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POSTER ABSTRACT PRESENTATIONS

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

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Abstract P-13: Cryo-ET Structural Analysis of Polyribosomes from HeLa Cells

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Background: Polyribosomes are complexes consisting of several ribosomes simultaneously translating a single mRNA molecule. In early electron microscopy studies, it was shown that eukaryotic polyribosomes in cells can have various shapes – rings, double rows, and a shape interpreted as helical, based on 2D-images and cross-sections.

Methods: Modern methods of cryo-electron microscopy and tomography (cryo-EM and cryo-ET) allows to analyze the 3D organization of polyribosomes in close to native conditions. We used the cryo-ET approach to investigate the structural organization of cytoplasmic polyribosomes in the lysates of HeLa cells.

Results: Cryo-EM confirmed the presence of ring and double-row structures of polyribosomes in the lysates. Using cryo-ET and averaging of polysomal ribosomes (subtomogram averaging), we determined the relative orientations of the ribosomes in the polyribosomes and traced the deduced mRNA path within every individual polyribosome. Thus, the circular path of the mRNA chain was for the first time demonstrated for ring-shaped polyribosomes from eukaryotic cells. A linear zigzag mRNA chain pathway was found for double row polyribosomes. Besides, relaxed helical and helical-like polyribosomes were detected in lysate. In addition, the structure of polyribosomes in lysate of HeLa cells growing in stressful (heat shock) conditions was analyzed. When the polyribosome profile resumed after heat shock, the ratio of different polyribosome conformations was found similar to that in normal conditions. A densely-packed helical configuration, which is characteristic of polyribosomes with reduced translational activity (Myasnikov *et al.*, *Nat. Commun.*, 2014, 5:5294), was not detected in lysates from cells grown in both normal and stressful conditions.

Conclusion: The structural organization of polyribosomes in lysates of HeLa cells growing in normal and stressful conditions was analyzed by cryo-ET. The revealed linear configurations of polyribosomes are in agreement with helical, helical-like, flat zigzag configurations of polyribosomes

found *in situ* in human glioma cells (Brandt *et al.*, *Mol.Cell*, 2010, 39:560-569). The circular topology of ring-shaped polyribosomes derived from eukaryotic cells was confirmed by cryo-ET method for the first time.

Key Words: structural organization of polyribosomes • cryo-electron tomography • HeLa

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