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Single-molecule Force Measurements Show That Early-binding R-proteins Assist 23s Rrna Folding Co-transcriptionally

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Mechanical overstretching of individual RNA-DNA hybrids is used as a novel in vitro assay to prepare a co-transcriptional RNA structure and study its interaction with proteins. Dual optical traps hold two microscopic beads linked by an RNA-DNA molecular construct that is designed such that the RNA strand progressively peels off and folds when trap-to-trap distance increases. Subsequent distance reduction leads to duplex re-annealing. RNA structure and its interaction with proteins are probed by continuously measuring force during this peeling/re-annealing cycle.

Focusing on the early stage of E.coli large ribosomal subunit assembly (domains I-II of 23S rRNA and early-binding r-proteins uL4, uL13, bL20, uL22, uL24), we find that these five r-proteins stabilise the 23S rRNA structure: this property is notably characterised in our experiments by the observation that full re-annealing is less frequent when the r-proteins are present than when they are absent. Our results also show that the five early-binding r-proteins bind the 23S rRNA co-transcriptionally, corroborating the classical assembly gradient hypothesis (K. H. Nierhaus (1991) The assembly of prokaryotic ribosomes. Biochimie 73, 739-755).

This work was supported by a Human Frontiers Research Grant to Ulrich Bockelmann, Takuya Ueda, Knud Nierhaus and Erwin Peterman.