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) 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

Recent Selected Publications

Shimamura, R.; Ohashi, Y.; Taniguchi, Y.Y.; Kato, M.; Tsuge, T.; Aoyama, T., Arabidopsis PLDζ1 and PLDζ2 Localize to post-Golgi Membrane Compartments in a Partially Overlapping Manner, *Plant Mol. Biol.*, DOI: 10.1007/s11103-021-01205-0 (2021).

Kuroda, R.; Kato, M.; Tsuge, T.; Aoyama, T., Arabidopsis Phosphatidylinositol 4-phosphate 5-kinase Genes PIP5K7, PIP5K8, and PIP5K9 are Redundantly Involved in Root Growth Adaptation to Osmotic Stress, *Plant J.*, **106,** 913-927 (2021).

Aki, S.S.; Yura, K.; Aoyama, T.; Tsuge, T., SAP130 and CSN1 Interact and Regulate Male Gametogenesis in *Arabidopsis thaliana*, *J. Plant Res.*, **134,** 279-289 (2021).

Kato, M.; Tsuge, T.; Maeshima, M.; Aoyama, T., Arabidopsis PCaP2 Modulates the Phosphatidylinositol 4,5-bisphosphate Signal on the Plasma Membrane and Attenuates Root Hair Elongation, *Plant J.*, **99,** 610-625 (2019).

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).

Cleavage Factor I Regulates the Diversity of 3′ UTR ends in mRNA Processing

In eukaryotes, gene expression is exercised by the transcription of DNA into mRNA in the nucleus. During this process, mRNA precursor (pre-mRNAs) undergoes a series of processing events, including its' cleavage at the 3′ end that then leads to addition of a polyadenosine tail. Cleavage and polyadenylation at the 3′ end of the pre-mRNA not only plays an important role in the transport and stability of the transcript but also in the transcription and the translation events. Once pre-mRNAs are processed to mature, they are subjected to protein synthesis and other regulatory functions in the cytoplasm.

Cleavage Factor I (CFI) is a nuclear protein complex that functions in the pre-mRNA 3′ end processing machinery that determines the polyadenylation site, among potential selections. Mammalian CFI is a tetramer composed of two CFI 25s and another two subunits of either CFI 59 and/or CFI 68. In *Arabidopsis thaliana*, a model plant for dicots, AtCFI 25a, AtCFI 25b, AtCFI 59, AtCFI 68 were identified as orthologs. The loss of function of *AtCFI 25a*, but not *AtCFI 25b*, showed pleiotropic developmental defects such as, small rosette leaf, reduced lamina pigmentation, undeveloped stamen with less pollen grains, abnormally elongated papillary cells, short primary root, and less secondary roots, when compared to wild type (Figure 1). Furthermore, the length of the silique and fertility was severely reduced in *atcfi 25a* (Figure 2). Hence, *AtCFI 25a* function was essential for proper development of plant architecture.

3′ RACE analyses on mRNAs of *AtCFI 25b*, *AtCFI 59*, and *AtCFI 68* revealed that *AtCFI 25a* function was essential to maintain the diverse pattern of 3′ UTR length, observed in the wild-type plants (Figure 3). This suggested a self-regulating mechanism of the plant CFI. Preliminary results indicate that this function of *AtCFI 25a* in 3′ end processing of pre-mRNA, seemed to affect a number of genes that are involved in various processes of development to different extents. It is interesting to note that the loss of CFI function in plants not only leads to proximal selection of the given transcript, as had been proposed in mammals, but fails to maintain the diversity of 3′ UTR end selection. This is a novel finding *via* taking advantage of the plant model system. Although further investigation is in progress, we hypothesize that gene expression is modulated through the selection of diverse cleavage and polyadenylation sites on the given transcript, which in turn, not only adds diversity to the limited DNA template resource, but also facilitates multiple level of regulation in development and environmental responses.

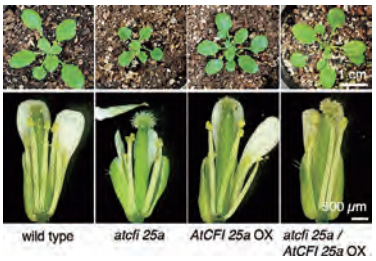

Figure 1. *AtCFI 25a* function is essential for plant morphogenesis. Null *atcfi 25a*, loss of function plants of *AtCFI 25a,* display pleiotropic defects, such as smaller aerial structure (upper panels) and deformed flower organs (lower panels). Overexpression of *AtCFI 25a* in wild-type background causes no obvious phenotypic difference when compared to wild type, however it partially complements the abnormality observed in *atcfi 25,* when expressed in *atcfi 25a* background. Some petals and sepals were removed to show the flower structure. Lengths of scale bars are noted in the figure.

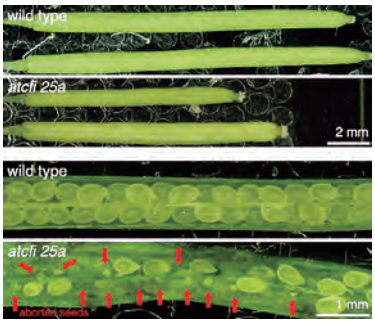

Figure 2. *atcfi 25a* displays low fertility due to abnormal flower organ development. Morphological comparison of wild type and *atcfi 25a* show that *AtCFI 25a* function is essential for maintaining proper length of the silique (upper panels) and seed formation (lower panels). Red arrows show the aborted seeds leading to low fertility. Lengths of scale bars are noted in the figure.

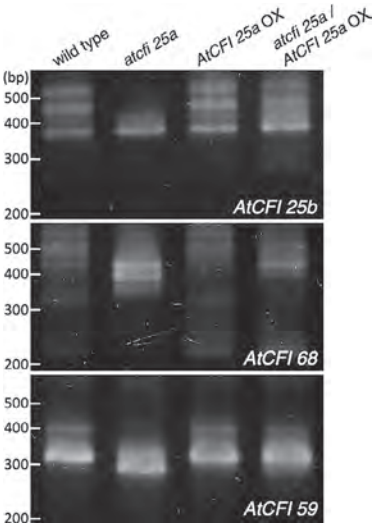

Figure 3. Comparison of cleavage and polyadenylation sites of wild type, *atcfi 25a*, *AtCFI 25a* OX, and *atcfi 25a-3* / *AtCFI25a* OX for genes encoding putative CFI subunits. 3′ RACE amplifications of *AtCFI 25a*, *AtCFI 25b*, *AtCFI 68*, and *AtCFI 59* are shown as comparable gel patterns on agarose gel after electrophoresis. Note that the of the 3′ UTR length pattern is less diverse in *atcfi 25* while those in *AtCFI 25a* OX and *atcfi 25a-3* / *AtCFI25a* OX is similar to wild type. Bands were confirmed to represent 3′ UTR of each gene through sequencing. Subjected samples: 7 DAS seedlings.