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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-20: Sub 3Å Resolution Cryo-EM Structure of Eukaryotic Small (40S) Ribosomal Subunit

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Background: The ribosome is a molecular machine that translates mRNAs into proteins. In eukaryotes, ribosome consists of small (40S) and large (60S) subunits. Translation in eukaryotes is a complicated molecular process that involves the formation of various molecular complexes consisting of ribosomal subunits and protein factors. Cryo-EM approaches such as single particle analysis are widely used for structural analysis of components and intermediates of the translation machinery. However, the process of translation in plants is still poorly characterized at a structural level. Here, we present the structure of *Triticum aestium* small ribosomal subunit obtained at sub 3Å resolution that can be used for further structural studies of the translation process in plants.
Methods: The structures of the 40S subunits purified from wheat germ extract were obtained using high-resolution single particle cryo-EM. For cryo-EM sample preparation were used Quantifoil R 1.2/1.3 grids coated with an additional 2 nm amorphous carbon film were glow-discharged for 30 seconds at 15 mA using PELCO easiGlow (Ted Pella). 3 µL of the sample were applied onto the grids, blotted for 3 sec at 10°C and 100% humidity, and plunge-frozen in liquid ethane using Vitrobot Mark IV (Thermo Fisher).
Cryo-EM data were collected using a C_s-corrected Titan Krios (Thermo Fisher) transmission electron microscope, equipped with a Falcon II direct electron detector. Data were acquired with defocus range of -0.6 to -2.0 at a nominal magnification of 75,000x, giving a calibrated pixel size of 0.86 Å/pixel. The micrographs were recorded as movie stacks. The exposure time for each stack

was 1.6 s, corresponding to a total electron dose of ~84 $e^-/Å^2$ fractionated into 32 frames (~2.6 $e^-/Å^2$ per single frame). A total of 5521 movie stacks was collected. Raw cryo-EM data preprocessing was performed with Warp software (Tegunov *et al*., 2019). All further data processing steps were performed using the cryoSPARC v3.2.0 software (Punjani *et al*., 2017).

Results: For final cryo-EM map refinement, 140,000 particles were used resulting in 2.7 Å resolution estimated using an FSC=0.143 gold-standard threshold. The obtained structural data clearly demonstrate the peculiarities of the spatial organization of the 40S ribosomal subunit, like the motility of the head relative to the body revealed by 3D variability analysis.

Conclusion: The resulting structure was solved at a significantly higher resolution compared to the previously published structure of a plant ribosome (Armache *et al*., 2010) and will be used as a reference for further studies of translation initiation in plants.

Key Words: cryo-EM • 40S ribosomal subunit

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