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 by using genetic, and biochemical approaches.

I. Genetic approach

Several factors regulating leaf development along the adaxia-abaxial axis are identified in *Arabidopsis thaliana* and other plants. The adaxial- and abaxial-specific tissue differentiation and the lamina expansion are determined by the precise expression of the adaxial marker genes, such as *HD-Zip III* including *PHABULOSA* (*PHB*), and the abaxial marker genes, such as *FILAMENTOUS FLOWER* (*FIL*) and *YABBY*. Using the reporter gene-system, we visualized the activity pattern of microRNA165 (miR165), which targeted the *HD-Zip III* messenger RNA, and showed that miR165 from *MIR165A* locus, which was expressed in the abaxial epidermis, act non-cell-autonomously in a present-or-absent manner on the abaxial-side cells, indicating that miR165 is likely to move toward the adaxial side. When replacing either the miRNA precursor or mature miRNA sequence of miR165 with the corresponding parts of other miRNA, it was revealed that the formation of the miR165 activity pattern depends both on the precursor backbone and the mature miRNA sequence of miR165. Thus we proposed that the rigid patterning of miR165 activity is confined by the *MIR165A* precursor sequence (Tatematsu *et al*., submitted).

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. This result indicates that the boundary between the expression domain of *FIL* and that of *PHB* gradually shifted from the adaxial side to the abaxial side during leaf development. We also found that several mutants defective in the adaxial-abaxial cell differentiation and the lamina expansion showed altered speed of the boundary shifting, indicating that the boundary shifting correlates with the precise leaf development. Furthermore, genetic analysis and chemical-treatment experiments revealed that the state of expression of chloroplast genome-encoding genes might be a factor determining the speed of the boundary shifting between the *PHB*- and the *FIL*-expression domains (Tameshige *et al*., 2013).

II. Biochemical approach

We are taking a biochemical approach to isolate small peptides, which have a role in the intercellular signaling system of the SAM, from the apoplastic region of the curds of cauliflower (*Brassica oleracea* L. var. *botrytis*). We obtained a putative lipid transfer protein (LTP), which increased the number of SAMs in Arabidopsis seedlings 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. When expressed under constitutive active promoter in Arabidopsis, the transgenic plants showed increased number of SAMs near the shoot apex. Moreover, we analyzed RNAi knockdown lines of Arabidopsis *LTP*, and found that some seedlings of the knockdown lines had filamentous leaves and lost the SAM. Thus, we concluded that LTP proteins in apoplasts are responsible for SAM formation and maintenance (Yabe, Tatematsu, Tsuchida *et al*., manuscript in preparation).

Publication List

〔Original papers〕

- Ikeuchi, M., Tatematsu, K., Yamaguchi, T., Okada, K., and Tsukaya, H. (2013). Precocious progression of tissue maturation instructs basipetal initiation of leaflets in *Chelidonium majus* subsp. *asiaticum* (*Papaveraceae*). Am. J. Bot. *100*, 1116-1126.
- Miyashima, S., Honda, M., Hashimoto, K., Tatmeatsu, K., Hashimoto, T., Sato-Nara, K., Okada, K., and Nakajima, K. (2013). A comprehensive expression analysis of *Arabidopsis MICRORNA165/6* gene family in embryogenesis revealed a conserved role in meristem specification and a non-cell-autonomous function. Plant Cell Physiol. *54*, 375-384.
- Takeda, S., Iwasaki, A., Matsumoto, N., Tatematsu, K., and Okada, K. (2013). Physical interaction between floral organs controls petal morphogenesis in *Arabidopsis thaliana*. Plant Physiol. *161*, 1242-1250.
- Tameshige, T., Fujita, H., Watanabe, K., Toyokura, K., Kondo, M., Tatematsu, K., Matsumoto, N., Tsugeki, R., Kawaguchi, M., Nishimura, M., and Okada, K. (2013). Pattern dynamics in adaxial-abaxial specific gene expression are modulated by a plastid retrograde signal during Arabidopsis leaf development. PLoS Genetics *9*, e1003655.

〔Review article〕

- Nakata, T., and Okada, K. (2013). The leaf adaxial-abaxial boundary and lamina growth. Plants *2*, 174-202.

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