LABORATORY OF PLANT DEVELOPMENT AND PHYSIOLOGY

Specially Appointed Associate Professor
KAWADE, Kensuke

Adjunct Professor (BIO-NEXT Project, OIIB): *TSUKAYA, Hirokazu*
Postdoctoral Fellow: *NOZAKI, Mamoru*
Visiting Graduate Student: *TOMOI, Takumi*
Technical Assistant: *YAMAGUCHI, Chinami*
Secretary: *HACHISUKA, Midori*

Development and metabolism are intertwined with one another during organogenesis. This interaction is essential to maintain the metabolic state in a developmental context-dependent manner. There is also a growing awareness that metabolism plays instructive roles in developmental processes. The emerging picture depicts metabolism as a critical system not only for sustaining physiological conditions, but also regulating developmental patterning by coordinating various cellular processes. However, it remains largely unclear how this interaction is achieved in multicellular organisms. We aim to reveal as-yet-unknown relationships between developmental and metabolic processes and their biological meaning by elucidating molecular mechanisms of the system. To address this, we use trans-omics approach including metabolome and transcriptome analyses using *Arabidopsis thaliana* as a model, in conjunction with standard molecular genetics and biochemistry techniques.

I. Cytochrome P450 epoxidase for embryonic patterning

To uncover hidden relationships between development and metabolism, we performed quantitative phenome screening using *A. thaliana* mutants of orphan cytochrome P450 genes. We identified the *cyp77a4* mutant, which exhibits irregular embryonic patterning as evidenced by defects, such as a cup-shaped cotyledon morphology (Figure 1). Although CYP77A4 is the first cytochrome P450 reported to be able to catalyze the epoxidation of fatty acids in plants, its function in development is completely unknown.

Through the use of auxin-related reporters, we determined that CYP77A4 is essential for polar auxin transport via proper localization of PIN1 (an auxin efflux carrier). Interestingly, unlike other enzyme mutants defective in auxin dynamics in ubiquitous tissues, the *cyp77a4* mutant was associated with defects specifically in embryos. Furthermore, our double mutant analysis clarified that *CYP77A4* and *CYP77A6* (the phylogenetically closest gene to *CYP77A4*) are functionally independent. Based on these findings, we proposed that the metabolic requirement for polarity establishment via auxin dynamics differ between tissues, and that in embryos this depends on a CYP77A4-dependent metabolic pathway. These findings may augment our understanding of fatty-acid epoxidation by uncovering a novel developmental function of the epoxidase (Kawade et al., *submitted*).

Figure 1. Irregular arrangement of cotyledons in the *cyp77a4* mutants. (A-E) WT-like (A), abnormally arranged (B), single (C and D), and cup-shaped (E) cotyledons in the *cyp77a4-3* mutants. Bars = 2 mm.

II. Developmental signal intertwined with metabolism

ANGUSTIFOLIA3 (AN3) is a transcriptional co-activator, which promotes cell proliferation in leaves. We recently showed that AN3 forms an expression gradient along a proximal-to-distal developmental axis to regulate cell proliferation dynamics in time and space (Kawade *et al*., 2017; Figure 2). To gain further insights into how AN3 contributes to tissue patterning, we conducted transcriptome and metabolome analyses using the *an3* mutants. We found that AN3 regulates a transcriptional network for oxygen homeostasis. Our metabolic profiling indeed detected characteristic features of redox disturbance in the *an3* mutants. Although cell proliferation is an essential process for tissue patterning, reactive oxygen species are generated through energy production. It would be interesting to assume that the AN3 signal may solve this dilemma, cell proliferation *vs*. oxidative stress, in plant development.

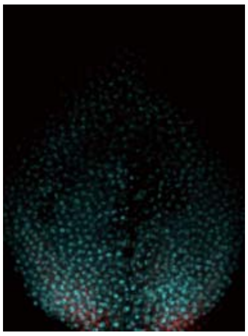

Figure 2. A merged image of the leaf primordia (around 200-μm length) expressing genetically engineered mobile or immobile AN3 (cyan or red, respectively). The distribution of the mobile AN3 gradually spreads along the leaf proximal-to-distal axis (from down to top), which is broader than that of the immobile one.

Publication List:

〔Original papers〕

- Kawade, K., Tanimoto, H., Horiguchi, G., and Tsukaya, H. (2017). Spatially different tissue-scale diffusivity shapes ANGUSTIFOLIA3 gradient in growing leaves. Biophys. J. *113*, 1109-1120.
- Kawade, K., and Tsukaya, H. (2017). Probing the stochastic property of endoreduplication in cell size determination of *Arabidopsis thaliana* leaf epidermal tissue. PLoS ONE *12*, e0185050.
- Sawada, Y., Tsukaya, H., Li, Y., Sato, M., Kawade, K., and Hirai, M.Y. (2017). A novel method for single-grain-based metabolic profiling of Arabidopsis seed. Metabolomics *13*, 75.