## DIVISION OF CELL MECHANISMS

*Professor*
NISHIMURA, Mikio

*Associate Professor*
HAYASHI, Makoto

| | |
|---|---|
| *Assistant Professors* | MANO, Shoji |
| | YAMADA, Kenji |
| *Technical Staff* | KONDO, Maki |
| *Postdoctoral Fellows* | ARAI, Yuko |
| | KAMIGAKI, Akane |
| | OIKAWA, Kazusato |
| | SINGH, Tanuja |
| *Graduate Students* | OGASAWARA, Kimi |
| | KANAI, Masatake |
| | GOTO, Shino |
| | CUI, Songkui |
| | SHIBATA, Michitaro |
| *Technical Assistants* | NAKAMORI, Chihiro |
| | YOSHINORI, Yumi |
| | SUZUKI, Iku |
| | FUKAZAWA, Mitsue |
| | KATO, Kyoko |
| | NISHINA, Momoko |
| | SATO, Yori |
| *Secretaries* | UEDA, Chizuru |
| | KUBOKI, Yuko |

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 while mitochondria acquire the ability to oxidize glycine. Glyoxysomes, which are peroxisomes engaged in the degradation of reserve oil 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 *Arabidopsis* genome. Custom-made DNA microarray covering all these genes was used to investigate expression profiles of the peroxisomal genes in various organs. It revealed that peroxisomes in root cells plays 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) (Figure 1). The overall results provide us new insights into plant peroxisomal functions.

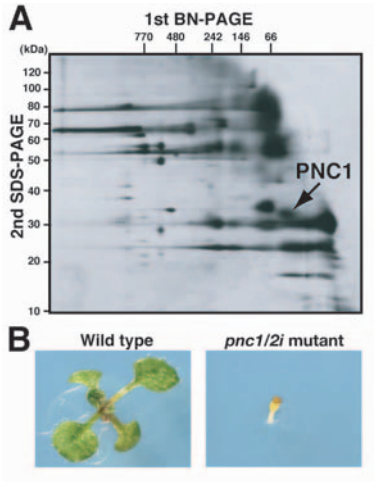


Figure 1. Proteomic identification and characterization of a novel peroxisomal adenine nucleotide carrier 1 (PNC1). (A) 2-D map of proteins in the digitonin-soluble fraction of purified peroxisomes. A 2-D gel in which the first dimension was Blue Native (BN)-PAGE and the second was SDS-PAGE is shown. Protein spots were detected by silver staining. (B) Phenotype of the *pnc1/2* RNAi knockdown mutant. Seedlings grown on medium without sucrose under constant illumination for 7 days.

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. 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. Of these, 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 continue to investigate the detailed molecular functions of other *PEX* genes.

## 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 a different pattern of GFP fluorescence than the parent plant, GFP-PTS1, in which peroxisomes with normal sizes and numbers can be visualized with GFP.

Of these *apm* mutants, *APM1* gene (whose defect causes the elongation of peroxisomes and mitochondria) encodes dynamin-related protein 3A (DRP3A), one member of the dynamin family. Two other *apm* mutants, *apm2* and *apm4*, showed GFP fluorescence in the cytosol as well as in peroxisomes. We demonstrated that both mutants are defective in PTS1- and PTS2-dependent protein transport to peroxisomes, and that *APM2* and *APM4* encode proteins homologous to PEX13 and PEX12, respectively. It was revealed that APM2/PEX13 and APM4/PEX12 are components of the protein-translocation machinery on peroxisomal membranes, and that they are involved in protein transport from the cytosol into the peroxisome. Other *APM* mutants are currently under investigation in order to identify the components responsible for peroxisome biogenesis 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 (5 μm long and 0.5 μm wide) surrounded by ribosomes. ER bodies 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 PYK10, a $\beta$-glucosidase with an ER retention signal. *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 and regulates the expression of *PYK10*. *Arabidopsis nai2* mutant has no ER bodies and reduces the accumulation of PYK10. *NAI2* encodes a member of a unique protein family that is only found in the Brassicaceae. NAI2 localizes to the ER body. In the *nai2* mutant, PYK10 becomes uniformly diffused throughout the ER (Figure 2). These findings indicate that NAI2 is a key factor that enables ER body formation and the accumulation of PYK10 in ER bodies.

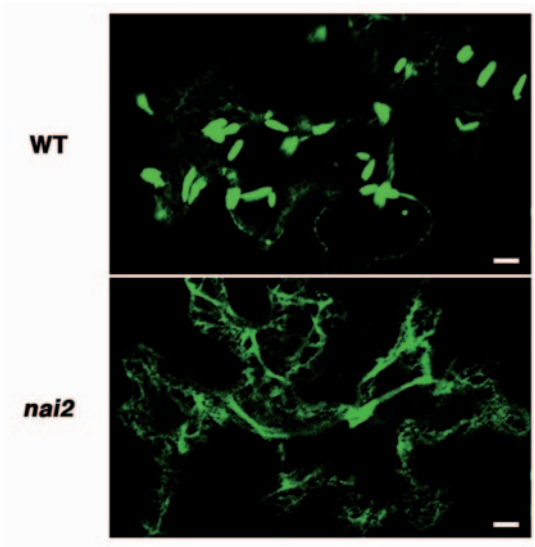


Figure 2. Localization of GFP-PYK10 in *nai2* mutant. GFP-PYK10 fusion gene was transiently expressed in the epidermal cells of 6-day-old wild-type (WT) and *nai2* seedlings. GFP-PYK10 is localized in ER bodies in wild-type plants; it becomes uniformly diffused, however, in ER in the *nai2* mutant. Bars = 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 is responsible for the maturation of various types of vacuolar proteins. 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. 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. 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. 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.

Recently, we found that 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 interacted with HSP90. 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. 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 characterizations are now being investigated.

## Ⅶ. The Plant Organelles Database 2 (PODB2) – Release of Version 2 for plant organelles dynamics and methods 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." We released Version 2 (PODB2), which incorporated time-lapse and 3D images. PODB2 consists of 4 individual parts: the organelles movie database, the organellome database, the functional analysis database, and external links (Figure 3). The organelles movie database contains the videos for organelle movements and 3D structures. 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 PODB2 (http://podb.nibb.ac.jp/Orgenellome).

## Publication List

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### 〔Review articles〕


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