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– Molecular Biology –

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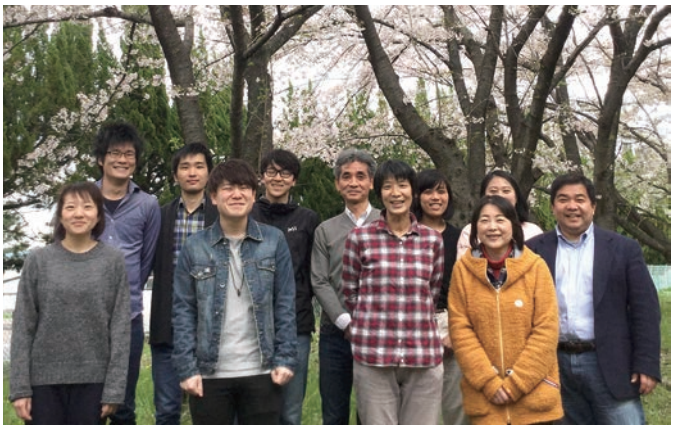
Scope of Research

This laboratory aims at clarifying molecular bases of regulatory mechanisms for plant development, especially plant morphogenesis, with techniques of forward and reverse genetics, molecular biology, and biochemistry. Current major subjects are: 1) phospholipid signaling in cell morphogenesis, 2) the transcriptional network for cytokinin responses, 3) COP9 signalosome modulating signal transduction in the nuclei, and 4) the endoreduplication cell cycle in cell differentiation.



KEYWORDS

Morphogenesis
Signal Transduction
Phospholipid Signaling
COP9 Signalosome
RNA

Selected Publications

Wu, Z.; Zhu, D.; Lin, X.; Miao, J.; Gu, L.; Deng, X.; Yang, Q.; Zhu, D.; Cao, X.; Tsuge, T.; Dean, C.; Aoyama, T.; Gu, H.; Qu, L.-J., RNA Binding Proteins RZ-1B and RZ-1C Play Critical Roles in Regulating Pre-mRNA Splicing and Gene Expression during Development in *Arabidopsis*, *Plant Cell*, **28,** 55-73 (2016).

Lin, Q.; Ohashi, Y.; Kato, M.; Tsuge, T.; Gu, H.; Qu, L.-J.; Aoyama, T., GLABRA2 Directly Suppresses Basic Helix-loop-helix Transcription Factor Genes with Diverse Functions in Root Hair Development, *Plant Cell*, **27,** 2894-2906 (2015).

Wada, Y.; Kusano, H.; Tsuge, T.; Aoyama, T., Phosphatidylinositol Phosphate 5-kinase Genes Respond to Phosphate Deficiency for Root Hair Elongation in *Arabidopsis thaliana*, *Plant J.*, **81,** 426-437 (2015).

Hayashi, K.; Nakamura, S.; Fukunaga, S.; Nishimura, T.; Jenness, M. K.; Murphy, A. S.; Motose, H.; Nozaki, H.; Furutani, M.; Aoyama, T., Auxin Transport Sites are Visualized *in Planta* Using Fluorescent Auxin Analogs, *Proc. Natl. Acad. Sci. USA*, **111,** 11557-11562 (2014).

Kato, M.; Aoyama, T.; Maeshima, M., The Ca^{2+}-binding Protein PCaP2 Located on the Plasma Membrane is Involved in Root Hair Development as a Possible Signal Transducer, *Plant J.*, **74,** 690-700 (2013).

Biological Functions of Phosphoinsitide Signaling in Plant Cells

Phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] regulates signal transduction for not only total cellular responses but also intracellular localizing events. It modulates the functions of a variety of actin regulatory proteins and regulators of exocytotic machinery on the plasma membrane by directly interacting with its effector proteins. In many cases, PtdIns(4,5)P_2 signaling pathways are tightly connected to those of small GTPases belonging to the Rho and Arf families in their upstream and downstream cascades. PtdIns(4,5)P_2 is also expected to play a pivotal regulatory role in the polarized expansion of plant cells. Indeed, PtdIns(4,5)P_2 localizes to the apical plasma membrane and cytoplasmic space of not only root hairs but also pollen tubes. We are studying on signaling functions of PtdIns(4,5)P_2 and its producing enzymes, PIP5Ks, in *Arabidopsis thaliana*. Recently, fluorescent molecular probes for phosphoinositides were drastically improved. Taking advantage of those probes, we are observing specific and dynamic localization patterns of PtdIns(4,5)P_2 in various types of *Arabidopsis* cells (Figure 1).

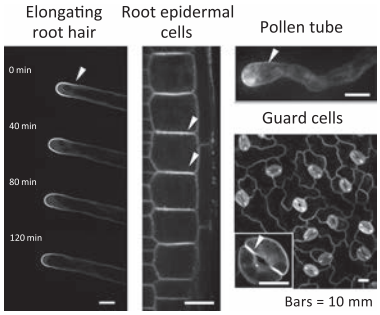


Figure 1. Dynamic localization patterns of PtdIns(4,5)P_2 markers (*UBQ10* promoter-driven fluorescence protein-fused 2xPH$^{PLC\delta}$). Arrowheads indicate loci where PtdIns(4,5)P_2 markers are preferentially localized.

Function of an *Arabidopsis thaliana* Brix Family Protein Gene in Female Gametogenesis

Male and female gametophytes, also called pollen grains and embryo sacs, respectively, include a few haploid cells embedded in the sexual reproductive organs of angiosperms. During gametogenesis of the female gametophyte in *Arabidopsis*, first, a hypodermal archesporial cell differentiates into a megaspore mother cell, which undergoes meiosis to produce four haploid megaspores. Three of these megaspores at the micropylar pole go through programmed cell death, while the chalazal-most one survives and then undergoes three consecutive rounds of nuclear division to give rise to an eight-nucleate, cenocytic embryo sac. Next, nuclear migration and cellularization occur, resulting in a seven-celled embryo sac: three antipodal cells at the chalazal pole, one diploid central cell, an egg cell and two synergid cells at the micropylar end (Figure 2).

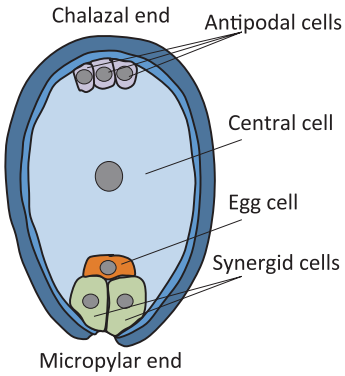


Figure 2. Schematic illustration of a seven-celled embryo sac.

AtSNAIL1 shares high sequence identity with yeast Ssf1 and Ssf2 proteins. The yeast strain *ssf1*-Δ *ssf2*-Δ was lethal. Reduction of the SSF gene products resulted in arrested cell division cycles and a decrease in the cell-mating efficiency. Further investigation showed that Ssf1 was required for synthesis of 5.8S and 25S rRNA and, thus, essential for the synthesis of the large ribosomal subunit. We found that the disruption of the *AtSNAIL1* gene caused retarded progression of mitotic division cycles during female gametophyte development *in vivo*, and thus led to severe reduction of female transmission efficiency in *snail1* (Figure 3). Furthermore, we found that the mutation in *SNAIL1* caused delay and failure in protein synthesis in the synergid cell. These indicate that functionally conserved *AtSNAIL1* is essential for reproductive development in *Arabidopsis*, possibly by affecting ribosome biogenesis.

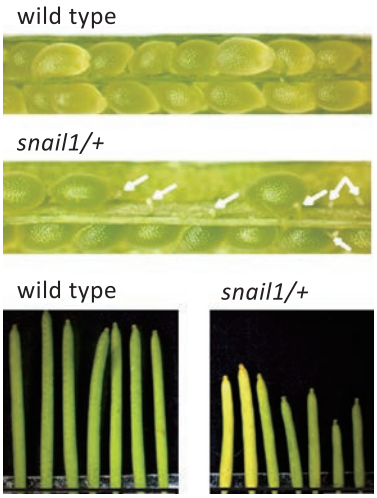


Figure 3. Seed sets (upper panels) and siliques (lower panels) of wild-type and *snail1*/+ heterozygous plants. Arrows indicate unfertilized ovules in the *snail1*/+ seed set.