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 "Organelle Differentiation as the Strategy for Environmental Adaptation in Plants" 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 are the functional transformations of many constitutive organelles. 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. 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 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, called *PEX* genes, for peroxisomal biogenesis factors. 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 (Figure 1). 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 *PEX* genes regulating for peroxisomal protein import. These analyses revealed that PEX5, a receptor for PTS1, is involved in both lipid metabolism and photorespiration by regulating the import of

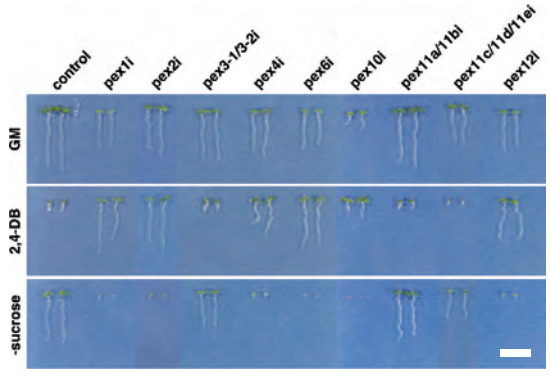

Figure 1. Effects of 2,4-DB and sucrose on growth of knockdown mutants. The name of each transgenic plant at the top of the panels indicates a silenced *PEX* gene or a combination of silenced *PEX* genes. Homozygous T3 progenies of these transgenic plants and parental plants (control) were grown for 7 days on growth medium (GM) containing 0.25 μg/mℓ of 2,4-DB (2,4-DB) or growth medium without sucrose (-sucrose) under constant illumination. Bar = 10 mm.

both PTS1- and PTS2-containing proteins. In contrast, PEX7, a receptor for PTS2, is involved only in lipid metabolism by regulating the 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 sizes and numbers can be visualized with GFP.

Of these *apm* mutants, *apm1* mutants contain slightly larger peroxisomes with long string-like tails. Interestingly, the division of mitochondria is also disturbed in *apm1* mutants. We revealed that *APM1* encodes dynamin-related protein 3A (DRP3A), one of a member of the dynamin family, and that DRP3A has a role in the division of both peroxisome and mitochondria on each membrane. Two other *apm* mutants, *apm2* and *apm4*, showed GFP fluorescence in the cytosol as well as in peroxisomes, indicating the decrease of efficiency of PTS1-dependent protein transport to peroxisomes. Interestingly, both mutants are defective in PTS2-dependent protein transport as well. *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, 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. Other *apm* mutants are under investigation so that we can identify the 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. *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.

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, and is responsible for the maturation of various type of vacuolar proteins. Plant VPE homologues are separated into three subfamilies: seed type, vegetative type, and seed-coat type. We revealed a novel function of VPE in various programmed cell death (PCD) in plants. 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* δ*VPE* expresses specifically and transiently in two cell layers of the seed coat that causes PCD accompanying cell shrinkage. In a δ*vpe* mutant, shrinkage of these cell layers was delayed. 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 began to characterize HSP90s, using a specific inhibitor of HSP90 or transgenic plants expressing mutated *Arabidopsis* HSP90. HSP90 inhibitor induced heat-inducible genes and heat acclimation in *Arabidopsis* seedlings (Figure 2). HSP90 inhibitor induced the 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 (AtHsfA1d, AtHsfA7a and AtHsfB1) interacted with *Arabidopsis* HSP90.2. Heat shock reduced cytosolic HSP90 activity in vivo. Thus, it appears that in the absence of heat shock, HSP90 actively suppresses HSF function. Upon heat shock, HSP90 is transiently inactivated, which leads to HSF activation. Plant survival requires the ability to acclimate to heat. This data indicates 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 characterization is now under experiment.

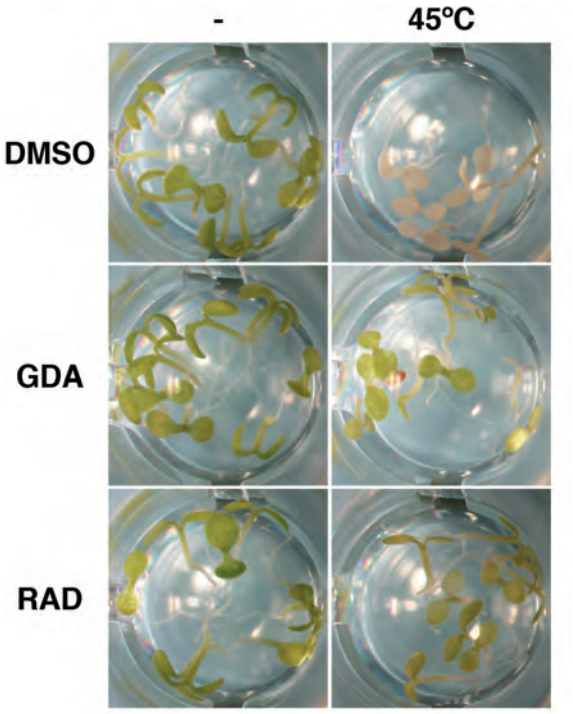

Figure 2. Pretreatment with HSP90 inhibitors induces the heat shock response and high temperature tolerance. Seedlings of *Arabidopsis* were treated with 50 μM of geldanamycin (GDA) or radicicol (RAD), HSP90 inhibitors, for 6 h, and they were washed three times with fresh medium. After plants were allowed to recover at 22 °C for 2 h, they were incubated at 22 °C (-; left) or 45 °C (right) for 1 h. As a negative control, plants were treated with dimethyl sulfoxide (DMSO), chemical solvent. The panels show the 2 days after the treatment.

Ⅶ. The Plant Organelles Database (PODB) – Databases of plant organelles visualized with fluorescent and nonfluorescent probes, and protocols for functional analysis

The Plant Organelles Database (PODB) was built to promote a comprehensive understanding of organelle dynamics and is maintained by the Scientific Research of Priority Areas on "Organelle Differentiation as the Strategy for Environmental Adaptation in Plants." This database consists of 3 individual parts: the organellome database, the functional analysis database, and external links to other databases and Web pages (Figure 3). The organellome database provides images of various plant organelles that were visualized with fluorescent and nonfluorescent probes in various tissues of several plant species at different developmental stages. The functional analysis database is a collection of protocols for plant organelle research. This public database is open to all researchers. We expect that this database will be a useful tool to help researchers gain greater knowledge of plant organelles.

Figure 3. The graphical user interfaces of PODB (http://podb.nibb.ac.jp/Orgenellome).

Publication List

〔Original papers〕

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- Morita, Y., Arai, H., Sugimoto, T., Takeuchi, T., Yamane, T., Maeda, T., Yamamoto, Y., Nishi, K., Asano, M., Shirahama-Noda, K., Nishimura, M., Uzu, T., Hara-Nishimura, I., Koya, D., Kashiwagi, A., and Ohkubo, I. (2007). Legumain/asparaginyl endopeptidase controls extracellular matrix remodeling through the degradation of fibronectin in mouse renal proximal tubular cells. FEBS Lett. *581*, 1417-1424.
- Nito, K., Kamigaki, A., Kondo, M., Hayashi, M., and Nishimura, M. (2007). Functional classification of Arabidopsis peroxisome biogenesis factors proposed from analyses of knockdown mutants. Plant Cell Physiol. *48*, 763-774.
- Yamada, K., Fukazawa, M., Hayashi, M., Suzuki, I., and Nishimura, M. (2007). Cytosolic HSP90 regulates the heat shock response that is responsible for heat acclimation in Arabidopsis thaliana. J. Biol. Chem. *282*, 37794-37804.

〔Review article〕

- Kuroyanagi, M., Hatsugai, N., Nishimura, M., and Hara-Nishimura, I. (2007). Vacuolar processing enzyme, a key molecule in both pathogen-induced and phytotoxin-induced cell death in higher plants. Mol. Plant Microbe Interact. 5, 208-214.