Glycerol Production in *Saccharomyces cerevisiae* – When, Why, and Where?

Katarina Granath

Department of Cell and Molecular Biology, Göteborg University
Medicinaregatan 9C, Box 462, SE-405 30 Göteborg

Abstract: The glycerol producing pathway in *Saccharomyces cerevisiae* is catalyzed by two pairs of iso-enzymes: the NAD^+-dependent glycerol 3-phosphate dehydrogenases Gpd1 and Gpd2, and the glycerol 3-phosphatases Gpp1 and Gpp2. Expression of the *GPD1* and *GPP2* genes are induced in response to hyperosmotic stress, resulting in production of glycerol that accumulates in the cell as an osmolyte. Expression of the *GPD2* and *GPP1* genes are, on the other hand, induced by anaerobic conditions. The Gpd2 iso-form is crucial for the regeneration of NAD^+ under anoxic conditions, when NADH can not be oxidized by respiration. The anaerobic growth inhibition of a *gpd1*Δ*gpd2*Δ mutant, which has a blocked glycerol production, is reversed by addition of an alternative electron acceptor that regenerates NAD^+, such as acetaldehyde. Bisulfite, which inhibits the reduction of acetaldehyde to ethanol, increases the cytosolic NADH levels and stimulates expression of *GPD2* even in the presence of oxygen. We therefore propose that the redox state of the cell, as reflected in the $NADH/NAD^+$ ratio, serves as a signal for transcriptional activation of the *GPD2* gene to increase the redox mediated glycerol production. Gpd2 also plays a crucial role in respiratory deficient strains. A respiratory defective *cox18*Δ strain lacking the *GPD2* gene does not grow on minimal media. This growth defect is, however, relieved by the addition of acetoin or acetaldehyde, which oxidizes cytosolic NADH, or by the addition of the amino acids lysine or glutamic acid, the synthesis of which gives rise to mitochondrial NADH production. The amino acid effect pointed to NADH accumulation in the mitochondrial matrix as a primary reason for the observed growth inhibition. We demonstrate that the N-terminal of Gpd2 targets GFP-fusions to the mitochondria and propose a role for Gpd2 in the mitochondrial inter-membrane space, where the enzyme is given an optimal position for generating an NADH gradient across the mitochondrial inner membrane that will drive efflux of reducing equivalents from the mitochondrial matrix to the cytosol via the ethanol-acetaldehyde shuttle. The Gpd1 enzyme could not substitute for Gpd2 in the *cox18*Δ mutant, despite enhanced Gpd1 levels. This appears due to an increased targeting of Gpd1 to the peroxisomal compartment in the respiratory deficient mutant, and a consequent decrease of its capacity to oxidize cytosolic NADH. We suggest that the peroxisomal Gpd1 takes part in a G3P shuttle that ensures re-oxidation of NADH produced by fatty acid β-oxidation, exporting the reducing equivalents to the cytosol. We have also characterized Yig1, a novel protein that is involved in modulating expression of *GPD2* and *GPP1* and that has a cytosolic and nuclear distribution. We propose that the Yig1 mediated control serves to adjust the cellular G3P pool to facilitate energy conservation via the mitochondrial G3P shuttle, when energy sources are scarce or when fatty acids are being used as the energy source.

Key words: *GPD1, GPD2, GPP1, GPP2*, redox, glycerol, mitochondria, *YIG1*, peroxisomes

Göteborg 2004 ISBN 91-628-6248-0