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 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 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 microbodies 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*. Peptide MS fingerprinting analyses allowed us to identify novel proteins exists 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 a new insight into plant peroxisomal functions.

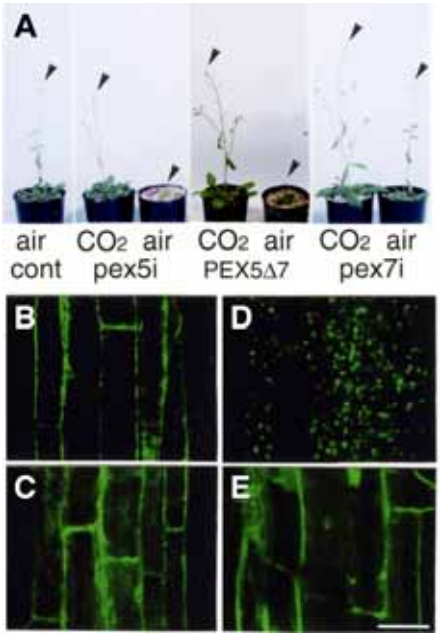

Figure 1. Differential contribution of two peroxisomal protein receptors to the maintenance of peroxisomal functions. Effect of CO_2 on the growth of pex5i, PEX5Δ7 and pex7i transgenic plants(A). Arrows indicate the top of an inflorescence apex. Import of PTS1-containing protein is inhibited in pex5i (B) but not in pex7i (D), while import of PTS2-containing protein is inhibited both in pex5i (C) and pex7i (E).

Bioinfomatic analysis of *Arabidopsis* genome predicted the presence of 15 kinds of genes for peroxisomal biogenesis factors, called *PEX* genes. We comprehensively investigated whether 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 (Figure 1A, 1B, 1C). In contrast, PEX7, a receptor for PTS2, is involved only in photorespiration by regulating import of PTS2-containing protein (Figure 1A, 1D, 1E).

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.

It was revealed that one of these mutants, *apm1*, whose peroxisomes and mitochondria are long and reduced in number, is defective in DRP3A (Dynamin-related protein 3A). This finding shows that APM1/DRP3A protein is involved in both peroxisomal and mitochondrial division. We revealed that other mutants, *apm2* and *apm4*, exhibit the GFP fluorescence in not only peroxisomes but also the cytosol, and determined that both APM2 and APM4 are responsible for matrix protein transport on peroxisomal membranes. Analyses of other *apm* mutants and identification of *APM* genes will identify components necessary 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. *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, 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 embryogenic type. Seed type VPE is responsible for the maturation of seed storage proteins. On the other hand, the function of vegetative and embryogenic type VPEs was obscure. Recently, we revealed a novel function of VPE in various types of programmed cell death (PCD) in plants. The

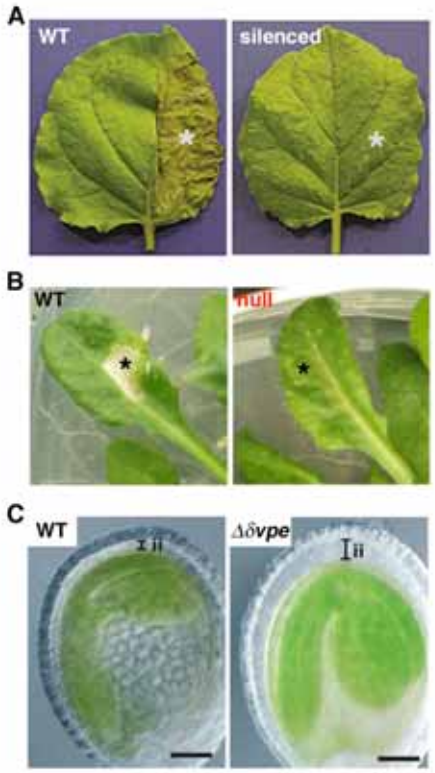

Figure 2. *VPE* deficiency suppresses various types of programmed cell death (PCD) in plants. (A) The non-silenced (WT) and *VPE*-silenced (silenced) *Nicotiana benthamiana* plants were infected with tobacco mosaic virus on halves of their leaves (indicated by asterisks). The photographs were taken after 24 hours. (B) Leaves of wild-type (WT) and VPE-null mutant (null) *Arabidopsis* plants were infiltrated with FB1, a fungal toxin (indicated by asterisks). The photographs were taken after 5 days. (C) Thickness of inner integuments (ii) of the seed coats is reduced in wild-type (WT) seed at the early stage, whereas it is not reduced in the *δvpe* mutant seed of *Arabidopsis*. PCD accompanies the shrinkage of two cell layers of the seed coat in wild type seeds.

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*-silenced tobacco plants (Figure 2A). Fumonisin B1 (FB1), a fungal toxin, induced cell death in *Arabidopsis*. The features of FB1-induced cell death were completely abolished in the *Arabidopsis* VPE-null mutant, which

lacks all VPE genes (Figure 2B). *Arabidopsis* δVPE was recently identified as an embryogenic type VPE. δVPE specifically and transiently expressed in two cell layers (ii2-ii3) of the seed coat at an early stage of seed development. At this stage, PCD accompanying cell shrinkage occurs in ii2-ii3. In a *δvpe* mutant, shrinkage of these layers was delayed (Figure 2C). 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 in *Arabidopsis*. The evolutional and functional characterization is now under experiments.

VII. Organellome database – Databases of plant organelles visualized with fluorescent protein, and protocols for functional analysis

The organellome database is a specialized database project dedicated to plant organelle research. This database is maintained by the Scientific Research of Priority Areas on “Plant Organelles”. To support this plant organelle research, we have been constructing a database consisting of three individual databases: the organellome database, the functional analysis database and external links about transcriptomics and proteomics. This database will be 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.

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