LABORATORY OF PLANT DEVELOPMENT AND PHYSIOLOGY

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There has been growing evidence that metabolic regulation has specific impacts on plant development. The picture emerging depicts the metabolism as a dynamic system that controls and/or supports developmental progression. Despite these advances, it remains largely unclear how metabolism is regulated behind developmental process. We aim to uncover as-yet-unknown relationships between developmental and metabolic processes in plants and their biological meaning by elucidating molecular mechanisms for the system. To address this, we primary 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. Functional screening of orphan metabolic enzymes from phenome data

To explore as-yet-unknown relationships between developmental and metabolic processes, we carried out functional screening of *Arabidopsis thaliana* mutants of orphan genes encoding metabolic enzymes. We examined 12 non-biased traits including leaf size, primary root length, seed color, *etc*. A large number of mutants grew normally compared to wild type (WT), probably due to gene functional redundancy. However, we found that cotyledon size is smaller and more variable in one mutant line. A more severe morphological defect in this mutant is irregular arrangement of cotyledons. Although bilateral arrangement of cotyledons was observed in WT, this mutant forms non-bilateral, single or cup-shaped cotyledon (Figure 1). This defect in cotyledon morphology/arrangement could be detected from early embryo development. Confocal microscopy using various auxin-related markers revealed that cellular polarity is often disordered by this mutation, resulted in abnormal distribution of auxin in the mutant embryos. To identify the *in vivo* metabolic target of this enzyme, metabolome analysis using gas-chromatography mass-spectrometry (GC-MS) will be performed.

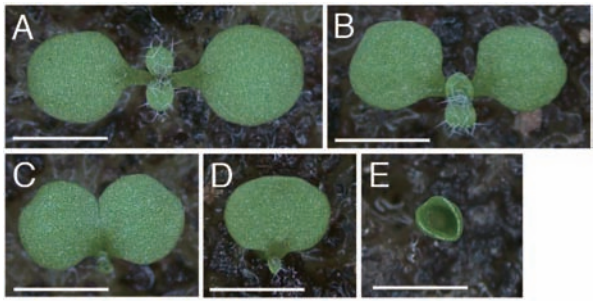

Figure 1. Irregular arrangement of cotyledons in the enzyme mutant. Wild type has two bilateral cotyledons (A). In contrast, our enzyme mutant shows abnormally-arranged (B), fused (C), single (D) and cup-shaped cotyledons (E). Bars = 2 mm.

II. Characterization of developmental signal intertwined with metabolism control

We recently uncovered that one of the plant developmental signals forms an expression gradient along the leaf proximal-to-distal axis to determine the cell-proliferation domain (Kawade et al., *submitted*). By metabolome and transcriptome analyses, we have uncovered that this developmental signal regulates not only cell proliferation but also leucine metabolism and/or tricarboxylic acid (TCA) cycle (Figure 2). When we cultured an *Arabidopsis thaliana* mutant of this developmental signal on culture media with higher leucine, this mutant showed hypersensitivity to this amino acid, resulted in growth arrest. Interestingly, we found that redox condition in this mutant was perturbed at the transcriptome level. We are now re-confirming the change in metabolic profile using liquid chromatography-mass spectrometry (LC-MS) analysis in collaboration with the Functional Genomics Facility in NIBB, and also examining the mutant's redox status to clarify an interaction of leucine metabolism and/or TCA cycle with redox homeostasis. This study would provide new insights into how a developmental signal coordinately regulates primary metabolism and redox condition, and then how this metabolic homeostasis has functional importance on developmental processes including cell proliferation.

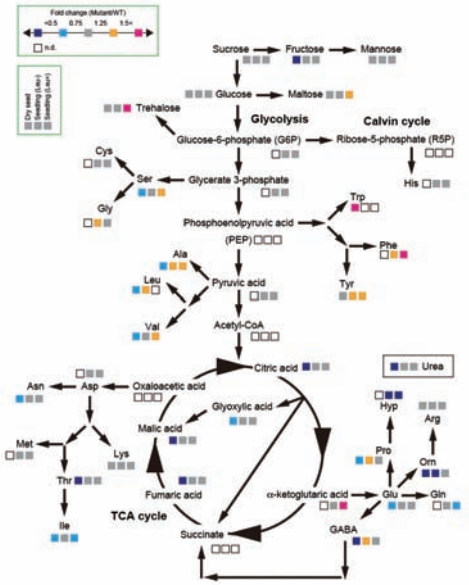

Figure 2. Metabolic change in the mutant of the developmental signaling detected by GC-MS metabolomics technique. Colors indicate change in metabolite content in dry seed, normally cultured seedling and seedling treated with leucine (left to right). Sugars, amino acids and organic acids in the TCA cycle are mainly summarized here.