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Epigenetic control of LTR retrotransposons in plant germline and somatic cells

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Abstract

Plant genomes include heterochromatic loci that consist of repetitive sequences and transposable elements. LTR retrotransposon is the major class of transposons in advanced plants in terms of proportion in plant genome. The elements contribute not only to genome size but also to genome stability and gene expression. A number of cases have been reported transposon insertions near genic regions affect crop traits such as fruit pigments, stress tolerance, and yields. Functional LTR retrotransposons produce extrachromosomal DNA from genomic RNA by reverse transcription that takes place within virus-like-particles (VLPs). DECREASED DNA METHYLATION 1 (DDM1) plays important roles in maintaining DNA methylation of heterochromatin affecting all sequence contexts, CG, CHG, and CHH. Previous studies showed that *ddm1* mutant exhibits massive transcription of retrotransposons in Arabidopsis, but only few of them were able to create new insertions into the genome. RNA-dependent RNA POLYMERASE 6 (RDR6) is known to function in restricting accumulation of transposon RNA by processing the transcripts into 21-22 nt epigenetically activated small interfering RNA (easiRNA). We purified VLPs and sequence cDNA to identify functional LTR retrotransposons in Arabidopsis *ddm1* and *ddm1rdr6* plants. Over 20 LTR copia and gypsy families were detected in *ddm1* and *ddm1rdr6* sequencing libraries and most of them were not reported for mobility. In *ddm1rdr6*, short fragments of ATHILA gypsy elements were detected. It suggests easiRNAs might regulate reverse transcription steps. The highest enriched element among transposon loci was previously characterized *EVADE* element. It has been reported that active *EVADE* element is more efficiently silenced through female germline than male germline. By genetic analyses, we found *ddm1* and *rdr6* mutation affect maternal silencing of active *EVADE* elements. DDM1-GFP protein accumulated in megaspore mother cell but was not found in mature egg cell. The fusion protein was also found in early embryo and maternal DDM1-GFP allele was more dominantly expressed in the embryo. We observed localization of DDM1-GFP in Arabidopsis and DDM1-YFP in maize and found the proteins accumulated in dividing zone of root tips. Currently we are looking at cell cycle dependency of DDM1 expression using maize system. Among 10 AGO proteins in Arabidopsis, AGO9 is specifically expressed in egg cell and shoot meristematic cells. In addition, mutation of AGO9 and RDR6 caused failure in maternal silencing, implying 21-22 nt easiRNA pathway is important for retrotransposon silencing in female gametophyte or/and early embryo. On the other hand, canonical 24 nt sRNA-directed DNA methylation (RdDM) pathways did not contribute to maternal silencing as confirmed by this study. Heat-activated LTR retrotransposon, ONSEN, was not silenced by DDM1 but the silencing mechanisms require RdDM pathways in somatic cells. We will propose distinct mechanisms of LTR retrotransposons in germline and somatic stages.

Keywords: epigenetic pathways, LTR retrotransposon, transcriptional silencing, VLP DNAseq

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