

**Mitochondrial oxidation of cytosolic NADH and regulation of catabolic fluxes in
*Saccharomyces cerevisiae***

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Abstract

Keeping a cytoplasmic redox balance is a prerequisite for living cells in order to maintain a metabolic activity and enable growth. During growth of *Saccharomyces cerevisiae*, an excess of NADH is generated in the cytosol. Aerobically it has been shown that the external NADH dehydrogenase, Nde1p and Nde2p, as well as the glycerol-3-phosphate (G3P) shuttle, comprising the cytoplasmic glycerol-3-phosphate dehydrogenase, Gpd1p, and the mitochondrial glycerol-3-phosphate dehydrogenase, Gut2p, are the most important mechanisms for mitochondrial oxidation of cytosolic NADH.

A large part of the work included in this thesis has focused on the importance and regulatory properties of the G3P shuttle. It was shown that the G3P shuttle was active during growth on a reduced substrate, such as ethanol. Furthermore, it was found that Gut2p was more efficient in producing ATP compared to the external NADH dehydrogenase, *i.e.* the amount of ATP produced per amount of oxygen consumed (P/O ratio) was higher for the G3P shuttle. However, the ATP producing capacity per unit of time for the NADH dehydrogenase was superior to Gut2p. The importance of the G3P shuttle seemed to be most significant at low growth rates. In fact, the amount of Gut2p decreased with increasing dilution rate in a glucose limited chemostat culture. Two regulatory mechanisms that influence the activity of the G3P shuttle were identified in this study. It was observed that the efficiency (K_m/V_{max}) of Gut2p was increased when the external NADH dehydrogenase was deleted. Similarly, if Gut2p was deleted, the efficiency of Nde1/2p was enhanced. It was concluded that due to close physical contact between the two membrane dehydrogenases, deletion of one influenced the kinetic properties of the other. Additionally, an inhibitory effect exerted by ATP on the enzymes activities was detected for Nde1/2p, Gut2p as well as Gpd1p.

Not only seems ATP to be involved in the regulation of mitochondrial oxidation of cytosolic NADH. ATP also seems to have a role in controlling the glycolytic rate during different conditions. It was found that total metabolic activity was positively correlated to intracellular ATP concentrations in starved cells. A similar result was obtained when permeabilised spheroplasts of *S. cerevisiae*, isolated from glucose limited chemostat cultures, were used. A positive correlation between glycolytic flux and ATP was recorded for ATP concentrations below approximately 1.5 mM. It was suggested that this was due to an insufficiency of ATP required for the initial step of glycolysis, where ATP is a substrate. In contrast, at ATP concentrations above about 1.5 mM a negative correlation between glycolytic flux and ATP level was found for the permeabilised spheroplasts. It was observed that the main targets for this inhibition were exerted on phosphofructokinase, which was inhibited by ATP values above 1-2 mM. In addition, pyruvate kinase seemed to be affected at ATP concentrations above 2.5 mM. Like shown by other laboratories as concerns growing cells, nitrogen- and carbon-starved cells of *S. cerevisiae* showed no correlation between glycolytic flux and glycolytic protein levels. In conclusion, regulation of enzyme activity seems to dominate glycolytic rate control.

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