Transcriptome Profiling and Deciphering the Regulatory Role of Global Transcription Factor *fadR*

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1 Introduction

fadR is a transcription factor which has a Helix-turn-Helix motif [5], one of most common prokaryotic transcription factor. It regulates metabolic pathway such as the fatty acid biosynthesis and degradation pathways, glyoxylate pathway and possible role in regulation of amino acid biosynthesis directly or indirectly [1, 2, 3, 4]. By use of cDNA microarray one can search for regulation of transcription factors and its impact on metabolism using microarray data analysis and sequence information.

By comparing wild-type W3110 strain, *fadR* null mutant strain, subjecting them in various conditions (i.e., different carbon sources such as oleic acid), it is possible to identify the functional role of *fadR* [1, 3]. Also candidate genes that could possibly be regulated by *fadR* can be predicted using sequence information, which assist microarray data analysis.

Expression profile of wild-type W3110 strain and *fadR* null mutant strain (WFR) was compared in LB media (peptone 10g/L, yeast extract 5g/L, NaCl 5g/L) using *E. coli* cDNA microarray with 2850 ORFs. Genes related to fatty acid biosynthesis of WFR, compared to W3110, was down-regulated and genes related to fatty acid degradation were up-regulated. Through analyzing the transcriptome of *Escherichia coli*, genetic regulation and physiology of the cell related to *fadR* regulation could be explained. Understanding of the cell physiology and genetic regulation through cDNA microarray analysis may find its use in metabolic engineering and deciphering genetic regulation in metabolism.

2 Method and Results

Bacterial Strains & Culture Condition

From *E.coli* W3110, *fadR* null mutant strain(WFR) was constructed by replacing *fadR* gene with Cm^r. Wild-type W3110 and WFR were cultured at 37°C and 250 rpm in 100 ml culture flask. Cells were subcultured several times to ensure exponential growth. Cells were harvested at mid-exponential growth phase.

DNA Chip Experiment

Total of 2,850 open reading frames (ORFs) including all functionally known genes were amplified by polymerase chain reaction (PCR). The primers for PCR were purchased from Sigma Genosys

(N.S.W., Australia). The resulting 2,850 gene probes were arrayed on poly-L-lysine coated slides using a robotic microarrayer developed previously. Total RNA was isolated by Qiagen Rneasy columns as manufacturer's protocol. Fluorescence labeled DNA was made during reverse transcription of total RNA by using a random hexamer. The DNA microarray was scanned by GenePix 4000B (Axon Instruments, Inc.). Signal intensities and local background were determined by GenePix Pro 3.0. Following background subtraction, signal intensities were calculated as the percentage of total signal as a means of normalization.

Among the 2,850 spotted genes, 322 genes were found to be up-regulated by above 1.5-fold while 241 genes were down-regulated. Among them, *fabB, fabD,* which are related to fatty acid biosynthesis pathway, was down-regulated and *fadL, fadA* genes, which are related to fatty acid degradation pathway, were up-regulated. *aceA, aceK* genes, related to glyoxylate shunt, were up-regulated which coincides with the literatures [1, 2, 3, 4].

fadR Sequence Motif Search in the *Escherichia coli* Whole Genome

Through BLASTN using FadR DNA-binding consensus sequence, several possible FadR regulatory binding sites were identified. Among them were *fadA, fadB, fadL* and others.

3 Discussion

When comparing *fadR* null mutant strain (WFR) and wild-type strain grown in LB media, fatty acid biosynthesis related genes were up-regulated and genes related to fatty acid degradation were down-regulated. Also glyoxylate pathway related gene was up-regulated. Transcriptome profile of *fadR* null mutant strain and wild-type in different media, respectively were obtained as well.

Based on microarray experimental data, *fadR* regulation in fatty acid biosynthesis and degradation is observed and coincides with literatures [1, 2, 3, 4]. Data also reveals the regulation of *fadR*, regulating metabolism in different conditions.

cDNA microarray has vast potential to decipher the complex genetic network of living organisms. It is obvious that cDNA microarray technology can find various applications in metabolic engineering which makes possible to decipher the genetic regulation in metabolic pathways.

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References

[1] Cronan J.E. and Subrahmanyam, S., FadR, transcriptional co-ordination of metabolic expediency, *Molecular Microbiology*, 29:937–944, 2002.

[2] DiRusso, C.C., Heimert, T.L., and Metzger, A.K., Characterization of FadR, a global transcriptional regulator of fatty acid metabolism in *Escherichia coli*, *Journal of Biological Chemistry*, 267:8685–8691, 1992.

[3] DiRusso, C.C. and Nystrom T., The fats of *Escherichia coli* during infancy and old age: regulation by global regulators, alarmones and lipid intermediates, *Molecular Microbiology*, 27:1–8, 1998.

[4] Gui L., Sunnarborg, A., and LaPorte D., Regulated expression of a repressor protein: FadR activates *iclR*, *Journal of Bacteriology*, 178:4704–4709, 1996.

[5] Van Aalten, D.M., DiRusso, C.C., and Knudsen, J., The structural basis of acyl coenzyme A-dependent regulation of the transcription factor FadR, *EMBO Journal*, 20:2041–2050, 2001.