

# Division of Biochemistry

## – Molecular Biology –

<http://www.scl.kyoto-u.ac.jp/~molbio/index.html>



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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

Phospholipid Signaling

RNA

Signal Transduction

COP9 Signalosome



### 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).

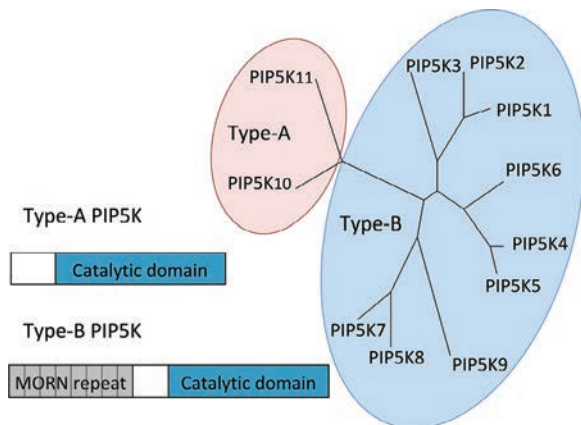
Hayashi, K.; Nakamura, S.; Fukunaga, S.; Nishimura, T.; Jenness, M. K.; Murphy, A. S.; Motose, H.; Nozaki, H.; Furutani, M.; Aoyama, T., Auxin Transport Sites are Visualized in *Planta* Using Fluorescent Auxin Analogs, *Proc. Natl. Acad. Sci. USA*, **111**, 11557-11562 (2014).

Kato, M.; Aoyama, T.; Maeshima, M., The Ca<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).

## Functional Differentiation of Phosphatidylinositol 4-Phosphate 5-Kinases in Higher Plants

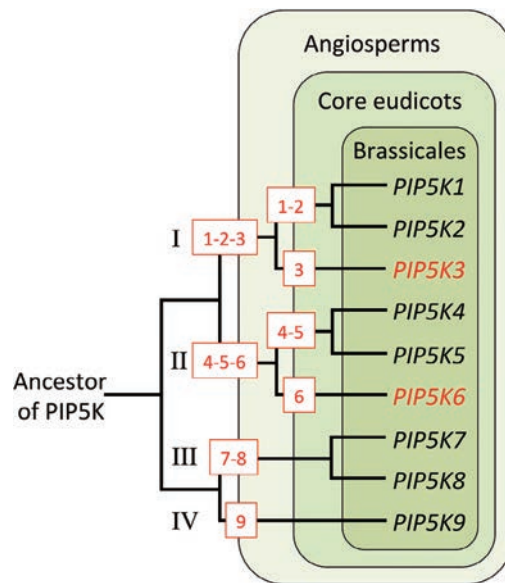
Phosphatidylinositol 4,5-bisphosphate [PIP(4,5)P<sub>2</sub>] acts as a signaling molecule and is involved in the regulation of a wide variety of intracellular events. PIP(4,5)P<sub>2</sub> modulates the functions of a variety of actin regulatory proteins and regulators of membrane traffic machinery from and to the plasma membrane by directly interacting with its effector proteins. In addition, PtdIns(4,5)P<sub>2</sub> signaling pathways are frequently connected to those of small GTPases belonging to the Rho and Arf families in their upstream and downstream cascades.

PtdIns(4,5)P<sub>2</sub> is expected to play a pivotal regulatory role in the polarized expansion of plant cells. Indeed, PtdIns(4,5)P<sub>2</sub> 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<sub>2</sub> and its producing enzymes, Phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks), in the model plant *Arabidopsis thaliana*. *A. thaliana* encodes two type-A and nine type-B Phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks), of which



**Figure 1.** PIP5Ks Encoded in *A. thaliana*. Domain structures and a phylogenetic tree of *A. thaliana* type-A and type-B PIP5Ks are shown.

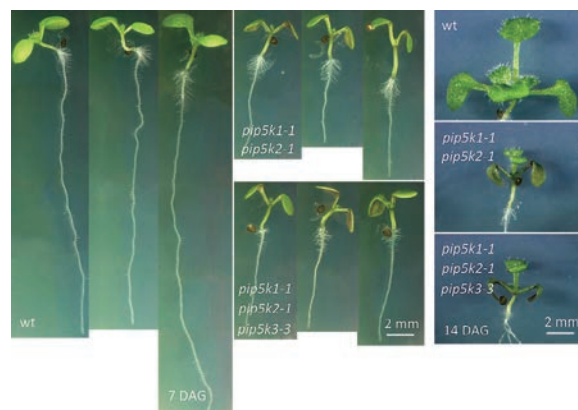
type-B PIP5Ks have a plant-specific structure with the MORN motif for membrane localization (Figure 1). We performed evolutionary analysis of type-B PIP5Ks using their amino-acid sequences available in public databases, and found that four clades of type-B PIP5Ks (I, II, III and IV, containing *Arabidopsis* PIP5K1-3, PIP5K4-6, PIP5K7-8, and PIP5K9, respectively) are conserved in angiosperms (Figure 2), suggesting that each clade is in charge of some essential function in plant development or survival in angiosperms. To elucidate biological functions of the each clade, we performed genetic analysis of *Arabidopsis thaliana* type-B PIP5K genes (*PIP5K1* to 9). Although each single mutant of their genes resulted in only a mild



**Figure 2.** Schematic Phylogenetic Tree of Angiosperm Type-B PIP5Ks. A phylogenetic tree of angiosperm type-B PIP5Ks is schematically shown. Four clades (I, II, III, and IV) are conserved in angiosperms.

phenotype in plant development if any, some of multiple mutants showed severe defects.

To elucidate biological functions of clade-I PIP5Ks, we performed genetic analysis of *A. thaliana* type-B PIP5K genes using their multiple mutants. Seedlings of the *pip5k1pip5k2* double mutant showed a severe dwarfism (Figure 3), and their mature plants were infertile because of abnormal flower development. However, the dwarfism was not enhanced in *pip5k1pip5k2pip5k3* triple mutant seedlings. Root hair elongation was affected in both the *pip5k2* and *pip5k3* mutants, and the effect was enhanced in the *pip5k2pip5k3* double mutant. These results indicate that *PIP5K1* and *PIP5K2*, but not *PIP5K3*, redundantly have an essential function in plant growth and that *PIP5K2* and *PIP5K3* have a redundant function in root hair elongation.



**Figure 3.** Phenotypes of *pip5k1*, *pip5k2*, and *pip5k3* Multiple Mutant Seedlings. Seedlings of the wild type (wt), *pip5k1pip5k2* double, and *pip5k1pip5k2pip5k3* triple mutants 7 and 14 days after germination (DAG) are shown.