

## 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  $\beta$ -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) based on them having a different pattern of GFP fluorescence from the parent plant, GFP-PTS1, in which peroxisomes with normal sizes and numbers can be visualized with GFP.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2013. The former title is indicated by an asterisk (\*).

†: This laboratory was closed on 31 March, 2014.

Of these *apem* mutants, the *APEM1* gene encodes dynamin-related protein 3A, which is involved in division of both peroxisomes and mitochondria. *APEM2* and *APEM4* were revealed to encode proteins homologous to PEROXIN 13 (PEX13) and PEX12, respectively, and both proteins are responsible for protein translocation on peroxisomal membranes. In addition, we revealed that *APEM3* and *APEM9* are the peroxisomal membrane proteins. *APEM3* is a transporter, whose defect causes enlargement of peroxisomes, and *APEM9* is the plant-specific PEX to tether the PEX1-PEX6 complex on peroxisomal membranes. Recently, we found that *APEM10* encodes the Lon protease 2, which modulates peroxisome degradation processed by autophagy.

From an analysis using *peroxisome unusual poisoning* (*peup*) 1, *peup2* and *peup4* mutants, which were isolated as showing abnormal position and an increase in number of peroxisomes, we revealed that autophagy selectively degrades oxidized 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.

#### 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. Undamaged rosette leaves have no ER bodies, but accumulate ER bodies after wounding or jasmonic acid

treatment. This suggests that ER bodies function in the defense against herbivores. ER bodies in seedlings include  $\beta$ -glucosidase PYK10. When plant cells are damaged, PYK10 forms large protein aggregates. The aggregate formation increases glucosidase activity, 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. *NAI2* interacts with MEB1 and MEB2. *NAI2* deficiency relocates MEB1 and MEB2 to the ER network. The results suggest that *NAI2* facilitates the accumulation of MEB1 and MEB2 on the ER body membrane (Figure 2). These findings indicate that *NAI2* is a key factor that enables ER body formation. We are now investigating the function of *NAI2* on ER body formation by heterologously expressing it in onion and tobacco cells.

#### 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 HSP90.2. Thus, it appears that in the absence of heat shock, HSP90 actively suppresses

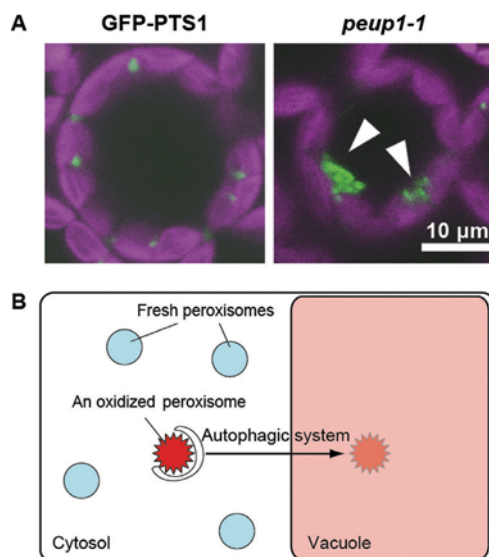


Figure 1. PEUP1/ATG2 is involved in autophagy to degrade oxidized peroxisomes. (A) Compared to the parent plant, GFP-PTS1, the *peup1-1* mutant contains peroxisome aggregates (arrowheads). Chloroplasts and peroxisomes are shown in magenta and green, respectively. (B) The scheme of the quality control of peroxisomes by autophagic system. Oxidized peroxisomes are selectively removed from the cytosol via autophagy.

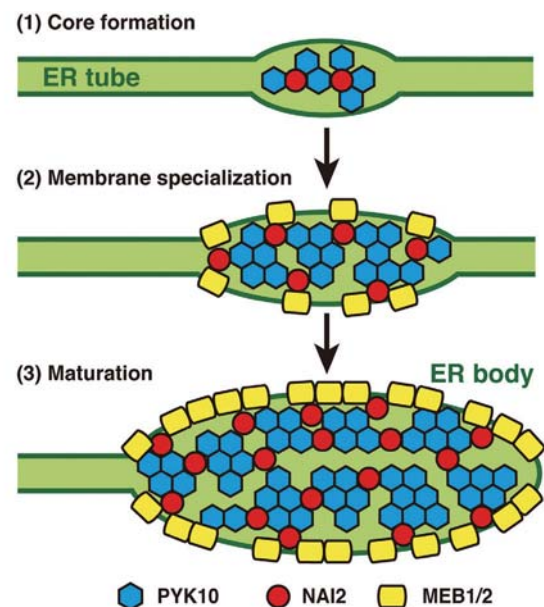


Figure 2. Models of ER body formation. PYK10 and NAI2 may physically interact to form ER body structures from the ER. NAI2 forms a complex with MEB1 and MEB2 to generate the ER body-specific membrane.

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 evolutionary 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 organelle 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 (Figure 3). The perceptive organelles database shows organelles' dynamics in response to environmental stimuli. The organelles movie database contains time-lapse images and 3D structure rotations. The organelle 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. The amount of included data is increasing day by day. In addition, we updated the website, Plant Organelles World, which is based on PODB3 as an educational tool to engage members of the non-scientific community. We expect that PODB3 and Plant Organelles World will enhance the understanding of plant organelles



Figure 3. The graphical user interface of the electron micrograph database in PODB3 (<http://podb.nibb.ac.jp/Organelle>).

among researchers and the general public who want to explore plant biology.

## Publication List

### [Original papers]

- Cui, S., Fukao, Y., Mano, S., Yamada, K., Hayashi, H., and Nishimura, M. (2013). Proteomic analysis reveals that the Rab GTPase RabE1C is involved in the degradation of the peroxisomal protein receptor PEX7 (peroxin 7). *J. Biol. Chem.* 288, 6014-6023.
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- Tameshige, T., Fujita, H., Watanabe, K., Toyokura, K., Kondo, M., Tatematsu, K., Matsumoto, N., Tsugeki, R., Kawaguchi, M., Nishimura, M., and Okada, K. (2013). Pattern dynamics is adaxial-abaxial specific gene expression are modulated by a plastid retrograde signal during *Arabidopsis thaliana* leaf development. *PLoS Genetics* 9, e1003655.
- Tamura, K., Iwabuchi, K., Fukao, Y., Kondo, M., Okamoto, K., Ueda, H., Nishimura, M., and Hara-Nishimura, I. (2013). MyosinXI-i links the nuclear membrane to the cytoskeleton to control nuclear movement and shape in *Arabidopsis*. *Curr. Biol.* 23, 1776-1781.
- Yamada, K., Nagano, A.J., Nishina, M., Hara-Nishimura, I., and Nishimura, M. (2013). Identification of two novel endoplasmic reticulum body-specific integral membrane proteins. *Plant Physiol.* 161, 108-120.

### [Original paper (E-publication ahead of print)]

- Mano, S., Nakamura, T., Kondo, M., Miwa, T., Nishikawa, S., Mimura, T., Nagatani, A., and Nishimura, M. The Plant Organelles Database 3 (PODB3) update 2014: integrating electron micrographs and new options for plant organelle research. *Plant Cell Physiol.* 2013 Sep. 18.

### [Review articles]

- Cui, S., Mano, S., Yamada, K., Hayashi, M., and Nishimura, M. (2013). Novel proteins interacting with peroxisomal protein receptor PEX7 in *Arabidopsis thaliana*. *Plant Signal. Behav.* 8, e26829.
- Mano, S., Kimori, Y., Takeda, T., Miwa, T., Nishikawa, S., Mimura, T., Nagatani, A., and Nishimura, M. (2013). Using image-based resources: databases for plant organelle dynamics and applications based on image information. In *Introduction to Sequence and Genome Analysis*. Edited by Mishra, A., Nawaz, Z. and Shahid, Z. pp.83-109. iConcept Press.