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Title (English)

Reconstitution of critical modification reactions on *E. coli* 23S ribosomal RNA

Title (Swedish)

Abstract

Several post-transcriptional RNA modifications are critical for *E. coli* ribosomal activity. In this project, six post-transcriptional modification reactions at the peptidyl transferase centre of the 23S ribosomal RNA (rRNA) were studied. These modifications were detected by standard gel assays by virtue of associated pauses in reverse transcription. The four methylations paused the reverse transcriptase directly, while the two pseudouridines only caused pauses after being chemically modified. Four of these modifications were synthesized *in vitro* using purified, over-expressed enzymes and *in vitro*-synthesized, unmodified 23S rRNA. The reactions with RlmM were most notable as they established that this enzyme did not require a modified RNA substrate and that only domain V of the substrate was necessary for efficient modification. This work furthers our understanding of ribosome biogenesis, and these reactions may be useful for creating a functional ribosome *in vitro* for mutagenesis experiments and for building a minimal cell.

Keywords

Escherichia coli, 23S rRNA, Post-transcriptional modification, *In vitro*, Peptidyl transferase centre, domain V, Methylation, Pseudouridine, Reverse transcription

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