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"

ORAL ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF VIRUSES AND CHAPERONINS

DOI: 10.21103/IJBM.11.Suppl_1.OR9

Abstract OR-9: Cryo-EM Structure of the Reconstituted Human γ-Tubulin Ring Complex

Marina Serna[1], Fabian Zimmermann[2], Artur Ezquerra[1], Rafael Fernandez-Leiro[2], Jens Luders[2], Oscar Llorca[1]

[1]*Structural Biology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain*
[2]*Mechanisms of Disease Programme, Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain*

Background: Microtubules (MTs) are essential cytoskeletal polymers that provide structural support for the cell and play important roles in cell division, motility, and intracellular transport. The γ-tubulin ring complex (γTuRC) is the major MT nucleator in animal cells. The molecular mechanism by which the γTuRC promotes MT nucleation remains poorly understood although a template-based mechanism, remains the most widely accepted (Moritz *et al*., 2000, Kollman *et al*., 2010). According to this model γTuRC, a 2 MDa multi-subunit protein complex, forms a lock washer-like structure, in which γ-tubulin molecules are arranged in a ring-shaped structure that serves as a template for the assembly of αβ-tubulin heterodimers.
Methods: We have set up an *in vitro* system to purify the human γTuRC using infected insect cells with recombinant baculoviruses. This complex sample was subjected to cryo-EM analysis and single-particle reconstruction.
Results: We have demonstrated that RUVBL1-RUVBL2 AAA-ATPase complex (RUVBL) controls the assembly and composition of γTuRC in human cells both in vivo and in vitro. Likewise, RUVBL assembles γTuRC from a minimal set of core subunits in a heterologous co-expression system. Purified, reconstituted γTuRC has nucleation activity and resembles native γTuRC (Consolati *et al*., 2020, Liu *et al*., 2020, Wieczorek *et al*., 2020), as revealed by its cryo-EM structure at ~4.0 Å resolution.

Conclusion: We have been able to identify novel mechanistic and structural features that determine the intricate, higher-order γTuRC architecture (Zimmermann, Serna *et al*., 2020).

Key Words: Cryo-EM • RUVBL• γTuRC • microtubules

**Corresponding author*: *Marina Serna. E-mail: mserna@cnio.es*

International Journal of Biomedicine. 2021;11 Suppl 1: S10.
doi: 10.21103/IJBM.11.Suppl_1.OR9