Contribution ID: **41** Type: **Poster contribution**

Identification, structural and biochemical characterization of a RsmD-like Methyltransferase from Mycobacterium tuberculosis.

Thursday, 5 July 2012 10:30 (1h 30m)

As many as 29 post- translational modifications in 16S and 23S ribosomal RNA of Escherichia coli are known; 10 of which are located in the 16S RNA and 19 are present in 23S RNA. These modifications are brought about by specific methyltransferases. Nine of ten methylated nucleotides of Escherichia coli 16 S rRNA are conserved in Mycobacterium tuberculosis. All the 10 different methyltransferases are known in E. coli, whereas only TlyA and GidB have been identified in mycobacteria. We have identified Rv2966c of M. tuberculosis as an ortholog of RsmD protein of E. coli based on its structure and activity. rv2966c can complement rsmD-deleted E. coli cells confirming this role for the enzyme. Recombinant Rv2966c can use 30 S ribosomes purified from rsmD-deleted E. coli as substrate and methylate G966 of 16 S rRNA in vitro. Three-dimensional structure of the protein shows the protein to consist of two independent domains; a short hairpin domain at the Nterminus and a C-terminal domain with the S-adenosylmethionine-MTfold. We show that the N-terminal hairpin is a minimalist functional domain that helps Rv2966c in target recognition. Deletion of the N-terminal domain prevents binding to nucleic acid substrates, and the truncated protein fails to carry out the m2G966 methylation on 16 S rRNA. We have shown that 30 S ribosome is required for the activity of Rv2966c but the role of ribosome on methyltransferase activity remains an area of interest for future research. Details of this w

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Session Classification: Poster Session

Track Classification: Biological / Pharmaceutical Research