LABORATORY OF PLANT ORGAN DEVELOPMENT

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Plant organs, leaves, flowers, and roots show impressive, symmetrical shapes, based on an ordered arrangement of differentiated cells. The organs are formed from a group of undifferentiated cells located at the tip of the stem or the root. In the case of leaves, the process of organogenesis starts with the formation of a leaf primordium in the peripheral zone of the shoot apical meristem (SAM) at a fixed position, following an order called phyllotaxis. Cells in the primordium then proliferate and differentiate according to three spatially fixed axes: the apical-basal axis, the lateral (central-marginal) axis, and the adaxial-abaxial (foreside-backside) axis. In the course of proliferation and differentiation, plant cells are believed to exchange information with neighboring or separated cells in order to regulate organ architecture. We are trying to understand the mechanisms of information exchange between plant cells during the development of lateral organs, such as leaves, sepals, petals, stamens and carpels by using genetic, biochemical, microsurgical and one-cell gene induction approaches.

I. Genetic approach

Recent studies of *Arabidopsis* mutants show several genes are involved in the axes-dependent control of lateral organ development. The adaxial- and abaxial-specific tissue differentiation in the leaf primordium is determined by the precise expression of the adaxial marker genes, *HD-Zip III* including *PHABULOSA* (*PHB*), and the abaxial marker genes, *FILAMENTOUS FLOWER* (*FIL*) and *YABBY*. We showed that the adaxial-specific expression of PHB is regulated by the action of microRNA165/166 (miR165/166) which targeted the *HD-Zip III* messenger RNAs. We also revealed that expression of *MIR165/166* genes is observed only in the abaxial side. One of the *MIR165/166* genes, *MIR165A*, is expressed preferentially in the abaxial epidermal cells (Figure 1). We also found that *MIR165A* is enough to suppress the expression of PHB in the cells located in the abaxial side. These results suggested that miR165/166 might suppress the expression of PHB in some non-cell-autonomous manner.

To examine the mechanisms of establishment of the adaxial-abaxial axis, we isolated novel mutants which show altered patterns of *FIL* promoter:*GFP* expression, and named them *enlarged fil-expression domain* (*enf*). One of them, *enf1*, forms leaves with enlarged and reduced *FIL*-expression domains. In the extreme cases, leaves are filamentous. This phenotype indicates that *ENF1* is involved in the fixation or maintenance of the position of the adaxial-abaxial boundary. We revealed that the *ENF1* gene encodes an enzyme associated with primary metabolism, and that *ENF1* is strongly expressed in leaf primordia although its expression was not found in the SAM. This indicates that *ENF1*-catalized metabolite and/or its derivatives affect the axis-dependent cell fate in leaf primordia. In contrast, another mutant, *enf2*, has leaves with an enlarged *FIL*-expression domain, and the *ENF2* gene encoded a plastid-localized unknown protein. Chloroplast development was repressed in a severe allele of the *enf2* mutant. Exogenous application of chloroplast development inhibitors to *Arabidopsis* seedlings mimics the defects of the *FIL*-expression pattern by *enf2* mutation. These results suggest that chloroplast development is required for normal differentiation of leaf tissues.

A line of unique oblong cells is found at the marginal edge of leaves. We noticed that a homeobox-related gene, *PRESSED FLOWER* (*PRS*) and its homolog, *WOX1*, are required for forming the margin-specific cells. *prs wox1* double mutants completely lack the margin-specific cells in leaves, and interestingly, the abaxial side-specific epidermal cells, which are smaller than the adaxial side-specific epidermal cells, "invade" the adaxial side surface. The results indicate that the margin-specific cells act as a physical barrier separating the epidermal cells of the adaxial-side surface from those of the abaxial-side surface.

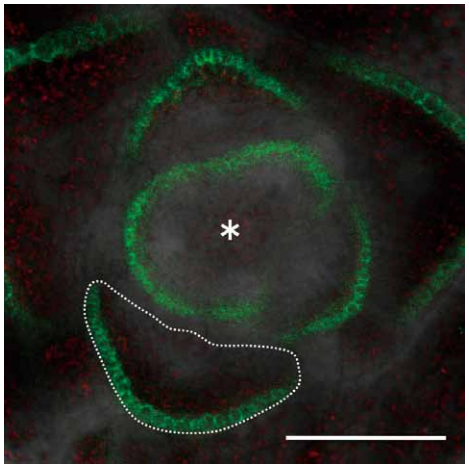

Figure 1. Expression domain of the *MIR165A* promoter:*GFPer* in Arabidopsis leaf primordia. Transverse section of the transgenic plant is shown. *MIR165A* is expressed mainly in the cells at the abaxial epidermis. GFP signal and autofluorescence of chloroplasts are indicated by green and red, respectively. White asterisk is the position of the SAM, and white dashed line outlines a leaf primordium. Scale bar is 100 μm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

To reveal how floral organs fix their forms through the developing processes, we analyzed mutants named *folded petals* (*fop*) (Figure 2). In the early stage of flower development, *fop* petals are similar to those of wild type, but the petals cannot grow through the narrow space between the sepal and anther in the flower buds. In *fop* mutants, petals can grow straight when the sepals are removed in the early stage of flower development. We also revealed that FOP proteins are related to wax/cutin synthesis or transport. Thus, we concluded that secreted wax/cutin on the petal epidermis might be important for the precise development of petals.

Figure 2. *fop* flowers (right) have folded petals (white arrow heads), while petals of wild type (left) elongate straightly.

II. Biochemical approach

We are taking a biochemical approach to study of the intercellular signaling system by analyzing small peptides as candidates for intercellular signaling ligands, which are present in the apoplastic region of the SAM. Small peptides were collected from apoplast fractions of floral buds of *Arabidopsis apetala1 cauliflower* double mutants and were analyzed by peptide sequence methods or LC-MS/MS methods. We chose about 30 peptides as candidates, and prepared synthetic peptides based on the obtained sequences. When applied to *Arabidopsis* seedlings, several peptides caused morphological defects in the SAM, vascular tissue, and root development. We are currently examining the mechanisms involved. We are also isolating small peptides from the apoplastic region in the heads of cauliflower (*Brassica oleracea* L. var. *botrytis*). Recently, we obtained several purified fractions of the apoplastic region, which affect axis-dependent leaf developments after exogenous application to *Arabidopsis* seedlings, and are analyzing the peptide sequences by LC-MS/MS methods.

III. Microsurgical approach

We are also carrying out microsurgical approaches using novel laser-ablating microscopy to investigate the cell-to-cell signaling system working during leaf development. When we ablated a small number of cells at the peripheral of the SAM of young *Arabidopsis* seedlings a few days after germination, some of the newly generated rosette leaves changed to a filamentous structure lacking the adaxial-abaxial identity. This suggests a flow of signal(s) from the SAM to the leaf primordia has a role of fixing the abaxial-adaxial regions in that primordia. We are also examining the leaf serration mechanism by ablating some cells at the margin in serrated leaves of several plants.

IV. One-cell gene induction approach

As a new tool for examining the intercellular communication system, we are developing a one-cell gene-induction system *in planta* using the InfraRed Laser Evoked Gene Operator (IR-LEGO) system, and showed gene expression in only a single cell of the root. Currently, we are endeavoring to examine cell-to-cell communication in the SAM using this system.

Publication List

〔Original papers〕

- Ishiguro, S., Nishimori, Y., Yamada, M., Saito, H., Suzuki, T., Nakagawa, T., Miyake, H., Okada, K., and Nakamura, K. (2010). The Arabidopsis *FLAKY POLLEN1* gene encodes a 3-hydroxy-3-methylglutaryl-coenzyme A synthase required for development of tapetum-specific organelles and fertility of pollen grains. Plant Cell Physiol. *51*, 896-911.
- Tsugeki, R., Ditengou, F.A., Palme, K., and Okada, K. (2010). *NO VEIN* facilitates auxin-mediated development in Arabidopsis. Plant Signal. Behav. *5*, 1249-1251.
- Yuguchi, M., Yokouchi, T., Tominaga-Wada, R., Kuromori, T., Shinozaki, K., Okada, K., and Wada, T. (2010). Phenome analysis of root development in *Arabidopsis*. Plant Biotech. *27*, 345-347.
- Preston, J.*, Tatematsu, K.*, Kanno, Y., Hobo, T., Kimura, M., Jikumaru, Y., Yano, R., Kamiya, Y., and Nambara, E. (2009†). Temporal expression patterns of hormone metabolism genes during imbibition of Arabidopsis thaliana Seeds: a comparative study on dormant and non-dormant accessions. Plant Cell Physiol. *50*, 1786-1800. (*: Equally contributed)

〔Review paper〕

- Okada, K. (2010). Start of Arabidopsis research in Japan: a personal memoir of the seedling stage 1985-2000. J. Plant Research *123*, 267-273.

† a paper that was not listed in the 2009 Annual Report