Structures of ribosomes lacking up to 10 rRNA modification enzymes

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During ribosome assembly, rRNA is posttranscriptionally modified. In bacteria this is performed by site-specific enzymes. Curiously, most single-gene knockouts of modification enzymes in *E. coli* have little or no phenotype. In this project we aim to elucidate how systematic knockout of up to 10 rRNA modification enzymes affects the structure, stability and function of the local rRNA region around the modification sites as well as the translation efficiency of the mature ribosome. The Δ9 and Δ10 cells show reduced growth rate and purified actively translating ribosomes from these cells show reduced activity *in vitro*. The lack of modifications could be confirmed by LC-MS and cryogenic electron-microscopy single-particle analysis. Cryo-EM structures of 70S ribosomes from Δ9 and Δ10 show altered structure around the peptidyl transfer center, in particular at the tRNA binding sites. The reduced translational efficiency of the ribosomes seems to be a consequence of structural heterogeneity leading to lower tRNA occupancy and alternative, non-catalytic, conformations of the tRNA CCA ends. The observed ensemble of structures highlights the intrinsic driving force of RNA to form base stacks, and suggest that RNA modifications collectively favor the native/active ribosome conformation over an otherwise large accessible conformational landscape.