論文の内容の要旨

Molecular genetic studies on leaf development in *Oryza sativa*

（イネの葉の形態形成に関する分子遺伝学的研究）

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Leaves are important for photosynthesis and show diverse morphology in angiosperms. Although molecular genetic mechanism underlying leaf morphogenesis have been understood deeply in eudicots such as *Arabidopsis thaliana*, the knowledge of leaf development in monocots is still insufficient. In this dissertation, I focused on two curling leaf mutants, *curled later1* (*cul1*) and *halfpipe-like leaf1* (*hal1*), in order to get new insights for leaf development in a model monocot, *Oryza sativa* (rice). In chapter 2, I revealed that *CUL1* encoding a large subunit of Elongator complex is required for leaf development at late stage of vegetative phase and for proper maintenance of the shoot apical meristem (SAM). In chapter 3, I characterized the leaf phenotype of *hal1*.

***CURLED LATER1* encoding the largest subunit of the Elongator complex has a**

unique role in rice development

In chapter 2, I first characterized the *cul1* mutant with narrow and curled leaves. By histological analysis, I revealed that the development of both the bulliform cells and sclerenchyma cells were impaired in *cul1* leaves and that these defects were a cause of curling leaf phenotype. These leaf phenotypes were not observed in early-middle stages of the vegetative phase and appeared specifically at late stage. It is therefore likely that *cul1* displayed a heteroblastic change. In general, the heteroblastic change is observed in the transition from the juvenile to adult phase in many plants. The heteroblastic change in *cul1* was likely to occur at the late adult phase. In this sense, the *CUL1* seems to play a unique role in leaf development in rice.

I also revealed that the SAM was smaller in *cul1* than in wild-type, suggesting that *CUL1* function is associated with meristem activity to some extent. Consistent with this, the leaf initiation rate was slow in *cul1*, as compared with wild-type. Unlike leaf phenotype, the partial defect in the SAM appeared independently of growth stage. I also suggested that the appearance of leaf phenotype at the late adult phase seems to be associated with the transition of meristem fate, that is, from the vegetative meristem to the reproductive meristem.

I revealed that *CUL1* encodes a yeast ELP1-like protein, which is the largest subunit of the Elongator complex. Elongator is conserved in eukaryotes such as yeast, animals and plants, whereas its roles are diversified among various organisms. In Arabidopsis*,* Elongator is known to regulate leaf and root development, hormone action and environmental responses. In other plant, however, the function of Elongator complex is still largely unknown. To get further evidence that Elongator is required for rice development, I disrupted *OsELP3* encoding the catalytic subunit of the Elongator complex by CRISPR-Cas9 technology. Knockout lines of *OsELP3* produced curled narrow leaves with impaired bulliform and sclerenchyma cells, similar to the *cul1* mutant. In addition, the curled narrow leaf phenotype appeared at late stage of the vegetative phase. Therefore, these results confirm the importance of Elongator activity in normal leaf development at later vegetative phase in rice. Furthermore, this result showing that the *cul1* mutant and *OsELP3* knockout line shared mutant phenotypes suggests that the proteins encoding by these two genes act together as a component of the Elongator complex.

To explore the effect of the *cul1* mutation on gene expression, I carried out transcriptome analysis. As a result, the genes involved in protein quality control, such as ubiquitin-associated protein degradation and molecular chaperon, were highly up-regulated specifically in the *cul1* shoot apex at the later vegetative phase (*cul*-L shoot apex). This result suggests that the genes responsible for protein quality control is induced by the accumulation of abnormal proteins in the *cul1*-L apex probably due to the loss of Elongator activity. Therefore, it seems likely that the Elongator is required for the protein progression of translational processes. The defects in leaf development in *cul1* might be related to the impaired translation of the proteins, which plays essential roles in the differentiation of bulliform and sclerenchyma cells.

Characterization of *half-pipe-like leaf1* mutant that exhibit curled leaf phenotype

In chapter 3, I focused on a semi-dominant mutant, *hal1-d*, which produced the adaxially curled leaves. Histological analysis revealed that the bulliform cells of *hal1-d* leaf were small and abnormal shape. The obseversion of semi-transparent leaves by TOMEI methods showed that the bulliform cell files were often indistinguishable from other cell files in *hal1-d*, suggesting that the bulliform cells were failed to growth. Therefore, the defective development of the bulliform cells were likely to be a main cause of curling leaf phenotype in *hal1-d* mutant. The size of the leaf blade and the spikelet were also influenced by the *hal1-d* mutation.

Conclusion and perspectives

The Elongator complex plays multiple roles in diverse organisms. The loss-of-function of the genes encoding Elongator subunit exhibits various phenotypes depending on diverse species. In addition, the studies on Elongator have not yet reported in plants, except for Arabidopsis. In this dissertation, I revealed that the Elongator complex is required for leaf development and meristem activity, and associated with heteroblasty in rice. The role of Elongator in rice development seems to be different from that of Elongator in Arabidopsis development to some extent. Therefore, the function of Elongator is likely to have been diversified even within angiosperms. Further studies on Elongator in rice would be expected to provide important information on not only elucidation of leaf and meristem development in rice but also the conservation and diversification of Elongator function in angiosperms.