The physiological response of Saccharomyces cerevisiae to temperature stress
Postmus, J.

Citation for published version (APA):
Postmus, J. (2011). The physiological response of Saccharomyces cerevisiae to temperature stress

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 5

General discussion
**SYSTEMS BIOLOGY APPROACH TO UNCOVER ADAPTATION TO INCREASED ENVIRONMENTAL TEMPERATURES**

In this thesis, we aimed to investigate the regulatory processes involved in adaptation of *Saccharomyces cerevisiae*’s metabolism to environmental temperature challenges using a systems biology approach. In systems biology, the organization and interactions of molecular and cellular networks in determining biological function are studied [1, 2]. To that end, it is imperative that responses at relevant levels of organisation (in this case genes, proteins, and metabolites) are quantified.

Initially, we used batch fermentations to study the physiological responses upon an increase in the cultivation temperature. Due to their ease of use, these cultivation systems were initially deployed to scan effects of temperature challenges on yeast growth and yield parameters. However, for more detailed quantitative studies they do not suffice; in batch fermentations temperature and growth rate are coupled, making it impossible to distinguish the effects of temperature from those of changes in growth rate on metabolism. To overcome these problems, chemostat cultivations with low specific growth rates were used, leading to well-defined conditions suitable for quantitative analysis of many cellular and molecular levels of organisation.

Throughout this thesis we have studied the efficacy of regulation analysis, introduced by Ter Kuile and Westerhoff [3]. Regulation analysis is a mathematical method to quantitatively dissect the role of hierarchical and metabolic aspects of regulation, in which the hierarchical component is defined as the regulation caused by changes in the gene-expression cascade, while metabolic regulation is defined as regulation caused by changes in the way the enzyme interacts with the rest of metabolism. It can be derived that hierarchical regulation ($\rho_h$) and metabolic regulation ($\rho_m$) together sum up to 1 (see [3]).

To determine $\rho_h$, the total amount of active enzyme needs to be quantified. Since changes in $V_{\text{max}}$ are proportional to changes in corresponding active enzyme amounts, we used maximal activities of enzymes in cell extracts, measured in assay conditions mimicking the in vivo situation, to determine $\rho_{h}$.

We extended regulation analysis with an extra component, namely the direct effect of temperature on catabolic rates. The temperature coefficient can be separated from the hierarchical coefficient when the direct effect of temperature on the $V_{\text{max}}$ is independent from that of the gene-expression cascade, which we determined to be true for almost all enzymes studied. In cases where this condition was not fulfilled, we determined a combined hierarchical coefficient using $V_{\text{max}}$ data from enzyme extracts from cells grown at two cultivation temperatures, each assayed at the corresponding cultivation temperature. Thus,
the direct flux regulatory effect of temperature, together with hierarchical and metabolic regulation sums up to 1. One can question the description of this term as “regulation”, since it describes the contribution of temperature to a flux change, and effect of temperature on an existing biomolecule. However, within the framework of this study, we have found it useful to be able to dissect this direct effect from the hierarchical and metabolic terms. Whether yeast has evolved to “use” these temperature properties, or only to cope with them, remains unanswered.

The flux changes that we observed took 1-2 doublings (at a D of 0.1) to establish (our unpublished data). However, we did not study in detail the transient changes that most likely occur within the first hour after temperature up- or downshift. These changes, and their manner of regulation, can also be dissected using a time resolved version of regulation analysis [4]. On the timescales that we have studied, with 2 hours as the first time point, no flux changes occurred. This uncovers one shortcoming of regulation analysis: one cannot (reliably) analyze the regulation of flux changes that are zero or only small. This precludes the analysis of regulation with the express purpose of homeostasis.

**LIFE IS MORE EXPENSIVE AT HIGH TEMPERATURES**

In the previous section the methodological aspects of the systems biology approach used in this thesis were discussed. The next sections will deal with the experimental findings concerning yeast physiology at supra-optimal temperatures.

Surviving the initial temperature increase and growing at supra-optimal temperatures is requires adaptation to the physicochemical effects of the stress. Physically, temperature affects the fluidity of membranes [5, 6], which may result in increased permeability and ion leakage [7-9]. Also, temperature affects proper folding of proteins, exemplified by the fact that denaturation and aggregation occurs more easily at high temperatures [10, 11]. Chemically, temperature directly affects reaction rates.

Membrane composition is changed when cells are grown at varying temperatures, to preserve appropriate membrane fluidity [12, 13]. Additionally, large numbers of heat shock genes are induced, of which many code for protein chaperones with a role in the prevention of protein denaturation [14-17]. The latter implies the cost of maintaining protein folding is higher at these temperatures. Indeed, both in C- and N-limited chemostats, glycolytic flux was increased at higher temperatures (chapter 2 and 3). However, in C-limited cultivations metabolism was changed from respiratory metabolism at 30°C to respiro-fermentative at 38°C. Direct analysis of the flux to ATP in **chapter 4** showed that the ATP flux increased with the increase in temperature, in C-limited chemostats. Similarly, in N-limited chemostats the ATP required for cell maintenance is almost doubled when at 39°C compared to 28°C [18].
In order to restore the balance between growth and maintenance the glycolytic flux should increase too. Indeed, in batch fermentations large transient increases of glycolytic fluxes are observed immediately upon increasing the environmental temperature. The final flux values at a new steady state are (slightly) above the initial ones, depending on the temperature (see for instance [19]). This redistribution allows the cell to produce ATP to match the new energy requirements. The same transient pattern was observed at the transcriptional level: Immediately after shifting to a new culture temperature, cells respond with large changes in gene expression in attempt to adapt to the perturbation. Finally, over time the expression declines and transcript levels tend to return to the pre-stress level for many genes, although not for all [20, 21].

REGULATION OF TEMPERATURE INDUCED FLUX INCREASE
We aimed at understanding of the regulation of temperature-induced changes. We used steady state cultivations, and therefore only fully adapted cells were studied. Although, we did not observe changes in glycolytic flux 2 hours after the temperature increase, transient flux changes may be overlooked. Still, comparing steady states gave remarkable insights.

In Chapter 3 we studied the regulation of the temperature induced flux increase in nitrogen-limited chemostats. Under these conditions the increase in temperature from 38°C to 30°C induced a flux increase of 2-fold, without a qualitative change in metabolism. Regulation analysis revealed that the 2-fold was regulated differently for all enzymes. In contrast, in chapter 2 the same temperature change induced a 6-fold flux increase and a qualitative switch in metabolism occurred. Remarkably, in this case the flux increase through the enzymes was mainly facilitated by changes in the metabolic environment of the enzymes. Whether this apparent difference is related to the absolute fluxes, with N-limited chemostats at 30°C already having a glycolytic flux as high as C-limited chemostats at 38°C, or rather to the specifics of the conditions, remains to be answered. That regulation depends on the cultivation conditions or growth history was previously suggested by van Eunen et al. [22]. They determined that regulation of glycolytic flux in response to nitrogen starvation was predominantly metabolic in cells grown under respiratory conditions, whereas in cells grown under respiro-fermentative conditions the hierarchical coefficient was dominant.

The hierarchical component is affected by the outcome of regulation of the gene-expression cascade, including transcription, translation, post-translational modifications and degradation. By taking these processes into account, hierarchical regulation can be dissected quantitatively into these separate mechanisms. Daran-Lapujade et al. used this extended regulation analysis to study the contribution of these processes to the flux increase in yeast cells confronted with oxygen depletion and benzoic acid induced stress [23], and found that post-translational steps, including protein degradation, play a major role. We used
proteomics to quantitatively determine the abundance of the proteins responsible for the catalysis of the various steps in glycolysis, and found a lack of correlation between protein abundance and enzyme activity, suggesting that post-translational modifications or interactions affecting the activity of the proteins are of crucial importance.

Interestingly, under C-limited conditions the direct effect of temperature on the catalytic rates was very limited. The expected 2-fold increase in activity as consequence of a 10°C temperature increase ($Q_{10}$) was not observed. This might suggest that the properties of the enzymes somehow dampen the effect of temperature possibly to prevent an overshoot in metabolism at supra-optimal temperatures. In contrast, under N-limited conditions enzymes of the lower part of glycolysis showed rate increases close to the expected $Q_{10}$. This revealed that isoenzymes are differently affected by the physical environment. In addition, the temperature dependence of the activity of some glycolytic enzymes obtained from cells cultured at either 30°C or 38°C differed. In Chapter 3 we quantified the abundance of various isoenzymes at the protein level using quantitative proteomics. Indeed, isoenzymes of at least one protein enzyme could explain the difference in temperature sensitivity of enzyme catalytic rate. For other enzymes, other explanation must be found. Possibly, a condition specific presence of regulatory proteins or allosteric effectors plays an important role.

**TEMPERATURE SENSITIVITY OF MITOCHONDRIA**

In chapter 2 we observed an unexpected switch from respiratory to respiro-fermentative metabolism at high temperature. Also on plate cultivations, yeast could not grow on non-fermentable carbon sources at 38°C, whereas both in liquid cultures and on plates respiratory growth was normal at 37°C. In Chapter 4 we analysed the temperature dependence of mitochondrial respiration. The defect was fully reversible in individual cells, and took multiple generations to establish. Using electron microscopy we showed that the morphology of the mitochondria, in particular the structure of the cristae, was severely perturbed. In line with a hypothesis that metabolic switches are the consequence of trade-offs between energy costs and benefits of the expression of enzymes or pathways[24] we suggest that in de range of 30°C to 37°C the maintenance energy increases, as observed from an increasing $q_{ATP}$. In this same temperature range, the efficiency of respiration is increased close to the theoretical maximum. At even higher temperatures, energy yield of the respiratory metabolism is no longer still high enough to support the investment in mitochondria, and a shift to fermentative metabolism is observed. Alternatively, the shift in metabolism can also be the effect of loss of function of one or more key enzymes. Observations by Moro and Muga [25] support the latter concept. At temperatures above 37°C, they observed a steep temperature dependent reversible conformational change and dimer dissociation of Mge1p, the nucleotide exchange factor for mHsp70. This protein is
essential for mitochondrial protein import. Since mitochondrial DNA does not encode for all necessary mitochondrial proteins [26], many essential, nuclear encoded, proteins need to be imported from the cytosol. Without functional mitochondrial import machinery, over time, mitochondria will loose function. However, overexpression of Mge1p did not result in suppression of the respiratory growth deficiency at high temperatures (our unpublished data).

These different interpretations of the observations do not preclude one another.

Trying to explain the observed increase in respiratory chain efficiency, we noted that in vitro, Gut2p is found to have a higher P/O ratio compared to the other NADH dehydrogenases [27]. This indicates that the increased efficiency that we observed in our experiments might be due to the activity of Gut2p. Indeed, a \textit{GUT2} deletion strain did not show increased efficiency at high temperatures, suggesting that a switch from the NADH dehydrogenases to de glycerol-3-phosphate shuttle leads to a higher \textit{in vivo} respiratory chain efficiency. This leaves us with two new questions. First, respiratory growth generates NADH in the mitochondria. To be available for Gut2p, NADH needs to be in the cytosol. Yet, the redox couples NAD$^+$/NADH can not pass the inner mitochondrial membrane. Therefore, to maintain the redox balance, NADH molecules must be reoxidized in the compartment in which they are produced. Several shuttles couple oxidation of mitochondrial NADH to the external NADH dehydrogenases, such as the ethanol-acetaldehyde shuttle [28], the malate-oxaloacetate shuttle, the malate-aspartate shuttle and the highly unlikely malate-pyruvate shuttle (for review on these shuttles see [29] and references therein). Whether these shuttles operate under our conditions remains elusive. Therefore, it is still obscure how the mitochondrial NADH becomes available for Gut2p to increase the respiratory efficiency.

Another interesting consequence of different in vivo respiratory chain efficiencies comparing Gut2p and the NADH dehydrogenases is that, while in both cases 2 electrons are donated to ubiquinone, somehow these electrons do not lead to the same proton translocation. This can be explained by the small but significant differences in the use of scalar protons (chapter 4, table 4.4), or by more sophisticated differences, such as local ubiquinone pools, or ETC subcomplexes. This, however, is food for further study.

**PERSPECTIVES**

From a biotechnological point of view, understanding of pathways and their regulation is crucial for the fine-tuning of fluxes by genetic modifications. Previous attempts to increase glycolytic flux were unsuccessful. Among these attempts were modulation of single rate-limiting enzymes and simultaneous modulation of multiple enzymes or complete parts of the pathway [30-32]. This thesis shows that depending on the cultivation conditions the flux of a pathway can be increased by subtle changes in the ratio of substrates, products and effectors (\textit{chapter 2}). In the face of a temperature perturbation multiple regulatory
processes play an important role in regulating the flux (chapter 4). As a general rule, before trying to increase the metabolic flux through a pathway it needs to be realized that flux regulation is exerted in a complex way and often at multiple levels of cellular organisation. Thus stress response analysis should start with a careful dissection of the levels of cellular organisation most involved in regulation. Subsequent detailed studies can then be focused on these.
REFERENCES


