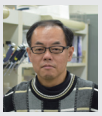
LABORATORY OF ORGANELLE REGULATION



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Because plants spread their roots in the ground, they must survive in a given environment. To adapt to their environment, they utilize various signals generated from environmental changes as being necessary for their survival. The flexibility of plant organelles is the basis for environmental adaptation in plants.

The aims of this laboratory are to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, especially peroxisomes and oil bodies, as well as to understand the integrated functions of individual plants through organelle dynamics.

I. Molecular mechanisms of peroxisome dynamics and functions in plant cells

Peroxisomes are single-membrane bounded organelles, which are ubiquitously present in eukaryotic cells, and are involved in various biological processes such as lipid metabolism and photorespiration. These functions are dramatically changed during certain developmental stages and when confronted with environmental changes. For example, light induces the transformation of peroxisomes from glyoxysomes, which are peroxisomes engaged in the degradation of reserve oil stored in the oil body via β-oxidation and the glyoxylate cycle, to another type of peroxisome, leaf peroxisomes, that function in several crucial steps of photorespiration. In addition to functioning in vegetative tissues such as leaf and root cells, it has been revealed that peroxisomes play essential roles in reproductive processes. Studies using Arabidopsis mutants defective in peroxisomal functions demonstrate that peroxisomes contribute to pollen fertility, pollen tube elongation, and male–female gametophyte recognition. Gene expression, alternative splicing, protein transport, protein degradation and degradation of peroxisomes themselves control these peroxisomal functions.

To better understand peroxisome biogenesis and functions, we isolated a number of Arabidopsis mutants that displayed aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual positioning (*peup* mutants) based on them having a different pattern of GFP fluorescence compared to their parent plant, GFP-PTS1, in which peroxisomes with normal sizes, numbers and distribution could be visualized with GFP. As of writing, we have reported the function of APEM1, APEM2, APEM3, APEM4, APEM9 and APEM10. Based on these results we were able to update the model for protein transport, proliferation and quality control of peroxisomes via autophagy, using these *apem* mutants in concert with the analyses of *peup1*, *peup2* and *peup4* mutants, which were defective in Autophagy-related 2 (ATG2), ATG18a and ATG7, respectively (Figure 1).

We are currently investigating other *apem* and *peup* mutants. From these analyses, we will be able to identify the components responsible for peroxisome biogenesis, functions and maintenance, and to address the mechanism at the molecular level.

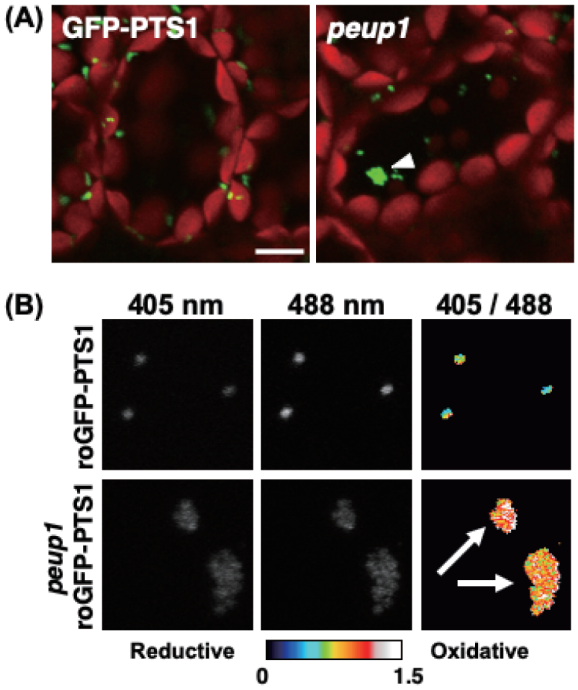

Figure 1. Phenotype of Arabidopsis *peup1* mutant. (A) GFP fluorescence in leaf cells was observed in the parent plant, GFP-PTS1, and *peup1* mutant. *peup1* demonstrates aggregated peroxisomes (arrowhead). Bar indicates 10 μm. (B) Fluorescence intensities from peroxisome-targeted reduction-oxidation-sensitive GFP (roGFP) with two excitation wavelengths were measured, and the 405/488 nm ratio was calculated in the transgenic plants. The 405/488 nm ratio of the peroxisome aggregates in the *peup1* roGFP-PTS1 mutant showed that the peroxisome aggregates were more oxidative than peroxisomes in the wild type.

II. Accumulation mechanism of seed storage oils and proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves in food and industrial materials. Storage reserves vary among different types of plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybeans major reserve are proteins. Storage oils and storage proteins are synthesized in the endoplasmic reticulum (ER) and accumulated in oil bodies and protein bodies, respectively, during the same period of seed development.

We are analyzing the molecular mechanisms controlling oil and protein contents in seeds. Based on the analysis of the temporal sequence of oil and protein synthesis during seed development in *Arabidopsis thaliana*, which produces seeds containing approximately 30% oil and 30% protein, we revealed that the extension of *WRINKLED1* (*WRI1*), a tran-

scription factor in fatty acid biosynthesis, expression during the mid-phase of seed development significantly enhanced seed oil content, and caused an enlargement of seed size.

We are also currently investigating the mechanisms of oil accumulation in other plant species. In the soybean (*Glycine max*. L), we identified four lipases, GmSDP1s, on the oil body membrane. The analyses of GmSDP1s revealed that plant seeds have the mechanism required for the quality control of fatty acids by degrading particular fatty acids in oil bodies (Figure 2).

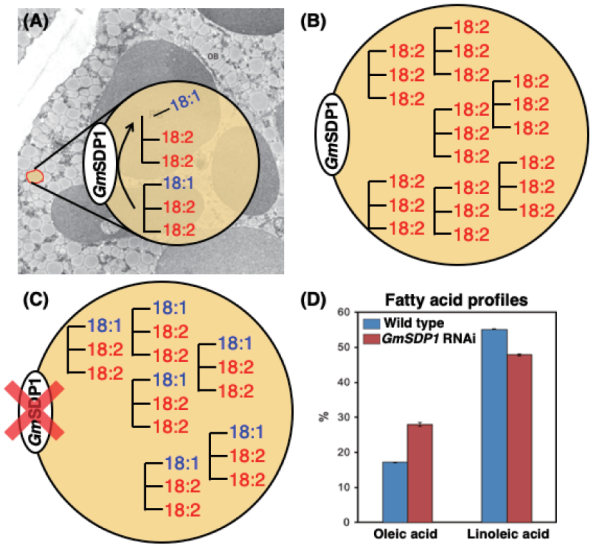

Figure 2. Quality and quantity control of storage oils in oil bodies by GmSDP1 in soybean (*Glycine max* L.). (A) Storage oils are accumulated as triacylglycerol (TAG) in oil bodies. Four Glycine max SUGAR DEPENDENT-1s (GmSDP1), which are types of lipase, have an activity for releasing fatty acids from TAG. GmSDP1s degrade oleic acid (18:1), so that the content of linoleic acid (18:2) in seeds increases (B), whereas the content of oleic acid rises in GmSDP1-knockdown plants compared to the wild-type plants due to reductions in the degradation of oleic acid (C, D).

III. Development of Gateway-technology vectors for plant research

Gateway cloning is a popular technology which allows the simultaneous generation of multiple constructs containing a range of fusion genes. We have developed various types of Gateway cloning-compatible vectors to improve resources in the plant research field. As of writing, we have provided vector sets to detect multiple protein-protein interactions in vivo using multi-color bimolecular fluorescence complementation, and the binary vectors to facilitate tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha*. We will continue developing other useful Gateway cloning-compatible vectors to contribute to the plant research community.

IV. Construction of The Plant Organelles Database 3 (PODB3) and Plant Organelles World

The Plant Organelles Database 3 (PODB3) was built to promote a comprehensive understanding of organelle dynamics. This public database is open to all researchers. PODB3 consists of six individual sections: the electron micrograph database, the perceptive organelles database, the organelles movie database, the organellome database, the functional analysis database, and external links. The function of each database is as follows:

- The electron micrograph database provides information on the ultrastructures in plant cells
- The perceptive organelles database shows organelles dynamics responding to environmental stimuli
- The organelles movie database contains time-lapse images and 3D structure rotations
- The organellome database is a compilation of static image data of various tissues of several plant species at different developmental stage.
- The functional analysis database is a collection of protocols for plant organelle research

Through these databases, users can easily grasp plant organelle dynamics. Plant Organelles World, which is built based on PODB3, is an educational tool for engaging members of the non-scientific community to explore plant biology. We hope that both PODB3 and Plant Organelles World are of help to researchers as well as the general public.

Publication List:

〔Original papers〕

- Goto-Yamada, S., Oikawa, K., Bizan, J., Shigenobu, S., Yamaguchi, K., Mano, S., Hayashi, M., Ueda, H., Hara-Nishimura, I., Nishimura, M., and Yamada, K. (2019). Sucrose starvation induces microautophagy in plant root cells. Front. Plant Sci. *10*, 1604. doi: 10.3389/fpls.2019.01604
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- Sultana, M.M., Dutta, A.K., Tanaka, Y., Aboulela, M., Nishimura, K., Sugiura, S., Niwa, T., Maeo, K., Goto-Yamada, S., Kimura, T., Ishiguro, S., Mano, S., and Nakagawa, T. (2019). Gateway binary vectors with organelle-targeted fluorescent proteins for highly sensitive reporter assay in gene expression analysis of plants. J. Biotech. *297*, 19-27. doi: 10.1016/j.jbiotec.2019.03.015
- Watanabe, E., Mano, S., Nishimura, M., and Yamada, K. (2019). AtUBL5 regulates growth and development through pre-mRNA splicing in Arabidopsis thaliana. PLoS One *14*, e0224795. doi: 10.1371/journal.pone.0224795
- Yamauchi, S., Mano, S., Oikawa, K., Hikino, K., Teshima, K.M., Kimori, Y., Nishimura, M., Shimazaki, K.I., and Takemiya, A. (2019). Autophagy controls reactive oxygen species homeostasis in guard cells that is essential for stomatal opening. Proc. Natl. Acad. Sci. USA *116*, 19187-19192. doi: 10.1073/pnas.1910886116