Plant Systems Biology
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Introduction to systems biology

Frank J. Bruggeman[1,2], Jorrit J. Hornberg[1], Fred C. Boogerd[1]
and Hans V. Westerhoff[1,2]

[1] *Molecular Cell Physiology, Institute for Molecular Cell Biology, BioCentrum Amsterdam, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1085, NL-1081 HV, Amsterdam, The Netherlands*
[2] *Systems Biology Group, Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, School of Chemical Engineering and Analytical Science, University of Manchester, 131 Princess Street, Manchester M1 7ND, UK*

Abstract

The developments in the molecular biosciences have made possible a shift to combined molecular and system-level approaches to biological research under the name of *Systems Biology*. It integrates many types of molecular knowledge, which can best be achieved by the synergistic use of models and experimental data. Many different types of modeling approaches are useful depending on the amount and quality of the molecular data available and the purpose of the model. Analysis of such models and the structure of molecular networks have led to the discovery of principles of cell functioning overarching single species. Two main approaches of systems biology can be distinguished. *Top-down* systems biology is a method to characterize cells using system-wide data originating from the Omics in combination with modeling. Those models are often phenomenological but serve to discover new insights into the molecular network under study. *Bottom-up* systems biology does not start with data but with a detailed model of a molecular network on the basis of its molecular properties. In this approach, molecular networks can be quantitatively studied leading to predictive models that can be applied in drug design and optimization of product formation in bioengineering. In this chapter we introduce analysis of molecular network by use of models, the two approaches to systems biology, and we shall discuss a number of examples of recent successes in systems biology.

From a molecular to a systems perspective in biology

In the last century many of the molecular details of living organisms have been deciphered. The identification of molecular constituents was greatly speeded up by genome sequencing. Many of the processes occurring in cells have been characterized. For simple organisms, such as *Escherichia coli* or yeast, large parts of the metabolic network structure, the operon structure and their transcriptional regulators are now known [1–3].

This knowledge allows for combined molecular and system-level studies applying a synergistic approach involving modeling, theory, and experiment under the name of *Systems Biology*. Dynamics of entire cells cannot yet be modeled with detailed kinetic models but we anticipate that this may happen within a decade or two. Detailed stoichiometric models of entire organisms have already been studied [1, 4–6]. Those cannot deal with the dynamics of cells for they do not contain any kinetic data; they focus on distributions of steady-state flux or study network organization. However, the dynamics of a number of subsystems of cells have already been modeled in great detail (e.g., [7–12]). Such models describe the molecular mechanisms operative in cells. They contain all the molecular knowledge available of the systems under study; they are near replica of the real system. We term such models *silicon-cell models*. They allow for a 'completeness' test of our knowledge (e.g., [7, 9, 10]). This form of scientific rigidity is unprecedented in biology. In addition, those models allow for analysis of the system *in silico* in ways not (yet) achievable in the laboratory (e.g., [13, 14]). More importantly, they may allow for rational strategies of drug design in medicine and optimization of product formation in bioengineering (e.g., [11, 15, 16]). Also more qualitative models are of importance in systems biological approaches to illustrate principles (re-) occurring in molecular networks [17, 18]. Such models may be model reductions of complicated silicon-cell models to facilitate explanation of phenomena by focusing on the core mechanism responsible for some phenomenon of interest. In other cases, such models may be approximations of the real system to describe phenomena too complicated to grasp without usage of mathematical modeling [14, 18, 19].

Systems biology aims to provide a firm link between the molecular disciplines in biology, such as genetics, molecular biology, biochemistry, enzymology, and biophysics, and the disciplines within biology that study entire organisms, i.e., cell biology and physiology [20, 21]. It does so by quantitatively characterizing the molecular mechanisms in organisms on a molecular and system level. Such combined molecular and system-level studies are therefore a sort of unification; they 'unify' the molecular characterization of organisms with their physiological – behavioral or functional – characterization. That is, they indicate how the properties of organisms are brought about by the properties of their molecular constitution and organization and how the system can be altered molecularly to have it behave as desired.

Many associate this kind of strategy with reduction, i.e., that properties of organisms are reduced to properties of molecules; that properties of organisms are *just* properties of molecules. We disagree with such kinds of statements [22]. Rather, the type of reduction achieved here is that of mechanistic explanation [23, 24]. Properties of organisms that are unique to organisms – not found on the level of single molecules or simpler systems thereof – are explained in terms of the molecular mechanisms that manifest those properties. Accordingly, organisms display emergent behaviors not displayed by any of their molecules in isolation, such as adaptation, growth, robustness, and natural selection [22, 25]. Those emergent system properties do depend on the properties of the molecular constituents *but* even more

so on how they interact in the organism to function in mechanisms. Without the latter knowledge the emergent properties are not understood.

From a nested-level-of-organization point of view, systems biology is an *interlevel* approach to biology rather than an *intralevel* approach, which is more characteristic of molecular biology and genetics [22]. Comparing to physics, systems biology shares more similarities with statistical thermodynamics than with macroscopic thermodynamics, which is more a mirror image of physiology or molecular biology. Contrast the temperature of a system of particles, perceived in statistical thermodynamics as the average kinetic energy of the particles, which is an intrinsically interlevel concept, with the interpretation of the ideal gas law (pV=nRT) in macroscopic thermodynamics that merely expresses a relation among system properties and is therefore intralevel. Interlevel approaches are not so common in science [26] but are central to studies of complex systems [23, 27].

Organismal properties are not properties of molecules but of networks of molecules

A characterization of a (resting) bag of billiard balls leads to a list of many properties. None of them depend on how the billiard balls are organized within the bag. Many of them are retrievable by superposition of the properties of isolated individual billiard balls. Actually, according to any reasonable sense of organization, the billiard balls in the bag cannot be considered organized relative to each other. Even if all blue ones are on top it does not matter, for many of the characterizing properties of a bag of billiard balls do not depend on the color of the balls. This example, simple as it may be, indicates a number of interesting points. For instance, not all systems have properties that depend on the organization of their constituents. One could then argue that this is obviously so since the billiard balls are all the same; therefore one cannot speak of organization in this case. But changing their color does not have an effect, indicating that only some properties of parts matter for the systems characterization in terms of its organization – or in terms of its mechanisms.

Obviously, cells are *not* comparable to a bag of billiards balls in any meaningful biological sense. Cells *do* display behaviors that depend on their molecular organization. They consist of molecules of different types that occur in different abundances depending on conditions and history. Those molecules engage in interactions of high specificity; not all molecules interact and if some of them do interact then often by varying degree. The interactions and their effects are not retrievable from the isolated molecules without considering cells as molecular networks; that is, without integrating all the molecular properties, for instance by using mathematical models [22, 25]. This does not mean that all properties of cells depend on their molecular organization. For instance, their mass, total energy and the number of molecular constituents do not.

Let's consider a simple molecular network to make the dominant role of molecular organization in determining the properties of cells more transparent. Along the way, we shall introduce a number of general characteristics of cells perceived as

molecular networks. The network we consider consists of enzyme 1 and 2. Enzyme 1 produces *X* out of *S* whereas enzyme 2 has *X* as a substrate and produces *P*:

$$S \xleftarrow{\text{enzyme 1}} X \xleftarrow{\text{enzyme 2}} P$$

We shall describe it in terms of a kinetic model (e.g., [28]); a type of modeling used often in systems biology; for examples see JWS online at www.jjj.bio.vu.nl [29, 30]. The system properties of interest are the concentration of *X* and the flux *J* through the pathway at steady state. Steady state is defined as the state where *X* remains constant while a net flux runs through the pathway. In contrast, an equilibrium state is defined as a net flux of zero while *X* is constant. Both enzymes have many different properties but only their kinetic properties matter for *X* and *J* at steady state; that is, their 3D-structure, gene sequence, or weight do not matter.

In terms of kinetic properties, the rate with which enzyme 1 produces *X* and enzyme 2 consumes *X* is described by the following reversible Michaelis-Menten rate equations [31]:

$$v_1 = \frac{V_{MAX,1} \cdot S/K_{1,S} \cdot \left(1 - X/\left(S \cdot K_{eq,1}\right)\right)}{1 + S/K_{1,S} + X/K_{1,X}} \tag{1a}$$

$$v_2 = \frac{V_{MAX,2} \cdot X/K_{2,X} \cdot \left(1 - P/\left(X \cdot K_{eq,2}\right)\right)}{1 + X/K_{2,X} + P/K_{2,P}} \tag{1b}$$

The maximal rates of the enzymes are denoted by $V_{MAX,1}$ and $V_{MAX,2}$, respectively. The affinity of the two enzymes for their substrates and products are given by Michaelis-Menten constants: $K_{1,S}$, $K_{1,X}$, $K_{2,X}$, and $K_{2,P}$. $K_{1,S}$ indicates that in the absence of *X*, the first enzyme operates at half-maximal rate if $S = K_{1,S}$ whereas if $S >> K_{1,S}$ the rate of the first enzyme is maximal. Both reactions are inhibited by their products: by a thermodynamic term, involving an equilibrium constant, $K_{eq,1}$ for enzyme 1 or $K_{eq,2}$ for enzyme 2, and by a kinetic term involving a Michaelis-Menten constant. The equilibrium constants are determined by the standard free energies of the substrates and products of a reaction and do not depend on the properties of an enzyme (e.g., [32]).

The rate of change in the concentration of *X* is described by an ordinary differential equation:

$$\frac{dX}{dt} = v_1 - v_2 \tag{2}$$

The concentration of *X* increases, i.e., $dX/dt > 0$, if $v_1 > v_2$ and *vice versa*. This is a kinetic model of the simple network we are studying. To determine the dynamics of the concentration of *X* as function of time, given some initial concentration of *X*, a

computer is most helpful. This type of kinetic modeling approach, using experimentally determined kinetic parameters and network structure, has proven very promising. Many of such type of models can be found on the JWS online website (at www.jjj.bio.vu.nl) [29, 30].

In thermodynamic equilibrium ($v_1 = v_2 = 0$), one finds that: $X = S \cdot K_{eq,1} = P / K_{eq,2}$. Apparently, the kinetic properties of the enzyme do not matter! This is a general result for systems in thermodynamic equilibrium irrespective of the complexity of the network [33]. This changes in a steady state. To attain a steady state, the concentrations of *S* and *P* should remain fixed (set by the experimentalist) and their ratio (*P/S*) should not be chosen equal to the product of the equilibrium constants of the two reactions. In the steady state, $v_1 = v_2 \neq 0$ and the concentration of *X*, i.e., $\bar{X}$, is a solution from the algebraic equation $v_1 - v_2 = 0$. We will not give the analytical solution here as it is given by a rather complicated equation that depends on all the kinetic properties. Graphically, the steady-state concentration of *X* and the flux *J* can be found by determining the intersection of the rate functions v_1 and v_2 as function of *X* for a given set of kinetic parameters. It is not hard to imagine that all kinetic parameters now effect $\bar{X}$ and *J*, for the shape of the rate curves of enzyme 1 and enzyme 2, and therefore their intersection, depends on them. The steady-state flux *J* now equals $v_1(\bar{X})$.

For illustrative purposes, let us consider a biologically unrealistic form of rate equations for enzyme 1 and 2; that is, mass-action kinetics:

$$v_1 = k_1^+ S - k_1^- X, \; v_2 = k_2^+ X - k_2^- P \tag{3}$$

The '*k*' coefficients are referred to as elementary rate constants. The steady-state concentration of *X* now equals:

$$\bar{X} = \frac{k_1^+ S + k_2^- P}{k_1^- + k_2^+} \tag{4}$$

Already in this simple example, with unrealistic kinetics and over-simplified network structure, we find that all the kinetic parameters of the reactions and a characterization of the environment, the fixed concentrations of *S* and *P*, determine the steady state concentration of *X*. The mathematical function describing the dependency of the steady state concentration of *X* on those parameters, i.e., Eq. 4, is also dependent on the network structure. This illustrates that only by integration of all those pieces of information, i.e., characterization of the environment, properties of reactions, and network structure, the steady-state system properties can be retrieved. Examples of such studies can be found on the online modeling website JWS online (www.jjj.bio.vu.nl).

To investigate whether all molecular properties of the network are equally important we return to the description of the system having biologically relevant kinetics. Suppose we want to determine whether enzyme 1 and 2 are as important for controlling the steady-state concentration of *X* by investigating the fractional change in $\bar{X}$ upon a fractional in the enzyme amount of enzyme 1 and 2 by changing their

V_{MAX}'s. This we accomplish for enzyme 1 by taking the total fractional derivative of the steady-state condition for X, i.e., $v_1(\bar{X}, V_{MAX,1}) - v_2(\bar{X}) = 0$:

$$\frac{\partial \ln v_1}{\partial \ln \bar{X}} \frac{d \ln \bar{X}}{d \ln V_{MAX,1}} + \frac{\partial \ln v_1}{\partial \ln V_{MAX,1}} - \frac{\partial \ln v_2}{\partial \ln \bar{X}} \frac{d \ln \bar{X}}{d \ln V_{MAX,1}} = 0 \tag{5}$$

In terms of metabolic control analysis (MCA) [32, 34–36], those differentials are identified as control coefficients ('C' with proper subscript and superscript) and elasticity coefficients ('ε' with proper subscript and superscript):

$$C_1^X = \frac{d \ln \bar{X}}{d \ln V_{MAX,1}}, \; \varepsilon_X^{v_1} = \frac{\partial \ln v_1}{\partial \ln \bar{X}}, \; \varepsilon_X^{v_2} = \frac{\partial \ln v_2}{\partial \ln \bar{X}} \tag{6}$$

This gives an expression for the dependence of the concentration control coefficient of the first enzyme on the steady-state concentration of X in terms of elasticity coefficients (note that: $\partial \ln v_1 / \partial \ln V_{MAX,1} = 1$):

$$C_1^X = \frac{-1}{\varepsilon_X^{v_1} - \varepsilon_X^{v_2}} \tag{7}$$

Typically, the elasticity coefficient of the first enzyme for X shall be negative: X inhibits the rate of its producing enzyme. It activates the rate of the second enzyme. This leads to a positive control coefficient for enzyme 1, which can be intuitively understood: a higher activity of the first enzyme should lead to a higher concentration of X to allow for a higher rate of enzyme 2. For the second enzyme, we obtain (after the same operation as in Eq. 6 with respect to $V_{MAX,2}$):

$$C_2^X = -C_1^X \tag{8}$$

Interestingly, the sum of the concentration control coefficients equals zero! This can be understood by considering that, if in steady state, $v_1(\bar{X}) - v_2(\bar{X}) = 0$, both rates are changed by the same factor α, the value of $\bar{X}$ shall remain unchanged. The steady-state flux will change with factor α, however; illustrating that the flux control coefficients of the two enzymes obey the following law:

$$C_1^J + C_2^J = 1 \tag{9}$$

The flux control coefficient of enzyme 1, i.e., C_1^J, is defined as:

$$\begin{aligned} C_1^J &= \frac{d \ln J}{d \ln V_{MAX,1}} = \frac{d \ln v_1}{d \ln V_{MAX,1}} = \varepsilon_X^1 C_1^X + 1 \Rightarrow \\ C_1^J &= \frac{-\varepsilon_X^2}{\varepsilon_X^1 - \varepsilon_X^2} \end{aligned} \tag{10}$$

Interestingly, it has been proven mathematically that those two summation theorems (Eq. 8 and 9) hold irrespectively of the complexity of the network (having r reactions) and for all concentrations and fluxes [34, 35, 37]:

$$\sum_{i=1}^{r} C_i^X = 0, \ \sum_{i=1}^{r} C_i^J = 1 \tag{11}$$

This can be understood by the same kind of reasoning as was given above. Networks with a level-structure or cascade-structure have additional summation theorems [38, 39].

Within the network studied so far two other theorems exist. They are referred to as connectivity theorems and relate control coefficients and elasticity coefficients:

$$C_1^X \varepsilon_X^1 + C_2^X \varepsilon_X^2 = -1, \ C_1^J \varepsilon_X^1 + C_2^J \varepsilon_X^2 = 0 \tag{12}$$

Those relationships can be easily verified using Eq. 7, 8, 9 and 10. Those two equations can be easily understood by considering one of the assumptions of MCA: it assumes that the steady state is (asymptotically) stable with respect to fluctuations [32]. This stability means that the time-averaged concentration X in steady state, despite of thermally fluctuating reaction rates, equals $\bar{X}$ (and that the time-averaged flux equals J) with a variance depending on the distance from thermodynamic equilibrium and the non-linearity of the system at steady state [32, 40, 41]. The connectivity theorems express exactly this stability property for they indicate the outcome of the dissipating response of the system to restore any change in $\bar{X}$ and J upon a perturbation in $\bar{X}$ induced by thermally fluctuating reaction rates. In contrast to the summation theorems, the connectivity theorems do depend on the structure of the network [37, 42–44]. Together the summation and connectivity theorems allow one to derive control coefficients in terms of elasticity coefficients [42].

This section illustrated that many of the interesting properties of cells studied in cell biology and physiology are related to the properties of the molecules, the environment, and the network structure in a complicated nonlinear fashion. The exact dependency only becomes evident by integrating all those properties using models. This we illustrated using metabolic control analysis. Models then may indicate the existence of general relationships reminiscent of laws in physics [45].

Two approaches to systems biology: top-down and bottom-up

Two approaches to systems biology can be distinguished. *Top-down systems biology* starts with data, often generated by system-wide methods, and analyses this data using network models of various types and degrees of detail to discover molecular mechanisms, modules, and patterns of functional behavior (e.g., [4, 46–50]). Typically, the data analyzed originate from metabolomics, flux analysis, proteomics, transcriptomics, or combinations thereof. The following chapters will provide detailed information of how such data are acquired. This approach relies more on in-

duction than *bottom-up system biology*. Top-down systems biology extracts information from the data rather than deducing it from pre-existing knowledge. In bottom-up systems biology experimentation is done not on the entire system level but on smaller subsystems and typically small quantitative heterogeneous datasets are used, containing steady-state and transient metabolite and flux data. The experiments are done on the basis of detailed models of the system to both validate and improve the model or to investigate hypotheses inspired by model analysis. The models used are typically silicon-cell models (e.g., [7–12, 51, 52]). Top-down systems biology is an interesting approach for determination of the network structure and the identification of the molecular mechanisms operative within cells that have not yet been fully characterized [53]. This approach may lead to a more complete picture of the molecular network inside cells. In later stages, top-down systems biological studies may develop into bottom-up approaches as soon as the network has been more carefully characterized. Bottom-up systems biology builds on pre-existing molecular data and allows for analysis of their systemic consequences for the cell [20].

Examples of systems biology research[1]

One aspect of systems biology is the analysis of the structure of the molecular networks and its consequences for the cell. In much the same way as genome sequencing has lead to the emergence of the theoretical analysis of genomes (bioinformatics), has the availability of the entire metabolic, signaling, and gene networks of cells led to the development of theoretical analyses of networks [6, 54]. Many interesting properties of molecular networks haven been discovered [54–56]. Most noticeably are small world organization [57, 58], modularity [59, 60], motifs [61–63], flux balance analysis, extreme pathway and elementary mode analysis [6, 64–67]. All these methods analyze large-scale molecular networks and induce general information regarding their structure and functional consequences. This is one exciting branch of systems biology that is anticipated to develop further and discover many new insights into the molecular organization of cells. Reviews on this aspect of systems biology can be found elsewhere [6, 54].

Another aspect of systems biology is the construction of kinetic models of molecular network functioning as was introduced briefly in the previous section [12, 17, 20]. The history of kinetic model construction and analysis is already long. The first models of metabolism were created in the 1960s and 1970s [68, 69]. Those models suffered mostly from a lack of sufficient system data. The introduction of desktop computers, the development of theory for the analysis of dynamics of nonlinear systems (e.g., [70]), and the development of non-equilibrium thermodynamics (e.g., [71, 72]) lead to the analysis of simplified models – core models – illustrating complex dynamics of molecular networks [19, 73–76]. As understanding progressed, those core models were interchanged for detailed models describing com-

[1] The models mentioned in this section can all be investigated online at the JWS online website (www.jjj.bio.vu.nl)

plex dynamics, e.g., compare core models of glycolysis [74, 75] with detailed models [77, 78]. The more detailed models are of interest in bioengineering as they may facilitate rational approaches to optimization of product formation [10, 11, 51, 79].

Hoefnagel et al. [11] developed a kinetic model of pyruvate metabolism in *Lactococcus lactis* to optimize the production rate of acetoin by this organism. All the rate equations of enzymes, as they were characterized in the literature, were incorporated in a kinetic model. They showed that two enzymes (lactate dehydrogenase (LDH) and NADH oxidase (NOX)), previously not identified as important for acetoin production, had most control on the acetoin production flux. By deleting LDH and overexpressing NOX in experiment they were able to redirect carbon flux to acetoin; 49% of pyruvate consumption flux in the mutant *versus* ~0% in the wild type. This result was of importance for industry.

Glycolysis is a catabolic pathway (Fig. 1A) that is present in all kinds of cells. Teusink et al. [80, 81] constructed a kinetic model of yeast glycolysis that was quite helpful in solving the puzzle of an unexpected phenotype of a particular mutant strain and at the same time lead to a surprising new insight about glycolysis. *Saccharomyces cerevisiae* strains with a lesion in the *TPS1* gene, which encodes trehalose-6-phosphate (Tre-6-P) synthase, cannot grow with glucose as the sole carbon and free energy source. Although this enzyme appeared to have little relevance to glycolysis – it was considered to function in the formation of storage carbohydrates and the acquisition of stress tolerance – it turned out to be crucial for growth on glucose. Using the detailed kinetic model of *S. cerevisiae* glycolysis it was shown that the turbo design of the glycolytic pathway (Fig. 1B), apart from being useful in allowing for rapid growth, also represents an inherent risk. A yeast cell investing ATP in the first part of glycolysis and producing a surplus of ATP in the downstream (lower) part of glycolysis runs the risk of an uncontrolled glycolytic flux. In the model, this resulted in the accumulation of hexose monophosphate and fructose-1,6-bisphosphate to levels that are considered toxic when established in the real yeast cell. The formation of trehalose-6-phosphate prevented glycolysis from going awry by inhibiting hexokinase (Fig. 2A), the first ATP-consuming step of glycolysis and thereby restricting the flux of glucose into glycolysis [80]. The importance of the trehalose branch of glycolysis for growth on glucose could only be discovered through the systems biological approach of combining experimental data with kinetic modeling as outlined above. Detailed models can also be used to calculate the outcome of experiments that are not yet achievable, too laborious or too costly to perform as a pilot experiment. Glycolysis in *Trypanosoma brucei* takes place in a special organel, the glycosome, except for the steps by which 3-phosphoglycerate is converted into pyruvate. In contrast to the situation described above for *S. cerevisiae*, the first step catalyzed by hexokinase is not at all regulated in trypanosomes. The glycosome is surrounded by a membrane (Fig. 2B). Bakker et al. [13] were able to calculate the effect of the removal of the glycosomal membrane in *T. brucei*. At the time, this experiment could not be performed experimentally. However, they could remove the membrane in a detailed kinetic model that was validated earlier [7]. The removal of the membrane was of interest because the biological advantage

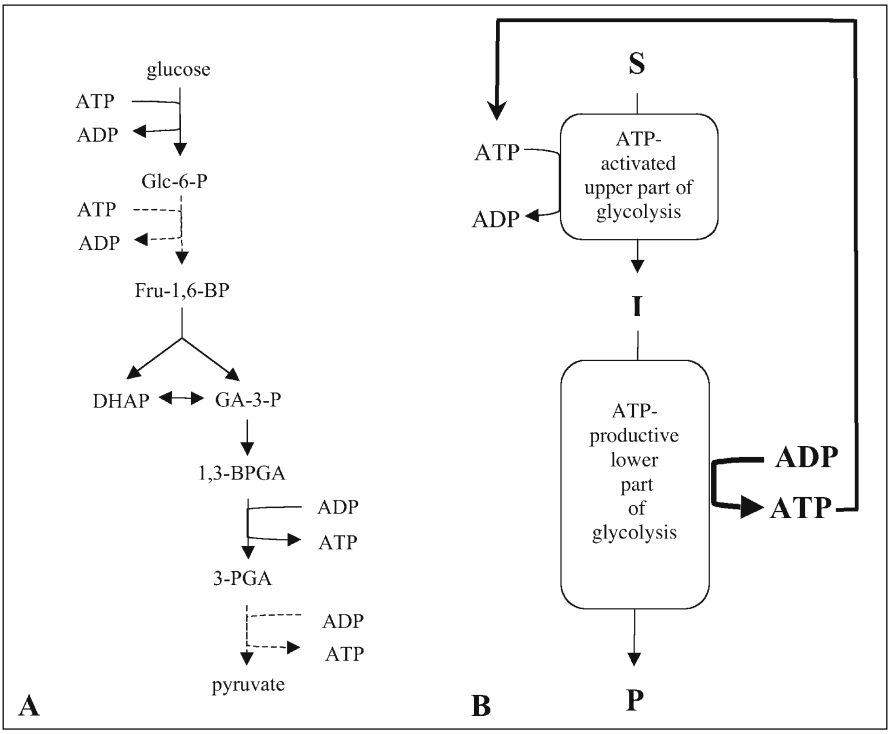

Figure 1. The dangerous turbo design of glycolysis. (A) A simplified scheme of glycolysis. Solid lines represent reactions catayzed by a single enzyme; dashed lines represent multiple sequential reactions. Glc-6P, glucose 6-phosphate; Fru-1,6-BP, fructose 1,6 bisphosphate; DHAP, dihydroxyaceton phosphate; GA-3-P, glyceraldehyde 3-phosphate; 1,3-BPGA, 1,3-bisphosphoglycerate; 3-PGA, 3-phosphoglycerate. (B) The turbo design of glycolysis. Generalized scheme for glycolysis in which the upper part from substrate S to intermediate I combines the ATP-consuming reactions and the lower part from I to product P combines the ATP-producing reactions. The surplus of ATP produced in the lower part is depicted in bold capitals and the boosting effect on the upper part is indicated by thick lines.

of the glycosome was hypothesized by others to enable this organism to have an extremely high glycolytic flux. Bakker et al. [13] showed that yeast – which does not have glycosomes – can have fluxes as high as *T. brucei*. In addition, they showed that the removal of the glycosomal membrane did not cause a physiologically significant change in the glycolytic flux. Rather, the removal of the glycosome caused accumulation of glucose-6-phosphate and fructose-1,6-bisphosphate up to 100 mM. This would certainly represent a pathological situation for *T. brucei* involving phosphate depletion and possibly osmotic swelling. As it turned out, the glycosomal membrane makes sure that the upper part of glycolysis is not accelerated by the ATP produced by the lower part of glycolysis, because the surplus ATP producing step in the lower part of glycolysis (by pyruvate kinase) actually resides outside of the glycosome. Thus the glycosome is another implementation of a protective device

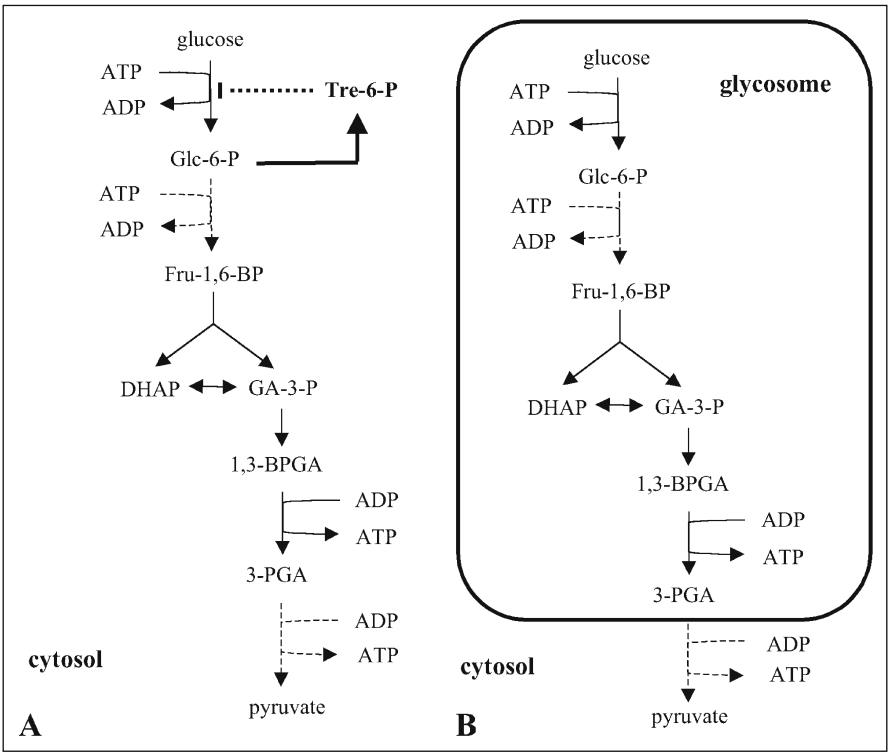

Figure 2. Two different solutions to the turbo design problem. (A) The trehalose branch in *S. cerevisiae*. The scheme is the same as the one shown in Figure 1A, except for the addition of the trehalose shunt in bold. Tre-6-P, trehalose 6-phosphate. The inhibition of hexokinase by Tre-6-P is indicated by a thick dashed line. (B) The glycosome in trypanosomes. Again, the scheme is the same as the one shown in Figure 1A, except for the addition of the glycosomal membrane in bold. The conversion of 3-PGA to pyruvate takes place outside of the glycosome.

against the potentially dangerous 'turbo' design of glycolysis. These two examples of models of glycolysis demonstrate the power of (bottom-up systems biological) kinetic models; when precise and detailed knowledge of the kinetics of the molecular components is available, so-called computer experimentation can be carried out which serves as an adequate substitute for true experimentation.

Regulation of metabolic flux is governed by many different mechanisms. They may function at the level of metabolism, transcription, translation, or at the level of degradation of mRNA or protein. At the level of metabolism, contributions to the regulation of enzymatic conversion rates are made by substrates and products, by effectors through allosteric feedback or feedforward loops, or by covalent modification. Recently a quantitative mathematical tool has been developed in our laboratory, referred to as hierarchical regulation analysis, that allows for the quantitative determination of the importance of all those mechanisms that contribute to the regulation of flux, given experimental data [82–84].

The regulation of the ammonium-assimilation flux by *Escherichia coli* is governed by a complicated mechanism involving multiple covalent modifications, feedback, substrate/product effects, gene expression and targeted protein degradation [85, 86]. This system has for a long time been a paradigm of flux regulation by way of covalent modification. We have recently integrated all molecular data of this network into a detailed kinetic model describing the short-term metabolic regulation of ammonium assimilation [12]. We confirmed many of the hypotheses postulated in the literature on how this system should function. We identified that covalent modification of glutamine synthetase is the most important determinant of the ammonium assimilation flux upon sudden changes in ammonium availability using hierarchical regulation analysis. Removal of the covalent modification of glutamine synthetase caused accumulation of glutamine and severe impairment of growth as was shown experimentally by others [87]. It was confirmed that indeed gene expression of glutamine synthetase alone can lead to regulation of ammonium assimilation; the ammonium assimilation flux was not sensitive to changes made in the level of any of the other enzymes. Finally, we predicted that one advantage of all this complexity is to allow *E. coli* to keep its ammonium assimilation flux constant despite of changes in the ammonium concentration and to change from an energetically unfavorable mode of ammonium uptake to a more favorable alternative as the ammonium level is increased.

The analysis and construction of models incorporating signal transduction networks at a high level of molecular detail has recently been pioneered because of their high potential in drug design [8, 15, 52, 88–90]. We have investigated one of the largest and most complete model of a signal transduction network for its control properties [90]. We determined the control coefficients of all the processes in the network on three characteristics of the transient activation profile of extracellular signal regulated kinase (ERK), which is a member of the mitogen activating protein kinase (MAPK) family. The model contained 148 reactions and 103 variable concentrations and it is an enlarged version of the model published by Schoeberl et al. [89]. To our surprise, we found that less than 10% of the reactions had a large control on ERK activation. We identified RAF as a candidate oncogene and indeed it was found frequently mutated in tumors. To cope with the enormous size of signal transduction network some systems biologists are presently developing theoretical methods for model reduction [91–93]. Such strategies may greatly facilitate understanding, analysis, and experimental design.

In model-driven experimentation, usage of simplified models that illuminate principles of system functioning and guide experimentation (experimental design) are extremely helpful. This approach is nicely illustrated by a series of papers by the group of Ferrell and co-workers [94–97] and Alon and co-workers [98–102]. In Pomerening et al. [97], Ferrell and co-workers investigate the core oscillator driving the cell cycle in *Xenopus laevis*. They study the entry into mitosis and the subsequent return to interphase by following the dynamics of the formation and degradation of the complex cdc2-cyclinB. The interphase-mitosis transition (mitosis: M-phase) is accompanied by synthesis and accumulation of cyclin-B and the subsequent formation of cdc2-cyclinB complex. The degradation of this complex is mediated by

APC-catalyzed degradation of cyclin-B and signals the exit of the M-phase and reentry into interphase. In addition, two net positive feedbacks play a role: via Myt1-Wee1 and cdc25. It was shown experimentally [103] that in the absence of the degradation of cyclin-B by APC the resulting network is bistable. In the presence of cyclin-B degradation, the network displays the oscillations characteristic for the cell cycle; more specifically, it functioned as a relaxation oscillator. Using a semi-detailed model (based on [18, 103]), the authors modeled the network in the absence and the presence of the degradation of cyclin-B and found bistability and oscillations, respectively. Then they investigated the effects of the two net positive feedbacks by inhibiting them. This caused the core oscillator to engage in damped oscillations rather than prolonged oscillations indicating the essentiality of the positive feedback for proper functioning of the cell cycle. The model they used was only quasi-detailed at best but still it had sufficient detail and reflection of reality facilitating model-driven experimentation. In our studies on MAPK signaling, we took a similar approach [45]. We used a simple core model of the MAPK pathway to investigate the difference between inhibition of phosphatases and kinases on the activation profile of ERK. We found that the core model could qualitatively predict the experimental data. It showed that phosphatases tend to control both the amplitude and duration of signaling whereas kinases tend to control only the amplitude. Those results were backed up by theory leading to new theorems in control analysis for signal transduction [45]. Another successful application of the use of simple models to drive experimentation is found in the work by Alon and co-workers [98–102]. They are characterizing the functional properties of motifs, small intracellular networks that occur more frequently in biological networks than in networks of similar size with a random structure. So far they focused mostly on gene circuitry and their activation by transcription factors. The reasoning behind the search and characterization for motifs is that if they occur significantly more frequently in biological networks their design is predicted to have a functional relevance for the cell. They have been successful in showing the functional significance of a number of these motifs. Synthetic biology takes the opposite approach. It tries to design new networks using simple models and implement those in cells to facilitate their analysis, as biosensors, and to endow them with new properties. One successful approach of synthetic biology has been the analysis of noise [104–111]. Noise occurs naturally in all physical systems. In cells noise, perceived as fluctuating copy numbers of molecules in cells, occurs because of fluctuating reaction rates due to local thermal fluctuations [40]. The magnitude of the fluctuations relative to the average copy number determines their influence and importance on intracellular dynamics. The effects of noise are most pronounced when the copy number of molecules are small, < 50 molecules/cell, but may become high even in systems with high average copy numbers, ~1,000s molecules/cell, if the system is sufficiently nonlinear [41, 112].

Conclusion

Systems biology is a rational continuation of successful experimental biology initiated by the molecular biosciences. It represents a combined molecular and systems approach to decipher how molecules jointly bring about cell behavior by cooperating in mechanisms. Those mechanisms can be studied individually (or in a small number) in bottom-up approaches of systems biology using either detailed models or core models. Top-down approaches of systems biology hope to identify such mechanisms and characterize them more roughly first before bottom-up approaches can home in on them in more detail. When the two approaches are combined a rational approach to discovery and characterization of molecular mechanisms, and therefore of cells, results that supplements pure molecular approaches.

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