Division of Biochemistry
– Molecular Biology –

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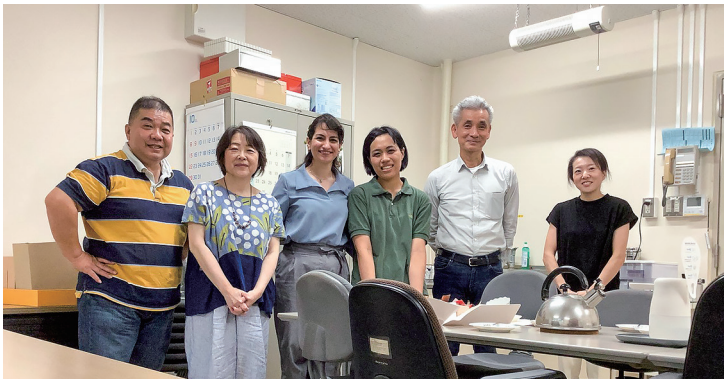
Scope of Research

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: 1) phospholipid signaling in cell morphogenesis, 2) transcriptional network for cell morphogenesis, 3) COP9 signalosome modulating signal transduction in the nuclei, and 4) RNA processing mediated gene expression regulation.



KEYWORDS

Morphogenesis
Phospholipid Signaling
mRNA Processing
Signal Transduction
COP9 Signalosome

Recent Selected Publications

Kato, M.; Watari, M.; Tsuge, T.; Zhong, S.; Gu, H.; Qu, L.-J.; Fujiwara, T.; Aoyama, T., Redundant Function of the *Arabidopsis* Phosphatidylinositol 4-Phosphate 5-Kinase Genes *PIP5K4-6* is Essential for Pollen Germination, *Plant J.*, (in press).

Akagi, C.; Kurihara, Y.; Makita, Y.; Kawaguchi, M.; Tsuge, T.; Aoyama, T.; Matsui, M., Transcriptional Activation of Ribosome-Related Genes at Initial Photoreception is Dependent on Signals Derived from Both the Nucleus Ans the Chloroplants in *Arabidopsis thaliana*, *J. Plant Res.*, **136**, 227-238 (2023).

Watari, M.; Kato, M.; Blanc-Mathieu, R.; Tsuge, T.; Ogata, H.; Aoyama, T., Functional Differentiation among the *Arabidopsis* Phosphatidylinositol 4-Phosphate 5-Kinase Genes *PIP5K1*, *PIP5K2* and *PIP5K3*, *Plant Cell Physiol.*, **63,** 635-648 (2022).

Zhang, X.; Nomoto, M.; Garcia-León, M.; Takahashi, N.; Kato, M.; Yura, K.; Umeda, M.; Rubio, V.; Tada, Y.; Furumoto, T.; Aoyama, T.; Tsuge, T., CFI 25 Subunit of Cleavage Factor I is Important for Maintaining the Diversity of 3′ UTR Lengths in *Arabidopsis thaliana* (L.) Heynh, *Plant Cell Physiol.*, **63,** 369-383 (2022).

Redundant Function of the *Arabidopsis* PIP5K Genes for Pollen Germination

Pollen is the male gametophyte with a functional structure for transferring sperm cells to the egg apparatus in seed plants. Pollen undergoes unique cell biological processes of pollen development to support its structural development and functional performance. After pollination, a pollen grain first establishes a cell polarity focusing on the future germination site, and then, a pollen tube germinates and elongates through tip growth toward the embryo sac according to a series of guidance signals. Although these complicated processes accurately progress, underlying regulatory mechanisms remain largely elusive, especially for the germination process involving the cell polarity.

Phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], one of phosphoinositides serving as a signaling molecule in eukaryotic cells is localized mainly to the plasma membrane frequently with a polarized pattern. Through the functions of its interacting proteins, PtdIns(4,5)P_2 regulates various cell biological processes, including cytoskeletal organization, membrane trafficking, and signal transduction for gene expression. While the metabolic pathways of phosphoinositides link together to form a complicated network, the phosphorylation of PtdIns(4)*P* by phosphatidylinositol 4-phosphate 5-kinase (PIP5K) is thought to be a key pathway responsible for the production of PtdIns(4,5)P_2 in higher plants, where some of the phosphoinositide metabolic pathways found in animals or fungi are missing.

Among the 11 PIP5K genes of *Arabidopsis thaliana*, *PIP5K4*, *PIP5K5*, and *PIP5K6* have been intensively studied on their functions in pollen tube growth, and strongly suggested to have an indispensable function for the elaborate pollen system of angiosperms. However, this idea remains to be verified, mainly because comprehensive genetic analysis of the genes using their loss-of-function mutants has not been done. We performed a comprehensive genetic analysis of the genes and revealed that their redundant function is essential for pollen germination. Pollen with the *pip5k4pip5k5pip5k6* triple mutation was sterile. PIP5K4-YFP, PIP5K5-YFP, and PIP5K6-YFP, which could rescue the sterility of the triple mutant pollen, preferentially localized to the tricolpate aperture area and the future germination site on the plasma membrane prior to germination (Figure 1). Triple mutant pollen grains under the germination condition, in which spatiotemporal localization of the PtdIns(4,5)P_2 fluorescent marker protein 2xmCHERRY-2xPH^{PLC} as seen in the wild type was abolished (Figure 2), exhibited swelling and rupture of the pollen wall, but neither the conspicuous protruding site nor site-specific deposition of cell wall materials for germination. These data indicate that PIP5K4–6 and their product PtdIns(4,5)P_2 are essential for pollen germination, possibly through the establishment of the germination polarity in a pollen grain.

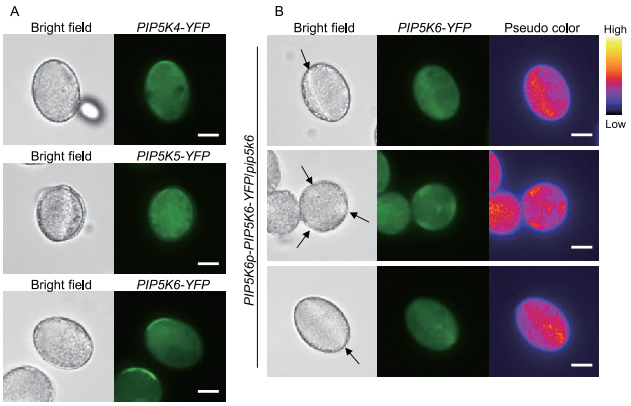


Figure 1. Subcellular localization of YFP-fused PIP5K4–6 proteins prior to pollen germination. (A) Bright field (left) and YFP fluorescence (right) images of pollen grains expressing PIP5K4-YFP, PIP5K5-YFP, and PIP5K6-YFP prior to pollen germination are shown. (B) Bright field (left) and YFP fluorescence (middle) images, and index-color images of YFP fluorescence (right) of pollen grains expressing PIP5K6-YFP prior to pollen germination are shown. Arrows on bright field images in (A) indicate pollen apertures. Scale bars: 10 μm.

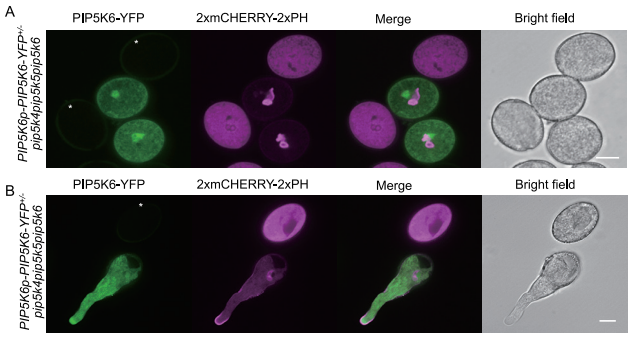


Figure 2. Subcellular localization of PtdIns(4,5)P_2 marker protein and PIP5K6-YFP in the *pip5k4*$^-$*pip5k5*$^-$*pip5k6*$^-$ pollen. Pollen grains from transgenic plants heterozygously and homozygously containing *PIP5K6p-PIP5K6-YFP* and *UBQ10p-2xmCHERRY-2xPH*^{PLC}, respectively, in the *pip5k4pip5k5pip5k6* background were untreated (A) or treated (B) with pollen germination medium. Fluorescence images of PIP5K6-YFP (left) and 2xmCHERRY-2xPH^{PLC} (second left), merged images (second right), and bright field images (right) are shown. Asterisks indicate *pip5k4pip5k5pip5k6* pollen grains without the *PIP5K6p-PIP5K6-YFP* transgene. Scale bars: 10 μm.