International Journal of Biomedicine | June 2021 - Volume 11, Issue Suppl_1: Abstracts from the Third Russian International Conference "Cryo-electron microscopy 2021: achievements and prospects"

POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: EM RESEARCH RELATED TO MEDICINE

DOI: 10.21103/IJBM.11.Suppl_1.P31

Abstract P-31: Assembly of the Complex of the 30S Ribosomal Subunit and the Ribosome Maturation Factor P from *Staphylococcus aureus* for Structural Studies by Cryo-Electron Microscopy

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Background: *Staphylococcus aureus* (*S. aureus*) is one of the main human pathogens causing numerous nosocomial soft tissue infections and is among the best-known causes of bacterial infections. The bacterial 70S ribosome consists of two subunits, designated the 30S (small) and 50S (large) subunits. The small subunit (30S) consists of 16S ribosomal RNA (rRNA), from which the assembly of 30S begins, and 21 ribosomal proteins (r-proteins). The ribosome maturation factor P (RimP protein) binds to the free 30S subunit. Strains lacking RimP accumulate immature 16S rRNA, and fewer polysomes and an increased amount of unassociated 30S and 50S subunits compared to wild-type strains are observed in the ribosomal profile. Structural studies of the 30S subunit complex and the ribosome maturation factor RimP will make it possible in the future to develop an antibiotic that slows down or completely stops the translation of *Staphylococcus aureus*, which will complicate the synthesis and isolation of its pathogenic factors. Here we present the protocol of the *in vitro* reconstruction of *S. aureus* 30S ribosome subunit in a complex with RimP for further structural studies by cryo-electron microscopy.

Methods: Recombinant RimP protein from *S. aureus* was expressed in *E. coli* and purified by Ni-NTA chromatography and size exclusion chromatography. Reconstitution of the 30S–RimP complex was performed by mixing RimP protein with 30S ribosome. Unbound RimP protein was removed by Amicon Ultra Concentration (Merk KGaA, Darmstadt, Germany) with a cut-off limit of

100 kDa. The presence of RimP protein in the resulting 30S-RimP complex was confirmed by SDS-PAGE, and the quality of the final sample was analyzed by the negative staining EM.
Results: Finally, by *in vitro* reconstruction, the 30S-RimP complex from *S. aureus* was obtained for further structural studies by cryo-electron microscopy.

Key Words: ribosome • cryo-electron microscopy • ribosome maturation factor

This work was supported by the Russian Foundation for Basic Research (project № 20-34-70021)

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International Journal of Biomedicine. 2021;11 Suppl 1: S25.
doi: 10.21103/IJBM.11.Suppl_1.P31