The role of the *GPD2* encoded glycerol 3-phosphate dehydrogenase in redox metabolism of *Saccharomyces cerevisiae*

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ABSTRACT

The co-factor NAD^+ is the major oxidant in both cytosol and mitochondria. Since only catalytic amounts of NAD^+ are present in the cell, sustained oxidation of important substrates depends on a rapid re-oxidation of the NADH produced in the reactions. In the presence of oxygen multiple mechanisms involving the respiratory chain can serve this purpose. However, under anaerobic conditions glycerol production remains the only mechanism available for yeast to oxidise surplus NADH. The reductive step in the glycerol production pathway is catalysed by the glycerol 3-phophate dehydrogenase encoded by the two iso-genes *GPD1* and *GPD2*. The encoded enzyme is a pacemaker for glycerol production in yeast. Expression of *GPD1* is induced by hyperosmotic stress to produce glycerol for intracellular osmoregulation. In addition, Gpd1p is involved in aerobic redox regulation by being implicated in the glycerol 3-phosphate shuttle that delivers reducing power from the cytosol to the respiratory chain. *GPD2* on the other hand, is important for anoxic glycerol production in response to redox imbalance. Cells lacking the *GPD2* gene exhibit an anaerobic growth defect and we investigated how the reductive stress that *gpd2Δ* mutants experience affects global protein production. We developed a method suitable for anaerobic screening and radiolabelling of samples and used 2D-PAGE analysis to identify proteins showing altered expression in cells exposed to reductive stress. The most prominent candidate was the Tdh1p, an iso-form of glyceraldehyde 3-phosphate dehydrogenase that has been proposed to act as a metabolic break allowing cells to adapt to stressful conditions. Using transposon mutagenesis as well as classic deletion screens, we searched for genes affecting the expression of the redox controlled *GPD2* gene. This investigation highlighted the connection between a dysfunctional respiratory chain and *GPD2* expression. Aiming at elucidating this relation further, we examined the roles of the *GPD* iso-genes in a respiratory deficient strain. We observed that the severe growth defect observed in non-respiratory *gpd2Δ* cells appears mainly due to and intra-mitochondrial redox imbalance emanating primarily from NADH generated in amino acid biosynthesis. This growth defect could be reversed if Gpd2p was partly localized to mitochondria. We demonstrated that the N-terminus of Gpd2p targets GFP fusions to mitochondria and propose that Gpd2p acts in the mitochondrial inter-membrane space in close connection with the ethanol-acetaldehyde shuttle to allow efflux of reducing equivalents from the mitochondria to the cytosol under non-respiratory conditions. Consistent with recent reports, we also localized Gpd1p to the peroxisomes where it may participate in a G3P shuttle to export reducing power to the cytosol. We propose a distinct sub-cellular localization of the *GPD* iso-ezymes under non-respiratory conditions where they significantly contribute to organellar redox regulation of *S. cerevisiae*.

Key words: *GPD1, GPD2*, NADH, redox, glycerol, mitochondria, metabolism, *S. cerevisiae*

Göteborg 2004 ISBN 91-628-6080-1