【Grant-in-Aid for Scientific Research (S)】

Broad Section G

Title of Project : Full elucidation of sorting mechanisms in and around the Golgi apparatus by super-resolution live imaging

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Research Project Number : 18H05275 Researcher Number : 90142140
Keyword : membrane traffic, Golgi apparatus, sorting and transport

【Purpose and Background of the Research】

Understanding of the mechanisms of membrane trafficking is now being totally innovated by state-of-the-art super-resolution live imaging microscopy. We have recently developed a new method with an extremely high spatiotemporal resolution, which can track dynamic 4D behaviors of even vesicles in cytoplasm. With this technology, we will tackle fundamental questions underlying the transport processes in the secretory pathway, from the ER to the Golgi apparatus and further to the *trans*-Golgi network. Experts on yeast, plant and animal cells will compare corresponding transport processes and extract common mechanisms and different features and draw comprehensive models, which will lead to thorough understanding of molecular mechanisms.

【Research Methods】

By making full use of SCLIM2 we developed, we will investigate the following problems.
[Yeast cells] 1) cargo capture from the ER by *cis*-Golgi; 2) cargo delivery between Golgi cisternae; 3) spatiotemporal regulation of sorting in the TGN.
[Plant cells] 1) cargo capture from the ER by GECCO; 2) cargo delivery in the Golgi stack; 3) spatiotemporal regulation of sorting in the TGN.
[Animal cells] 1) cargo capture from the ER by ERGIC; 2) cargo delivery in the Golgi stack and the TGN; 3) roles of the Golgi in nerve axons.

【Expected Research Achievements and Scientific Significance】

SCLIM, super-resolution confocal live imaging microscopy, has been a powerful tool to examine dynamic processes of membrane trafficking. We have further improved its performance by raising the sensitivity and the speed of detection system (Figure 1). By single-photon counting and a new deconvolution algorithm we developed, it now enables us to visualize assembly of coat proteins on the organelle membranes, sorting and packaging of cargo, handover of cargo between compartments, etc. which we have been eager to unveil.

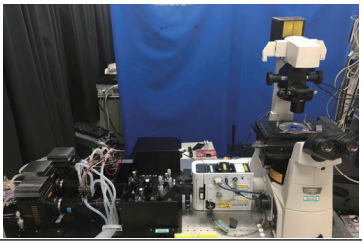

Figure 1 SCLIM2

【Publications Relevant to the Project】

- Ito, Y., Uemura, T., and Nakano, A. (2018). Golgi Entry Core Compartment functions as the COPII-independent scaffold for ER-Golgi transport in plant cells. *J. Cell Sci.* 131:jcs203893.
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- Kurokawa, K., Suda, Y. and Nakano, A. (2016). Sar1 localizes at the rims of COPII-coated membranes *in vivo*. *J. Cell Sci.* 129:3231-3237.
- Kurokawa, K., Okamoto, M., and Nakano, A. (2014). Contact of *cis*-Golgi with ER exit sites executes cargo capture and delivery from the ER. *Nat. Commun.* 5:3653.
- Uemura, T., Suda, Y., Ueda, T., and Nakano, A. (2014). Dynamic behavior of the *trans*-Golgi network in root tissues of Arabidopsis revealed by super-resolution live imaging. *Plant Cell Physiol.* 55:694-670.
- Suda, Y., Kurokawa, K., Hirata, R., and Nakano, A. (2013). Rab GAP cascade regulates dynamics of Ypt6 during the Golgi maturation. *Proc. Natl. Acad. Sci. U. S. A.* 110:18976-18981.

【Term of Project】 FY2018-2022

【Budget Allocation】 148,300 Thousand Yen

【Homepage Address and Other Contact Information】

https://rap.riken.jp/en/labs/sprg/lcmirt/