

Title

Detection of early-introduced RNA post-transcriptional modifications of an early-stage large subunit ribosomal intermediate

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Abstract

Protein production by ribosomes is fundamental to life. The ribosome requires numerous modifications to be assembled correctly. The ribosomal RNA (rRNA), which is post-transcriptionally modified, provides one part of ribosome assembly complex. Thus, a complete understanding of ribosome assembly requires the determination of the RNA post-transcriptional modifications in all the ribosome assembly intermediates. There are 26 RNA post-transcriptional modifications in 23S rRNA of the mature *Escherichia coli* (*E. coli*) large ribosomal subunit. The levels of these modifications have been investigated extensively only for a small number of large subunit intermediates and under a limited number of cellular and environmental conditions. The 27S intermediate is one of three large subunit intermediates accumulated in *E. coli* cells lacking the DEAD-box RNA helicase DbpA and expressing the helicase inactive R331A DbpA construct. In this study we determined the level of incorporations of 2-methyl adenosine, 3-methyl pseudouridine, 5-hydroxycytosine and seven pseudouridines in an early-stage *E. coli* large subunit assembly intermediate with a sedimentation coefficient of 27S. Through extensive bioinformatics analysis employing Illumina next-generation sequencing and tools such as ShapeMapper, modifications on the 27S intermediate were identified. Utilizing discoveries from earlier intermediates the current list of modifications, the known modified sites, at positions 1915, 1919, and 1921, were concluded to not be present in the 27S intermediate and to be incorporated at the later stages of ribosome assembly.