DIVISION OF CELL MECHANISM

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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 the 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 of Priority Areas on ìOrganelle Differentiation as the Strategy for Environmental Adaptation in Plantsî was started to clarify the molecular mechanisms underlying organelle differentiation.

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, many constitutive organelles are functionally transformed. Etioplasts differentiate into chloroplasts and mitochondria acquire the ability to oxidize glycine. Glyoxysomes, which are peroxisomes engaged in the degradation of reserve oil 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 are known to 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 *Arabidopsis* genome. Custom-made DNA microarray covering all these genes was used to investigate expression profiles of the peroxisomal genes in various organs. In parallel, we 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 proteins existing in either glyoxysomes or leaf peroxisomes. Some of these proteins contain no obvious PTS1 and PTS2. Combination of the transcriptomic and proteomic analyses is providing us with a new insight into plant peroxisomal functions.

Bioinfomatic analysis of *Arabidopsis* genome predicted the presence of 15 kinds of genes for peroxisomal biogenesis factors, called *PEX* genes. We 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, i.e. *PEX* genes regulating for peroxisomal morphology and peroxisomal protein import. These analyses revealed that PEX5, a receptor for PTS1, is involved in both lipid metabolism and photorespiration by regulating import of both PTS1- and PTS2-containing proteins. In contrast, PEX7, a receptor for PTS2, is involved only in photorespiration by regulating import of PTS2-containing proteins.

III. Identification of novel components essential for peroxisome biogenesis

To better understand peroxisome biogenesis, we isolated a number of *Arabidopsis* mutants having aberrant peroxisome morphology (*apm* mutants) based on the different pattern of GFP fluorescence from the parent plant, GFP-PTS1, in which peroxisomes with normal size and number can be visualized with GFP (Figure 1A).

Two of these *apm* mutants, *apm2* and *apm4* mutants, showed GFP fluorescence in the cytosol as well as in peroxisomes (Figure 1B, 1C), indicating the decrease of efficiency of PTS1-dependent protein transport to peroxisomes. Interestingly, both mutants are defective in PTS2-dependent protein transport as well. Both mutants exhibit dwafism (Figure 1D), and this phenotype was

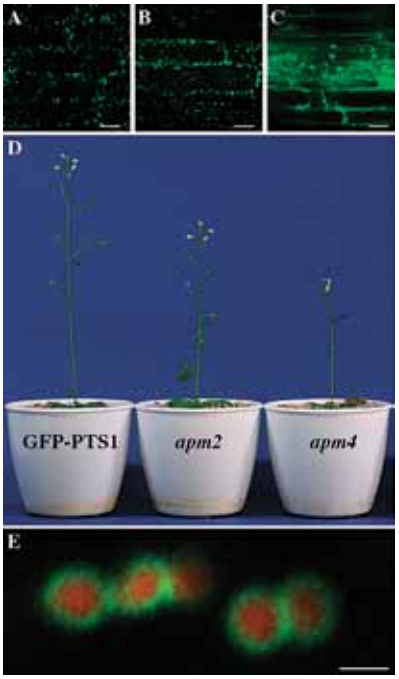

Figure 1. Contribution of APM2 and APM4 proteins for protein transport to peroxisomes. GFP fluorescence in cells of *apm2* (B) and *apm4* (C) mutants is observed in the cytosol as well as in peroxisomes, whereas the parent plant, GFP-PTS1, shows the fluorescence in only peroxisomes (A). These results show the decrease of efficiency of protein transport in both mutants. Both mutants exhibit dwarfism compared to the GFP-PTS1 (D). *APM2* and *APM4* genes encode Perxin 13 (PEX13) and PEX12, respectively, which are involved in protein transport on peroxiomal membranes (E, green and red signals represent PEX12-GFP and RFP-PTS1, respectively). Bars indicate 20 μm for (A) to (C), and 1 μm for (E).

related to the frequency of appearance of GFP fluorescence in the cytosol, apparently because protein transport was more severely decreased in *apm4* than in *apm2* mutant. *APM2* and *APM4* were found to encode proteins homologous to PEX13 and PEX12, respectively. It was revealed that APM2/PEX13 and APM4/PEX12 are localized on peroxisomal membranes (Figure 1E), and that APM2/PEX13 interacts with PEX7. In addition, we found that PEX5 binds to, and does not move on peroxisomal membranes in both mutants. These results show that APM2/PEX13 and APM4/PEX12 are components of the protein-translocation machinery on peroxisomal membranes. Analyses of other *APM* genes will help to identify components responsible for peroxisome biogenesis and address the regulation of its mechanism.

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 (5 μm long and 0.5 μm wide) that are surrounded by ribosomes. ER bodies were widely distributed in the epidermal cells of whole seedlings. Rosette leaves had no ER bodies, but accumulated ER bodies after wounding or jasmonic acid treatment. This suggests that ER bodies function in the defense against herbivores. ER bodies include PYK10, a β-glucosidase with an ER retention signal, in seedlings. We have isolated a couple of *Arabidopsis* mutants that have a defect in ER body formation. *Arabidopsis nai1* mutant has no ER bodies in whole plants and does not accumulate PYK10 (Figure 2). *NAI1* encodes a transcription factor that has a basic-helix-loop-helix (bHLH) domain. Transient expression of *NAI1* induced ER bodies in the *nai1* mutant. These results provide direct evidence that NAI1 plays a role in the formation of ER bodies. We are trying to isolate additional components that are involved in ER body formation.

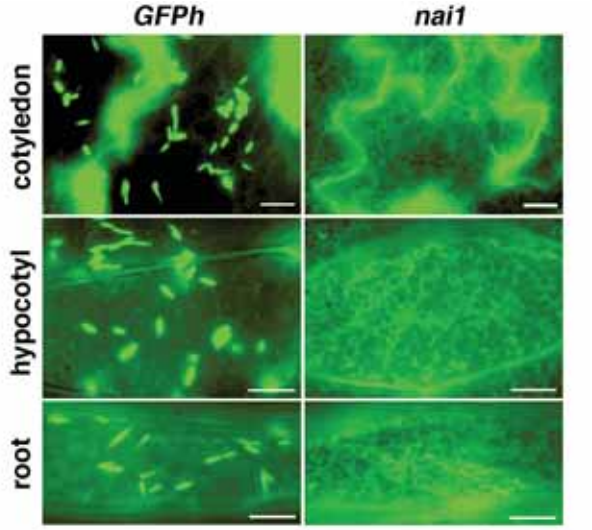

Figure 2. Fluorescent images of transgenic *Arabidopsis* (*GFPh*) and *nai1* mutant that express ER-localized GFP. Five-day-old seedlings were inspected with a fluorescence microscope. The GFPh seedlings had many ER bodies (rod -shape structures) in cotyledons, hypocotyls and roots. On the contrary, the *nai1* seedlings had no ER bodies in these organs. Bars= 10 μm.

V. Vacuolar processing enzyme responsible for programmed cell death in plants

The vacuolar processing enzyme (VPE) belongs to the cysteine protease family found in higher plants and animals. VPE exhibits substrate specificity toward asparagine and aspartic acid residues, the amino acid well conserved at the processing sites of vacuolar proteins. Plant VPE homologues are separated into three subfamilies: seed type, vegetative type, and seed-coat type. Seed type VPE is responsible for the maturation of seed storage proteins. On the other hand, the function of vegetative and seed-coat type VPEs was obscure. Recently, we revealed a novel function of VPE in various programmed cell death (PCD) in plants. The evidence from extensive studies indicates that caspase activity is involved in plant PCD. VPE is identified as the proteinase that exhibits caspase activity in plants. The plant hypersensitive response (HR), a type of defense strategy, constitutes well-organized PCD. No HR occurs on the tobacco mosaic virus-infected leaves of VPE-deficient

tobacco plants. Fumonisin B1 (FB1), a fungal toxin, induced cell death in *Arabidopsis*. The features of FB1-induced cell death were completely abolished in the VPE-null *Arabidopsis* mutant. *Arabidopsis* δVPE expresses specifically and transiently in two cell layers of the seed coat that causes PCD accompanying cell shrinkage. In a *δvpe* mutant, shrinkage of these cell layers was delayed. An ultrastructural analysis showed that the disintegration of the vacuolar membranes occurs before the cell death in these PCDs. These results suggest that VPE is involved in vacuolar collapse, which triggers PCD. Plants evolve a death strategy mediated by a vacuolar system, which is not seen in animals. Interestingly, a vacuolar enzyme is the key player in a plant-specific cell death system.

VI. Role 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 but that are not, themselves, components of the final oligomeric structure. To clarify the roles of molecular chaperones on cell differentiation, we have purified and characterized chaperonin and Hsp70s and analyzed their roles in the translocation of proteins into chloroplasts.

Previously, we characterized a mitochondrial co-chaperonin (Cpn10), chloroplast co-chaperonins (Cpn20 and Cpn10) and a small heat shock protein from *Arabidopsis*. Recently, we started to characterize HSP90s, using a specific inhibitor of HSP90 or transgenic plants expressing mutated *Arabidopsis* HSP90. Preliminary data suggests that HSP90 is involved in various cellular signaling, such as heat shock and hormone responses, in *Arabidopsis*. The evolutional and functional characterization is now under experiment.

VII. The Plant Organelles Database ñ Databases of plant organelles visualized with fluorescent probes, and protocols for functional analysis

Figure 3. The plant organelles database (http://podb.nibb.ac.jp/Orgenellome).

The plant organelles database is a specialized database project dedicated to plant organelle research and is maintained by the Scientific Research of Priority Areas on ìO rganelle Differentiation as the Strategy for Environmental Adaptation in Plantsî. This database consists of three individual databases: the organellome database, the functional analysis database and external links about transcriptomics and proteomics. The organellome database provides information of various plant organelles visualized with fluorescent probes. The functional analysis database contains useful protocols for analyses of plant organelles and integrated functions. This database is opened to all researchers as a public database. We expect that this database is going to be a useful analytical tool for plant organelle research.

Publication List:

Original papers

Li, L., Shimada, T., Takahashi, H., Ueda, H., Fukao, Y., Kondo, M., Nishimura, M., and Hara-Nishimura, I. (2006). MAIGO2 is involved in exit of seed storage proteins from the endoplasmic reticulum in *Arabidopsis thaliana*. Plant Cell *18*, 3535-3547.

Mano, S., Nakamori, C., Nito, K., Kondo, M., and Nishimura, M. (2006). The Arabidopsis *pex12* and *pex13* mutants are defective in both PTS1- and PTS2-dependent protein transport to peroxisomes. Plant J. *47*, 604-618.

Shimada, T., Koumoto, Y., Li, L., Yamazaki, M., Kondo, M., Nishimura, M., and Hara-Nishimura, I. (2006). AtVPS29, a putative component of a retromer complex, is required for the efficient sorting of seed storage proteins. Plant Cell Physiol. *47*, 1187-1194.

Ueda, H., Nishiyama, C., Shimada, T., Koumoto, Y., Hayashi, Y., Kondo, M., Takahashi, T., Ohtomo, I., Nishimura, M., and Hara-Nishimura, I. (2006). AtVAM3 is required for normal specification of idioblasts, myrosin cells. Plant Cell Physiol. *47*, 164-175.

Review articles

Hatsugai, N., Kuroyanagi, M., Nishimura, M., and Hara-Nishimura, I. (2006). A cellular suicide strategy of plants: vacuole-mediated cell death. Apoptosis *11*, 905-911.

Hayashi, M., and Nishimura, M. (2006). Arabidopsis thaliana-A model organism to study plant peroxisomes. Biophys. Biochim. Acta *1763*, 1382-1391.