Role of Golgi vesicle tethering and fusion machinery in protein glycosylation in human cells

Vladimir LUPASHIN [1], Farhana Taher SUMYA[1], Zinia D'SOUZA [1], Amrita KHAKUREL [1], Irina POKROVSKAYA [1]

[1] University of Arkansas for Medical Sciences, Little Rock, AR, USA,

vvlupashin@uams.edu

Golgi is the central organelle in the secretory pathway. It hosts glycosylation machinery that modifies macromolecules passing through Golgi compartments. The identity of Golgi compartments and proper glycosylation is achieved by the retrograde flow of components of Golgi glycosylation machinery from TGN to cis-Golgi. This recycling is performed by vesicles formed at trans compartments by vesicle coats and then tethered and fused to cis compartments by vesicular tethers and SNARE molecules. We used gene editing, degron-assisted rapid protein degradation, TurboID proximal biotinylation, mass-spectrometry analysis, electron and superresolution microscopy to decipher the role of COG and GARP vesicle tethering complexes and SNAREs in Golgi glycosylation in human cells. We found that the TGN-located GARP complex is necessary for Golgi glycosylation, indicating that enzymes recycle beyond the Golgi stack and have to be retrieved from endosomal compartments in a GARP-regulated pathway. Detailed analysis of COG complex uncovers the essential role of all COG subunits in the recycling and stability of Golgi glycosylation machinery. Acute deletion of COG4 results in the accumulation of several distinct populations of vesicles carrying different Golgi glycosyltransferases and sugar transporters. Mass-spec analysis of Golgi SNAREs led to the discovery of two novel SNARE complexes and revealed the remarkable plasticity in the intra-Golgi SNARE-mediated fusion machinery, uncovering a novel response mechanism to the failure of "classical" intra-Golgi vesicle tethering/fusion machinery.

This work was supported by the National Institute of Health grant R01GM083144 for Vladimir Lupashin

Bibliographic references:

Sumya FT, Pokrovskaya ID, D'Souza Z, Lupashin VV. (2022) Traffic. (2):52-75.
Khakurel A, Kudlyk T, Pokrovskaya I, D'Souza Z, Lupashin VV. (2022) Front Cell Dev Biol. (10):1066504.
D'Souza Z, Sumya FT, Khakurel A, Lupashin V. (2021) Cells. (12):3275.
Sumya FT, Pokrovskaya ID, Lupashin V. (2021)Front Genet.(12):733048.
Khakurel A, Kudlyk T, Bonifacino JS, Lupashin VV. (2021) Mol Biol Cell. (17):1594-1610.