# Functional analyses of Arabidopsis Cleavage Factor I

# （シロイヌナズナ Cleavage Factor I の機能解析）

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This thesis presents novel findings on the function of Arabidopsis Cleavage Factor I (AtCFI) in Arabidopsis. After general introduction in Chapter one, Chapter 2 reveals evidences that CFI would most likely form a protein complex in plants. Chapter 3 and Chapter 4, describe the novel function of AtCFI in maintaining proper diversity of 3' UTR lengths by showing results for, *AtCFI 25a* and *AtCFI 25b*, *AtCFI 59* and *AtCFI 68*, respectively. Finally, Chapter 5, summarizes the molecular function of CFI in the plant kingdom, and discusses possible models for its biological role.

## Chapter one: General introduction

Cleavage and polyadenylation at the 3' end of the pre-mRNA is essential for mRNA function, by regulating its translatability, stability, and translocation to the cytoplasm. Cleavage Factor I (CFI) is a component of the pre-mRNA 3' end processing machinery that determines the polyadenosine attachment site in higher eukaryotes. In mammals, CFI consists of two identical 25 kDa subunits (CFI 25) and another two larger subunits of either 59 or 68 kDa (CFI 59 and CFI 68). Although plants possess these homologs, the structure and mechanism of plant CFI remains elusive.

## Chapter two: CFI in Arabidopsis

By utilizing the model plant system, *Arabidopsis thaliana* (L.) Heynh., AtCFI 25a was shown to interact with AtCFI 59, AtCFI 68, and itself, through *in planta* experiments of BiFC and Tandem Affinity Purification (TAP). Together with *in vitro* interaction studies, it was shown that there is high probability that AtCFI would form a complex consisted of AtCFI 25a, AtCFI 59, and AtCFI 68. In addition, AtCFI 25b, a homolog of AtCFI 25a, also interacted with AtCFI 68, suggesting that multiple forms of AtCFI complexes may exist in plants. AtCFI was also shown to interact with AtFIP1, another cleavage and polyadenylation factor, implying that various cleavage and polyadenylation factor complexes work together in plants.

It is interesting to note that AtCFI was found to interact with AtCSN1, a subunit of the COP9 signalosome protein complex (CSN), that functions not only in signal transduction but also in ubiquitin-dependent proteolysis. This would open doors to a hypothesis that AtCFI function, that plays a role in the cleavage and polyadenylation process of pre-mRNA, could be inter-linked to proteolysis and signal transduction.

## Chapter three: Function analysis of *AtCFI 25*

Transcripts of *AtCFI 25a* and *AtCFI 25b* were detected in different plant organs with little variation in amount. Promoter activities of *AtCFI 25a* and *AtCFI 25b* were high in meristematic domains and floral organs, with a slight difference of *AtCFI 25a* being higher in the young anther and pollen, while *AtCFI 25b* in young carpel. Although loss-of-function mutants for *AtCFI 25b* (*atcfi 25b*) showed no obvious differences in comparison to wild-type plants (WT), loss-of-function mutants for *AtCFI 25a* (*atcfi 25a*) displayed smaller rosette leaves, longer stigmatic papilla, smaller anther, earlier flowering, and lower fertility. *atcfi 25a atcfi 25b* double mutant showed phenotype similar to that seen in *atcfi 25a*, suggesting that *AtCFI 25b* might have functions other than in plant developmental processes, which are yet to be determined.

The molecular function of *AtCFI 25a* and *AtCFI 25b* was investigated by analyzing the 3' ends on transcripts of genes coding for AtCFI subunits, in loss-of-function mutants. 3' Rapid Amplification of cDNA Ends (3' RACE) method revealed that the diversity of the 3' UTR lengths of *AtCFI 25a* in *atcfi 25b* did not differ from those of WT. In contrast, the diversity of the 3' UTR lengths of the *AtCFI 25b*, *AtCFI 59*, and *AtCFI 68* was altered in *atcfi 25a* and *atcfi 25a atcfi 25b* double mutant. This suggests that *AtCFI 25a* function is essential for maintaining proper diversity of 3' UTR lengths in certain transcripts. Similar analyses for some phase transition and plant development related genes further revealed that, *AtCFI 25a* function was also important to maintain proper 3' UTR ends of these genes, to different extents.

## Chapter four: Function analysis of *AtCFI 59* and *AtCFI 68*

Transcripts of *AtCFI 59* and *AtCFI 68* were detected in different plant organs with little variation in amount. Promoter activities of *AtCFI 59* and *AtCFI 68* were high in meristematic domains and floral organs, with a slight difference of *AtCFI 59* being higher in stigma, anther, root and root tip, while *AtCFI 68* mainly in leaf veins, anther, pistil, pollen, and primary root. The loss-of-function mutants for *AtCFI 59* (*atcfi 59*) showed a slightly shorter silique and main root, while the loss-of-function mutants for *AtCFI 68* (*atcfi 68*) showed no obvious differences, compared to WT. Interestingly, *atcfi 59 atcfi 68* double mutant displayed a similar phenotype to *atcfi 25a* mutant and *atcfi 25a atcfi 25b* double mutant. Taken together, similar phenotypes were observed when either *AtCFI 25a* was knocked out, or when *AtCFI 59* and *AtCFI 68* were simultaneously knocked out. This suggests that there is a high probability that AtCFI functions as a protein complex in plants, having AtCFI 25a as an essential subunit and AtCFI 59 and AtCFI 68 playing a redundant role, to execute CFI function in 3' end maintenance. This would explain why only *atcfi 25a* and *atcfi 59 atcfi 68* double mutant displayed pleiotropic morphological phenotypes.

The redundant function of *AtCFI 59* and *AtCFI 68*, was also confirmed at the molecular level through 3' RACE analyses. The diversity of the 3' UTR lengths of the *AtCFI 25a* and *AtCFI 25b* was altered in *atcfi 59 atcfi 68* double mutant, but not in *atcfi 59* and *atcfi 68* single mutants. Interesting to note that the 3' UTR length diversity of *AtCFI 68* in *atcfi 59,* but not *AtCFI 59* in *atcfi 68*, showed differences, when compared

to WT, indicating that *AtCFI 59* might play a major role through its functional redundancy with *AtCFI 68*.

## Chapter five: General discussion

This thesis discloses for the first time, that CFI most likely functions as a complex in plants. *AtCFI 25a*, *AtCFI 59* and *AtCFI 68* function was essential for maintaining proper diversity of the 3' end lengths of transcripts coding for CFI subunits, suggesting a self-regulation of the CFI machinery in plants. This was shown through reverse genetics and molecular analyses. Furthermore, *AtCFI 25a* function was also important to maintain proper 3' UTR ends for some phase transition and plant development related genes, to different extents.

Interestingly, AtCFI was found to have overrepresentation of interacting proteins from the proteasomal regulation proteins, in TAP experiments. Together with the interaction of AtCFI with AtCSN, a hub for signal transduction that regulates gene expression through proteolysis, facts point to a scenario where AtCFI might play more than a role of merely fine-tuning the 3' ends of pre-mRNAs. Although there are much discoveries to be made, here I argue possible schemes where AtCFI function could inter-link pre-mRNA 3' end processing to proteolysis and signal transduction.