DEPARTMENT OF CELL BIOLOGY

Chairman: Mikio Nishimura

The department consists of two regular divisions and three adjunct divisions. The department conducts studies on molecular dynamics of the cell in higher plants and animals such as organelle differentiation, autophagy, cell motility, cytokinesis and neural development.

DIVISION OF CELL MECHANISMS

Professor:	*Mikio Nishimura*
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Research Associates:	*Tomoo Shimada (-Feb. 28)*
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	Hiroshi Hayashi
	Kenji Yamada
	Naoto Mitsuhashi (Oct. 1-)
Graduate Students:	*Naoto Mitsuhashi (-Sept.30)*
	Kazumasa Nito
	Etsuko Watanabe
	Youichiro Fukao (April 1-)
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	Rie Takei (April 1-)
	Akiko Komori (May 23-)
Visiting Scientists:	*Yasuko Koumoto (-Feb. 28)*

Higher plant cells contain several distinct organelles that play vital roles in cellular physiology. During proliferation and differentiation of the cells, the organelles often undergo dynamic changes. The biogenesis of new organelles may occur, existing organelles may undergo a transformation of function, while other organelles may degenerate. Because the dynamic transformation of organellar function (differentiation of organelles) is responsible for flexibility of differentiation events in higher plant cells, the elucidation of regulatory mechanisms underlying organelle transformation are currently studied in this division.

I. Regulation at the level of protein transport to microbodies during the microbody transition.

Dramatic metabolic changes which underlie the shift from heterotrophic to autotrophic growth occur in greening of seed germination. Accompanying these metabolic changes, many constitutive organelles are functionally transformed. For example, etioplasts differentiate into chloroplasts and mitochondria acquire the ability to oxidize glycine. Glyoxysomes which are microbodies engaged in the degradation of reserve oil via b-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 microbody transition of leaf peroxisomes to glyoxysomes occurs during senescence. The functional transformation between glyoxysomes and leaf peroxisomes is controlled by gene expression, alternative splicing, protein translocation and protein degradation.

To investigate the roles of microbody membrane proteins in the reversible conversion of glyoxysomes to leaf peroxisomes, we characterized several membrance proteins of glyoxysomes. One of them was identified as an ascorbate peroxidase (pAPX) that is localized on glyoxysomal membranes. Its cDNA was isolated by immunoscreening. The deduced amino acid sequence encoded by the cDNA insert does not have a peroxisomal targeting signal (PTS), suggesting that pAPX is imported by one or more PTS-independent pathways. Subcellular fractionation of 3- and 5-d-old cotyledons of pumpkin revealed that pAPX was localized not only in the glyoxysomal fraction, but also in the ER fraction. A magnesium shift experiment showed that the density of pAPX in the ER fraction did not increase in the presence of Mg^{2+}, indicating that pAPX is not localized in the rough ER. Immunocytochemical analysis using a transgenic Arabidopsis which expressed pumpkin pAPX showed that pAPX was localized on peroxisomal membranes, and also on a unknown membranous structure in green cotyledons. The overall results suggested that pAPX is transported to glyoxysomal membranes via this unknown membranous structure.

II. Microbody defective mutant of Arabidopsis.

It has been suggested that the functional conversion between glyoxysomes and leaf peroxisomes is controlled by gene expression, protein translocation, and protein degradation. A genetic approach is an effective strategy toward understanding the regulatory mechanism(s) of peroxisomal function at the level of gene expression, protein translocation, and protein degradation. We isolated and characterized 2,4-dichlorophenoxybutyric acid (2,4-DB)-resistant mutants. It has been demonstrated previously that 2,4-dichlorophenoxybutyric acid (2,4-DB) is metabolized to produce a herbicide, 2,4-D, by the action of peroxisomal fatty acid β-oxidation in higher plants. To isolate mutants that have defects in peroxisomal fatty acid β-oxidation, we screened mutant lines of Arabidopsis seedlings for growth in the presence of toxic levels of 2,4-DB. Genetic analysis revealed that these mutants can be classified as carrying alleles at three independent loci, which we designated ped1, ped2, and ped3, (where ped stands for peroxisome defective). The *ped1* mutant lacks the thiolase protein, an enzyme involved in fatty acid β-oxidation during germination and subsequent seedling growth. *Ped2* gene was identified by positional cloning and complementation analysis. The amino acid sequence of the predicted protein product is similar to that of human Pex14p, which is a key component of the peroxisomal protein import machinery. Therefore, we decided to call it *At*Pex14p. Analyses of the *ped*2 mutant revealed that *At* Pex14p controls intracellular transport of both peroxisome targeting signal (PTS)1- and PTS2-containing proteins into three different types of microbodies, namely glyoxysomes, leaf peroxisomes and unspecialized peroxisomes. Mutation in the *PED2* gene results in reduction of enzymes in all of these functionally differentiated microbodies. The reduction in these enzymes induces pleiotropic defects, such as fatty acid degradation, photorespiration and the morphology of peroxisomes (Fig. 1). These data suggest that the *AT* Pex14p has a common role in maintaining

Figure 1. Reduced activity of photorespiration in *ped* 2 mutant.

Effect of CO2 on the growth of *ped*2 mutant. Wild-type *Arabidopsis* (WT/air) and *ped*2 mutant (*ped*2/air) were grown for 8 weeks in a normal atmosphere (36 Pa CO2) under constant illumination (100 μ E/m^2/s). The *ped*2 mutant was also grown for 8 weeks in an atmosphere containing 1000 Pa CO_2 (*ped*2/CO_2) under constant illumination (50 μ E/m^2/s).

physiological functions of each of these three kinds of plant microbodies by determining peroxisomal protein targeting.

III. Transport of storage proteins to protein storage vacuoles is mediated by large PAC (precursor-accumulating) vesicles.

Novel vesicles that accumulate large amounts of proprotein precursors of storage proteins were purified from maturing pumpkin seeds. These vesicles were designated precursor-accumulating (PAC) vesicles and have diameters of 200 to 400 nm. We characterized them to answer the question of how seed protein precursors are accumulated in the vesicles to be delivered to protein storage vacuoles. They contain an electron-dense core of storage proteins surrounded by an electron-translucent layer, and some vesicles also contained small vesicle-like structures. An immunocytochemical analysis revealed numerous electron-dense aggregates of storage proteins within the endoplasmic reticulum. It is likely that these aggregates develop into the electron-dense cores of the PAC vesicles and then leave the endoplasmic reticulum. Immunocytochemical analysis also showed that complex glycans are associated with the peripheral region of PAC vesicles but not the electron-dense cores, indicating that Golgi-derived glycoproteins are incorporated into the PAC vesicles. These results suggest that the unique PAC vesicles might mediate a transport pathway for insoluble aggregates of storage proteins directly to protein storage vacuoles.

In order to investigate the mechanism of the PAC vesicle formation, we constructed chimeric genes that encode fusion proteins consisting to both various lengths of polypeptides derived from pumpkin 2S albumin and a selectable marker enzyme, phosphinothricin acetyltransferase and expressed in Arabidopsis. A fusion protein expressed by one of the chimeric genes is accumulated as a proprotein-precursor form, and localized in novel vesicles of vegetative cells, that show distinct features that well much to the PAC vesicles. Despite of the accumulation of the fusion protein, the transgenic Arabidopsis is still sensitive to phosphinothricin. Phosphinothricin acetyltransferase contained in the fusion protein is obviously compartmentalized in the PAC vesicles that do not permit the detoxification of this herbicide. These results indicate that PAC vesicle can be induced in vegetative cells by ectopic expression of the protein that is destined to be compartmentalized into the PAC vesicles. Arabidopsis mutants that defect vesicular transport of the fusion protein are screened and characterized by using the transgenic plants.

In order to characterize the organelles in the vacuolar-sorting pathway, we constructed chimeric genes that encode various GFP fusion proteins and expressed in Tobacco BY-2 cells. The organelles in the vacuolar-sorting pathway were able to be visualized in vital conditions and were used for the characterization of the vacuolar-sorting pathway.

IV. Vacuolar processing enzymes in protein-storage vacuoles and lytic vacuoles.

Vacuolar processing enzyme (VPE) has been shown to be responsible for maturation of various seed proteins in plant vacuoles. *Arabidopsis* has three VPE homologues; βVPE is specific to seeds and αVPE and γVPE are specific to vegetative organs. We expressed the γVPE in a *pep4* strain of the yeast *Saccharomyces cerevisiae* and found that γVPE has the ability to cleave the peptide bond at the carbonyl side of asparagine residues. An immunocytochemical analysis revealed the specific localization of the γVPE in the lytic vacuoles of *Arabidopsis* leaves. These findings indicate that γVPE functions in the lytic vacuoles as the βVPE does in the protein-storage vacuoles. The βVPE promoter was found to direct the expression of the β-glucuronidase reporter gene in seeds of transgenic *Arabidopsis* plants. On the other hand, both the *αVPE* and *γVPE* promoters directed the expression in senescent tissues, but not in young intact tissues. The mRNA levels of both *αVPE*

and *γVPE* were increased in the primary leaves during senescence in parallel with the increase of the mRNA level of a senescence-associated gene (*SAG2*). Treatment with wounding, ethylene and salicylic acid up-regulated the expression of *αVPE* and *γVPE*. Our results suggest that vegetative VPE might regulate the activation of some functional vacuolar proteins that are known to respond to these treatments.

In order to clarify function of VPEs in vivo, their anti-sence Arabidopsis were generated and the characterization is under experiments.

V. Role of molecular chaperones in organelle 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 organelle differentiation, we have purified and characterized chaperonin and Hsp70s and analyzed their roles in the translocation of proteins into chloroplasts. In addition to mitochondrial chaperonin10 homologues, we isolated cDNAs for chloroplastic chaperonin 10 homologues from Arabidopsis thaliana. One of the cDNA insert was 958 bp long and encoded a polypeptide of 253 amino acids. The other cDNA insert was 603 bp and encoded a polypeptide of 139 amino acids. The former was comprised of two distinct Gro-ES domains whereas the latter had one Gro-ES domain. Functional analyses of these chaperonin homologues in differentiation of plastids are in progress.

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