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– Molecular Biology –

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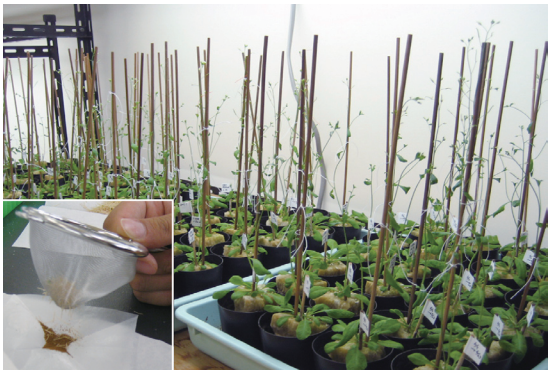
Scope of Research

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 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

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).

Kusano, H.; Testerink, C.; Vermeer, J. E. M.; Tsuge, T.; Shimada, H.; Oka, A.; Munnik, T.; Aoyama, T., The *Arabidopsis* Phosphatidylinositol Phosphate 5-kinase PIP5K3 Is a Key Regulator of Root Hair Tip Growth, *Plant Cell*, **20,** 367-380 (2008).

Menon, S.; Tsuge, T.; Dohmae, N.; Takio, K.; Wei, N., Association of SAP130/SF3b-3 with Cullin-RING Ubiquitin Ligase Complexes and Its Regulation by the COP9 Signalosome, *BMC Biochem.*, **9,** 1 (2008).

Taniguchi, M.; Sasaki, N.; Tsuge, T.; Aoyama, T.; Oka, A., ARR1 Directly Activates Cytokinin Response Genes that Encode Proteins with Diverse Regulatory Functions, *Plant Cell Physiol.*, **48,** 263-277 (2007).

Imai, K. K.; Ohashi, Y.; Tsuge, T.; Yoshizumi, T.; Matsui, M.; Oka, A.; Aoyama, T., The A-type Cyclin CYCA2;3 Is a Key Regulator of Ploidy Levels in *Arabidopsis* Endoreduplication, *Plant Cell*, **18,** 382-396 (2006).

Mechanism of Spliceosomal Protein in Its Requirement for Pollen Development

In flowering plants, the male gametophyte plays a vital role in plant fertility through the generation and delivery of the sperm cells to the embryo sac for double fertilization. During male gametogenesis, diploid pollen mother cells undergo meiotic division to produce tetrads of haploid microspores. The microspores are then released from tetrads and undergo an asymmetric cell division to produce bicellular pollen grains that contain a generative cell and a much larger vegetative cell. The smaller generative cells continue through another round of mitosis to produce twin sperm cells, composing the tricellular pollen grains.

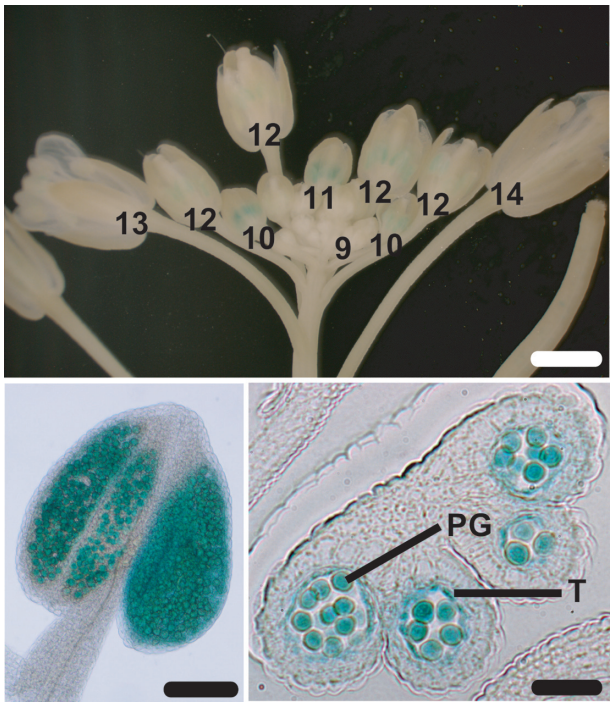


Figure 1. GUS staining of *AtSAP130a* pro-GUS plants revealed strong promoter activity in the theca (below left), specifically at stages 10 to 12 of flower development (above). Stages are indicated with numbers. Transverse sections of anther show strong GUS signals in the tapetum and pollen grains (below right). T, tapetum; PG, pollen grain. Bars = 1 mm (above), 100 µm (below left), or 50 µm (below right).

We found that the *Arabidopsis* spliceosomal protein, SPLICEOSOME-ASSOCIATED PROTEIN 130 (AtSAP130), was required for proper pollen development. Although SAP130 is essential for mRNA splicing and the formation of the pre-spliceosome, its detailed function remains unclear. AtSAP130 is encoded by two genes, *AtSAP130a* and *AtSAP130b*. Activities of the promoters of these genes overlapped at specific stages of anther and pollen development (Figure 1). Plants with reduced expression of the *AtSAP130* genes, induced by RNA interference (*AtSAP130* RNAi), showed developmental defects in the pollen during the transition from microspore to bicellular stages of male gametogenesis (Figure 2).

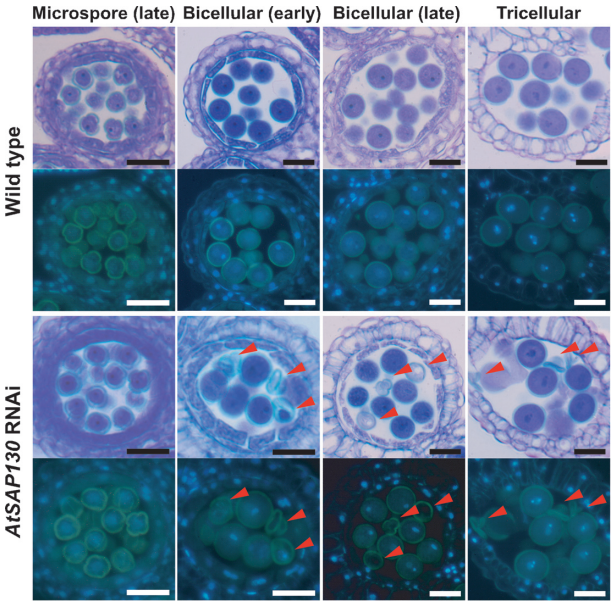


Figure 2. Histological analyses of pollen development in wild type and *AtSAP130* RNAi plants. Upper panels show the transverse light-field images of the anther locules stained with toluidine blue, while lower panels show the corresponding UV-field images stained with DAPI. Red arrowheads indicate deformed non-viable pollen grains detected at the bicellular stage and later. Bars = 25 µm.

In attempt to identify genes responsible for the pollen deficiency in the *AtSAP130* RNAi plants, we analyzed the expression of key genes in anther and pollen development. *QRT1* and *QRT3*, which have been shown to be required for microspore separation, preferentially accumulated less mRNA in *AtSAP130* RNAi plants (Figure 3). Although the two *QRT* genes might not be the direct cause for the pollen defect we observed in the *AtSAP130* RNAi plants, this could be due to the partial but not complete knock-down of the *QRT* genes. Taken together, these results suggested that *AtSAP130a* and *AtSAP130b* play an indispensable role in specific spatiotemporal events during pollen development.

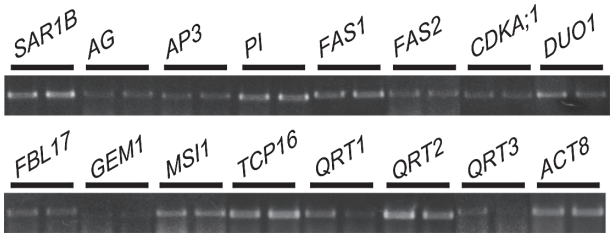


Figure 3. Expression analysis of key genes involved in pollen development. Semi-quantitative RT-PCR was performed on 14 representative genes expressed in the anther and post-tetrad stage of pollen development. *SAR1B* was also analyzed as a putative target, based on a former report in yeast. *ACT8* was used as a control.

Previously, we revealed that SAP130 binds to COP9 signalosome (CSN) in human and *Arabidopsis*. CSN regulates signal transduction through its control on proteolysis. We are exploring the venue where the interaction between CSN and SAP130 would bridge regulations of mRNA maturation and proteolysis.