Partner in der Forschung

ZENTRUM ANGEWANDTE CHEMIE
Institut für Technische Chemie

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Dependency of the gene regulation in glycolysis on the glucose concentration

B. Grote, C. Klockow, F. Stahl, B. Hitzmann
Leibniz University of Hannover, Institute for Technical Chemistry

Introduction

Glycolysis is the center of energy generation and the most important way for substrate uptake. In addition, it supplies the cell with important precursors for anabolism. In this contribution we focus on the transcriptional regulation in dependence on extracellular glucose concentration, which ranges between 0.05 g/L and 0.5 g/L. Yeast shows an oxidative metabolism at lower and an oxidative-reductive metabolism at higher glucose concentrations. The experiments included both metabolic states, which reflects in the gene expression.

Methods

Experiments

Fed batch cultivations of *Saccharomyces cerevisiae* in a mineral medium and with different glucose set points were carried out. After 6-7 h, when the metabolism reached a steady state, samples were taken, the total RNA isolated, labelled and reverse transcribed to cDNA and hybridised on whole genome yeast microarrays (MWG Yeast 2 Array).

Data processing and analysis

The data processing consists of the following steps:

- image processing (commercial software ImaGene)
- substraction of local background from signal (median)
- removal of badly shaped spots (Matlab)
- all signals below a chosen threshold are given a value of 1 (Matlab)
- array-to-array standardisation (Matlab)
- addition of Cy3 and Cy5 signals (Matlab)

New Method for Visualisation

It is still a great challenge to visualise expression data from series, where no neutral reference exists, in a conclusive way. Here, we present a new method.
Basically, the mean of the expression data for all time points, but for each gene separately, is set to a value of 50 %, as shown in this formular:

Relative Expression:

$$s_{\text{stand,ih}} = \frac{s_{ih}}{\overline{s_i}} \cdot 50$$

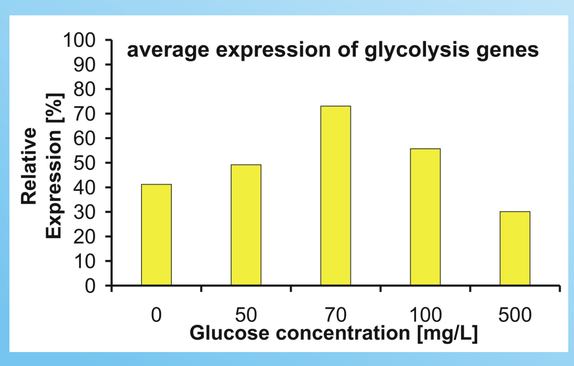
Advantages:

- enhanced comparability from gene to gene
- effects of different stability of mRNA, efficiency of translation and activity of enzyme are eliminated, as these factor should be the same for one gene under different conditions

=> gene expression from different genes can be averaged

Results

Averaged Gene Expression for Glycolysis



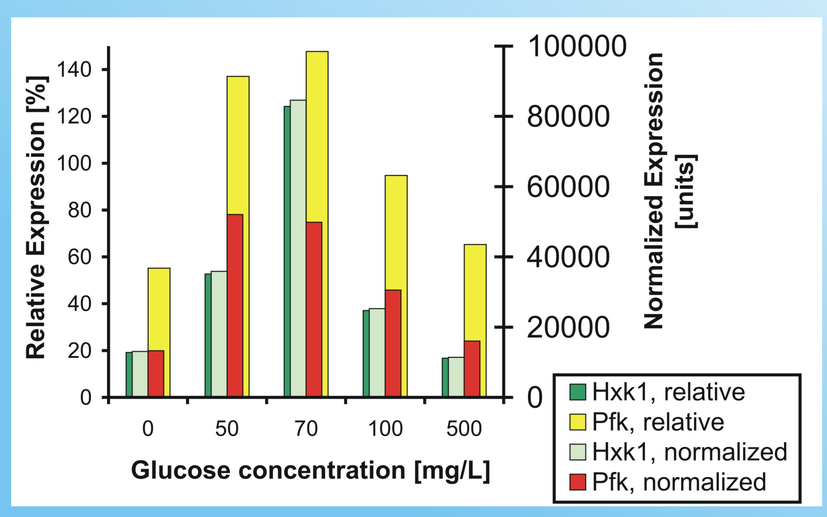
Relative Expression Data

- low expression in the absence of glucose and at low concentrations (50 mg/L)
- highest relative expression at 70 mg/L
- expression decreases at higher concentrations (500 mg/L)

Expression Data

- signal intensity ranges between 3000 and 100 000

Hxk1, Pfk1 & Pfk2 - Key Genes of Glycolysis



Data for Pfk1 & Pfk2 was summerized

Biological Results

- maximum of expression at 50- 70 mg/L
- repression at 500 mg/L
- similar to regulation of whole pathway

New Visualisation Method

- the Normalized and the Relative Expression data show the same course
- a correlation analysis was performed: the correlation coefficient for Relative and Normalized Expression data has a value of 1 for every gene

Conclusions

- A new method for visualisation of expression data was developed.
- Expression of genes of the gylcolysis runs through a maximum at 50- 70 mg/L glucose. The expression reduces significantly for concentrations like 500 mg/L.

Possible explanations:

- As more glucose is available, the glycolysis is more active.
- As substrate concentration increases to 500 mg/L, less enzyme is needed for the same reaction velocity due to increased substrate concentration.
- A different, additional pathway is used for the glucose uptake at 500 mg/L. The pathway is not yet identified, but Pentose Phosphate Pathway and synthesis of amino acids or polysaccharides can be excluded.