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Genome-Wide Expression Profiling of Carbon Metabolism in the Methylotrophic Yeast *Hansenula polymorpha*

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The thermotolerant methylotrophic yeast *Hansenula polymorpha* has gained increasing interest as a useful system for fundamental research and applied purpose. *H. polymorpha* has been a favorable model to study the genetic control mechanism of methanol metabolism and peroxisome biogenesis. It has recently become one of the promising microbial hosts for the production of recombinant proteins on an industrial scale[1]. Moreover, peculiar physiological characteristics of *H. polymorpha*, such as resistance to heavy metals, oxidative stress, and heat, make this yeast attractive for several biotechnological purposes[2]. Successful exploitation of the industrial potential of *H. polymorpha* requires solid knowledge of the cellular systems at the global level.

In an effort to obtain comprehensive information on gene function and regulatory networks in this yeast, we have developed whole-genome microarrays of *H. polymorpha* using information from the manual annotation of *H. polymorpha* complete genome sequence, which predicted 5,848 open reading frames (ORFs)[3]. Each *H. polymorpha* ORF was PCR-amplified using gene-specific primer sets, of which the forward primers have 5'-aminolink. The PCR products were printed in duplicate or triplicate onto the aldehyde-coated slide glasses to link only the coding strands to the surface of the slide via covalent coupling between amine and aldehyde groups. This generated single-stranded array elements minimizing the interference of complementary strands during hybridization (http://www.kribb.re.kr/metabolic/).

The whole-genome microarrays were used to investigate systematically the catabolite regulation of carbon metabolism in *H. polymorpha*. As methylotrophic yeast, it can grow on methanol, a cheap and pure substance, as the carbon and energy source. The metabolism of methanol in methylotrophic yeasts is induced by methanol but subject to strong repression by glucose, which is the primary and preferred fuel for eukaryotic microorganisms. We analyzed the temporal change of gene expression accompanying the carbon source shift from glucose to methanol and observed that genes involved in methanol metabolism and peroxisome biogenesis were highly induced within 2 hours after shift to methanol in *H. polymorpha*, supporting prior knowledge on methylotrophic yeasts. Interestingly, the expression profiles also showed that several genes involved in glycoxylate cycle and pentose-phosphate pathway were significantly induced, whereas quite a few genes encoding the enzymes of the tricarboxylic acid (TCA) cycle were decreased in the cells cultivated on methanol. This finding strongly supports the notion that during methylotrophic growth the main source of

NADH generation is not the TCA cycle but the oxidation pathway of methanol to CO_2 via formate.

We have also analyzed the gene expression profiles during glucose utilization and compared it to the temporal transciptome profiles accompanying the metabolic shift from fermentation to respiration in *Saccharomyces cerevisiae*[4]. It was observed that in *H. polymorpha* many genes involved in TCA cycle were not repressed in glucose-rich medium, unlike the case in *S. cerevisiae*, indicating that respiration in *H. polymorpha* is repressed only partially in the presence of high concentrations of glucose. It was also noteworthy that many methanol-inducible genes, especially genes encoding enzymes involved in methanol utilization pathway, were highly expressed as glucose was depleted from the growth medium, demonstrating that the main regulation mechanism of the methanol metabolism is a depression/repression rather than an induction/repression by carbon sources. These findings provide invaluable information on the metabolic fate of glucose and methanol in the methylotrophic yeast *H. polymorpha*, which will be usefully applied for pathway engineering and process optimization in exploiting this yeast as a cell factory.

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1. Guengerich L, Kang HA, Behle B, Gellissen G, Suckow M (2004) A platform for heterologous gene expression based on the methylotrophic yeast *Hansenula polymorpha*. In Kück U (ed) The mycota II. Genetics and Biotechnology (2nd edition), Springer-Verlag, Heidelberg, pp 273-287
2. Gellissen, G. (Ed.). 2002. *Hansenula polymorpha*-Biology and Applications. Wiley-VCH, Weinheim.
3. Ramezani-Rad, Hollenberg MCP, Lauber J, Wedler H, Griess E, Wagner C, Albermann K, Hani J, Pionteck M, Dahlems U, and Gellissen G.. 2003. The *Hansenula polymorpha* (strain CBS4732) genome sequencing and analysis. *FEMS Yeast Res.* **4:** 207-215.
4. DeRisi, JL, Iyer VR, and Brown P.O. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science.* **278:** 680-686.