Functional studies of genes involved in glycerol metabolism of *Saccharomyces cerevisiae*, with emphasis on the two *GPP* isogenes for glycerol 3-phosphatase

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Abstract

This thesis describes the purification of two highly specific glycerol 3-phosphatases, identification and cloning of the corresponding genes and characterization of their role in the physiology and glycerol metabolism of the yeast *Saccharomyces cerevisiae*. Purification by gel filtration and ion-exchange chromatography revealed the existence of two isoforms. N-terminal microsequencing of purified peptides led to the identification of two previously uncharacterized genes, *GPP1/RHR2* and *GPP2/HOR2*. The genes encode proteins with a molecular weight of 28 kD, showing 95% amino acid identity and belonging to a family of low molecular weight phosphatases. Mutants deleted for *GPP1* and *GPP2* are devoid of glycerol 3-phosphatase activity and show a strongly restricted glycerol production. Over-expression studies indicated that the enzymes are not rate limiting in glycerol production. A *gpp1 gpp2* double mutant is hypersensitive to high osmolarity and does not grow under anaerobic conditions in agreement with the requirement of glycerol production for intracellular osmoregulation and anoxic redox regulation. Expression of the major isogene, *GPP1*, is transiently induced by hyperosmotic stress and anoxic conditions, whereas the basal *GPP1* expression level is strongly affected by protein kinase A activity. Expression of *GPP2* is strongly induced by osmotic stress in a high osmolarity glycerol (HOG) pathway dependent way, while the expression level is unaffected by anoxic conditions. The phenotype of the *gpp* single mutants indicates the *GPP* genes can substitute well for each other. However, a *gpp1* mutant shows poor anaerobic growth. We also observed that a *gpp1 gpp2* double mutant is sensitive oxidative stress generated by paraquat, indicating glycerol metabolism or glycerol production can protect against oxidizing conditions. The *gpp1 gpp2* mutant also exhibits increased specific activity of fructose 1,6 bisphosphate aldolase and glycerol 3-phosphate dehydrogenase. These observations together with its massive accumulation of glycerol 3-phosphate as well as other changes in metabolite levels suggest a regulatory system diverting an increased flux into the blocked glycerol pathway to compensate for lost glycerol production.

SGD1 was isolated by complementation of an osmosensitive mutant and encodes an essential nuclear protein. Increased dosage of *SGD1* suppresses the osmosensitive phenotype of HOG pathway mutants and partly restores the osmostress induced transcriptional activation of the HOG pathway dependent *GPD1* gene, encoding NAD^+ dependent glycerol 3-phosphate dehydrogenase. Sgd1p shows weak homology to Spt7p, a subunit of the nucleosomal SAGA histone acetylation complex, suggesting Sgd1p might have a role in chromatin remodeling.

Keywords: *Saccharomyces cerevisiae*, glycerol, glycerol 3-phosphate, Gpp1p, Gpp2p, Sgd1p, osmotic stress, oxidative stress, anaerobic conditions, metabolism, PKA, HOG pathway.

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