The physiological response of Saccharomyces cerevisiae to temperature stress
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SUMMARY
In order to adequately respond to constantly changing environmental conditions unicellular organisms, like *Saccharomyces cerevisiae*, have developed response mechanisms. The effects of changed environmental conditions have been studied extensively, but traditionally focussed on only one expression level, like the transcriptome or proteome. This way, several molecular mechanisms in response to changed environmental conditions were uncovered.

In order to understand the whole picture how yeasts responds to changed environmental conditions, it should be considered as an integrated interaction of genes, proteins and metabolites and therefore all these levels should be quantified. Studying the cell as a system, Systems Biology, has become a popular field of study with the advancement of high-throughput analytical methods, increased computer power and traditional molecular biological tools.

In this thesis we used a Systems Biology approach to unravel what regulatory processes are involved in the adaptation of yeasts energy producing pathway, glycolysis to increased cultivation temperatures.

**Chapter 1** of this thesis summarizes the current knowledge on the molecular mechanisms underlying the adaptive response to environmental stress with a focus on the response to changes in environmental temperature.

We discuss the modes of energy metabolism in baker’s yeast. Previous studies have shown that shifting from one to another energy generating strategy affects the energy production, which is required for growth and maintenance. In some stressful conditions maintaining a large set of enzymes put additional burden on the maintenance energy and therefore it might be cheaper to shift to an alternative metabolism. The cost-benefit trade-off between the pathways is discussed. Next, we discuss how the cell senses temperature changes and the subsequent signal transduction pathways it uses in order to activate transcription factors. Additionally, these mechanisms influences the delicate balance between energy required for growth and required to maintain cellular function.

The last part of the introduction focuses on a Systems Biology approach that is required to understand the integrated response to nutrients and stress with the heavy focus on glycolysis. We used a mathematical tool to quantify and dissect the contribution of gene-expression and metabolic components to the changed glycolytic flux, which is called regulation analysis.

**Chapter 2** describes the way we adapted and used regulation analysis in order to study to what extent the temperature-induced flux increase is facilitated by changes in enzyme concentration (hierarchical), caused by the transcription cascade, by the direct effect of temperature on catalytic rates or by changes in the environment of the enzyme (metabolic).
We observed no change in glycolytic flux in the temperature range of 30ºC to 37ºC, but observed a 6- to 10-fold flux increase through the glycolytic enzymes in cultivations at 38ºC compared to 30ºC cultivations. Besides, a shift from respiratory to respirofermentative metabolism was observed. Regulation analysis was used to reveal to what extent the hierarchical, metabolic and temperature coefficient contributed to the flux increase. It revealed that the flux increase through the individual enzymes was mainly brought about by the changed interaction with the rest of the metabolism. We confirmed the small hierarchical coefficient by quantifying the transcript level of the glycolytic enzymes. The intracellular metabolite levels were quantified to confirm the large metabolic contribution. Remarkably, the effect of temperature on the catalytic rates of the glycolytic enzymes was moderate, indicating that the enzyme rate increases are somehow buffered at these temperatures.

In chapter 3 we studied the effect of temperature on cultivations limited for nitrogen. As a result, the cultivations have a different metabolism. While in carbon-limited cultivations mainly respiration is used for energy production, except at high temperatures where the cultures are respirofermentative, in nitrogen-limited cultures over the whole temperature range the metabolism is respirofermentative. Again, we used temperature-dependent regulation analysis to uncover to what extend hierarchical, temperature and metabolic processes contributed to the flux change. The results were compared to the mode of regulation of the energy-limited cultivations from chapter 2. Regulation analysis revealed multiple modes of regulation. Almost all enzymes showed hierarchical regulation and the temperature dependence of the enzymes was larger compared to the carbon-limited cultivations. We set up a method to quantitatively analyze the proteome by mass spectrometry, looking for isoenzyme expression that could explain the difference in temperature dependence. The proteome level confirmed the increased hierarchical coefficient in nitrogen-limited cultivations, but the proteome data was not sufficient to explain the increased direct effect temperature on the glycolytic enzymes.

In chapter 4 we further investigated an observation made in chapter 2. We showed by using plate cultivations with various carbon sources that at 30ºC and at 37ºC yeast was able to grow on fermentable and non-fermentable carbon sources, whereas at 38ºC yeast was able to grow on glucose, but not on non-fermentable carbon sources. Surprisingly, this temperature effect on mitochondrial function was completely reversible. We studied this phenomenon thoroughly in carbon-limited chemostats. We observed fully respiratory metabolism from 30ºC to 37ºC. Ethanol production was observed at cultivation temperatures higher than 37ºC. Interestingly, the specific oxygen consumption rate was constant at all temperatures, even those above 37ºC. Since the biomass yield on ATP is suggested to
decrease upon an increase in temperature, we expected an increase in the glycolytic flux with increasing temperature. However, glycolytic fluxes and yield on glucose were not changed in the range of 30°C-37°C. This suggests that either the $Y_{ATP}$ does not decrease with increasing temperatures, or that the ATP production per glucose molecule is increased. In order to understand the contributions of respiration and fermentation to the energy required for growth, we developed a method to determine the efficiency of the respiratory chain in vivo. It showed that in the range of 30°C to 37°C the mitochondrial efficiency increased with increasing temperature and at 38°C we observed a efficiency of zero, indicating that the oxygen consumption is no longer coupled to energy production. Using a knockout strain we showed that Gut2p played a role in the increased efficiency. Morphological studies using electron microscopy showed that the mitochondria at 38°C were badly damaged. We discussed several mechanisms to explain the damage.

In conclusion, we used System Biology approaches to uncover the regulation of the flux through the enzymes upon temperature challenges and how mitochondria deal with temperature increases. We showed that yeast uses various ways to deal with the increased temperature depending on the cultivation conditions. These findings might affect the way we look at cellular metabolic networks and how to influence them.