Title:	Investigations into the regulation of human 5,10-methylene tetrahydrofolate reductase (hMTHFR) in S. cerevisiae
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Abstract:	Human Methylenetetrahydrofolate reductase (hMTHFR) is an enzyme of the one carbon metabolism pathway that plays a key role in methionine biosynthesis. It catalyzes the reduction of methylenetetrahydrofolate to methyltetrahydrofolate, the methyl donor for conversion of homocysteine to methionine. In humans, there are two isoforms of MTHFR (70 kDa and 77 kDa). Regulation of MTHFR activity is crucial for maintaining cellular pools of methionine and S-adenosylmethionine (AdoMet) and is very poorly understood. In this thesis we have investigated aspects of the hMTHFR regulation using two yeast assays. The first assay was a simple complementation of the yeast mutant met13Δ. We also used a more stringent assay where we used met13Δmet15Δ mutant in which there is a more severe defect with a higher requirement for methionine. We cloned the two isoforms of hMTHFR (70 kDa and 77 kDa) in a yeast expression plasmid and examined their function by complementation. Both were comparable. Regulatory domain mutant R392A and multiply mutated mutant with F388A, P389A, G391A, R392A, and G394A mutations of hMTHFR 70 in the AdoMet binding region were created but they did not exhibit any apparent growth differences. Patient mutations R377C, R377H, G387D and G390D in or near the AdoMet binding region of hMTHFR were created. These mutants also did not show any phenotype with the yeast assays. We also random mutagenized hMTHFR 70 by hydroxyl amine mutagenesis. Out of all the mutants selected, mutant 3 and mutant 4 were found to complement met13Δmet15Δ strain. Characterization of the mutations in mutant 3 or 4 responsible for this complementation still needs to be done. Further we tried to purify hMTHFR and some of the mutant proteins to evaluate their enzymatic activity in-vitro by using their his- tagged clones in E. coli expression plasmid. However, several attempts of purification were unsuccessful, reasons of which are not known.
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