The effect of altered expression of transcriptional regulators of catabolism on the transcription profile and physiology of Saccharomyces cerevisiae

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Summary

The yeast *S. cerevisiae*, also known as baker's yeast is a unicellular fungus that naturally occurs on various decomposing fruits. It is widely used in beer, bread and wine fermentations and serves as a model organism for higher eukaryotes. As such, the organism is widely studied and since 1996 the entire genome has been sequenced. A lot of research has been focused on the primary sugar metabolism and its regulation as well as on how this can be employed to our benefit. In the introduction (**Chapter 1**) the complex network of hexose transporters and signal transduction routes is described. *S. cerevisiae* has a preference for the hexose glucose. In order to effectively catabolize this sugar various mechanisms are used, which are all intricately linked, resulting in a complex network. Since *S. cerevisiae* is a Crabtree positive yeast, it will produce ethanol under aerobic conditions, whenever glucose is present in excess. Although advantageous in many applications, this has the disadvantage that the biomass that can be produced per unit of glucose is low due to the low energetic efficiency of the organism's catabolism. The research described throughout this thesis (**Chapter 2, 3 and 4**) characterizes two major transcriptional regulators which are important in maintaining the balance between fermentation and respiration in baker's yeast. Our knowledge as to the mechanism and the quantitative impact of this regulation may provide us with tools to improve the performance of the organism with respect to ethanol formation on the one hand and biomass formation on the other.

To begin with, non-limited batch conditions are investigated on both the physiological and transcriptional level (**Chapter 2**). There it is shown that by deleting the gene *HXK2* a large shift towards respiratory metabolism is obtained. On the physiological level, this mutant grows slower than the wildtype, but expresses a reduction in fermentation rate and an improved biomass yield on glucose. In accordance with these findings, genome-wide expression analysis shows an increase in genes involved in the TCA-cycle and oxidative phosphorylation. Additionally, many other genes that are normally not expressed under glucose excess conditions are shown to be expressed. These included genes in the glyoxylate cycle and genes needed for utilization of other carbon sources. Taken together, the analysis reveals that deletion of *HXK2* results in a strain that is relieved of glucose repression. Analysis of the *HAP4* overexpression mutant also shows that catabolism shifts towards respiration. On the physiological level the strain grows at a similar rate as the wild-type, with a slightly increased yield on biomass. Gene expression is increased with respect to genes involved in the TCA-cycle and oxidative phosphorylation, however not as strong as was seen in the *HXK2* deletion mutant. Unexpectedly, a downshift in expression of genes needed for maintaining proper zinc content in a cell is observed. Analysis of the double mutant, in which both *HXK2* was deleted and *HAP4* was overexpressed, reveals an even larger shift towards oxidative metabolism. The growth rate in this strain was higher than in the *HXK2* deletion mutant, and a near theoretical maximum biomass yield on glucose is achieved. Genome-wide expression analysis shows an even larger increase in expression of genes needed for the TCA-cycle and oxidative phosphorylation and again a downshift in genes needed for zinc homeostasis. Thus we conclude that the regulators Hap4p and Hxk2p function to some extent

synergistically: the double mutant expresses a physiological behaviour of enhanced respiratory catabolism and hence yield optimization.
Since these strains show an increase in respiration it was then investigated whether their ability to ferment was affected under conditions where respiration is not repressed (**Chapter 3**). In this chapter the mutants are tested for their fermentative capacity (the ability to ferment sugars to ethanol and CO_2 under anaerobic conditions), an important industrial parameter that indicates the dough-raising power of a yeast strain. The strains were grown continuously under aerobic glucose-limited conditions and subsequently transferred to an anaerobic environment. For the *HAP4* overexpression mutant no changes in behaviour upon transfer to anaerobic conditions are observed and nor did changes in growth rate affect this behaviour. However, the *HXK2* deletion mutant showed completely different behaviour, when transferred from the aerobic chemostat to an anaerobic environment. At low growth rates their ability to ferment over a short time is not affected compared to the wild-type. In contrast, at higher growth rates, this ability was significantly impaired. To investigate their reduced fermentative capacity on glucose at higher growth rates, gene expression, glycolytic enzyme activity and *in vitro* sugar uptake was measured. On the gene expression level hardly any changes in glycolytic gene expression during aerobic cultivation are observed. On the enzyme level, there is a reduction in maximum hexokinase activity in the mutant, but this could not account for the observed reduction in fermentation rate. Additionally, glucose uptake over a short time shows no clear difference. Another carbon source, maltose, which is abundant in bread dough, was tested. The *HXK2* deletion mutant was able to ferment this sugar effectively compared to the wild-type, irregardless of the growth rate.
The *HAP4* overexpression mutant was studied in more detail by comparing genome-wide expression profiles under four different physiological conditions (**Chapter 4**). The data used for the analysis where obtained by our colleagues in Delft and merged with those presented in chapter 2 and 3. Two glucose excess conditions (batch and nitrogen-limitation) and two glucose limited conditions (μ=0.1 and 0.32 h^{-1}) were analysed. The analysis shows an increase in genes under control of Hap4p under glucose repressed conditions. Besides the Mig1p and Cat8p elements being identified under glucose de-repressed conditions, two additional regulators were identified under varying conditions, namely Rsc1p and Zap1p. Both are involved in cation homeostasis: Rsc1p is a regulator for proper maintenance of iron content in the cell and Zap1p for zinc. The changes in genes under control of Zap1p are analysed in more detail. As changes in *ADH* expression were observed, a possible explanation of the changes in expression of Zap1p controlled genes emerged. *ADH4* is the only alcohol dehydrogenase which requires zinc. And indeed, it is shown that *ADH4* expression is correlated in most cases with up- or downregulation of Zap1p controlled genes. However, many other alternatives remain that can explain the changes in zinc regulation in the *HAP4* overexpression strain. Overexpression of *HAP4* shows the major effects on those genes involved specifically in respiration, which is a clear difference to what was seen in the *HXK2* deletion mutant, which shows a whole array of changes, including loss of glucose repression.
In **Chapter 5,** a theoretical framework is developed that relates the *overall* affinity constant for a substrate (K_s), effectively controlling the specific growth rate, to the *specific* affinity for that substrate of the transporter (K_m). This framework, based on metabolic control analysis was used to interpret data obtained from glucose-limited

chemostat cultures. If the transporter were to have full control, it follows that these two constants are identical. If, however, the transport step does not have full control, it leads to the counter-intuitive result that the affinity of the organism for a substrate can be higher then the affinity of the transporter. Indeed, it followed from that experimental data on chemostat cultures and *in vitro* glucose uptake assays that the affinity of the organism can be more than two times higher than the affinity of the transporter. Additionally, at specific glucose concentrations the control of the transporter on the specific growth rate could be calculated.