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"

POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF MEMBRANE PROTEINS

DOI: 10.21103/IJBM.11.Suppl_1.P12

Abstract P-12:Three-dimensional Structure of the Yeast Transmembrane Mechanosensor Wsc1

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Background: Wsc1 is the best studied of the five mechanosensors of the cell wall integrity (CWI) signal transduction pathway in *Saccharomyces cerevisiae.* In genetic and biophysical studies, Wsc1 functions were assessed either in living yeast cells or in crude cell extracts. So far, no attempts to purify the sensor and determine its structure have been reported.

Methods: The Wsc1-green fluorescent protein (GFP) fusion was expressed in *S. cerevisiae* following standard protocols. For solubilization, a 5% (w/v) solution of styrene-maleic acid (SMA) copolymer was added dropwise to the membrane suspension to get a final cell-to-SMA weight ratio of 1:2.5. The suspension was incubated for 30 min at room temperature (RT) and then for 16 hours at 4 °C, followed by centrifugation for 20 min at 134000 g at 4 °C. The supernatant was subsequently purified on affinity resin. Protein samples (3 µL) were placed onto the glow-discharged grid, stained twice, using 1% aquatic uranyl acetate solution for 30 s at RT, and air-dried. Micrographs were acquired using a transmission electron microscope Jem-2100 (Jeol, Japan) equipped with a 2K x 2K CCD camera Ultrascan 1000XP (Gatan, USA). The microscope operated at 200 kV in a low dose mode, with a magnification x40000 (2.5 Å/pix) and

defocus 0.5-1.9 μm. Images were acquired automatically with SerialEM software.
Results: We applied the 3:1 SMA copolymer to isolate Wsc1-GFP fusions into SMA/lipid particles (SMALPs) directly from native yeast membranes. We purified Wsc1-GFP-containing SMALPs by affinity chromatography; characterization by dynamic light scattering confirmed the presence of monodisperse nano-sized particles. The intensity-weighted diameter was estimated to be in the 10-nm range. The purified Wsc1-GFP-containing SMALPs were further analyzed by transmission electron microscopy (TEM). 46500 single particles were used to generate the 3D reconstructions of Wsc1-GFP. The resulting model showed the three functional parts of the Wsc1 sensor: the extracellular part, which has a clasp-like appearance, the transmembrane domain, and the short cytosolic part of Wsc1 fused to the GFP beta-barrel.
Conclusion: The 3:1 SMA copolymer enabled isolation of Wsc1-GFP fusion complexes into SMALPs directly from native yeast membranes. Image analysis of the TEM data showed the Wsc1 in its contracted conformation. We propose a model of conformational changes in Wsc1 in response to mechanical stress.

Key Words: Wsc1 • detergent-free extraction • SMA copolymer • membrane protein • transmission electron microscopy • 3D reconstruction

This work was funded jointly by German Research Foundation (DFG,STE640/15) to H.J.S., and RFBR grant no. 18-504-12045 to K.V.S

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International Journal of Biomedicine. 2021;11 Suppl 1: S16.
doi: 10.21103/IJBM.11.Suppl_1.P12