Structural and Functional Analysis of Transiently Expressed Metabolic Networks

Mina Oh[1,3] mina@kuicr.kyoto-u.ac.jp
Wolfram Liebermeister[2] lieberme@molgen.mpg.de
Minoru Kanehisa[1,3] kanehisa@kuicr.kyoto-u.ac.jp
Reinhart Heinrich[4] reinhart.heinrich@rz.hu-berlin.de
Oliver Ebenhöh[4] oliver.ebenhoeh@rz.hu-berlin.de

[1] Graduate School of Pharmaceutical Science, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan
[2] Max Planck Institute for Molecular Genetics, Berlin, Germany
[3] Bioinformatics Center, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
[4] Theoretical Biophysics, Institute of Biology, Humboldt University Berlin, Germany

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1 Introduction

Cells react to changes in their environment by a complex system of signaling pathways and regulatory mechanisms resulting in a genetic response involving up- and down-regulation of the relevant genes.

A well-studied example is the diauxic shift in yeast during which cells change their behavior from anaerobic fermentation to aerobic respiration due to the deprivation of external glucose. We use gene expression data [1] for *Saccharomyces cerevisiae* obtained during the diauxic shift at 9, 11, 13, 15, 17, 19 and 21 hours after application of glucose. We define the active subnetworks of the yeast metabolism at each of these time points. We then analyze the structure of these subnetworks using our previously developed method of network expansion [2]. In particular, we investigate the synthesizing capacities of the subnetworks when they are provided with different carbon sources and analyze how these capacities change during the diauxic shift.

This work aims at elucidating the effects of structural changes in metabolic networks, which are induced by a genetic response, on the metabolic functions of the networks.

2 Method and Results

The microarray data [1] includes information on the change of the mRNA level of each gene compared to a reference state. The genes were grouped into three categories, genes being up-regulated by a factor of at least two, genes being down-regulated by a factor of at least two, and those genes showing a change in transcriptional activity of not more than a factor of two. In the further analysis we consider only genes coding for a metabolic enzyme and associate each such gene with the reaction or reactions that the corresponding enzyme catalyzes.

We determine the active subnetworks at each time point as follows. For all genes whose corresponding mRNA level does not change by more than a factor of two, we assume that the gene products, i.e. the enzymes, are present at all time and we therefore include the corresponding reactions in each subnetwork. For the up-regulated genes, we assume that the corresponding enzymes are present in a sufficient concentration only at those time points for which the mRNA level is increased by a factor of more than two. Consequently, only for these time points, the associated biochemical reactions are included in the subnetwork. Similarly, for the down-regulated genes, we assume that the corresponding enzymes are not present in a sufficient concentration whenever the mRNA level has decreased by a factor of more than two and we include the associated reactions only for the other time points.

In the cases where several genes are associated with one reaction, we assume for the sake of simplicity that the activity of one of the genes is sufficient for the corresponding enzyme to operate.

This procedure results in the definition of seven different metabolic subnetworks, one for each time point.

Using the concept of scopes [3] based on the method of network expansion [2], we perform a structural analysis of all these subnetworks. The scope characterizes the synthesizing capacity of a metabolic network when it is provided with some external metabolites. Here, we particularly calculate the synthesizing capacities of all seven subnetworks when they are provided with different carbon sources. In Fig. 1, the numbers of metabolites which can be produced by each subnetwork from some selected carbon sources are plotted as a function of time. It can be seen that the synthesizing capacities for hexoses is strongly reduced as time proceeds. This is expected since, due to the lack of external glucose, the cells change their metabolism to the respiration of ethanol. The activation of the citric acid cycle explains the increase in synthesizing capacity for citrate. However, a surprising result is the strong increase in synthesizing capacity for pentoses such as xylulose.

3 Discussion

Some threshold values have been introduced ad hoc and it is to be expected that different values would result in slightly different subnetworks. However, first analyses indicate that the presented results are robust against small variations of the threshold values. For simplicity, we assumed that whenever several genes are mapped to the same reaction, the expression of one of the genes is sufficient for the reaction to occur. This is correct in the case of isoenzymes, however, the assumption has to be modified if the genes code for enzymes cooperating within one enzyme complex. We plan to include such detailed information in future analyses.

Despite the level of simplification and some arbitrariness in the choice of threshold values, we have shown that the method of network expansion is successfully applicable in combination with data obtained from microarray experiments. We could assess changes of the synthesizing capabilities of the expressed subnetworks during the diauxic shift.

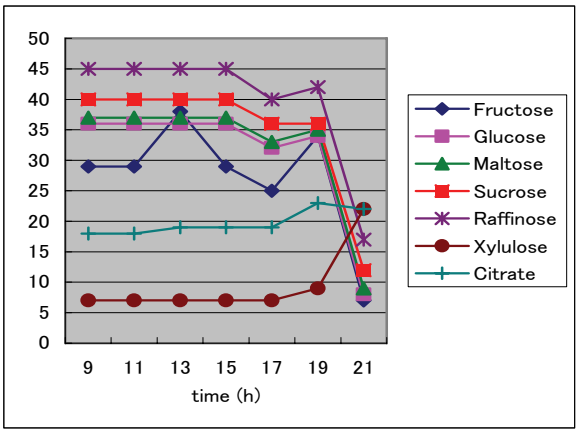

Figure 1: Synthesizing capacities for selected metabolites as a function of time during the diauxic shift .

Our calculations confirm expectations such as the decline of the synthesizing capacity for glucose. Other observations such as the increase of the synthesizing capacity for xylulose are rather surprising and require a further investigation.

The structural analysis of temporally changing metabolic networks using the concept of scopes is a promising method for understanding fundamental principles of the regulation and adaptation of cellular functions on the level of the underlying network structures.

References

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