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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*. Using the reporter gene-system, we visualized the function domain of microRNA165/166 (miR165/166), which targeted the *HD-Zip III* messenger RNA, and showed that miR165/166 act in the cells locating in the abaxial side and determine the adaxial-specific expression of PHB. One of the *MIR165/166* genes, *MIR165A*, is expressed in the abaxial epidermal cells. We revealed by the visualizing reporter genes that *MIR165A* is enough to repress the PHB expression in the cells located in the abaxial side. These results suggested that miR165 is likely to move cell to cell. We also analyzed the function domain of miR165 when primary transcript of *MIR165A* was expressed by the *FIL*-promoter. this revealed that miR165 can act in the entirety of the leaf primordia, suggesting that there is no physical barrier interfering with miRNA movement between the adaxial- and the abaxial-side.

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, indicating that *ENF1* is involved in the fixation or maintenance of the position of the adaxial-abaxial boundary (Toyokura et al., 2011). We revealed that the *ENF1* gene encodes SUCCINIC SEMALDEHYDE DEHYDROGENASE, which catalyzes the conversion of succinic semialdehyde (SSA) to succinate, and is strongly expressed in leaf primordia although its expression was not found in the SAM. Exogenous application of SSA at the side of the SAM induced the adaxial-characters on the abaxial side of newly formed leaves (Figure 1). These results indicate that SSA and/or its derivatives affect the axis-dependent cell fate in leaf primordia. We also isolated some suppressor mutants of *enf1*, which show normal leaf shapes. We determined the genes, which have the mutation for the suppression of *enf1* phenotype, using next-generation sequence methods, and revealed that one of them has the mutation in a gene which encodes a transaminase enzyme.

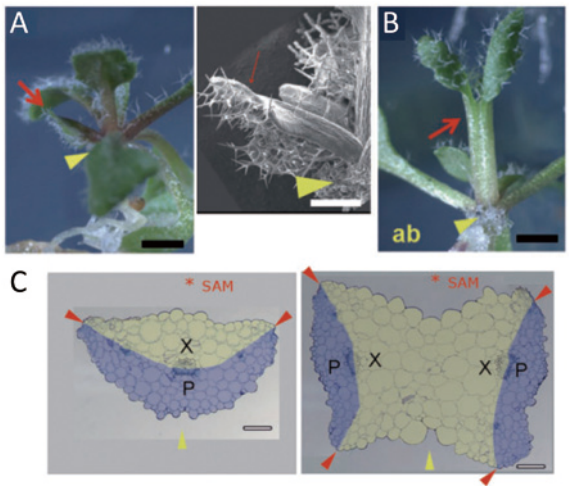

Figure 1. Effect of exogenous application of SSA on the adaxial-abaxial polarity in leaf primorda. We applied SSA-containing lanolin paste on the side of the Arabidopsis SAM, and checked the newly formed leaves after 1-2 weeks. Yellow arrowheads indicate the application site, and red arrows represent the newly formed leaves. (A) The lateral views of extreme effect (class I) of SSA taken by stereomicroscopy (left) and scanning electron microscopy (right). Newly formed leaves have the adaxial-characteristics on the abaxial-side. (B) The abaxial view of strong effect (class II) of SSA. Newly derived leaf developed with two laminas with upper sides facing each other. (C) The sections of the leaf petiols of the control leaf (left) and class II leaf (right). In class II leaves, the adaxial identity was observed not only on the side facing the SAM but also on the abaxial side.

In contrast, another mutant, *enf2*, has leaves with an enlarged *FIL*-expression domain, and the *ENF2* gene

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

encoded a plastid-localized unknown protein. Chloroplast development was repressed in a severe allele of the *enf2* mutant. Exogenous application of inhibitors for the gene expression of chloroplast genome-encoding genes to *Arabidopsis* seedlings mimics the defects of the *FIL*-expression pattern by *enf2* mutation. These results suggest that the expression of chloroplast genome-encoding genes is required for the determination of adaxial-abaxial polarity.

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. The analyses of *prs wox1* double mutants 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. Genetic analyses also showed that *PRS* and *WOX1* function in blade outgrowth downstream of the adaxial-abaxial polarity. The expression of *PRS* and *WOX1* was observed in two middle mesophyll layers and the marginal region of leaf primordia. We also revealed that the expression of *PRS* and *WOX1* are upregulated by the function of *FIL* and *YAB* genes, and repressed by those of the abaxial-specific genes, *KANADI*. We propose that the blade outgrowth and the adaxial-abaxial patterning during leaf developments are controlled by the middle domain-specific function of *PRS* and *WOX1* genes.

To reveal how floral organs fix their forms through development processes, we analyzed mutants named *folded petals* (*fop*). 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 the anther in the flower buds. In *fop* mutants, petals grow straight when the sepals are removed in the early stage of flower development. We found that the petal epidermal cells of *fop* petals bear epicuticular nanoridges like as those of wild type. FOP proteins are related to wax/cutin synthesis or transport. Thus, we proposed that wax/cutin components secreted by FOP proteins on the surface of the petal epidermis might act as a lubricant before the epicuticular nanoridge formation.

II. Biochemical approach

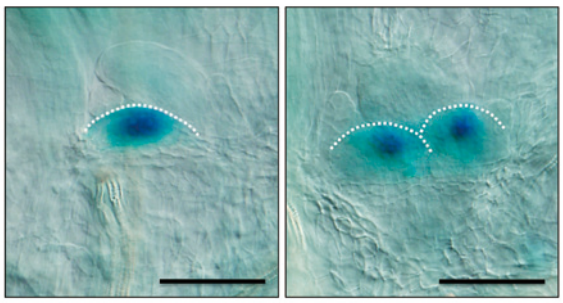

Figure 2. A purified fraction increasing the number of the SAMs in Arabidopsis seedlings. Purified fractions obtained from the apoplastic region of the curds of cauliflower were applied to Arabidopsis *CLV3* promoter:GUS transgenic lines. A purified fraction increased the number of the SAMs (Right) compared to the control (Left). GUS expression driven by the *CLV3* promoter indicates the position of the SAM. White dashed lines indicate the shape of the SAM. Bars indicate 200 μm.

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 the curds of cauliflower (*Brassica oleracea* L. var. *botrytis*). Through a biological assay using purified fractions, we obtained a fraction, which increased the number of SAMs after exogenous application of the fraction to Arabidopsis seedlings (Figure 2). Then we analyzed the peptide sequences included in the fraction by LC-MS/MS methods, and a putative lipid transfer protein was identified. We are preparing a recombinant protein of the candidate protein and examining effects of the recombinant protein on the development and growth of Arabidopsis seedlings.

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, some of the newly generated leaves changed to a filamentous structure lacking the adaxial-abaxial identity, suggesting that a flow of signal(s) from the SAM to the leaf primordia has a role of fixing the abaxial-adaxial polarity. We also examined the leaf serration mechanism by ablating some cells at the margin in serrated leave of *E. californica*, which form the leaflet acropetally. When leaflet incipient site is ablated, a new leaflet initiated at intact tissue near leaf tip, escaping from the ablated site, suggesting that a regular space of the leaflet initiation site is actively kept from the leaf tip.

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 InfarRed Laser Evoked Gene Operator (IR-LEGO) system, and showed gene expression in only a single cell of the root. When *WUSSEL* (*WUS*) gene, which functions in maintenance of the SAM, was ectopically expressed in the root, callus or a shoot-like structure was generated at the root tip. We observed induction of ectopic cell division when *WUS* was ectopically expressed in lateral-root-cap cells.

Publication List

〔Original papers〕

- Toyokura, K., Watanabe, K., Oikawa, A., Kusano, M., Tameshige, T., Tatematsu, K., Matsumoto, N., Tsugeki, R., Saito, K., and Okada, K. (2011) Succinic semialdehyde dehydrogenase is involved in the robust patterning of Arabidopsis leaves along the adaxial-abaxial axis. Plant Cell Physiol. *52*, 1340-1353.
- Ueda, M., Matsui, K., Ishiguro, S., Kato, T., Tabata, S., Kobayashi, M., Seki, M., Shinozaki, K., and Okada, K. (2011) Arabidopsis *RPT2a* encoding the 26S proteasome subunit is required for various aspects of root meristem maintenance, and regulates gametogenesis redundantly with its homolog, *RPT2b*. Plant Cell Physiol. *52*, 1628-1640.