Regulation Analysis by Combining the Information Obtained from the mRNA Expression, Protein Expression and Metabolic Fluxes

Chen Yang[1,2] yangc@ttck.keio.ac.jp

Qiang Hua[2] huaq@sfc.keio.ac.jp

Kazuyuki Shimizu[1,2] shimi@bse.kyutech.ac.jp

[1] Department of Biochemical Engineering and Science, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan

[2] Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0017, Japan

Keywords: regulatory mechanism, metabolic flux, mRNA expression, protein expression, *Synechocystis*

1 Introduction

In the post genome era, a challenge is to relate linear sequence information to nonlinear cellular phenotypes. The analyses of mRNA and protein expression are not sufficient for the description of the cellular phenotype, since they do not provide direct information about the cellular metabolism and physiology that are fundamental to the cells' behavior [1, 2]. Since metabolic flux analysis provides a detailed description of the cellular physiology, this method, in combination with the gene expression analysis at the transcript and protein levels, can be used to analyze the complex genotype-phenotype relationship of biological systems. In this work, we analyzed the gene expression patterns at both the mRNA and protein levels for *Synechocystis* grown under autotrophic, mixotrophic and heterotrophic conditions. Moreover, we quantified the intracellular metabolic fluxes in *Synechocystis* under different trophic conditions. By combining the information obtained from the transcript levels, protein abundance and metabolic fluxes, the regulatory mechanisms of some enzymes in the central metabolism involved in the different trophic cultures of *Synechocystis* were analyzed.

2 Methods

Synechocystis sp. PCC6803 was cultivated under autotrophic, mixotrophic and heterotrophic conditions. The gene expression patterns at the transcription and translation levels were measured by using the semi-quantitative reverse transcription - polymerase chain reaction (RT-PCR) and two-dimensional electrophoresis (2DE), respectively. The intracellular metabolic flux distributions for *Synechocystis* grown under different trophic conditions were also determined by using the carbon isotope labeling technique. *Synechocystis* was grown on ^{13}C-labeled glucose, and the labeling patterns of the amino acids in biomass hydrolysates were measured using two dimensional ^{1}H-^{13}C correlation nuclear magnetic resonance spectroscopy and gas chromatography/mass spectrometry. The estimation of the metabolic fluxes from the measured labeling data was achieved by a nonlinear least squares fitting approach.

3 Results and Discussion

By combining the information obtained from the transcript levels, protein abundance and metabolic fluxes, the regulatory mechanisms of some enzymes in the central metabolism involved in the different cultures of *Synechocystis* were analyzed.

Table 1: Changes in transcript levels, protein abundance and metabolic fluxes of *Synechocystis* in the autotrophic (A) and heterotrophic (H) cultures compared to those in the mixotrophic (M) culture.

	transcript ratio		protein ratio		flux ratio
gene	A vs M	H vs M	A vs M	H vs M	H vs M
rbcLS	1.0	0.5	0.9	0.5	0
prk	1.1	0.9	0.8	0.8	0
zwf	0.9	1.1	–	–	∞
devB	–	–	1.1	1.0	∞
gnd	1.0	1.6	–	–	∞
gap2	0.9	0.5	1.0	0.5	1.00
gap1	0.9	0.9	–	–	1.14
fbp	1.1	0.9	–	–	0
pfkA	1.1	1.0	–	–	12.7
fbaA	0.9	0.8	0.9	0.8	12.7
fda	–	–	1.1	0.9	12.7
glk	0.9	1.1			2.24
cfxE	–	–	0.9	0.8	2.00
ppc	0.9	1.0	–	–	1.70
icd	0.9	1.0	–	–	1.56
citH	0.8	1.1	–	–	1.78

It was found that with the difference in the energy source available to *Synechocystis*, the enzymes in the central metabolism were differently regulated according to different mechanisms. The expression of several genes such as *rbcLS*, *gap2*, *cpcBA*, *psaE* and *glnB* was light-regulated transcriptionally. Meanwhile, the gene *gnd* was regulated for the apparent flux requirement but by unknown mechanism. Furthermore, there are other genes whose expressions are independent of the presence of light. The reactions catalyzed by G6PDH, Fbp, PfkA and FbaA were not regulated through enzyme synthesis but via the change in the metabolite concentrations. The enzyme PRK was post-translationally regulated by light probably through the operation of ferredoxin/thioredoxin system. For the enzyme RubisCO, both the transcriptional and post-translational regulations existed. These findings demonstrate that it is important to use different levels of information obtained from the mRNA expression, the protein expression, and the metabolic flux distribution to understand the regulatory events in the complex cellular networks.

References

[1] Bailey, J. E., Lessons from metabolic engineering for functional genomics and drug discovery, *Nature Biotechnology*, 17:616–618, 1999.

[2] ter Kuile, B.H. and Westerhoff, H.V., Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway, *FEBS Lett.*, 500(3):169–171, 2001.