EPIGENOMICS

Dissecting the roles of Pol V

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RNA POLYMERASE IV (Pol IV) and Pol V are multi-subunit polymerases that are unique to plants and that evolved from Pol II; they mediate RNA-directed DNA methylation at CG, CHG or CHH (where H is any base other than G) sequences through the production of small RNA transcripts. Two recent studies in *Arabidopsis thaliana* describe the global localization and role of Pol V in this methylation and further characterize its putative role in gene silencing.

Zhong *et al.* carried out chromatin immunoprecipitation with massively parallel sequencing (ChIP-seq) against NUCLEAR RNA POLYMERASE E1 (NRPE1), which is a catalytic subunit of Pol V in *A. thaliana*. Pol V was found to be enriched at promoters and evolutionarily young transposons. Furthermore, genome-wide DNA methylation and small RNA profiling in wild-type and *nrpe1*-mutant plants showed that this pattern correlates with Pol-V-dependent DNA methylation and small RNA accumulation. To assess how this localization pattern of Pol V is achieved further, the authors generated mutants for the three proteins that constitute the putative

chromatin-remodelling complex DDR, which is thought to act upstream of Pol V and either to regulate Pol V activity or to stabilize Pol V association with chromatin. The DDR mutations all resulted in the loss of, or substantial reduction of, NRPE1 chromatin association, confirming the importance of the role of DDR in the localization of Pol V. The authors hypothesize that when a transposon jumps into a promoter region, Pol V becomes stably associated with both the promoter and the transposon, which results in the transposon being targeted for *de novo* DNA methylation and transcriptional repression.

Wierzbicki *et al.* also combined Pol V ChIP-seq data with small RNA sequencing (RNA-seq) and methylcytosine mapping in Pol IV, Pol V and Pol IV and Pol V double mutants. They found that approximately 60% of Pol-V-associated regions encompass regions of cytosine methylation and 24-nucleotide short interfering RNA (siRNA) complementarity, supporting the idea that cytosine methylation is guided by base pairing of Pol-IV-dependent siRNAs with Pol V transcripts. Twenty-seven per cent of Pol V peaks did not correspond to regions of cytosine methylation or 24-nucleotide siRNA biogenesis, which suggests that Pol V alone does not specify the sites of cytosine methylation.

Most of the overlap between Pol V and cytosine methylation

occurs across sites of CHH methylation. Interestingly, around 7% of Pol V peaks coincided with sites of CG or CHG motifs that were methylated, but CHH methylation and 24-nucleotide siRNAs were not detected. As they had previously identified loci that were silenced by Pol V in conjunction with maintenance methylases, Wierzbicki *et al.* suggested that these data support this additional role for Pol V in silencing at CG-methylated loci. Interestingly, the total number of methylated CHH motifs is similar in Pol IV mutants (*nrpd1*), Pol V mutants (*nrpe1*) and the Pol IV and Pol V double mutants (*nrpd2* and *nrpe2*) relative to wild-type plants. Thus, the authors suggest that Pol IV and Pol V are involved in targeting CHH methylation but not the overall activity of the cytosine methyltransferases. Most of the ectopic CHH methylation was shown to occur in the pericentromeric regions; interactions between other methyltransferases may be responsible for methylation concentration at these regions.

Together, these studies suggest that Pol V has a role in silencing recent transposons and is guided by DDR to methylation sites. However, methyltransferase activity is independent of both Pol IV and Pol V, which is suggestive of the action of another pathway in CHH methylation.

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ORIGINAL RESEARCH PAPERS Zhong, X. *et al.* DDR complex facilitates global association of RNA polymerase V to promoters and evolutionarily young transposons. *Nature Struct. Mol. Biol.* 5 Aug 2012 (doi:10.1038/nsm.2354) | Wierzbicki, A. T. *et al.* Spatial and functional relationships among Pol V-associated loci, Pol IV-dependent siRNAs, and cytosine methylation in the *Arabidopsis* epigenome. *Genes Dev.* 26, 1825–1836 (2012)

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