Title:	Studies on the regulatory domain of the yeast Methylene tetrahydrofolate reductase (MTHFR), a key enzyme in one-carbon metabolism
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Keywords:	Methylene Tetrahydrofolate Carbon metabolism
Issue Date:	28-Jul-2021
Publisher:	IISERM
Abstract:	Methylenetetrahydrofolate reductase (MTHFR), is an enzyme that links the folate cycle to the methionine cycle in one-carbon metabolism. Previous studies with the purified eukaryotic MTHFR have revealed that the enzyme is under allosteric inhibition by S-Adenosyl methionine (SAM). Although this allosteric regulation has been known for decades, the importance of this regulatory control to one-carbon metabolism has never been adequately understood, as mutants defective in this regulation have never been obtained. We describe in this thesis the identification of amino acid residues within the regulatory region of MTHFR critical for its regulation by SAM and the creation of mutations within this region to yield feedback insensitive, deregulated MTHFR. These mutants were exploited to investigate the effects of defective allosteric regulation. Genetic analysis revealed a strong growth defect in the presence of methionine. To understand the metabolic consequences we carried out biochemical and metabolite analysis. We observed that both the folate and methionine cycles were affected in these mutants, as was the transsulfuration pathway leading to decreased formation of glutathione and its precursors critical for redox homeostasis. The major consequences, however, appeared to be in the depletion of nucleotides. Folates are precursors to nucleotides, but folate supplementation led to only partial recovery. 13 C isotope labelling and metabolic studies revealed that the deregulated MTHFR cells undergo continuous transmethylation of homocysteine by CH 3 THF to form methionine, which drives SAM formation accentuating ATP depletion. SAM was also cycled back, leading to futile cycles of SAM synthesis and recycling. This also explains the need for MTHFR to be regulated by SAM, and the study has yielded valuable insights into one-carbon metabolism. Furthermore, these mutants would be powerful tools both in yeasts and in humans (which have a similar domain) to further dissect the regulation of one-carbon metabolism and its intersections with various pathways.
URI:	http://hdl.handle.net/123456789/3723
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