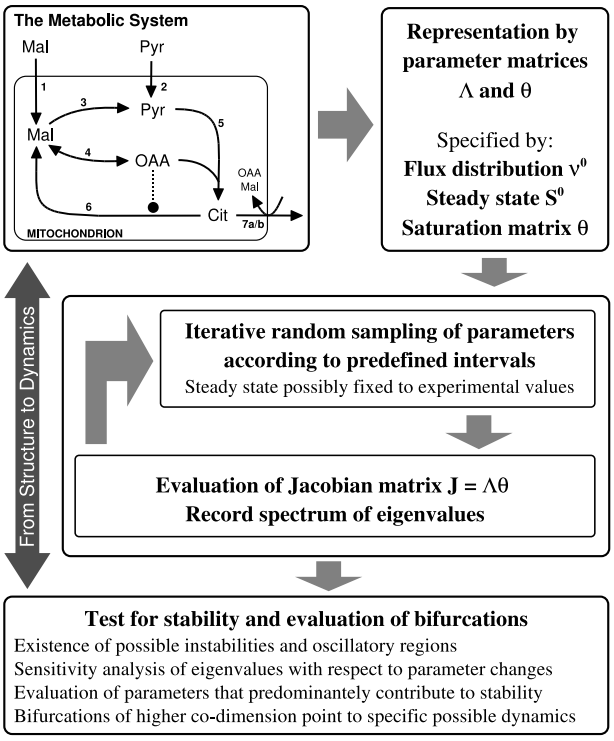


Fig. 6. From structure to dynamics of metabolic systems. The method builds upon creating a large ensemble of possible models (Jacobians), drawn randomly from the comprehensive parameter space, and a subsequent quantitative evaluation of the dynamic properties.

3. Transitions from structure to dynamics

Among the most important computational challenges in current systems biology is to infer the dynamic and functional properties of metabolic networks, given knowledge about the topological organization (Klipp et al., 2004; Sweetlove and Fernie, 2005; Steuer et al., 2007). To exemplify how structural kinetic modeling can contribute to this transition from structure to dynamics, we briefly reconsider the minimal model of the TCA cycle already depicted in Fig. 3. Clearly, a quantitative picture of the possible dynamics cannot be obtained by visual inspection of the pathway alone. In particular, the role of the inhibitory feedback with respect to the stability and dynamics of the system remains elusive when only assessed by intuitive reasoning.

However, structural kinetic modeling (SKM) allows to draw quantitative conclusions about the possible dynamics of the system, based on only a minimal amount of additional information. To this end, the approach builds upon generating large ensembles of possible models (Jacobians), and a subsequent quantitative evaluation of their dynamic properties. The schematic workflow is depicted in Fig. 6: Given the stoichiometry of a metabolic system and qualitative information about allosteric regulation, the method proceeds as follows:

- Set up the parametric representation of the matrices $\mathbf{\Lambda}$ and $\boldsymbol{\theta}_x^{\mu}$. The structure of both matrixes is fully determined by the stoichiometry and the possible allosteric regulation of the metabolic system.
- Each metabolic state $\{v^0,\mathbf{S^0}\}$ defines an instance of the matrix $\mathbf{\Lambda}$. However, the elements of the matrix $\boldsymbol{\theta}_x^{\mu}$ are usually unknown and only specified with respect to biochemically feasible intervals. The metabolic state may either be an experimentally observed state or itself an unknown quantity, sampled from a set of possible metabolic states.
- For each metabolic state, the elements of the matrix $\boldsymbol{\theta}_x^{\mu}$ define the metabolic capabilities at this state and are repeatedly sampled randomly from their respective intervals. The eigenvalues of the corresponding Jacobian are recorded and evaluated according to Fig. 4.
- The subsequent evaluation of the spectrum of eigenvalues allows to deduce the stability and the possible dynamics of the metabolic system at each metabolic state. Most importantly, various qualitative transitions in the dynamics (bifurcations) can be deduced from the Jacobian.

In the case of the minimal model of the TCA cycle, the metabolic state is assumed to be unknown. The matrix $\mathbf{\Lambda}$ is thus specified by $m = 4$ unknown steady state metabolite concentrations $\mathbf{S^0}$, sampled randomly from a uniform distribution $S_i^0 \in [0, 10]$ (in arbitrary units, measured relative to malate.), as well as a flux distribution v^0, sampled as random superpositions of EFMs, with the total import restricted to unity. To simplify the evaluation of the saturation matrix $\boldsymbol{\theta}_x^{\mu}$, all reactions are modeled as mass-action

kinetics. Thus $\theta_x^y = 1$ (mass-action, no saturation) for all reactions, with the exception of the inhibitory feedback of OAA on v_6 (ATP production). Given the structure of both matrices, each free parameter is repeatedly sampled from its respective interval and the resulting ensemble of Jacobians is evaluated.

Of foremost interest with respect to the possible dynamics is the largest positive real part λ_R^{max} of the eigenvalues, determining the local response and the stability of the system. Fig. 7 (*left*) shows the distribution of λ_R^{max} for fixed feedback strength $\xi = 1$ and all other parameters sampled randomly. In this case, about ~15% of the randomly generated models (Jacobians) are dynamically unstable, i.e. the maximal real part of the eigenvalues is larger than zero.

More general, Fig. 7 (*right*) shows the percentage of dynamically unstable models as a function of feedback strength ξ. In the absence of feedback, the system is at a steady state for all possible metabolic states. For increasing feedback strength, the percentage of unstable models increases – in each case, the instability is generated by a bifurcation of the saddle-node type.

The existence of saddle-node bifurcations implies that there are conditions under which the steady state will loose its stability, pointing to bi- or multistable behavior. Indeed, using numerical integration of a corresponding set of explicit differential equations, the bistable behavior of the system as a function of the feedback parameter is verified in Fig. 8.

It is emphasized that mathematical modeling itself does not allow any conclusions about the *actual* dynamic behavior of the biological system. However, by virtue of the translation of the pathway diagram into a mathematical representation, mathematical modeling allows to specify unambiguously the *dynamic capabilities* of the system. With respect to the TCA cycle depicted in Fig. 3, the analysis shows that the feedback mechanism can induce bistable behavior and that the probability of transitions to instability increases with increasing feedback strength – a result

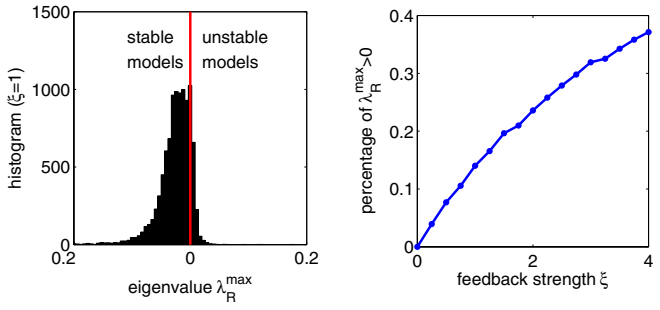


Fig. 7. The largest real part λ_R^{max} of the eigenvalues determines the stability of the TCA cycle model. *Left:* The distribution of λ_R^{max} for an ensemble of randomly sampled models (Jacobians) with fixed feedback strength $\xi = 1$. A fraction of ~15% of randomly sampled models is dynamically unstable ($\lambda_R^{max} > 0$), corresponding to the case shown in Fig. 4c. *Right:* The percentage of dynamically unstable models as a function of the feedback strength $\xi \in [0,4]$. In each case the instability arises from a bifurcation of the saddle-node type.

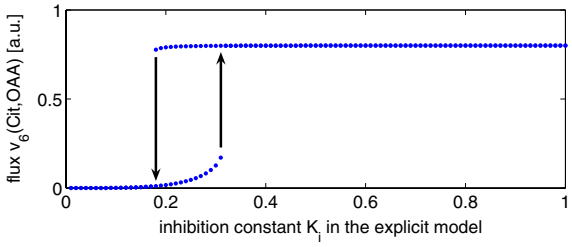


Fig. 8. The negative feedback can induce bistable behavior. Shown is the steady state of a corresponding explicit kinetic model of the TCA cycle, based on differential equations. For strong inhibition of v_6 by OAA (K_i low) almost all flux is directed towards the export reactions ($v_{7a/b}$) and $v_6 \approx 0$. As the inhibition decreases (K_i increases), the steady state looses its stability via a bifurcation of the saddle-node type and a large fraction of flux is rapidly redirected towards ATP production $v_6 \approx 3/4$. The resulting hysteresis curve is typical for bistable system and a systemic property of the pathway.

that could not have been inferred from the pathway diagram by intuitive reasoning alone.

Importantly, structural kinetic modeling holds several advantages as compared to an analysis in terms of explicit differential equations:

(i) The method is applicable to metabolic systems of almost arbitrary size and complexity. The set of generalized parameters determine the Jacobian matrix at each point in parameter space, necessitating no further computational efforts and avoiding explicit numerical integration of large systems of differential equations.

(ii) The results do not depend on the explicit functional form of the rate equations. Nonetheless, the Jacobian matrix fully determines the existence and location of a variety of bifurcations – there is no approximation involved.

(iii) The method allows to obtain a quantitative picture of the possible dynamic behavior, based on qualitative knowledge of the stoichiometry and regulation only. The generalized parameters have a straightforward biochemical interpretation and the existence and size unstable regions can be specified using intuitively accessible biochemical terms.

(iv) The method allows to reveal quantitative biochemical conditions under which certain dynamic behavior can be expected. A sensitivity analysis of the eigenvalues with respect to variations in parameters allows to evaluate which parameters and interactions predominantly determine the dynamics of the system.

The last aspect, sensitivity with respect to parameter changes, directly relates to the robustness of metabolic systems. In particular the role of feedback mechanisms in the maintenance of stability and the response to perturbations can be investigated. Questions of interest relate to (i) what are the possible dynamics of the system, i.e. what are its dynamic capabilities, and, (ii) is an observed dynamic

behavior generic, i.e., can it be expected to occur without fine-tuning of specific parameters and over a broad region in parameters space. The implications of robustness and stability in the analysis of metabolic systems are discussed in the following section.

4. Stability, dynamics and robustness

One of the basic assertions in most computational approaches to cellular regulation is that biological system share a common trait – robustness (Kitano, 2004; Stelling et al., 2004; Sweetlove and Fernie, 2005). In particular, as one of the fundamental organizing principles, biological systems must be able to maintain their function and stability even in the face of a constantly changing environment and perturbations that affect internal or external parameters of the system. Vice versa, as a modeling guideline, if a small perturbation or modification of a mathematical model, independent of its specific kind, would result in an immediate loss of function or qualitatively different behavior, the respective model must be regarded as highly implausible (Morohashi et al., 2002).

In the context of network approaches to cellular metabolism, robustness is mainly understood as the persistence of topological network properties, such as average pathlength or connectedness, upon removal of nodes and links (Albert et al., 2000). More quantitatively, flux balance approaches allow to evaluate the redistribution of fluxes upon perturbation or removal of enzymatic reactions, and thus likewise allow to asses the robustness of flux distribution and the capability of metabolic networks to synthesize desired products and substrates.

However, taking the dynamics of the system into account, the notion of robustness attains a broader interpretation and can be augmented by several aspects. Classic approaches relate to the persistence or stability of dynamic properties with respect to perturbations of parameters (Morohashi et al., 2002; Stelling et al., 2004) – a concept already signified in the definitions of metabolic control analysis. Here, the function and robustness of a metabolic system is discussed from a slightly different perspective, namely its dynamic stability. In particular, a flux distribution v^0 that satisfies the steady state condition $\mathbf{N}v^0 = \mathbf{0}$ must not necessarily be stable. Rather, large systems are known to be prone to instability (May, 1973; McCann, 2000) and only a concerted interplay between kinetic parameters and regulatory feedback loops can ensure the stability of a physiological state.

In this respect, the role of feedback mechanisms is ambivalent: a negative feedback may stabilize the system and ensure an optimal response to perturbations. However, if the strength of the feedback is too large, it may induce instability and eventually sustained oscillations (Steuer et al., 2006). In this context, it is worth pointing out that the two most prominent examples of metabolic oscillations, namely glycolytic and photosynthetic oscillations, are usually observed under non-physiological conditions. Though no conclusive evidence is available, this fact allows for the hypothesis that in both cases the physiological role of the feedback mechanisms is to ensure stability, rather than to bring about sustained oscillations. However, by shifting the system to non-physiological conditions, and thereby inducing a change in the relevant parameters, the feedback reverses its physiological role.

In fact, numerous numerical and theoretical studies indicate that instability and oscillations, rather than a stable steady state, is the generic dynamic behavior of large systems (McCann, 2000). A change in environmental conditions, moving the system far apart from its physiological state, brings about concomitant changes in substrate concentrations and saturation of biochemical reactions. As these parameters determine the Jacobian matrix, and thus the stability of the system, an uncontrolled change in these parameters must be expected to almost inevitably lead to instability.

At this point, the notion and implications of stability should be clarified. At the most basic level, dynamic stability implies that the system returns to its steady state after a small perturbation. More quantitatively, increased stability can be associated with a decreased amount of time required to return to the steady state. Note that, however, stability does not imply the absence of variability in metabolite concentrations. In the face of constant perturbations, the concentration and flux values will fluctuate around their steady state values, rather than attaining these values exactly (Steuer et al., 2003; Camacho et al., 2005; Steuer, 2006). Nonetheless, dynamic stability of the metabolic state is mandatory to ensure metabolite homeostasis and robustness against a fluctuating environment. In particular, the notion of dynamic stability also implies the absence of weakly damped intrinsic dynamics in a metabolic system. In this sense, dynamic stability is closely associated with the potential of a metabolic system to be regulated: Any weakly damped intrinsic dynamics, entailing the existence of resonance frequencies, will potentially interfere with regulation by time-dependent enzyme activities and other factors.

Given the sensitive dependence of stability on the kinetic parameters, the static picture of pathways and flux distributions, as often encountered in textbook diagrams and discussions about possible flux distributions, is highly misleading. A flux distribution, even when optimized for maximal biomass yield, is no static entity. Its stability, and thus its existence, depends crucially on the precise numerical values of kinetic parameters and regulatory interactions. However, as yet, most current discussions about biochemical feasible flux distributions pay no, or only little, attention to the dynamic properties of metabolic networks. Likewise, most current depictions of flux distributions and pathways comply with the apriori assumption of a stable system, only regulated by, i.e., changes enzyme activity, but lacking any intrinsic dynamics. Though this view is certainly reasonable under physiological conditions, given

that evolution has ensured a particular set of most robust parameters, it might not hold true for biotechnological modifications of the system. In this case, the concomitant control of dynamic properties of metabolic systems might play a much more crucial role in biotechnological applications than currently anticipated.

5. Discussion and outlook

Undoubtly, mathematical modeling of cellular metabolism will continue to play an increasing role to understand biological functions and mechanisms, and to provide identification of targets for biotechnological modifications. In this respect, mathematical modeling has many facettes. As yet, there is a rather clear distinction between detailed kinetic models of particular pathways on the one hand, and large-scale network or flux balance approaches on the other hand. However, both approaches are already beginning to merge into a more comprehensive description of cellular processes: Given recent improvements in measurement technologies (Sumner et al., 2003; Goodacre et al., 2004; Sauer, 2004), the 'bottom-up' development of kinetic models allows to cover a more comprehensive set of reactions and interactions, including transcriptional and post-transcriptional regulation, while large-scale 'top-down' approaches allow to include more and more quantitative and kinetic aspects into the description of the system. As yet, however, the dynamic properties of large-scale metabolic networks, such as stability and robustness against perturbations, while still allowing for the necessary flexibility in response to a changing environment, remain relatively unexplored. Though the theoretical and mathematical aspects of dynamic properties have been studied extensively in small and moderate sized systems, the implications of dynamics on large networks has received much less attention so far.

In this respect, one of the fundamental challenges remains to integrate heterogeneous datasets, consisting of metabolomic and proteomic and transcriptomic data, to build dynamic quantitative descriptions of metabolic processes and their regulation. Several initial studies already indicate a path towards such improved computational descriptions, utilizing different hierarchies of mathematical representations and allowing for the integration of heterogeneous and possibly incomplete data (Mendes et al., 2005; Liebermeister and Klipp, 2006). These data-driven approaches need to be supported by the development of improved computational representations of metabolic networks that make use of the specific structure of metabolic systems and allow to deduce dynamic and functional properties from structural knowledge. To this end, a previously proposed approach was described that aims to mediate between detailed kinetic models one the one hand and large-scale approaches to cellular metabolism on the other hand. Starting with the static network of biochemical reactions and interactions, the descriptions of the system is augmented by normalized kinetic parameters that allow for the direct construction of the Jacobian matrix of a metabolic system at each point in parameter space. The computational simplicity then allows to scan large regions of the parameter space and to categorize the possible dynamics based on the eigenvalues of the Jacobian. Involving no approximation or restrictions to particular rate functions, the approach allows to investigate the possible dynamics of large metabolic systems, even when the construction of an explicit kinetic model is not yet feasible.

Acknowledgements

The author thanks T. Gross, B. Blasius, A. Fernie, A. Nunes Nesi and M. van der Merwe for helpful discussions on the dynamics of complex systems and the organization of the TCA cycle in plants. Fig. 1 is inspired and adapted from a figure used by Khalem and Birney (2006).

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