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Form 3

Abstract of Thesis

Name (Zanariah Binti Hashim)	
Title	Metabolomics-based analysis of gene-metabolite correlations in yeast transcription factor knockouts (メタボロミックス技術に基づく出芽酵母転写調節因子遺伝子破壊株の遺伝子－代謝物の解析)

Abstract of Thesis

Cellular functions are determined by integrative interactions between various constituents, i.e. genes, transcripts, proteins, and metabolites. Thus, it is important to study these interactions to understand the whole biological system. Genetic perturbations are often used to investigate the contribution of individual components. One of such components is a transcription factor. Transcription factors (TFs) are the regulatory proteins that interact with DNA to either promote or suppress gene expression. Due to the importance of TFs in gene regulation, they have been extensively studied and several databases dedicated to TFs were established. However, our understanding is still lacking since many regulatory events that link gene expression to the final phenotypic changes remain poorly characterized. In particular, systematic analyses of global metabolic alteration following a TF perturbation have been largely unexplored.

This thesis regards the effects of transcription factor-related single gene deletion towards metabolic alteration, using yeast *Saccharomyces cerevisiae* as a model system. Specifically, the correlations between TF gene and metabolites were investigated. In Chapter 1, general introduction and research background are presented. In particular, yeast transcription factors and metabolomics techniques are discussed. In Chapter 2, metabolic profiling of two representative transcription factors Rtg1 and Rtg3 yeast knockout is demonstrated as a proof-of-principle of the utility of metabolomics approach in finding novel TF-metabolite correlations. These two proteins are positive regulators of the mitochondrial retrograde response (RTG), a signaling pathway activated under repressed mitochondrial function. Using a widely-targeted metabolomics approach, polyamine biosynthesis and other amino acid metabolism were found to be significantly altered in RTG-deficient strains, apart from the expected TCA and glyoxylate cycles. A characteristic decrease of 2-oxoglutarate preceding the decreases of other TCA cycle intermediates in RTG disruptants suggests that 2-oxoglutarate may play a pivotal role in controlling the flow and balance of TCA/glyoxylate cycles under RTG response.

In Chapter 3, a global metabolome analysis was performed for 154 TF deletion strains. Characterization using hierarchical clustering analysis (HCA) and differential analysis showed that the strains can be categorized according to their metabolic phenotype, and both known and unknown correlations were demonstrated. Differential strains (strains with large differences in metabolite levels compared with wild-type) and strain clusters that share a highly similar metabolic profile were identified. About 30% of the strains were classified as differential, while 27 individual clusters consisting of differential and non-differential strains were observed. The comprehensive metabolome dataset presented here may serve as an input for deeper investigations into transcription factors. Also discussed are issues regarding data normalization and correction of batch-to-batch variation, a prevalent problem in mid- to large-scale metabolomics studies. Finally, in Chapter 4, general conclusion and future perspective are presented. It is expected that metabolomics will be routinely performed, whether as a primary or complementary means in many gene regulation studies.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

細胞の機能は種々の複雑な要因（遺伝子，転写産物，タンパク質，代謝物等）の間の統合的な相互作用により決定される．それゆえ，細胞全体の生物学的システムを理解するためには，複雑な要因間の相互解析が重要となる．遺伝子に摂動を与えるという手法は，当該遺伝子の貢献を観測するためにしばしば用いられる．転写調節因子に対しては特に良く用いられる．転写調節因子は遺伝子の発現を調節するタンパク質であり，これまで幅広い研究の対象となってきた．しかしながら，複雑な遺伝子発現イベントの全容を解明するには至っていない．特に転写調節因子に摂動を与えた際の代謝物の総合的な変動の系統的解析については殆ど解明されていない．

本論文は出芽酵母（*Saccharomyces cerevisiae*）を実験材料として転写調節遺伝子の摂動が代謝変動に与える影響について研究した．特に，転写調節遺伝子と代謝物プロファイリングの関係について深く検討解析した．

第 1 章では，当該研究の背景について総合的に解説した．特に，酵母の転写調節因子について，ならびに，代謝プロファイリングについて議論した．

第 2 章では，代表的な二つの転写調節因子遺伝子，Rtg1 ならびに Rtg3 が破壊された酵母変異株の代謝プロファイリングを実施し，メタボロミクス技術を用いた手法が転写調節因子のと代謝物の関係解明に対して有用な手段であることを証明した．

第 3 章では，第 2 章で証明された有用手法を 154 個の転写調節因子破壊変異株に拡大適用した．階層的クラスター解析（HCA）等の多変量解析を実施し，30 種類弱の特異的なパターンに分類することに成功した．そのた，種々の新規知見を得ることに成功した．

第 4 章では，総合的な結論を記述するとともに，当該研究の将来性に言及した．

以上のように，本論文はメタボロミクス技術が転写調節遺伝子機能解析に極めて有用な新規技術であることの証明に成功した．よって本論文は博士論文として価値のあるものと認める．