## DIVISION OF PLANT ENVIRONMENTAL RESPONSES

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Plant organs have the ability to sense various vectorial stimuli such as light, humidity, gravity, *etc*. and to reorient their growth direction so as to enhance their chances of survival and acclimatize to their environments. These responses in plant organs are referred to as tropisms. Gravitropism is one of major determinant for the direction of the growth angle of plant organs. In gravity sensing cells (statocytes), plastids that accumulate starch in high-densities relocate toward the direction of gravity. Amyloplasts relocationis used as signal to physically trigger biochemical signal transduction, which in turn leads to the regulation of the polar auxin transport necessary for change in the growth direction of plant organs.

The above points have raised the following important questions: 1) How is amyloplast relocation converted into biochemical signals? 2) How does the signal affect the directional plant growth? We aim to understand the detailed molecular mechanism of gravity signaling by applying a genetical and molecular biological approach using model plant *Arabidopsis thaliana*. We are currently focusing on the function of novel gravity signaling factor *LAZY* (*LZY*) genes and its interactors.

## I. Analysis of molecular function *LZY* genes

To identify genes involved in gravity signaling in statocytes, we carried out transcriptome analysis using statocyte-deficient Arabidopsis mutants and found that several *LZY* genes are specifically expressed in the statocytes. We have shown that *LZY* genes are required for gravity signal transduction in statocytes following amyloplast relocation, thus determining the growth angle of plant organs (Figure 1). We are currently focusing on the analyses of the molecular function of LZY and the regulation of LZY protein levels.

### 1-1 *LZYs* are key gravity signaling factors in Arabidopsis roots and shoots

We have shown that the *lzy1 2 3* triple mutant exhibits greatly reduced gravitropism both in its primary roots and shoots. The differential auxin distribution in the organs, that is necessary for gravitoropic bending failed form in the mutant roots. Detailed observation indicated that development and behavior of amyloplasts in the triple mutant are normal. Furthermore, artificial expression of *LZY3* in statocytes clearly complemented the phenotype of gravitropism in the triple mutant. This experimental evidence strongly suggests that *LZYs* play an important role in gravity signaling after amyloplasts relocation.

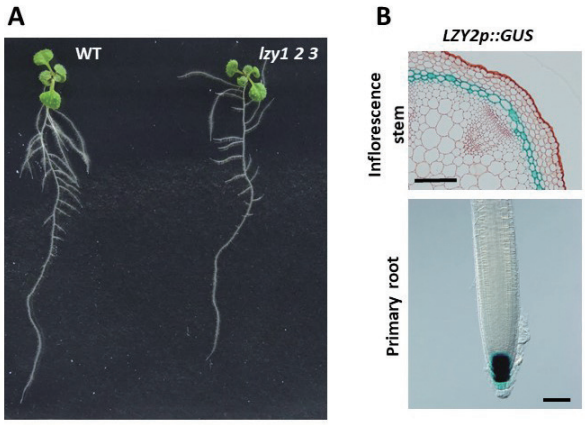


Figure 1. A, Phenotype of *lzy1 2 3* triple mutant. The mutant shows defect in growth angle of lateral roots. B, Expression analysis of *LZY2p:GUS*. Clear GUS staining are observed in endodermal cells in shoot and columella cells in root, respectively.

### 1-2 Subcellular localization of LZY3-mCherry in columella cells

We have found that a very low level of LZY proteins function within gravity signaling. Clear-see method barely visualized the fluorescence signal of LZY3-mCherry driven by native *LZY3* promoter in columella cells. Interestingly, LZY3-mCherry was localized on the plasma membrane at the bottom side of cells. The localization also appeared to change in response to the organ inclination. The plasma membrane localization of LZY3 was also confirmed by transient assay using Arabidopsis cultured cells (Figure 2). Analysis of amino-acid sequence of LZYs indicated no putative transmembrane domain. We are currently trying to reveal the mechanism of membrane localization of LZY3.

### 1-3 Protein level and tissue distribution of LZY3 affects growth angle of lateral roots

Under the control of the native LZY3 promoter, a feint *LZY3-mCherry* signal was detected in columella cells, indicating a very low expression level of LZY3 protein. To investigate the relationship between the expression level of LZY3 and gravitropic response, we generated a transgenic plant harboring LZY3-mCherry driven by the statocytes specific promoter combined with β-estradiol inducible system. We found that the protein level of LZY3-mCherry is able to be controlled in estradiol concentration dependent manner in columella cells. The proper induction of LZY-mCherry clearly complemented *LZYs* phenotype, whereas an excessive induction disturbed the direction of the growth angle in roots. Observations using a confocal microscope revealed that excessive LZY3-mCherry was localized on the plasma membrane uniformly in columella cells treated with a high concentration of β-estradiol. We found that the treatment of proteasome inhibitor MG132 significantly increased the LZY3-mCherry signal in the columella cells of plants carrying *LZY3p:LZY3-mCherry*. These results suggest that

the existence of proteasome-dependent regulatory mechanism in regulating the proper LZY3 protein level.

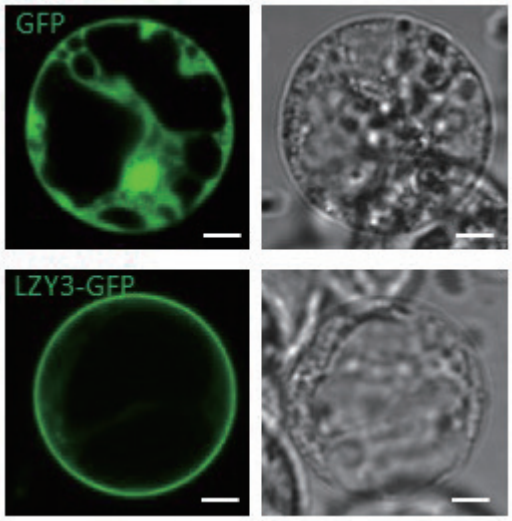


Figure 2. LZY3-GFP localization in protoplast of Arabidopsis cultured cells. Bar=10 $\mu$m

## II. RCC1-like domain (RLD) proteins as LZY-interactor.

We have so far tried to identify LZYs interacted protein using yeast two-hybrid system and immunoprecipitation coupled with mass spectrometry in order to elucidate the molecular function of LZYs. We identified RLD family proteins as candidates. The *RLD* family is composed of eight genes in Arabidopsis and contain PH domain, RCC1-like motif repeat, FYVE domain and BRX domain. Further analysis revealed that LZY C-terminal 14 amino acids interact with BRX domain in RLDs. Also, LZYs seems to recruit RLDs to the plasma membrane through their mutual interaction. Among RLD family genes, *RLD1* and *RLD4* contribute gravity signaling in columella cells. Because *rld1, 2, 3, 4* quadruple mutant seedlings show severe morphological defects, which resemble those of plant ARF-GEF *gnom* mutant, *RLDs* are possibly involved in membrane trafficking. We are analyzing the biochemical activity of RLD proteins, as well as characterizing the molecular function of *RLD5-8*.

### Publication List:

〔Original paper〕


- Yamamoto, T., Yoshida, Y., Nakajima, K., Tominaga, M., Gyohda, A., Suzuki, H., Okamoto, T., Nishimura, T., Yokotani, N., Minami, E., Nishizawa, Y., Miyamoto, K., Yamane, H., Okada, K., and Koshiba, T. (2018). Expression of RSOsPR10 in rice roots is antagonistically regulated by jasmonate/ethylene and salicylic acid via the activator OsERF87 and the repressor OsWRKY76, respectively. Plant Direct *2*, e00049.