Physiological responses of carbon fluxes to deletion of specific genes in Saccharomyces cerevisiae.

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Publication date
2000

Summary

This thesis is concerned with various topics all sharing the same goal: the understanding of regulation and distribution of carbon fluxes in *Saccharomyces cerevisiae*. For this study, two genes have been studied extensively, *ATP2* and *HXK2*. The first gene encodes the catalytic β-subunit of the F_1·F_0 ATP synthase. The F_1·F_0 ATP synthase catalyses the final step in oxidative phosphorylation and carbon metabolism, the transduction of free energy to the more manageable form of ATP. The second gene, *HXK2* encodes hexokinase II which is one of the three enzymes catalysing the first step in glycolysis, the conversion of glucose to glucose 6-phosphate. Besides its catalytic function, hexokinase II has been shown to play a profound role in the glucose repression pathway. A third aspect of this thesis concerns the development of techniques to recognize and identify unknown genes that reveal no obvious phenotype when deleted and are therefore called silent genes.

Chapter 1 gives an introduction to all topics dealt with in this thesis; genomics, carbon metabolism in general, molecular and physiological aspects of glucose repression, the resulting changes in distribution of the carbon fluxes over the fermentative and oxidative pathway. Additionally energetics in relation to oxidative phosphorylation are discussed and metabolic control analysis is shortly dealt with.

The yeast genome sequencing project has revealed approximately six thousand open reading frames (ORFs) in *Saccharomyces cerevisiae* or bakers yeast [69]. The successor of the yeast genome sequencing project, *i.e.* EUROFAN (European functional analysis network), is elucidating the function of hundreds of the ORFs for which no function was known. For more than half of the latter, initial screening on agar plates of strains each deleted in one gene, has revealed no obvious phenotype [47]. Consequently, the biological role of these genes remains a mystery. In Chapter 2, two new methods are described that can facilitate the recognition of gene products with a silent phenotype in yeast by carefully tracking changes in metabolite profiles of a knockout strain compared to its parent. The approaches are both based on the idea that the growth rate of a mutant may not be changed much, precisely because the concentrations of intracellular metabolites have altered so as to compensate for the effect of the mutation. By implication, mutants that are silent when scored on the basis of metabolic fluxes such as growth rate, should be "loud and clear" when scored on the basis of metabolite concentrations.

Chapter 3 deals with how carbon and energy metabolism of *Saccharomyces cerevisiae*

change in response to a deletion of the *ATP2* gene (encoding the catalytic β-subunits of the $F_1 \cdot F_0$ ATP synthase). Such a deletion strain, when growing on excess glucose, displayed unchanged intracellular metabolites and growth yields. Nevertheless, the deletion of the *ATP2* gene caused *in vivo* respiration to decrease by 10%, ethanol and glycerol production to increase by 15% and the specific growth rate to decrease by almost 20%. This shows that $F_1 \cdot F_0$ ATP synthase plays a significant role in metabolism even under glucose repression conditions but without detectable effects on the biomass yield. In glucose-limiting chemostats at a dilution rate of 0.1 h^{-1} the glucose consumption rate was more than 5 times larger in the *atp2* deletion strain than in the wild-type strain. The biomass yield of the mutant had remained the same as under the glucose excess conditions, whilst the biomass yield of the wild-type strain increased 4.5 fold.

For a quantitative determination of the control of $F_1 \cdot F_0$ ATP synthase on metabolism, its activity was modulated by genetic means in Chapter 4. It was shown that the response of the specific growth rate on non-fermentable carbon sources was strong and amounted to a response coefficient of 0.9, indicating that the enzyme is a crucial controller of cell physiology. The *in vivo* respiration rate depended on $F_1 \cdot F_0$ ATP synthase activity with a response coefficient of approximately 0.5. Surprisingly, a decreased $F_1 \cdot F_0$ ATP synthase activity and therefore decreased ATP synthesis capacity did not lead to a decrease in the ATP concentration in the cell. The decrease of ATP production by adding uncoupler to wild-type cells gave similar results: no effect on the intracellular ATP concentration was detected whereas the specific growth rate was decreased. Now, respiration was increased due to the reduced proton motive force over the mitochondrial inner membrane. Additionally, the intracellular pH was shown to be 0.5 unit lower in a strain with down-regulated $F_1 \cdot F_0$ ATP synthase activity. A possible explanation for these phenomena is that the rate of ATP hydrolysis by plasma membrane ATPase decreases to maintain the cellular ATP concentration, resulting in acidification of the cytosol. The decreased intracellular pH is likely to slow down metabolic fluxes as well.

In the following chapters physiological effects of the deletion of another interesting gene involved in carbon metabolism was studied, namely, *HXK2*. In Chapter 5A it became clear that an *hxk2* deletion strain grown in batch fermentors with excess glucose reveals fully oxidative metabolism during early to mid-exponential growth whereas its parent mainly grows fermentatively due to glucose repression. The oxidative metabolism resulted in according high yields of biomass and strongly reduced ethanol production. Despite the fact that the specific growth rate of the *hxk2* deletion strain is reduced compared to the wild-type strain (0.32 h^{-1} and 0.39 h^{-1} respectively), the *hxk2* deletion strain exhausted all available carbon sources before its parent. This is a consequence of a faster diauxic shift due to a derepressed metabolism and subsequent a relatively short period of growth on ethanol due to the reduced alcoholic

fermentation during the growth on glucose.

In Chapter 5B, the physiological studies of the *hxk2* deletion strain were continued in glucose limited continuous cultures. Surprisingly, the *hxk2* deletion strain revealed an enhanced maximal specific growth rate compared to the wild-type strain (0.46 h^{-1} and 0.40 h^{-1} respectively). Additionally, the critical specific growth rate was enhanced in this strain compared to the wild-type strain (0.35 h^{-1} and 0.29 h^{-1} respectively). This proves that the *hxk2* deletion strain has an increased oxidative capacity. The discrepancy between the maximal specific growth rate of the *hxk2* deletion strain in batches (0.32 h^{-1}) and continuous cultures (0.46 h^{-1}) may be explained by the residual glucose concentrations in the cultures but this needs further investigation.

Since glucose repression seems diminished in the *hxk2* deletion strain we studied the physiology of the *hxk2* deletion strain on growing on mixed substrates in Chapter 6. The *hxk2* deletion strain was able to co-consume sucrose/glucose and galactose/glucose. In the *hxk2* deletion strain, not only sugars were co-consumed together with glucose but also ethanol was consumed simultaneously with glucose. This indicates that the ethanol consumption pathway and perhaps even gluconeogenesis are derepressed.

The final experimental chapter, Chapter 7, deals with the increased demand for biotin by the *hxk2* deletion strain. The genes involved in biotin uptake and metabolism were very strongly up-regulated in the DNA array which gave us a hint that biotin was limiting in these cultures. The addition of extra biotin to the medium reduced the ethanol production by the *hxk2* deletion strain growing on glucose by an extra 40%. Biotin functions as a co-factor for some carboxylating enzymes, such as pyruvate carboxylase and acetyl CoA-carboxylase. Biotin deficiency causes these enzymes to reduce activity and as a consequence fermentation is initiated and the specific growth rate decreases.