International Journal of Biomedicine | June 2019 - Volume 9, Issue Suppl_1: Abstracts From the Second Russian International Conference "Cryo-electron microscopy 2019: achievements and prospects"

ORAL ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE AND FUNCTIONS OF THE TRANSCRIPTION AND TRANSLATION APPARATUS OF THE CELL

DOI: 10.21103/IJBM.9.Suppl_1.OR4

Abstract OR-4: Molecular Mechanism of Antibiotics Inhibiting Prokaryotic Translation

Andrey Konevega[1,2,3], Evgeny Pichkur[1,2,4], Alena Paleskava[2,3], Pavel Kasatsky[2], Elena Maksimova[2], Daria Vinogradova[2], Yury Polikanov[5], Alexander Myasnikov[2,6]

[1]NRC "Kurchatov Institute", Moscow, Russia; [2]NRC "Kurchatov Institute" - PNPI, Gatchina, Russia; [3]Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia; [4]FSRC "Crystallography and Photonics", Moscow, Russia; [5]Department of Biological Sciences, University of Illinois at Chicago, Chicago, USA; [6]St. Jude Children's Research Hospital, Memphis, USA and Centre for Integrative Biology (CBI), IGBMC, CNRS, Inserm, Université de Strasbourg, Illkirch, France

Background: About 50% of antibiotics used in the therapy of infectious diseases target bacterial 70S ribosomes. High resolution X-ray crystallographic studies allow us for determination of position of drug on the ribosome, but to elucidate the detailed molecular mechanism of inhibition it is necessary to study the dynamics of partial reactions of protein biosynthesis. Majority of high resolution structures were obtained on ribosomes from thermophilic bacteria *T. thermophilus*, whereas most of the functional studies were performed on reconstituted in vitro translation system from mesophilic organism *E. coli.* Despite of high homology among bacterial ribosomes, in many cases particular contacts observed are specific to thermophilic organism and is not allowing us to generalize the molecular mechanism of inhibition.

Methods: Functional ribosomal pre-translocation complexes containing deacylated $tRNA^{fMet}$ in the P site and fMetPhe-$tRNA^{Phe}$ or fMetVal-$tRNA^{Val}$ in the A site were incubated with specific inhibitors: spectinomycin, amicoumacin, dirithromycin. Functional and structural studies were performed by using pre-steady state stopped-flow fluorescent spectroscopy and time-resolved cryo-electron microscopy. Kinetic studies of partial reactions of elongation were performed with thermophilic elongation factors and mesophilic reconstituted translation system.

Results: Combination of structural data and pre-steady state kinetics reveals the details of molecular mechanism of inhibition of EF-G catalysed translocation and shows the intermediate conformations of ribosome-tRNA complexes during forward translocation. Heterologous translation system with substituted thermophilic elongation factors allows for differential studies of elongation cycle.

Conclusion: Time-resolved high resolution cryo-electron microscopy is a method of choice for structural characterization of active complexes in physiological conditions in the process of functioning. Reconstituted *in vitro* translation system from *E. coli* can be used both for structural and functional

studies, allowing merging of two types of data for extensive characterization of bacterial protein synthesis apparatus.

Key words: ribosome, antibiotics, structure, cryo-EM

Sources of Funding: This work was supported by the Russian Science Foundation Grant #17-14-01416. The authors acknowledge the support and the use of resources of the Resource Center for Probe and Electron Microscopy at the NRC "Kurchatov Institute".

International Journal of Biomedicine. 2019;9 Suppl 1: S7. doi: 10.21103/IJBM.9.Suppl_1.OR4