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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 the 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-abaxial boundary in the leaf primordium is determined by the precise expression of the adaxial marker gene, *PHABULOSA* (*PHB*), and the abaxial marker genes, *FILAMENTOUS FLOWER* (*FIL*) and *YABBY3* (*YAB3*). We showed that PHB is expressed in cells of the adaxial side and separated clearly from the abaxial side-specific *FIL* gene expressing cells, by the action of microRNA165/166 which targeted the *PHB*, *REV*, and *PHV* messenger RNAs. We also revealed by laser microdissection that specific expression of *MIR165/166* genes in the abaxial side is important for adaxial-abaxial boundary formation.

To examine the mechanisms of boundary formation, 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. The structure of the SAM is often aberrant in *enf1* mutants. 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* affects 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, suggesting that chloroplast development is required for normal differentiation of leaf tissues.

There are methylated tandem repeat sequences in the promoter region of another abaxial side-marker gene, *YAB3*. We revealed that the methylation of the region occurred in a Pol IV/Pol V-dependent manner. We also obtained results suggesting the methylated sequences occur at a higher frequency in the cells of the adaxial-side of leaves than in those of the abaxial side.

A line of unique oblong cells is found at the marginal edge of leaves (Fig. 1 left). 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 (Fig. 1 right). 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.

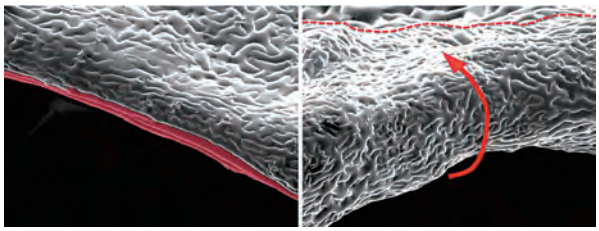

Figure. 1 Oblong cells located at the marginal edge of wild type *Arabidopsis* leaves (painted in red in left panel). In the *prs wox1*double mutant, the long margin-specific cells are absent, and the abaxial side-specific epidermal cells seem to "invade" the adaxial side surface (shown by the arrow in right panel). A red-dashed line indicates the border between the adaxial-side epidermal cells and those of the abaxial side.

II. Biochemical approach

We are taking another approach to study 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 collected from apoplast fractions of the heads of cauliflower (*Brassica oleracea* L. var. *botrytis*) and Arabidopsis *apetala1 cauliflower* double mutants were analyzed by peptide

sequence methods or LC-MS/MS methods. We chose about 30 peptides as the candidates, and prepared synthetic peptides based on the obtained sequences. When we applied the peptides to Arabidopsis seedlings, several peptides caused morphological defects in the SAM, vascular tissue and root development. We are currently examining the mechanisms involved.

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. Currently, we are ablating some of the margin-specific cells of leaf primordia to analyze the role of these cells.

IV. One-cell gene induction approach

As a new tool for examining the intercellular communication system, we are developing the one-cell induction system *in planta* using the InfraRed Laser Evoked Gene Operator (IR-LEGO) system. We irradiated root epidermal cells of a transgenic line carrying the Arabidopsis heart-shock promoter *hsp18.2*::*GUS* fusion gene. GUS expression was observed in irradiated cells (Fig. 2 left). Induction of a single cell is possible by focusing the irradiation (Fig. 2 right).

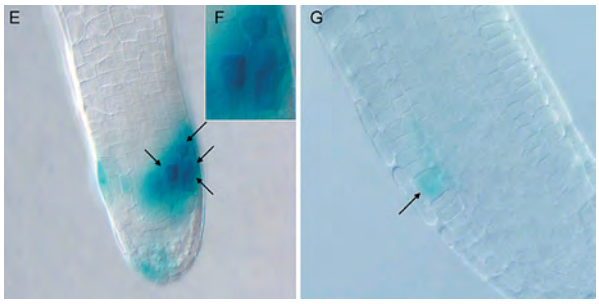

Figure. 2 GUS expression induced by IR laser irradiation to 4 target cells arranged in a diamond shape. The four irradiated cells (arrows) expressed GUS strongly (left). However, weak staining was found in the surrounding cells. Top right panel shows magnified image. GUS expression was restricted to the targeted single cell (arrow in Right) using different laser irradiation settings.

Publication List

〔Original papers〕

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- Tominaga-Wada, R., Iwata, M., Sugiyama, J., Kotake, T., Ishida, T., Yokoyama, R., Nishitani, K., Okada, K., and Wada, T. (2009) The GLABRA2 homeodomain protein directly regulates CESA5 and XTH17 gene expression in Arabidopsis roots. Plant J. *60*, 564-574.
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