# **Division of Biochemistry** – 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 phospholipid signalings in cell morphogenesis, the transcriptional network for cytokinin responses, COP9 signalosome modulating signal transduction in the nuclei, and the endoreduplication cell cycle in cell differentiation.



### **KEYWORDS**

Morphogenesis Signal Transduction Phospholipid

COP9 Signalosome Cytokinin

### **Selected Publications**

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<sup>2+</sup>-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).

Lin, Q.; Aoyama, T., Pathways for Epidermal Cell Differentiation *via* the Homeobox Gene *GLABLA2*: Update on the Roles of the Classic Regulator, *J. Integr. Plant Biol.*, **54**, 729-737 (2012).

Aki, S.; Nakai, H.; Aoyama, T.; Oka, A.; Tsuge, T., *AtSAP130/AtSF3b-3* Function is Required for Reproduction in *Arabidopsis thaliana*, *Plant Cell Physiol.*, **52**, 1330-1339 (2011).

Taniguchi, Y. Y.; Taniguchi, M.; Tsuge, T.; Oka, A.; Aoyama, T., Involvement of *Arabidopsis thaliana* Phospholipase Dζ2 in Root Hydrotropism through the Suppression of Root Gravitropism, *Planta*, **231**, 491-497 (2010).

### Phosphatidylinositol Phosphate 5-Kinase Genes Mediate a Phosphate-Deficiency Signal to Root Hair Elongation in *Arabidopsis thaliana*

Root system architecture is highly plastic and responsive to the surrounding growth conditions, ensuring that plants adapt to variegated underground environments. Soil phosphate (Pi) deficiency is a challenging growth condition that land-based plants encounter frequently. To cope with this difficulty, plants drastically alter their root system architecture by modifying the length and branching patterns of roots, and the length and density of root hairs, as well as their metabolic state throughout the whole plant. During Pi deficiency, most accessions of Arabidopsis thaliana suppress primary root growth via root apical meristem (RAM) exhaustion and impaired root cell elongation and enhance lateral root development, resulting in a short and bushy root system. These macro-scale responses in root system architecture enable exploration of the upper soil layers, where Pi tends to accumulate. In addition, in the micro-scale architecture of roots, Pi deficiency remarkably enhances root hair development and elongation for effective Pi absorption from soil (Figure 1).

Figure 1. Wild-type seedlings grown for 3 and 7 days after germination (DAG) under the Pisufficient and deficient conditions. Areal parts (upper row), and roots (lower row) of 3- and 7-DAG wild-type seedlings grown under the 1 mM and 3  $\mu$ M Pi conditions are shown. Bar = 1 mm (upper row), and 0.5 mm (lower row).



To elucidate the regulatory pathways specific to the root hair elongation response to Pi deficiency, we investigated the expression of type-B phosphatidylinositol phosphate 5-kinase (*PIP5K*) genes, as putative regulators of root hair elongation in *Arabidopsis*. Of the nine *Arabidopsis* B-type *PIP5K* genes, the *PIP5K3* and *PIP5K4* genes responded to Pi deficiency in steady-state transcript levels *via* PHR1binding sequences (PIBSs) in their upstream regions at young seedling stages. Both *pip5k3* and *pip5k4* single mutants, which exhibit short-root-hair phenotypes, remained responsive to Pi deficiency for root hair elongation. However, the *pip5k3pip5k4* double mutant exhibited shorter root hairs than the single mutants, and lost responsiveness to Pi deficiency at young seedling stages. In the tactical complementation line in which modified *PIP5K3* and *PIP5K4* genes with base substitutions in their P1BSs were co-introduced into the double mutant, root hairs of young seedlings had normal lengths under Pi-sufficient conditions, but were not responsive to Pi deficiency. From these results, we conclude that *PIP5K* genes connect a Pi-deficiency signal to the regulatory pathway for root hair elongation *via* their P1BSs.



Interestingly, the P1BS and sequences similar to the root hair cell-specific *cis*-element (RHE) in the *PIP5K3* upstream intergenic region are conserved in *Brassicaceae*, the family to which *Arabidopsis* belongs. It has been proposed that *Brassicaceae* plants take advantage of rapid root growth and root hair formation rather than mycorrhizal symbiosis as a strategy for nutrition uptake. *PIP5K3* might play a crucial role in enhancing the root hair elongation response to Pi deficiency in *Brassicaceae*, especially at young seedling stages, when macro-scale architectures of the root system are still immature.



Figure 3. Conserved cis-elements in the upstream intergenic regions of *Brassicaceae PIP5K3* orthologs.

Upstream intergenic sequences of *Arabidopsis thliana*, *Arabidopsis lyrata*, *Capsella rubella*, *Brassica rapa*, *Thellungiella parvula*, and *Thellungiella halophila* are aligned. Conserved nucleotides and *cis*-elements (RHE: 5'-GCACGT-3' and P1BS: 5'-GNATATNC-3') are marked in grey and black, respectively.