The Snf1 Protein Kinase in the Yeast Saccharomyces cerevisiae

In yeast, Saccharomyces cerevisiae, the Snf1 protein kinase is primarily known as a key component of the glucose repression regulatory cascade. The Snf1 kinase is highly conserved among eukaryotes and its mammalian homolog AMPK is responsible for energy homeostasis in cells, organs and whole bodies. Failure in the AMPK regulatory cascade leads to metabolic disorders, such as obesity or type 2 diabetes. The knowledge about the Snf1 protein kinase remains to be of much interest in studying yeast carbon metabolism and human biology. To investigate the effect of Snf1 kinase and its regulatory subunit Snf4 on the regulation of glucose and galactose metabolism, I physiologically characterized Δsnf1, Δsnf4, and Δsnf1Δsnf4 CEN.PK background yeast strains in glucose and glucose-galactose mixture batch cultivations (chapter 2). The results of this study showed that delayed induction of galactose catabolism was SNF1 or SNF4 gene deletion specific. In comparison to the reference strain, growth delay on galactose was found to last 2.4 times (7 hours) longer for the Δsnf4, 3.1 times (10.5 hours) longer for the Δsnf1, and 9.6 times (43 hours) longer for the Δsnf1Δsnf4 strains. The maximum specific growth rates on galactose were found to be two to three times lower for the recombinant strains compared to the reference strain (0.13 h⁻¹) and were found to be 0.07 h⁻¹ for the Δsnf1, 0.08 h⁻¹ for the Δsnf4 and 0.04 h⁻¹ for the Δsnf1Δsnf4 strain. In contrast to what is generally believed, the study showed that the Snf1 kinase was not solely responsible for the derepression of galactose metabolism. To investigate the regulatory role of Snf1 kinase on a global scale, the global scale mRNA, large-scale yeast quantitative proteome and metabolome datasets were generated. One of the largest yeast global quantitative proteome datasets (2388 proteins) to date was generated using Multidimensional Protein Identification Technology followed by quantitation using stable isotope labeling approach (chapter 3). The stable isotope labeling was compared to the spectral counting quantitative approach and the study showed that the stable isotopic labeling approach is highly reproducible among biological replicates when complex protein mixtures containing small expression changes were analyzed. Where poor correlation between stable isotopic labeling and spectral counting was found, the major reason behind the discrepancy was the lack of reproducible sampling for proteins with low spectral counts. To reconstruct a regulatory map of the yeast Snf1 protein kinase, I used the abundances of 5716 mRNAs, 2388 proteins, and 44 metabolites measured for the wild-type, Δsnf1, Δsnf4, and Δsnf1Δsnf4 strains. By integrating these measurements with global protein-protein-interactions, protein-DNA-interactions and a genome-scale metabolic model, I mapped the complete network of interactions around the protein kinase Snf1 (chapters 4, 5). Through these interactions, I identified how the Snf1 protein kinase regulated cellular metabolism on gene or protein level. The study revealed that the Snf1 protein kinase played a far more extensive role in controlling both carbon and energy metabolism than previously anticipated. Similar to the function of AMPK in humans, my findings showed that Snf1 was a low energy checkpoint. Our results suggested that it was possible to use yeast more extensively as a model system for studying the molecular mechanisms underlying the global regulation of AMPK in mammals.