ABSTRACT:

5,10-Methylene tetrahydrofolate reductase(MTHFR) is a cytoplasmic enzyme which catalyzes the conversion of 5,10-methylene tetrahydrofolate(CH2-THF) to 5,10-methyl tetrahydrofolate(CH3-THF) using NADPH as a reducing equivalent. This enzyme plays an important role in folate one carbon metabolism pathway. The polymorphisms of this enzyme associated with disruption of function cause neural tube defects and hyperhomocysteinuria in many individuals.This enzyme consists of N-terminal catalytic domain and C-terminal regulatory domain linked by a small stretch of amino acids called as linker region. The function is negatively regulated by the metabolite S-adenosyl methionine (SAM) produced downstream to methionine in the methionine cycle. It has been known that SAM binds to the regulatory domain and thus regulates the activity of the enzyme, but the exact SAM binding region is not known. The mutational studies on the conserved residues in the regulatory domain of S. cerevisiae MTHFR (MET13) show a deregulated phenotype. On the continuation of the previous finding of a deregulated mutant of MET13_R357A in the lab, the conservation of deregulation of MTHFR in higher organisms was examined in Homo sapiens MTHFR. The isoforms of Homo sapiens MTHFR shows interesting phenotype when supplemented with different sulphur sources. The second part of the work involves attempts at construction of a genetically encoded SAM sensor based on fluorescence. It has been shown that the insertion of an in sense molecular recognition domain in between the GFP responds on binding to the ligand. Based on this we are trying to create a GFP based SAM sensor by inserting the regulatory domain of the MTHFR protein in frame with the GFP protein.The response of GFP constructs to SAM on insertion of the regulatory domain of MTHFR shows a potential to be a SAM sensor.