Multi-site-specific 16S rRNA methyltransferase RsmF from *Thermus thermophilus*

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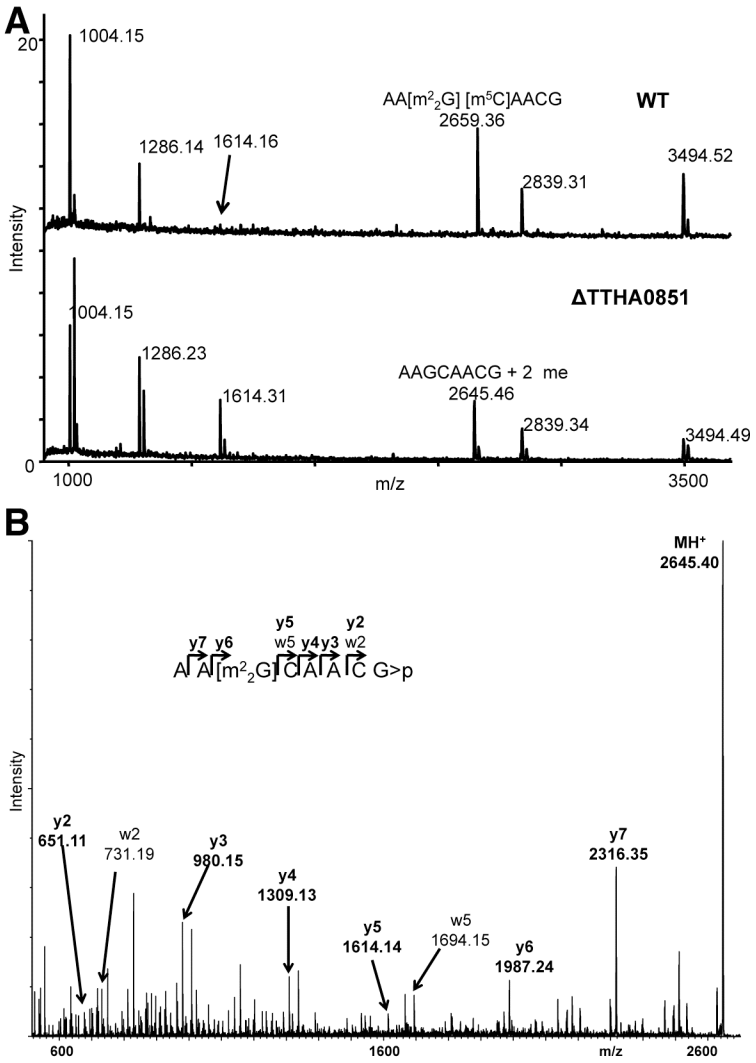
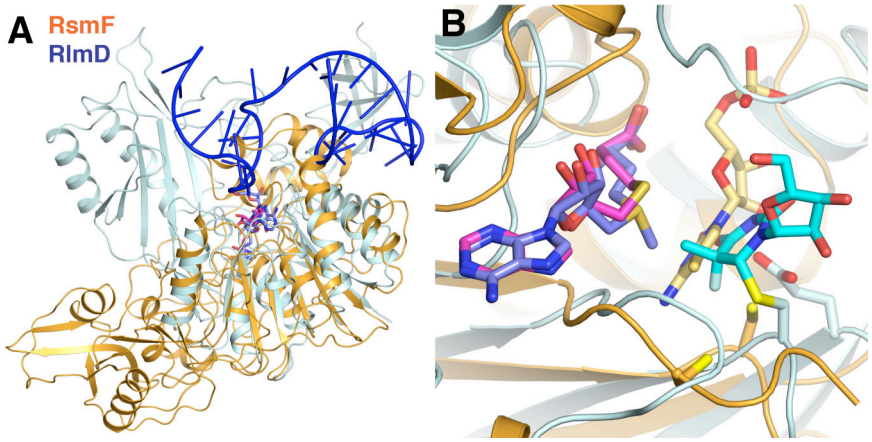
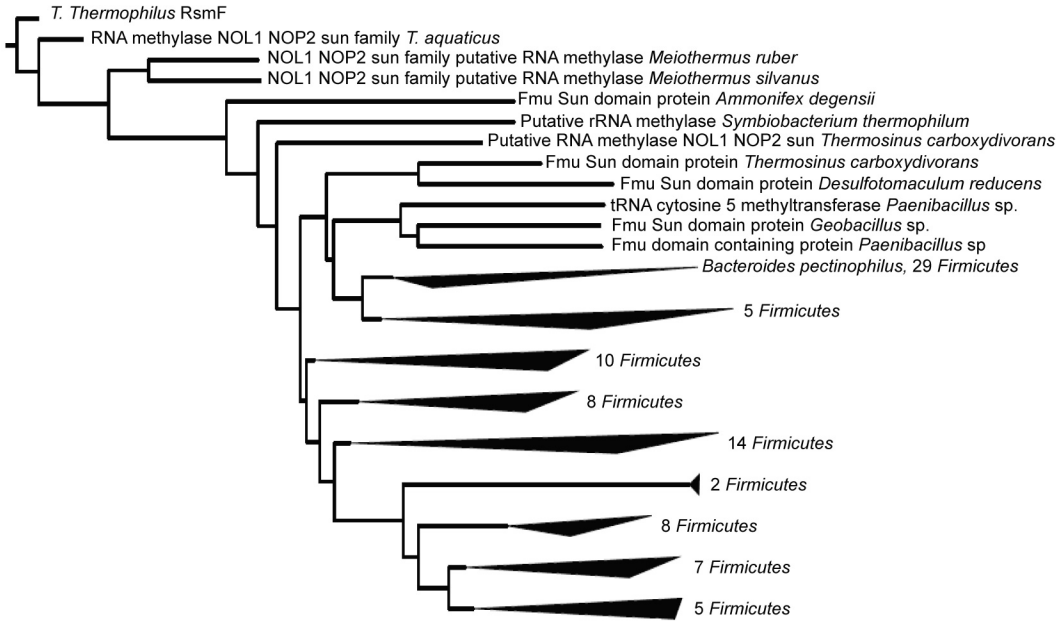


Supplementary Figure S1: (a) MALDI mass spectra of an Rnase T1-digested 16S subfragment (pos. 944-990) from wild-type cells (upper panel) and the TTHA0851 (putative *rsmB*) null mutant (lower panel). Expected digestion products are labeled; fragment affected by the gene disruption is given with sequence. The doublet nature of the signals in the lower panel results from a mixture of 2´-3´-cyclic phosphate and 3´-phosphate products upon RNase T1 digestion. me: methyl group. (b) MALDI tandem mass spectrometry of an RNase T1 fragment of 16S rRNA (pos. 964-970) from the TTHA0851 null mutant. Mass spectrometric fragments used to deduce the methylation status are labeled. Position of the backbone fragments (nomenclature according to (McLuckey et al. 1992)) in the sequence is shown. MH^+: precursor ion selected for fragmentation.



Supplementary Figure S2: Comparison with the other methyltransferases. (a) Comparison of the overall structure of RsmF (orange) with the substrate-bound RlmD structure (cyan/blue, PDB entry 2BH2). (b) Comparison of the active site region. AdoMet in RsmF is shown in blue sticks, S-adenosylhomocysteine in RlmD is shown in magenta sticks, the modeled m^5C in RsmF in light orange, and the covalently attached fluoromethyluridine in cyan sticks, respectively.



Supplementary Figure S3: Phylogenetic tree for *T. thermophilus* RsmF's closest homologues, drawn using the web based iTOL software (http://itol.embl.de/, Letunic I, Bork P. 2007. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* **23**:127-8).