Doctoral thesis/dissertation Digest Form

Thesis/dissertation Title: Studies on the functional role of histone methyltransferases ATXR5 and ATXR6 in DNA replication

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DNA replication is a key step in the cell cycle for producing a genetically identical set of chromosomes. Previous studies on replication timing revealed that in *Arabidopsis* and maize, euchromatic and pericentromeric heterochromatin regions replicate in the early S and the late S phase, respectively. However, how such ordered DNA replication in distinct chromatin domains is regulated and its physiological significance remains largely unknown in plants. Chromatin state is controlled by epigenetic modifications, such as DNA methylation, and histone methylation and acetylation. Our group has recently found that the level of histone monomethylation at H3K27 (H3K27me1), which promotes heterochromatin formation, oscillates during the cell cycle in the root apical meristem. In *Arabidopsis*, this methylation is catalyzed by the plant-specific histone methyltransferases, ARABIDOPSIS TRIHORAX-RELATED 5 (ATXR5) and its homolog ATXR6. The accumulation patterns of ATXR5/6 proteins during the cell cycle were well correlated with that of H3K27me1, suggesting that ATXR5/6 contribute to the oscillation of H3K27me1. The treatment with MLN4924, an SCF-type E3 ligase inhibitor, caused the elevated accumulation of ATXR5/6 proteins in the root tip, suggesting that the SCF-mediated ubiquitin-proteasome pathway is involved in the regulation of ATXR5/6 accumulation.

To examine the role of ATXR5/6 in cell division, *atxr5 atxr6* was generated through CRIPSR-Cas9 system and I observed shoot and root growth of the *atxr5* or *atxr6* single mutants, and the *atxr5 atxr6* (hereafter called *atxr5/6*) double mutants. *atxr5/6* exhibited smaller plant size and slower root growth than wild-type whereas *atxr5* or *atxr6* single mutants showed no difference. In addition, reduced root meristem size was observed in *atxr5/6*, indicating that *ATXR5* and *ATXR6* function redundantly in promoting cell division. Since

ATXR5/6 is known to be involved in monomethylation of replication-dependent histone H3.1, I speculated that reduced H3K27me1 level might affect the S-phase progression. I monitored the expression pattern of *pPCNA:PCNA-GFP*, a cell cycle marker, and measured cell cycle duration in *atxr5/6*. Indeed, in *atxr5/6*, the S-phase duration was shortened, and DNA replication timing was disordered among distinct chromatin domains. Moreover, chromosome mis-segregation was detected in mitotic nuclei of *atxr5/6* probably as a consequence of replication stalling in S phase, suggesting that mutations in *ATXR5/6* impair ordered DNA replication, and eventually cause genome instability. Consistently, the expression of DNA damage-responsive genes and the number of γ-H2AX foci, an indicator of DNA damage, were increased, and the stem cell death, one of the DNA damage responses, was observed in *atxr5/6* under normal growth conditions. These findings indicate that ATXR5/6-dependent H3K27me1 plays a crucial role in controlling DNA replication timing, contributing to the maintenance of genome integrity.

To uncover the mechanism underlying the cell cycle-dependent ATXR5/6 accumulation, I first monitored the ATXR5/6 protein accumulation pattern during the cell cycle in the presence of MLN4924. ATXR5/6 accumulation was enhanced by the treatment; especially, cell cycle-dependent oscillation of ATXR5 was totally disrupted. This result indicates that SCF-mediated proteolysis generates the oscillation of ATXR5/6 accumulation in the cell cycle. FBL17 is an F-box protein which is known to be involved in cell cycle regulation. I found that FBL17 controls ATXR5 only at the protein level, whereas it might regulate ATXR6 in both transcriptional and protein levels. Monitoring the FBL17 protein accumulation in the cell cycle showed the negative correlation to that of ATXR5/6. Since it is challenging to isolate ~1% *fbl17* homozygous plants, I then investigated cell cycle-dependent ATXR5 accumulation in the *fbl17* heterozygous lines. I found that the protein level of ATXR5 was significantly increased in the early S compared to wild-type, implying that partial loss of *FBL17* is capable of retaining ATXR5 protein. These results suggest the possibility that FBL17 targets ATXR5 for ubiquitin-proteasome pathway to ensure the H3K27me1 oscillation during the cell cycle. Taken together, my study proposed a model that the cell cycle-dependent control of H3K27me1 level via SCF^{FBL17}-mediated ATXR5/6 proteolysis works as a safeguard for the maintenance of genome integrity in

Arabidopsis root.