Title:	Studies on 6-phosphogluconate dehydrogenase, an enzyme of the pentose phosphate pathway of Saccharomyces cerevisiae
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Abstract:	The pentose phosphate pathway is the major source of NADPH in living cells. The two enzymes in this pathway involved in the generation of NADPH are Glucose 6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH). In this thesis, studies have been initiated on some aspects of the 6PGDH enzyme of Saccharomyces cerevisiae. 6-phosphogluconate dehydrogenase (6PGDH) is the third enzyme in the oxidative Pentose Phosphate Pathway (PPP) that catalyzes the production of Ribulose 5-phosphate from 6-Phosphogluconate [14]. During this process, 6PGDH generates NADPH by using NADP + as co-factor [14]. In Saccharomyces cerevisiae, GND1 gene encodes the major isoform of the protein, which performs 80% of the 6PGDH activity, whereas, GND2 gene encodes the minor isoform, which performs the remaining 20% activity[8]. Multiple sequence alignments of the 6PGDH enzymes across organisms, revealed six conserved cysteines from yeast to humans. As these cysteines did not appear to be in the active site of the enzyme, an important goal of this thesis was to investigate whether these cysteines might have some regulatory roles. We created different cysteine to serine mutants of 6PGDH encoded by GND1. To investigate the functionality of these mutants we carried out experiments to establish an in vivo functional assay. To establish this assay, WT and mutants of deletions of GND1 and GND2 were evaluated on both normal and stress conditions. We also evaluated the ability of NADPH deficient mutants to be complemented by GND1. Finally, as it was not clear from the literature whether the knockout of both GND1 and GND2 would be lethal, as an aid to the in vivo functionality assays, we made attempts to create a double knockout of both GND1 and GND2 using a plasmid shuffling method.
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