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POSTER ABSTRACT PRESENTATIONS

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Abstract P-27: The 30S Ribosomal Subunit Assembly Factor Rbfa Plays a Key Role in the Formation of the Central Pseudoknot and in the Correct Docking of Helix 44 of the Decoding Center

Elena M. Maksimova[1], Alexey P. Korepanov[1], Olesya V. Kravchenko[1], Timur N. Baymukhametov[2], Alexander G. Myasnikov[3,4], Konstantin S. Vassilenko[1], Zhanna A. Afonina[1], Elena A. Stolboushkina[1]

[1]*Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia*
[2]*National Research Center "Kurchatov Institute," Moscow, Russia*
[3]*Petersburg Nuclear Physics Institute named by B.P. Konstantinov of NRC "Kurchatov Institute," Gatchina, Russia*
[4]*Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA*

Background: Ribosome biogenesis is a complicated multi-stage process. In the cell, 30S ribosomal subunit assembly is fast and efficient, proceeding with the help of numerous assembly protein factors. The exact role of most assembly factors and mechanistic details of their operation remain unclear. The combination of genetic modification with cryo-EM analysis is widely used to identify the role of protein factors in assisting specific steps of the ribosome assembly process. The strain with knockout of a single assembly factor gene accumulates immature ribosomal particles which structural characterization reveals the information about the reactions catalyzed by the corresponding factor.
Methods: We isolated the immature 30S subunits (pre-30S subunits) from the *Escherichia coli* strain lacking the *rbfA* gene (Δ*rbfA*) and characterized them by cryo-electron microscopy (cryo-EM).
Results: Deletion of the assembly factor RbfA caused a substantial distortion of the structure of an important central pseudoknot which connects three major domains of 30S subunit and is necessary for ribosome stability. It was shown that the relative order of the assembly of the 3′ head domain and the docking of

the functionally important helix 44 depends on the presence of RbfA. The formation of the central pseudoknot may promote stabilization of the head domain, likely through the RbfA-dependent maturation of the neck helix 28. The cryo-EM maps for pre-30S subunits were divided into the classes corresponding to consecutive assembly intermediates: from the particles with completely unresolved head domain and unfolded central pseudoknot to almost mature 30S subunits with well-resolved body, platform, and head domains and with partially distorted helix 44. Cryo-EM analysis of Δ*rbfA* 30S particles revealing the accumulation of two predominant classes of early and late intermediates (obtained at 2.7 Å resolutions) allowed us to suggest that RbfA participate in two stages of the 30S subunit assembly and is deeper involved in the maturation process than previously thought.

Conclusion: In summary, RbfA acts at two distinctive 30S assembly stages: early formation of the central pseudoknot including the folding of the head, and positioning of helix 44 in the decoding center at a later stage. An update to the model of factor-dependent 30S maturation was proposed, suggesting that RfbA is involved in most of the subunit assembly process.

Key Words: ribosome assembly • cryo-EM • 30S subunit maturation • RbfA

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**Corresponding author: Elena M. Maksimova. E-mail:elena.maks.89@gmail.com*

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