

DIVISION OF CELLULAR DYNAMICS



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Membrane traffic between single membrane-bounded organelles plays integral roles in various cell activities in eukaryotic cells. Recent comparative genomics has indicated that membrane trafficking pathways are diversified among eukaryotic lineages, which is associated with lineage-specific acquisition of new trafficking pathways and secondary loss of preexisting trafficking routes. Our long-term goal is unraveling how plants have acquired their unique membrane trafficking systems during evolution, which will be achieved by comparative analyses using the model plant *Arabidopsis thaliana* and a model of liverworts, *Marchantia polymorpha*. We also aim to elucidate detailed molecular mechanisms and physiological functions of membrane trafficking in higher-ordered plant functions.

I. Diversification of membrane trafficking pathways associated with acquisition of novel machinery components

Although the basic framework of membrane trafficking is well conserved among eukaryotic lineages, recent comparative genomics has suggested that each lineage has acquired unique membrane trafficking pathways during evolution. RAB GTPases and SNARE proteins are evolutionarily conserved key regulators acting in tethering and/or fusion of membrane vesicles with target membranes. It has been proposed that lineage-specific diversification of these key factors is tightly associated with acquisition of lineage-specific membrane trafficking pathways, whose molecular basis remains unknown.

1-1. Characterization of RAB and SNARE proteins in the liverwort, *Marchantia polymorpha*

For information on the diversification of membrane trafficking pathways during land plant evolution, we systematically identified RAB GTPases and SNARE proteins in *Marchantia polymorpha*. Comparison of organization of these protein families with other plant lineages, followed by their functional analyses in *M. polymorpha*, indicated that diversification of membrane trafficking pathways in land plants has been achieved by 1) acquisition of novel machinery components, 2) relocating conserved machinery components

to distinct trafficking events, and 3) secondary loss of conserved machinery components, during evolution.

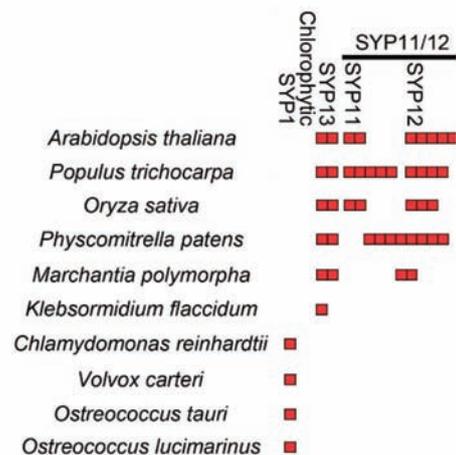


Figure 1. *SYP1* genes in the genomes of green plants. Genes are indicated as individual boxes. Numbers of *SYP1* genes increased during land plant evolution, suggesting functional diversification of *SYP1* members in land plants.

1-2. Analysis of the liverwort-specific organelle, oil body

Through analyses of SNARE members in *M. polymorpha*, we found that a member of the *SYP1* group is localized to the membrane of an organelle specific to liverworts, the oil body, whose origin and mechanisms of biogenesis remain unclear. We are currently analyzing the molecular function of the *SYP1* member, as well as characterizing membrane trafficking pathways responsible for oil body biogenesis. We are also trying to isolate mutants defective in oil body functions by a forward-genetic approach.

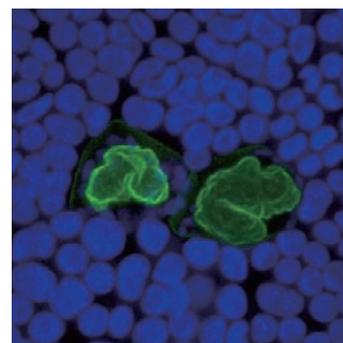


Figure 2. Oil bodies in a thallus of *M. polymorpha*, which are visualized by the YFP-tagged *SYP1* member.

1-3. Mechanisms and dynamics of vacuolar transport

The vacuole is the largest organelle in plant cells, and occupies over 90 % of mature plant cells. The vacuole fulfills various functions in plant physiology and development, which include protein degradation, protein storage, and regulation of turgor pressure. To fulfill these vacuolar functions, a wide variety of vacuolar proteins and other components must be correctly transported to the vacuole, which is mediated by membrane trafficking. To understand molecular mecha-

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2016. The former title is indicated by an asterisk (*).

nisms of vacuolar transport in plants, we analyzed molecular functions of RAB5 and RAB7, and found that these proteins act sequentially in the vacuolar transport pathway in Arabidopsis cells. Furthermore, we also found that two additional vacuolar transport pathways, the RAB5-dependent but RAB7-independent pathway and the AP-3-dependent pathway operate in vacuolar transport in Arabidopsis. We are now exploring details of molecular mechanisms of these vacuolar transport pathways, especially focusing our interest on the RAB5-dependent but RAB7-independent pathway, because this trafficking pathway has not been described in non-plant systems.

SNARE proteins are also key molecules of vacuolar trafficking. Defective functions in vacuolar SNAREs affect both vacuolar transport and vacuolar morphology, which are distinctly regulated in plant cells. The *sgr3-1* (*shoot gravitropism3*) mutant was isolated as a mutant defective in shoot gravitropism, which resulted from a point mutation in SYP22/VAM3, one of the SNARE proteins residing on the vacuole and acting in vacuolar transport. Intriguingly, *sgr3-1* exhibits abnormal vacuolar morphology, although vacuolar transport is not markedly affected in this mutant. Therefore *sgr3-1* should be a good tool for dissecting functions of the vacuolar SNARE. We are exploring vacuolar dynamics regulated by SYP22/VAM3 by analyzing the effect of the *sgr3-1* mutation in a detailed manner.

II. Significance of membrane trafficking in higher-ordered plant functions

2-1. Analyses of functions of the plant-specific RAB GTPase ARA6 in stress responses in *Arabidopsis thaliana*

ARA6 is a plant-unique RAB GTPase, whose close homologs are found in only green plant lineages. To elucidate why only plants harbor the ARA6 members, we analyzed functional significance of ARA6 in biotic and abiotic stress responses. We found that ARA6 is recruited to the extrahaustorial membranes formed by the fungal pathogen causing powdery mildew and the oomycete causing downy mildew, suggesting modulation of host membrane trafficking by pathogenic microbes (Inada *et al.*, 2016). We also found that proliferation of a pathogenic bacterium is repressed in the *ara6* mutant, which was due to an enhanced defense response mediated by elevated sugar concentration.

2-2. Membrane trafficking in plant gametogenesis

Gametogenesis in plants also involves membrane trafficking-mediated processes. We are now analyzing molecular mechanisms of gametogenesis in Arabidopsis and *M. polymorpha*, especially focusing our interests on secretory and degradative trafficking pathways during male gamete formation.

Publication List:

[Original papers]

- Ebine, K., Hirai, M., Sakaguchi, M., Yahata, K., Kaneko, O., and Saito-Nakano, Y. (2016). Plasmodium Rab5b is secreted to the cytoplasmic face of the tubovesicular network in infected red blood cells together with N-acylated adenylate kinase 2. *Malar. J.* 17, 323.
- Inada, N., Betsuyaku, S., Shimada, T.L., Ebine, K., Ito, E., Kutsuna, N., Hasezawa, S., Takano, Y., Fukuda, H., Nakano, A., and Ueda, T. (2016). Modulation of plant RAB GTPase-mediated membrane trafficking pathway at the interface between plants and obligate biotrophic pathogens. *Plant Cell Physiol.* 57, 1854-1864.
- Mbengue, M., Bourdais, G., Gervasi, F., Beck, M., Zhou, J., Spallek, T., Bartels, S., Boller, T., Ueda, T., Kuhn, H., and Robatzek, S. (2016). Clathrin-dependent endocytosis is required for immunity mediated by pattern recognition receptor kinases. *Proc. Natl. Acad. Sci. USA* 113, 11034-11039.
- Sakurai, H.T., Inoue, T., Nakano, A., and Ueda, T. (2016). ENDOSOMAL RAB EFFECTOR WITH PX-DOMAIN, an interacting partner of RAB5 GTPases, regulates membrane trafficking to protein storage vacuoles in Arabidopsis. *Plant Cell* 28, 1490-0503.
- Yoshinari, A., Fujimoto, M., Ueda, T., Inada, N., Naito, S., and Takano, J. (2016). DRP1-dependent endocytosis is essential for polar localization and boron-induced degradation of the borate transporter BOR1 in *Arabidopsis thaliana*. *Plant Cell Physiol.* 57, 1985-2000.

[Original papers (E-publication ahead of print)]

- Akita, K., Kobayashi, M., Sato, M., Kutsuna, N., Ueda, T., Toyooka, K., Nagata, N., Hasezawa, S., and Higaki, T. Accumulation of fluorescent proteins derived from a *trans*-Golgi cisternal membrane marker and paramural bodies in interdigitated apoplastic space in *Arabidopsis* leaf epidermis. *Protoplasma* 2016 Mar 9.
- Cui, Y., Zhao, Q., Xie, H.T., Wong, W.S., Gao, C., Ding, Y., Tan, Y., Ueda, T., Zhang, Y., and Jiang, L. MON1/CCZ1-mediated Rab7 activation regulates tapetal programmed cell death and pollen development in *Arabidopsis*. *Plant Physiol.* 2016 Oct 31.