## 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, and biochemical 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 epidermal and mesophyll 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 that *MIR165A* is enough to repress the PHB expression in the cells located in the abaxial side, indicating that miR165 is likely to move to cells at the adaxial side. When miR165 was produced by another primary miRNA transcript backbone, pri-miR319a, in the abaxial epidermal cells, the activity of miR165 was observed in the whole region of leaf primordia, showing that sequence and/or structure of *MIR165A* primary transcript is necessary to restrict the miR165-active region to the abaxial-side cells. Thus we concluded that the number of cells in which miR165 can move is determined depending on the *MIR165A* primary transcript (Tatematsu et al., in preparation).

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*). We revealed that one of them, *enf1*, has a mutation in a gene that encodes SUCCINIC SEMALDEHYDE DEHYDROGENASE, which catalyzes the conversion of succinic semialdehyde (SSA) to succinate, and that SSA and/or its derivatives affect the axis-dependent cell fate in leaf primordia (Toyokura et al., 2011, 2012). We also isolated some suppressor mutants of *enf1*, which show normal leaf shapes and expression domain of *FIL* promoter:*GFP*. 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.

Detailed analysis of temporal and spatial expression pattern of *FIL* in a developing leaf primordium using reporter gene combined with the Cre-LoxP recombinant system showed dynamic shift of the *FIL*-expression domain (Figure 1). This

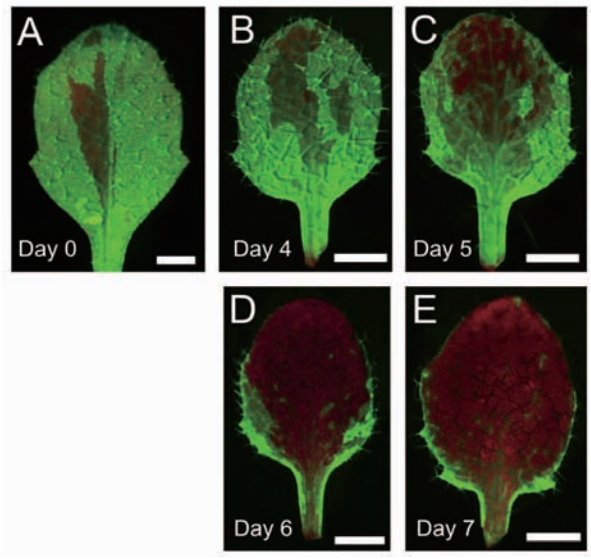


Figure 1. Cell lineages of *FIL*-expressing cells were visualized using a transgenic line having *FIL* promoter:*Cre-GR* and *35S:LoxP-ter-LoxP-YFP*. After dexamethasone (DEX) treatment, YFP is expressed constitutively in the *FIL*-expressing cells by Cre-LoxP recombinant system. We applied DEX at various timing after seed germination and checked YFP signal in the third leaves of 12-day-old seedlings. Photo was taken from the adaxial side. Green and red colors indicate YFP and autofluorescence of chlorophyll, respectively. Strong green colors indicate YFP expressed in the adaxial epidermal- and adaxial mesophyll cells, and weak colors indicate YFP expressed in the adaxial mesophyll cells, but not in the adaxial epidermis. The day of DEX treatment after seed germination is indicated at the bottom left of each panel. Bars represent 1 mm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

†: This laboratory was closed on 31 March, 2013.

result indicates that the boundary between the expression domain of *FIL* and that of *PHB* moves to the abaxial side during leaf development. Another enlarged-*FIL*-expression domain mutant, *enf2*, which has abaxialized leaves, showed slower moving of the boundary than that in wild type. We also indicated that the *ENF2* gene encoded a plastid-localized unknown protein, and that the state of expression of chloroplast genome-encoding genes might be a factor determining the rate of the boundary shifting between the *PHB*- and the *FIL*-expression domains. Using a simple mathematical model, we also predicted that general cellular functions, such as production and/or degradation of the determining genes, regulate the rate of the boundary shifting during leaf development (Tameshige et al., in press).

We examined the role of homeobox-related gene, *PRESSED FLOWER* (*PRS*) and its homolog, *WOX1*, and showed that they are required for forming the margin-specific oblong cells. Genetic analyses showed that *PRS* and *WOX1* control blade outgrowth and determine the expression domains of the adaxial- and the abaxial-determining genes in the marginal region of leaf primordia. Based on the observations we proposed the middle domain model (Nakata et al., 2012; Nakata and Okada 2012). To identify the genes acting in the downstream of *WOX1*, we carried out gene expression profiling using Arabidopsis genome array in the *WOX1* overexpressing transgenic plants. This analysis indicates that *WOX1* directly regulates the expression level of several adaxial and abaxial determining genes, suggesting that three domains, the adaxial-, the abaxial-, and the middle-domain, interact mutually during leaf developments. We also found that the expression of some auxin-inducible genes was repressed in the *WOX1* overexpression plants. To reveal the relationships between *WOX1* and auxin, we are analyzing the auxin responsibility and expression of auxin-related genes in the shoot apex of wild type and *prs wox1* seedlings (Nakata et al., in preparation).

To reveal how floral organs fix their forms through development processes, we analyzed a series of mutants named *folded petals* (*fop*) with stacked and folded petals when flowers open. We found that epidermal cells of the mutant petal show traces of stacking with the epidermal cells of sepals. 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 between the floral organs in a developing bud (Takeda et al., 2013).

## II. Biochemical approach

We are taking a biochemical approach to study of the intercellular signaling system by analyzing small peptides, which are present in the apoplastic region of the SAM. We purified fractions containing small peptides from apoplast fractions of the curds of cauliflower (*Brassica oleracea* L. var. *botrytis*), and found a putative lipid transfer protein (LTP) has a role in increasing the number of SAMs in Arabidopsis seedling when applied exogenously. The cauliflower *LTP* gene was highly expressed in the curd, and the expression of the Arabidopsis ortholog was observed in the L1 cell layers of the SAM (Figure 2A, B). When *LTP* expressed under constitutive active promoter in Arabidopsis, the transgenic plants showed increased number of SAMs near the shoot apex (Figure 2C). Moreover, to analyze the role of *LTP* gene *in planta*, we made RNAi knockdown lines of Arabidopsis *LTP*. In some seedlings of the knockdown lines, multiple cotyledon and/or filamentous leaves were observed. Thus, we concluded that LTP proteins in apoplasts are responsible for SAM maintenance and formation.

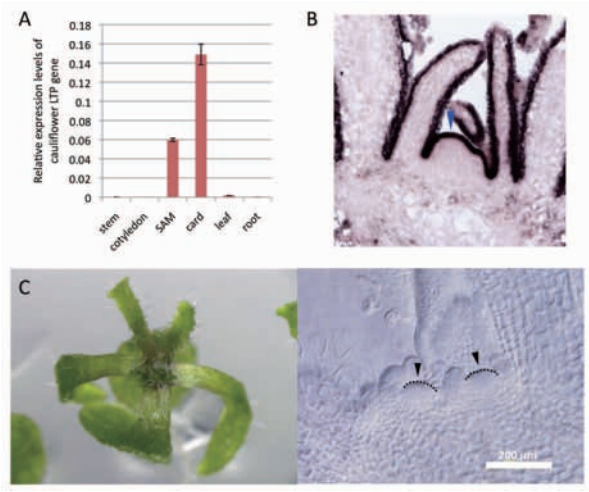


Figure 2. (A) Expression levels of cauliflower *LTP* genes in cauliflower organs analyzed by qRT-PCR methods. (B) Pattern of *in situ* hybridization of Arabidopsis *LTP* gene showing expression in epidermis of a wild type seedling. A blue arrowhead indicates the SAM. (C) (Left) 10-day-old seedling of Arabidopsis *LTP* overexpression plants. (Right) A longitudinal section of the seedling. Black arrowheads indicate the SAMs, and black dashed lines indicate shapes of the SAMs.

## Publication List

### 〔Original papers〕


- Endo, A.*, Tatematsu, K.*, Hanada, K.*, Duermeyer, L., Okamoto, M., Yonekura-Sakakibara, K., Saito, K., Toyoda, T., Kawakami, N., Kamiya, Y., Seki, M., and Nambara, E. (2012). Tissue-specific transcriptome analysis reveals cell wall metabolism, flavonol biosynthesis, and defense responses are activated in the endosperm of germinating Arabidopsis thaliana seeds. Plant Cell Physiol. *53*, 16-27. (*: Equally contributed)
- Nakata, M., Matsumoto, N., Tsugeki, R., Rikirsch, E. Laux, T., and Okada, K. (2012). Roles of the middle domain-specific *WUSCHEL-RELATED-HOMEOBOX* genes in early development of leaves in Arabidopsis. Plant Cell *24*, 519-535.
- Nakata, M., and Okada, K. (2012). The three-domain model: A new model for the early development of leaves in *Arabidopsis thaliana*. Plant Signal. Behav. *7*, 1423-1427.
- Sakai, T., Mochizuki, S., Haga, K., Uehara, Y., Suzuki, A., Harada, A., Wada, T., Ishiguro, S., and Okada, K. (2012). The WAVY GROWTH 3 E3 ligase family controls the gravitropic responses in Arabidopsis root. Plant J. *70*, 303-314.
- Toyokura, K., Hayashi, M., Nishimura, M., and Okada, K. (2012). Adaxial-abaxial patterning: A novel function of the GABA shunt. Plant Signal. Behav. *7*, 705-707.