Title

Toward An Understanding Of The Role Of The L1 Stalk In Translocation By The Escherichia Coli Ribosome

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Abstract

Translocation is the process by which the ribosome advances the mRNA:tRNA complex by a one-codon step during the elongation cycle of translation. In the pretranslocation state, the ribosome transitions between two metastable states, Global state 1(GS1 or classical state) and Global state 2 (GS2 or hybrid state). EF-G interacts with GS2 and catalyzes translocation. The dynamics of the L1 stalk of the 50S subunit E-site, which is composed of a portion of the 23S rRNA and protein L1, are finely tuned to the GS1 to GS2 transitions. Interaction of the L1 stalk with the elbow of the P/E tRNA both stabilizes the tRNA and facilitates its passage from the P/E to the E/E site. Disruption of these interactions should destabilize the hybrid state, or GS2, and reduce the efficiency of translocation.

Mutations were made in the 23S rRNA of the L1 stalk, with the aim of interfering with segments that contribute to its function. The segments targeted for mutagenesis were: 1. The L1 binding fold, which binds protein L1 and also interacts with the elbow of the deacylated tRNA. 2. Helix 76 (H76), the flexibility of which enables the dynamics of the L1 stalk. 3. Helix 79 (H79), which supports the L1 stalk and may coordinate the mobility of the stalk with the functional state of the ribosome. Mutants in which the L1-binding fold was likely to be disrupted, were mostly dominant lethal. Here, the loss of interaction of the L1 stalk with the tRNA may increase the thermodynamic barrier for the mutant ribosomes to attain GS2, such that the efficiency of translocation falls below a growth sustaining threshold.

Diminishing the flexibility of H76 did not produce any observable defect. These mutants, which can support growth in a strain lacking all wild-type rRNA, displayed only mild growth defects or mild subunit association defects.

Partial deletion of H79 also had no affect on the ability of the ribosome to support growth in the absence of wild-type rRNA, although it lead to defects in subunit association, suggesting a role for H79 in the formation of intersubunit bridge/s.

In general, mutants of the 23S rRNA component of the L1 stalk that supported cell growth as the sole source of rRNA did not affect the efficiency or accuracy of translocation, or of reverse translocation, in vitro. Although they may affect the rates of GS1 to GS2 transitions, these mutants do not appear to impair life-supporting translational rates.

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