論文の要旨

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論文題目　Comprehensive metabolomic analysis of the fission yeast, *Schizosaccharomyces pombe*
(分裂酵母の網羅的メタボローム解析)

Metabolomics is a modern branch of chemical biology that aims to detect and quantify hundreds of cellular metabolites simultaneously. The fission yeast, *Schizosaccharomyces pombe*, is a unicellular model organism widely applied in many areas of cell biology research. The fission yeast genome has been sequenced and numerous high-throughput transcriptomic and proteomic analyses have been performed. However, so far this organism has not been subject to serious metabolomic study.

In this thesis we developed the methodology for metabolomic analysis of *S. pombe*, optimized mainly for extraction and detection of polar metabolites. For sample measurements we employed liquid chromatography mass spectrometry, using a high-resolution Orbitrap mass detector. Out of ~6,000 peaks detected from a single sample, we were able to assign the origin of ~1,900. Among the assigned peaks, we identified 123 distinct metabolites, 108 of which were verified using pure standards. In the initial analysis, we examined the metabolomes of *S. pombe* cells grown in a synthetic culture medium at two different cultivation temperatures, 26ºC and 36ºC. Further, we demonstrated the feasibility of integration of genetics and metabolomics by analyzing a deletion mutant (*sib1*Δ) that lacked a non-essential enzyme, ferrichrome synthetase, and a temperature-sensitive mutant (*hcs1-143*) defective in an essential enzyme, HMG-CoA synthase.

Our next project focused on glucose, the ubiquitous source of cellular energy for most living organisms. We investigated cell division modes of the fission yeast under a wide range of glucose concentrations (0-111 mM). Surprisingly, *S. pombe* cells proliferated normally under highly diluted glucose level, similar to that of human blood (5.6 mM). Cell division became stochastic below 4.4 mM glucose, and a transition from division to quiescence occurred between 2.2 and 1.7 mM glucose. Under starvation (1.1 mM), cells were predominantly quiescent and became greatly resistant to oxidative stress. On the contrary, cells rapidly lost viability under fasting (0 mM), unless they were pre-treated with starvation. Using metabolomic analysis, we identified specific biomarker compounds corresponding to different glucose levels. Interestingly, cells maintained high levels of nucleotide triphosphates such as ATP even under starvation. Under fasting, cells rapidly lost antioxidant and energy compounds such as glutathione and ATP. However, in cells undergoing long-term starvation, these critical metabolites remained abundant.

Among the compounds strongly up-regulated under glucose starvation was ergothioneine, a curious sulfur-containing metabolite that is synthesized only by certain species of bacteria and

fungi, yet it accumulates in tissues of higher eukaryotes including humans. In the third study we identified the genes forming the ergothioneine biosynthetic pathway in fission yeast, through combined use of genetics and metabolomics. The first gene (*egt1*$^{+}$) was identified by sequence homology to a previously published gene in *Neurospora crassa*. The second gene (*egt2*$^{+}$) was found by metabolomic screening of four putative homologs of a corresponding bacterial gene. We confirmed the identity of the genes by metabolome analysis of deletion mutants, and constructed an *egt1*$^{+}$ overexpression system by replacing its native promoter with an inducible *nmt1*$^{+}$ promoter. Finally, we demonstrated that the same pathway can also synthesize a selenium-containing derivative of ergothioneine, selenoneine, if selenium is provided in the culture medium. The biosynthesis of selenoneine involved a novel intermediate metabolite, hercynylselenocysteine.

In conclusion, this thesis demonstrates the great potential of metabolomics as a modern, high-throughput, comprehensive method for studying basic cellular processes, including the effects of genetic and environmental perturbations.