DIVISION OF CELL MECHANISMS †

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Since plants spread their roots in the ground, they must survive in a given environment. To adapt to the environment, they positively utilize environmental changes in their life cycle as important signals that are necessary for their survival. Plant cells can induce, degenerate and differentiate their organelles to adapt to environmental changes. The flexibility of plant organelles is the basis of the strategy for environmental adaptation in plants.

The aim of this division is to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, and to understand the integrated function of individual plants through organelle dynamics. The Scientific Research on Innovative Areas “Environmental sensing of plants: Signal perception, processing and cellular responses” was started to clarify the molecular mechanisms underlying organelle differentiation and interaction.

I. Reversible transformation of plant peroxisomes

Dramatic metabolic changes that underlie the shift from heterotrophic to autotrophic growth occur in the greening of seed germination. Accompanying these metabolic changes are the functional transformations of many constitutive organelles. Etioplasts differentiate into chloroplasts while mitochondria acquire the ability to oxidize glycine. Glyoxysomes, which are peroxisomes engaged in the degradation of reserve oil stored in the oil body via β-oxidation and the glyoxylate cycle, are transformed into leaf peroxisomes that function in several crucial steps of photorespiration. After the functional transition of glyoxysomes to leaf peroxisomes during the greening of pumpkin cotyledons, the reverse transition of leaf peroxisomes to glyoxysomes occurs during senescence. Gene expression, alternative splicing, protein translocation and protein degradation control the functional transformation between glyoxysomes and leaf peroxisomes.

II. Transcriptomics, proteomics and phenomics of plant peroxisomes

Enzymes localized in plant peroxisomes are synthesized in the cytosol and function after their post-translational transport into peroxisomes. Almost all of the peroxisomal matrix proteins contain one of two targeting signals (PTS1 and PTS2) within the molecules. PTS1 is a unique tripeptide sequence found in the carboxyl terminus of the mature proteins. In contrast, PTS2 is involved in a cleavable amino terminal presequence of peroxisomal proteins that are synthesized as a precursor protein with larger molecular mass.

We identified 256 gene candidates of PTS1- and PTS2-containing proteins and another 30 genes of non-PTS-containing proteins from the *Arabidopsis* genome. Custom-made DNA microarrays covering all these genes were used to investigate expression profiles of the peroxisomal genes in various organs. They revealed that peroxisomes in root cells play a pivotal role in polyamine catabolism. We also made a two-dimensional protein map of glyoxysomes and leaf peroxisomes isolated from *Arabidopsis* and soybean. Peptide MS fingerprinting analyses allowed us to identify novel peroxisomal membrane proteins, i.e. voltage-dependent anion-selective channel and adenine nucleotide carrier 1 (PNC1). We also found that peroxisomal membrane ATP-binding cassette transporter promotes seed germination by inducing pectin degradation under the control of abscisic acid signaling. The overall results provide us with new insights into plant peroxisomal functions.

Bioinformatic analysis of the *Arabidopsis* genome predicted the presence of 15 kinds of genes, called *PEX* genes, for peroxisomal biogenesis factors. We demonstrated that PEX5 and PEX7 form a cytosolic receptor complex and recognize PTS1- and PTS2-containing proteins, respectively. PEX14 is a peroxisomal membrane docking protein that captures the receptor-cargo complex. We also comprehensively investigated whether or not these predicted *PEX* genes function in peroxisome biogenesis by generating knock-down mutants that suppress *PEX* gene expression by RNA-interference. Phenotypes of these mutants allowed us to identify the functional *PEX* genes, which can be classified into two groups: *PEX* genes regulating for peroxisomal protein import and *PEX* genes regulating for peroxisomal morphology. We continue to investigate the detailed molecular functions of other *PEX* genes. Of these, we recently proposed that function of PEX7 is maintained by a quality control mechanism involving RabE1c.

III. Identification of novel components essential for peroxisome biogenesis

To better understand peroxisome biogenesis and functions, we isolated a number of *Arabidopsis* mutants having aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual poisoning (*peup* mutants) based on them having a different pattern of GFP fluorescence from the parent plant, GFP-PTS1, in which peroxisomes with normal sizes, numbers and distribution can be visualized with GFP.

Up to date, we reported the function of gene-products of *APEM1*, *APEM2*, *APEM3*, *APEM4* and *APEM9*. Recently,

†: This laboratory was closed on 31 March, 2015.

we found that *APEM10* encodes Lon protease 2, which has roles in chaperon and proteinase, and that modulates peroxisome degradation processed by autophagy. Taken together with the analyses using *peup1*, *peup2* and *peup4* mutants, which were defective in Autophagy-related 2, (ATG2), ATG18a and ATG7, respectively, we were able to update the model for functional transformation of peroxisomes (Figure 1).

We are currently characterizing 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.

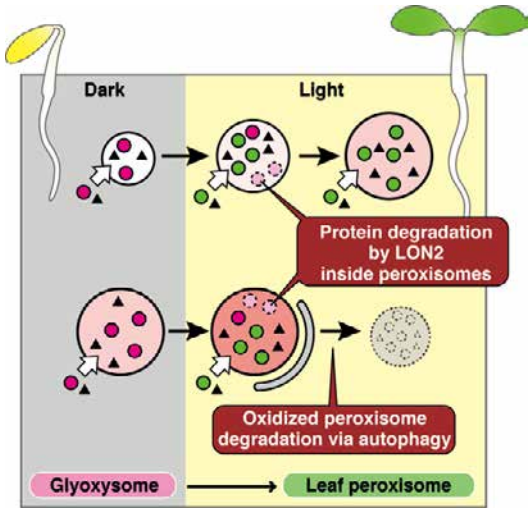

Figure 1. A model for the coordinated regulation of functional transformation of peroxisomes and peroxisome quality control by LON2/APEM10 and autophagy during functional transition.

IV. ER derived organelles for protein storing and defense strategy

Plant cells develop various types of endoplasmic reticulum (ER)-derived structures with specific functions. ER bodies are ER-derived compartments observed in *Arabidopsis*. They are rod-shaped structures surrounded by ribosomes, and are widely distributed in the epidermal cells of whole seedlings (Figure 2). Undamaged rosette leaves have no ER bodies, but accumulate ER bodies after wounding or jasmonic acid treatment. ER bodies accumulate β-glucosidase PYK10. When plant cells are damaged, PYK10 forms large protein aggregates. The aggregate formation increases glucosidase activity. These findings suggest that ER bodies function in the defense against pathogens and herbivores, possibly producing toxic products. *Arabidopsis nai1* mutants have no ER bodies in the entire plant and do not accumulate PYK10. *NAI1* encodes a transcription factor and regulates the expression of *PYK10* and *NAI2*. The *Arabidopsis nai2* mutant has no ER bodies and reduced accumulation of PYK10. *NAI2* encodes a unique protein that localizes to the ER body. Membrane protein of ER body 1 (MEB1) and MEB2 are integral membrane proteins of the ER body and have iron/manganese transport activity. These results suggest that the ER body has specific membrane proteins that are involved in defense against metal stress as well as pathogens and herbivores. We are now investigating ER body formation and function using ER body deficient mutants, and heterologously expressing NAI2 in onion and tobacco cells.

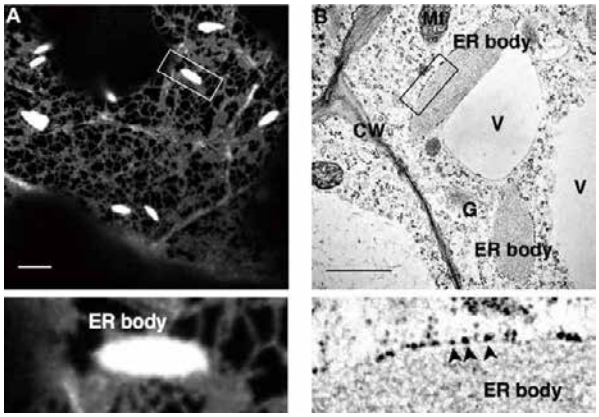

Figure 2. ER bodies in *Arabidopsis thaliana*. A confocal micrograph (A) and an electron micrograph (B) of cotyledon and root epidermal cells, respectively. Arrowheads indicate ribosomes on the surface of the ER body membranes. ER-localized GFP labels ER bodies as well as the typical ER network, and electron microscopy identifies ribosomes at the cytosolic surface of ER bodies, both of which indicate the luminal continuity between ER and ER bodies. Enlarged images of the squared regions are shown below. CW, cell wall; V, vacuole; Mt, mitochondrion; G, Golgi body; Bars,10μm (A) and 1μm (B).

V. Roles of molecular chaperones on cell differentiation

Molecular chaperones are cellular proteins that function in the folding and assembly of certain other polypeptides into oligomeric structures. We have found that HSP90 inhibitor induces genes with heat shock response element (HSE) motifs in their promoters, suggesting that heat shock transcription factor (HSF) is involved in the response. *Arabidopsis* HSFs interacted with HSP90. Thus, it appears that in the absence of heat shock, HSP90 actively suppresses HSF function. During heat shock, HSP90 is transiently inactivated, which leads to HSF activation. These data indicate that HSP90 regulates correct gene expression for heat acclimatization in plants. We also observed that HSP90 is involved in hormone responses in *Arabidopsis*. The evolutional and functional characterizations are now being investigated.

VI. Update 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 units: the electron micrograph database, the perceptive organelles database, the organelles movie database, the organellome database, the functional analysis database, and external links. The electron micrograph database, which was added as new content, provides information on the ultrastructures in plant cells. The perceptive organelles database shows organelle dynamics in response 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 stages. 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 based on PODB3 is an educational tool to engage members of the non-scientific community to explore plant biology. We hope that PODB3 and Plant Organelles World are of help to researchers and the general public.

Figure 3. The graphical user interface of the PODB3 (http://podb.nibb.ac.jp/Orgenellome).

Publication List

〔Original papers〕

- Goto-Yamada, S., Mano, S., Nakamori, C., Kondo, M., Yamawaki, R., Kato, A., and Nishimura, M. (2014). Chaperone and protease functions of LON protease 2 modulate the peroxisomal transition and degradation with autophagy. Plant Cell Physiol. *55*, 482-496.
- Mano, S., Nakamura, T., Kondo, M., Miwa, T., Nishikawa, S., Mimura, T., Nagatani, A., and Nishimura, M. (2014). The Plant Organelles Database 3 (PODB3) update 2014: integrating electron micrographs and new options for plant organelle research. Plant Cell Physiol. *55*, e1.
- Shibata, M., Oikawa, K., Mano, S., and Nishimura, M. (2014). Measurement of the number of peroxisomes. Bio-Protoc. *4*, e1284.
- Yoshimoto, K., Shibata, M., Kondo, M., Oikawa, K., Sato, M., Toyooka, K. Shirasu, K. Nishimura, M., and Ohsumi, Y. (2014). Organ-specific quality control of plant peroxisomes is mediated by autophagy. J. Cell Sci. *127*, 1161-1168.

〔Original paper (E-publication ahead of print)〕

- Motomura, K., Le, Q.T., Hamada, T., Kutsuna, N., Mano, S., Nishimura, M., and Watanabe, Y. Diffuse DCP2 accumulates in DCP1 foci under heat stress in *Arabidopsis thaliana*. Plant Cell Physiol. 2014 Oct 22.

〔Review articles〕

- Goto-Yamada, S., Mano, S., and Nishimura, M. (2014). The role of peroxisomes in plant reproductive processes. In Sexual reproduction in animals and plants. – Edited by Sawada, H., Inoue, N., and Iwano, M. Springer Japan, pp. 419-429.
- Goto-Yamada, S., Mano, S., Oikawa, K., Shibata, M., and Nishimura, M. (2014). Interaction between chaperone and protease functions of LON2, and autophagy during the functional transition of peroxisomes. Plant Signal. Behav. *9*, e28838.
- Nakano, R.T., Yamada, K., Bednarek, P., Nishimura, M., and Hara-Nishimura, I. (2014). ER bodies in plants of the Brassicales order: Biogenesis and association with innate immunity. Front. Plant Sci. *5*, 73, doi: 10.3389/fpls.2014.00073.
- Shibata, M., Oikawa, K., Yoshimoto, K., Goto-Yamada, S., Mano, S., Yamada, K., Kondo, M., Hayashi, M., Sakamoto, W., Ohsumi Y., and Nishimura, M. (2014). Plant autophagy is responsible for peroxisomal transition and plays an important role in the maintenance of peroxisomal quality. Autophagy *10*, 936-937.