

Analysis of metabolic control strategies

Karl Bayer

Department of Biotechnology

University of Natural Resources and Applied Life Sciences

Muthgasse 18, A-1190 Vienna, Austria

Phone: +43-1-36006-6220, Fax: +43-1-3697615

email: karl.bayer@boku.ac.at

ABSTRACT: Due to their complexity microbial cells have evolved multiple regulatory networks to control growth and replication and to cope with adverse conditions. Based on a hierarchical organisation and modularisation of the cell metabolism transcription of the particular genes can be instantly induced or inhibited. This tight and selective control is achieved by master regulators in combination with expression of highly specific transcription factors which in turn initiate specific reaction cascades. Robustness is another important feature of biological systems. The hierarchical organisation in combination with modularity, redundancy by development of alternative pathways and feedback control circuits are the key elements contributing to the increase of robustness. The evolved strategies have proven their aptitude in complex systems and need to be assessed in other areas of technology.

KEYWORDS: transcription factors, hierarchical, regulatory network, robustness, modularisation,

INTRODUCTION

Bacteria are omnipotent single cell organisms able to perform all biochemical reactions essential for their life, such as growth/replication, metabolism (anabolism/catabolism), DNA replication, transcription and translation, response sensor systems, differentiation, motility and communication. The great variety of biochemical reactions needs tight coordination such as the appropriate supply of metabolites and energy for synthesis processes and compensation of changing environmental conditions. Therefore regulation of cellular processes must be designed for a high number of parallel processes with built in strategies to orchestrate catabolic and anabolic reactions in the most efficient and economic way.

KEY ELEMENTS OF REGULATION

The fundamental reaction sequence to establish the “hardware” of cell metabolism is represented by the dogma of biochemistry, i.e. from DNA \rightarrow mRNA \rightarrow proteins (transcription, translation) (Fig. 1). The basic units of transcription control, the operons, consist of a polymerase binding site, a regulatory site (operator) and the sequence of the structural gene encoding the particular protein. Operons are controlled by repressors and activators. The expressed proteins are mainly involved in biocatalysis (enzymes), binding reactions (antibodies, receptors, DNA binding proteins) and formation of cellular structures. The amount of protein is controlled on the transcription level by induction and repression.

The main entity of transcription is the RNA polymerase (RNAP). The “core enzyme” shows unspecific binding to DNA, the promoter specificity is conferred by σ -factors, e.g. the σ_{70} -factor, which directs the RNAP holoenzyme to promoters with -35 and -10 boxes (Pribnow box). Binding of RNAP to the promoter results in a “close complex”. The operator sequence enables binding of the repressor.

Sigma factors

Since the rather simple combination of repressors and activators does not qualify to perform the needs of complex metabolic control cells have evolved means to achieve transcription of certain subsets of genes distributed all over the genome. Therefore specific proteins (= sigma factors) are expressed, which are able to recognise different promoter consensus sequences and bind to the RNA which in turn trigger the expression of specific proteins to confer either protection or catalyse degradation. Fine tuning is achieved by the amount of the expressed sigma factor or even by

expression of anti-sigma factors. The following table gives an overview of sigma factors involved in E. coli metabolism (Table I).

From the concept of specific sigma factors triggered during particular perturbations the hierarchical organisation of regulatory networks on different levels was derived. As mentioned, operons are the fundamental transcription units under the control of one promoter. On the next level, the so called regulon, operons distributed on the genome are controlled by the same regulatory protein (activator or repressor). Modulons, representing a further level comprise genes which are controlled by a general regulator, e.g. cAMP/CRP. Stimulons acting on the highest level are involved in the activation of global networks, e.g. triggered by environmental stimuli or by starvation due to nutrient limitation (stringent response).

In order to assess the aptitude the cellular regulatory design concepts for the control other complex systems it is important to analyse the components and the signalling pathways of the network. A general view on the design principles shows that the information content derived from the DNA can be mainly assigned to the sequence, whereas that of mRNA combines sequence and structural properties, such as codon usage to modulate elongation rate and thereby assist accurate folding. Finally, the multiple functions of proteins rely to a major part on the adoption of a particular structure. Therefore an overall view of the flow of information from DNA to protein shows a transition from a sequence to a structure dominated system in analogy from digital to analogous control. This "concept" enables great flexibility by synthesis and/or degradation of the particular catalysts, transcription factors, inhibitors on demand.

REGULATORY STRATEGIES

To comply with the multiple demands of metabolic control regulatory mechanisms can be activated on different levels such as transcription, mRNA stability, translation, substrate uptake, biomolecule structure.

As the regulatory tasks span from maintaining growth associated processes to overcoming versatile perturbations such as starvation, osmotic shock etc. cells have developed complex regulatory and signalling networks. An additional challenge of physiological regulation is brought up by the fact that stress response can be triggered by different elicitors whereby identical regulatory loops are activated.

One of the most remarkable phenomena of cellular regulation is the fact that different strengths of perturbations often lead to only slight changes of the overall physiological activity, cells act within a certain "solution space" defined by physical-chemical constraints. Effective regulation of the cell metabolism is provided by the concept of 'master regulators' serving as decisive information processing units (for review see [1]). To safeguard key processes of cellular life such as replication, translation and the core metabolism 'house keeping' genes are expressed under non limiting conditions at a approximately constant rate. To overcome severe perturbations even the activity these genes will be changed.

The 'master regulators' provide a link between complex signalling networks with downstream regulatory networks which are directly involved in the expression control of response associated regulatory cascades. These regulatory networks exhibit a hierarchical structure which enables the involvement of lower level secondary regulators for very specific and confined signal input.

Two component regulatory systems

Small molecules enter the cell and function as effectors. However, these signals are not transmitted directly to the regulatory protein but they are further processed in a signal transduction pathway. As an example a kinase located in the cell membrane is phosphorylated in response to an environmental signal. The phosphoryl-group is then transferred to a response regulator acting as DNA-binding protein, which blocks transcription in the phosphorylated state. In addition a phosphatase removes the phosphate whereby the response regulator is returned to the fully unphosphorylated state except the signal has not been re-established by the kinase activity.

Regulatory motifs and modules

The analysis of cellular regulation has shown that the understanding of control networks can be improved and simplified by identification of motifs representing small regulatory subnetworks, which can be clustered owing to their function, structure etc. (for details see [2]).

Regulatory motifs are described analogous to switches, amplitude filters, oscillators, noise filters, amplifiers, logic gates and memory units.

Interconnected motifs are further arranged into modules. An interesting aspect is brought up by Wolf and Arkin [2] with intracellular noise being produced largely at the protein translation stage to establish survival enhancing population

heterogeneity. Since each cell represents an independent unit out of the competing cellular processes evolutionary stable processes can be derived and transferred to other areas of industrial application.

ROBUSTNESS OF CELLULAR FUNCTIONS

In view of “what can we learn from biology” robustness is an important topic. In biology robustness is understood as the ability to maintain performance in the face of perturbations arising from environment and fluctuations of genetic stability (an excellent review see [3]). Other authors define robustness as “canalization” towards a specific outcome from uncertain starting conditions [4].

In order to be able to derive robustness design principles from biological systems an accurate definition is imperative. In the past stability, homeostasis and canalisation were often related to robustness. However, robustness must be seen as relative property and hence it is necessary to specify the unchanged properties and for which species of perturbations these properties are not changed. In engineering and biology robustness is always connected to functionality due to the fact that we have to deal mainly with global parameters. Therefore the assessment of robustness requires a more detailed specification of functions and perturbations.

As already mentioned biological systems tend to perform a more or less identical outcome in spite of considerable changes of the environment, the processes are operated within a defined solution space.

Important observations of biological systems showed a close connection between complexity and fragility. It is a drawback of complex systems that the increase of robustness of one feature made at the same time other features more fragile. Shielding certain functions of a system may require additional control loops and thereby increase fragility [5].

Basic mechanisms that confer robustness to biological and engineered systems

A survey of biological systems brought up the following mechanisms contributing to robustness:

Redundancy, feedback control, hierarchy, modularity and developmental control circuits.

Redundancy: in biological systems redundancy in terms of a backup system does not exist, because it would be eliminated during evolution. The concept is that other entities perform the required pathway. These capabilities can be acquired during “forced” evolution.

Feedback control: a feedback control circuit is an important strategy to increase robustness.

Modularity: cells constitute of “functional units” (modulons) with strong internal connections. The spatial separation of these units often leads to compartmentation. Thereby unwanted reactions of metabolites can be avoided and/or different environmental conditions can be maintained.

Hierarchy: different layers of integration and the creation of subsystems in conjunction with specific control tasks enables to span response reactions from global answers to very specific singular interventions. Cellular networks are clearly hierarchically organised and provide a sophisticated design to be applied with other technologies. The steadily increasing number of analytical platforms providing insight on molecular level will improve the monitoring of individual components and thereby facilitate the comprehension of their interplay.

Developmental control circuits: multicellular systems provide increased robustness due to specialisation of the different cells.

CONCLUSION

Due to the ever increasing knowledge of biological systems, in particular from the control of gene expression by the hierarchical organisation of regulatory and signalling networks, from modularity, feedback loops and from highly specific metabolic control we will be able to derive efficient design principles for other complex systems. Due to the rapid development of analytical platforms a more detailed view of cellular processes will be available in the near future and thus leading the way to quantitative description and prediction of the behaviour of an ever increasing number of different organisms.

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Figure 1: Principles of control

Table I: Sigma factors

Table I

Sigma factors in <i>E. coli</i>	Function
$\sigma 70$	Major sigma factor during normal growth
$\sigma 54$	Nitrogen assimilation
$\sigma 38$	Major sigma factor during stationary phase, also for genes involved in oxidative and osmotic responses
$\sigma 32$	Heat shock response (chaperones)
$\sigma 28$	Genes involved in flagella synthesis
$\sigma 24$	Response to misfolded proteins in periplasma
$\sigma 19$	For certain genes in iron transport

Figure 1

