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博士学位論文

要約

シロイヌナズナの葉の発生分化のエピジェネティック制御におけるASYMMETRIC LEAVES2と核小体タンパク質の分子遺伝学的研究

Molecular and genetic study of *ASYMMETRIC LEAVES2*
and nucleolar factors in epigenetic regulation of
Arabidopsis thaliana leaf development

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Leaves develop as lateral organs from the peripheral zone of a shoot apical meristem. Initially, a group of cells is patterned along the proximal-distal axis and then establishment of the adaxial-abaxial axis is crucial for further leaf development. Subsequent cell proliferation along the medial-lateral axis results in flat and mediolateral symmetric leaves. The ASYMMETRIC LEAVES1 (AS1)-AS2 complex is regarded as a key regulator for the proper confinement of the stem cell fate and then abaxial cell fate, resulting in the adaxial development in *Arabidopsis thaliana*. AS1-AS2 represses the expression of *AUXIN RESPONSE FACTOR3/ETTIN* (*ARF3/ETT*), which promotes abaxial identity, through at least 2 previously reported pathways (two pathway: direct repression and miR390-tasiR-ARF pathway) and it has been also showed that AS1-AS2 maintains the status of DNA methylation of CpG sites in the *ETT* coding region (called gene body methylation). The *ETT* gene is expressed during early leaf development, and then silenced as new developmental processes in the supposed adaxial side of leaf primordia. An epigenetic control of AS1-AS2 downstream gene *ETT* has been proposed, however, information on the silencing mechanisms has still remained elusive. It has been reported that cooperative action between the nucleolar proteins and AS1-AS2 is critical to repress *ETT*, which leads to adaxial development in leaf primordia at shoot apices. The molecular mechanisms of the cooperative action, such as DNA methylation, however, remain to be demonstrated. *AS2* encodes a nuclear protein made up of 199 amino acid residues that has a plant-specific AS2/LOB domain (so-called AS2D), which contains a zinc-finger-like motif (ZFL-motif) including four cysteine residues at the N-terminus. The ZFL-motif of AS2 is similar, in terms of the cysteine-repeat pattern, to some extent to the zinc-finger motifs of CxxC Finger Protein 1 (CFP1) and DNA methyltransferase1 (Dnmt1), which are members of a family of ZF-CxxC domain-containing proteins in vertebrates and have the capacity to bind non-methylated, hemi-methylated and/or methylated CpG. It, however, was not known that the AS2 protein binds to CpG sequences of the *ETT* gene. First, my results showed that AS1-AS2 is involved in the maintenance of CpG methylation at specific positions in the *ETT* gene body mediated by METHYLTRANSFERASE1 that is a homolog of Dnmt1 of vertebrates. Secondly, examination of mutants of *NUCLEOLIN1* (*NUC1*) and *RNA HELICASE10* (*RH10*) encoding nucleolus-localized proteins showed that NUC1 and RH10 are involved in the maintenance of the methylation levels of specific CpG

positions, suggesting the existence of different pathways. In addition, analyses with *as2 nuc1*, *as2 rh10* double mutants showed new patterns and significantly reduced levels of methylation compared to each single mutant, suggesting that some interactions between *AS2* and these nucleolar factors function in maintenance the CpG methylation status in *ETT* exon 6. Thirdly, I found that *AS2* binds specifically the sequence containing CpGs in exon 1 of *ETT/ARF3*, and that the binding requires the zinc-finger-like motif in *AS2* that is structurally similar to the zinc finger-CxxC domain in vertebrate DNA methyltransferase1.