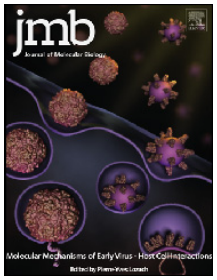
Communication



Structural Insights into the Role of Diphthamide on Elongation Factor 2 in mRNA Reading-Frame Maintenance

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Abstract

One of the most critical steps of protein biosynthesis is the coupled movement of mRNA, which encodes genetic information, with tRNAs on the ribosome. In eukaryotes, this process is catalyzed by a conserved G-protein, the elongation factor 2 (eEF2), which carries a unique post-translational modification, called diphthamide, found in all eukaryotic species. Here we present near-atomic resolution cryo-electron microscopy structures of yeast 80S ribosome complexes containing mRNA, tRNA and eEF2 trapped in different GTP-hydrolysis states which provide further structural insights into the role of diphthamide in the mechanism of translation fidelity in eukaryotes.

Introduction

During protein synthesis, mRNA and tRNA move in a coordinated and accurate way along the ribosome. In eukaryotes, this complex process of translocation is ensured by a translational GTPase, elongation factor 2 (eEF2), which is homologous to bacterial elongation factor EF-G [1]. According to structural and biochemical experiments, after peptidyl transfer reaction, the small ribosomal subunit (SSU) spontaneously rotates relative to the large ribosomal subunit (LSU). This rotation results in the movement of acceptor ends of tRNAs on LSU to P and E site while leaving main bodies of tRNAs bound to the A and P sites of SSU (A/P and P/E hybrid states, respectively) (see a review in Ref. [2]). In addition to this intersubunit ratcheting-like motion, large conformational changes occur inside SSU by itself. Thus, swivelling of the head domain and rearrangement of the shoulder promote further movement of tRNAs on SSU from their A and P sites to the P and E sites. These rearrangements are coordinated and catalyzed by eEF2, which hydrolyzes GTP and controls the precise movement of mRNA with tRNA through multiple intermediate states.

Main structural knowledge about dynamics of the eukaryotic elongation cycle, in the presence of mRNA and tRNA, has been provided by low to intermediate-resolution cryo-electron microscopy (cryo-EM) reconstructions that enabled the understanding of the major conformational rearrangements responsible for guiding the translocation process [3–5]. These snapshots were captured in different GTP hydrolysis states and suggested that eEF2 binds to a ratcheted ribosome as was originally discovered in bacteria (see a review in Ref. [2]). Notwithstanding, due to the limited resolutions obtained by these reconstructions, a detailed structural understanding of the mechanism by which eEF2 catalyzes translocation in eukaryotes remained