EXTENSION OF A MATHEMATICAL MODEL FOR CARBON CATABOLITE REPRESSION IN *E. coli* TO DESCRIBE GALACTOSE EFFLUX

A. Kremling, K. Bettenbrock and E.D. Gilles
MPI für Dynamik komplexer technischer Systeme
Leipzigerstr. 44, 39120 Magdeburg

Summary:

A mathematical model that is able to describe the phenomenon of glucose-lactose diauxie in *E. coli* was peviously presented [3]. Although the model reflects growth and gene induction of the wildtype strain and of some mutants very well it failed to describe the behavior of a *ptsG* mutant lacking the major uptake system for glucose. Experimental data showed that relativly high rates of efflux of glucose and galactose from the cells could account for the difference in model and reality. Here, we desribe the analysis of galactose export in *E. coli* and the extension of the model by inclusion of a galactose transport/export system.

Introduction

Mathematical modeling provides a powerful tool to understand and elucidate biological regulation. A modeling concept was introduced previously allowing the set up of models by aggregating defined modeling objects to higher structured functional units [3]. By applying this modeling concept a mathematical model to describe glucose and lactose transport was set up [1]. One important feature of the modeling concept is that functional units are characterized by a hierarchical organisation of the regulatory proteins involved. A new approach was introduced to calculate the transcription efficiency in such networks by assigning each regulatory protein to one level in hierarchy [2].

In *Escherichia coli* the expression of carbohydrate uptake systems and metabolizing enzymes is very well controlled in order to avoid the useless expression of proteins. For growth, some carbohydrates are preferred towards others, resulting in the sequential use of different carbohydrates in mixed cultures. The best examined example of this phenomenon is the diauxic growth of *E. coli* in cultures with glucose and lactose. Because of the extensive knowledge that has accumulated glucose-lactose diauxie of *E. coli* is a perfect model system of complex regulatory reaction networks. The main control in this system is exerted by the cAMP.Crp transcriptional activator complex. This complex is active in the regulation of a number of operons, most involved in the quest of food. The concentration of the alarmone cAMP inside the cell

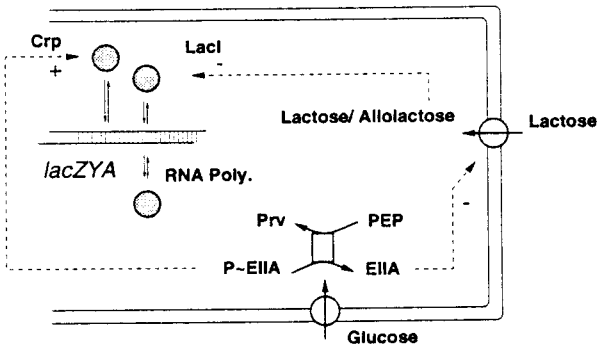

Figure 1: Scheme of lactose induction with proteins involved: *LacI*, *Crp* and RNA polymerase.

and thereby the concentration of the cAMP.Crp complex is regulated by complex mechanisms. These mechanisms are basically understood.Central in its regulation is the action of the phosphoenolpyruvate-dependent phosphotransferase systems (PTSs), especially the glucose PTS. If the PTSs are not active in the uptake of substrates, the PTS proteins including Crr which acts as the EIIA in glucose transport

accumulate in their phosphorylated form. P~Crr is needed for the activation of the enzyme adenylate cyclase (CyaA) that converts ATP into cAMP. This brings about that only in the absence of transport of PTS substrates an activation of CyaA is possible resulting in an increased level of cAMP inside the cell and in the formation of the cAMP.Crp activator complex. As a result operons like the *lac* operon that depend on the Crp.cAMP complex for transcription can be expressed exclusively if no PTS-substrates are present that is PTS-substrates in the medium repress transcription of the members of the *crp* modulon. This regulation has therefore been termed catabolite repression. The Crr protein is also active in regulating the activity of the lactose permease, LacY, another important step in the establishment of diauxie. The unphosphorylated form of Crr present if substrates are transported by the PTS is able to form a complex with LacY thereby inhibiting uptake of lactose from the culture medium. This phenomenon is referred to as inducer exclusion.

A mathematical model for catabolite repression in *E. coli*

According to the modular modeling concept the proposed model covers glucose, lactose and galactose uptake and metabolism as well as the central pathways for carbohydrates starting from glucose-6-phosphat [1]. In the central pathways all drain fluxes to the monomers are included. Figure 2 shows a scheme of the functional units. The global signal transduction unit describes the signal processing from

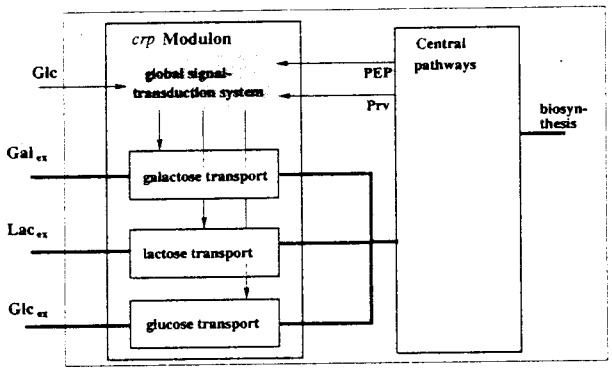

Figure 2: Structure of the entire model with relevant in- and outputs of the functional units. Global signal transduction system as well as pathways for lactose and glucose are described in [1]. Galactose pathway is described here.

the extracellular stimulus glucose to the target regulator protein Crp. It covers the PTS (regarded here as a sensory system), the synthesis of cAMP by the enzyme adenylate cyclase, and the interactions of cAMP with Crp and subsequent binding to the DNA binding sites. The control regions e.g. for the enzymes of lactose uptake and degradation show at least three binding sites for regulatory proteins, the polymerase, the repressor (LacI) and the cAMP.Crp binding site, reflecting the hierarchical organisation of the network. The model was validated quantitatively by performing experiments with isogenic mutant strains. Measured data and simulation resultus were compared. In addition the experimental data were used for parameter estimation.

Model extension

Motivation

By performing experiments for model validation one of the mutant strains, the *ptsG* mutant BKG47 displayed a behavior that was not well reflected by the model. The model introduced so far does not describe export of sugars. New measurement indicate, that galactose is exported in the wild type as well as in the mutant BKG47.

Transport/export of galactose

The following reaction equation holds:

$$Gal_{ex} \overset{r_1}{\rightleftharpoons} Gal \overset{r_2}{\rightleftharpoons} Glc6P \tag{1}$$

For reaction rates r_1 and r_2 a reversible Michaelis-Menten equation and a irreversible Michaelis-Menten equation was choosen, respectively. To analyse galactose export with the help of the mathematical model, the different types of exporters are modeled and included into the model: (i) transporter/exporter are constitutive; (ii) transporter/exporter are induced by galactose and (iii) protein synthesis is activated by cAMP. The results of simulations are compared with the measured data. Although there are only minor differences, the results indicate that the transporter/exporter induced by galactose and dependent on cAMP.Crp show the best behaviour. Modeling gene expression for the transporter/exporter and for parameter identification, the methods described in [2] and in [1] are used. Figure 3 shows the time course

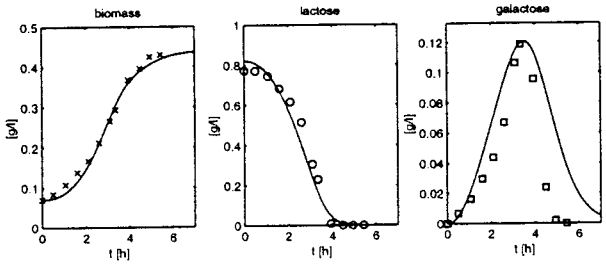

Figure 3: Time course of biomass, extracellular lactose and galactose for the PtsG strain with lactose as the only carbon source. Experimental results are indicated by 'o' and 'x'.

of biomass, lactose and galactose, if the strain is pregrown on glycerol. Two phases can be recognized. In the first phase galactose is excreted in the medium. In the second phase lactose is running out and the excreted galactose is taken up now. An explanation of this behavior could be an accumulation of galactose inside the cells. To validate the parameters, two further experiment with the wild type strain was performed using lactose and galactose or glucose and lactose in the medium (Figure 4). For all experiments a good agreement between simulation and experimental results are obtained.

Discussion

Experiments with mutant strains of *Escherichia coli* lacking important regulator proteins in signal transduction pathways show interesting dynamics. Although the molecular mechanism are not understood very well, mathematical modeling can help to better understand intracellular processes. A mathematical

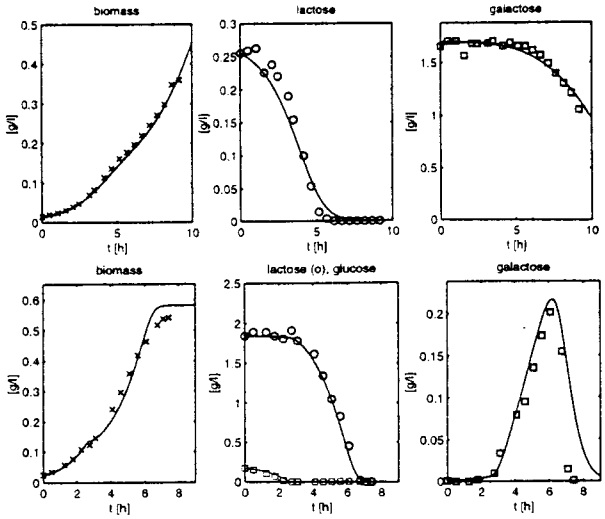

Figure 4: Upper figures: Time course of biomass, extracellular lactose and galactose for the wild type with lactose and galactose in the medium. Lower figures: Time course of biomass, extracellular lactose, glucose and galactose for the wild type with glucose and lactose in the medium.

model was extended to describe galactose efflux. Since only a few experiments are available up today, we do not expect a perfect agreement. The best agreement of experiments and simulations was observed if a galactose exporter is assumed that is inducible by galactose and which expression is dependent on the cAMP.CRP complex. Recent results have shown that in *E. coli* export of carbohydrates often seems to be achieved by specialized systems [4]. A further experimental analysis in required to answer this question and to determine the transporter.

References

[1] A. Kremling, K. Bettenbrock, B. Laube, K. Jahreis, J.W. Lengeler, and E.D. Gilles. The organization of metabolic reaction networks: III. Application for diauxic growth on glucose and lactose. *Metabolic Engineering*, 2000. submitted.

[2] A. Kremling and E.D. Gilles. The organization of metabolic reaction networks: II. Signal processing in hierarchical structured functional units. *Metabolic Engineering*, 2000. submitted.

[3] A. Kremling, K. Jahreis, J.W. Lengeler, and E.D. Gilles. The organization of metabolic reaction networks: A signal-oriented approach to cellular models. *Metabolic Engineering*, 2000. In press.

[4] J. Y. Liu, P. F. Miller, M. Gosnik, and E. R. Olsen. The identifikation of a new family of sugar efflux pumps in *Escherichia coli*. *Molecular Microbiology*, 31:1845-1851, 1999.