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Role of histone and DNA methylation in gene regulation

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Transcription is known to be regulated by given chromatin states, distinguished as transcriptionally active euchromatin and silent heterochromatin. In plants, silencing in heterochromatin is associated with hypermethylation of DNA and specific covalent modifications of histone H3. Several lines of evidence have suggested that maintenance of DNA methylation patterns at CG sequences is responsible for the formation of stable and thus heritable activity states termed epialleles. By contrast, histone modification and DNA methylation outside CGs confer the flexibility of transcriptional regulation necessary for plant development and adaptive responses to the environment. Recent studies have refined our understanding of the biological significance of and the molecular mechanisms involved in the interplay between DNA and histone H3 methylation.

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Introduction

The term 'epigenetics' defines heritable states of gene activity not encoded in the DNA sequence. Any given epigenetic state appears to be correlated with levels and patterns of DNA methylation, post-translational modifications of histone proteins, the presence of histone variants, and chromatin compaction. Similar to the situation in mammals, DNA methylation in plants affects cytosine residues preceding guanines (CG), but plant cytosines are also modified at CNG (where N is any nucleotide) and asymmetrical sequence contexts CHH (where H is any nucleotide but G). This obviously provides increased combinatorial power to the 'DNA methylation code'. In plants as in animals, histone-tails are modified by the addition of various covalent attachments, including methyl, acetyl, phosphor, sumo, ubiquitin and ADP-ribose. Moreover, modification levels may vary. For example, lysine residues (K) can be monomethylated (Kme1), dimethylated (Kme2) or trimethylated (Kme3). The combination of histone modifications is thought to constitute a 'histone code' that, in association with information encoded by DNA methylation, determines the structure of chromatin and its transcriptional competence [1,2]. For example, dimethylation of lysine 9 and lysine 27 of histone H3 (H3K9me2, H3K27me2) in plants, together with hypermethylation of DNA, are linked to the transcriptional repression characteristic of heterochromatin, whereas dimethylation of lysine 4 of the histone H3 (H3K4me2) and hypomethylation of DNA are associated with active transcription taking place in euchromatin.

In this review, we will focus on selected aspects of chromatin-mediated suppression of transcription, considering recent advances in understanding molecular mechanisms linking such epigenetic modifications to particular states of gene expression. For clarity, we will divide these states arbitrarily into those that must be constantly controlled/modulated and those that once formed can be autonomously propagated or even passed to subsequent plant generations. To conclude, we will attempt to define functional links between molecular mechanisms determining these two types of transcriptional regulation.

Non-autonomous epigenetic regulation of transcription

Two well-studied but biologically unrelated processes depend on the tight epigenetic regulation of transcription during plant development. Both need to be re-established in each plant generation. One of these, vernalization, is discussed in a separate chapter of this volume (by Elisabeth Dennis) and the second, genomic imprinting, will be briefly reviewed here.

Imprinting refers to the differential expression of alleles depending on their parental origin. In plants, imprinting is confined to the seed endosperm and since the endosperm is a terminal tissue the outcome of imprinting cannot be transmitted to the progeny and must, therefore, be re-established at each plant generation. Imprinted genes are usually silent in vegetative tissues and, with the exception of *PHERES1* (*PHE1*), their expression relies on release of the silencing of the maternal alleles.

Two distinct mechanisms involving DNA or H3K27 methylation appear to maintain the silenced status of the imprinted loci. Analysis of mutants and transgenic lines with reduced activity of the *DNA METHYLTRANSFERASE 1* (*MET1*) gene, which is required to maintain CG methylation, revealed that CG methylation is involved in silencing of *FWA* and *FERTILIZATION INDEPENDENT*

SEED2 (*FIS2*) during male gametogenesis and endosperm development [3]. Moreover, *FWA* and *FIS2* are expressed during female gametogenesis and their activation depends on expression of *DEMETER* (*DME*) [3,4]. *DME* encodes a DNA glycosylase catalyzing the excision of methylated cytosines [5], thereby antagonizing *MET1* activity. *FWA* and *FIS2* are silent in the sporophyte and MET1 maintains their DNA hypermethylation [3,4].

Unlike *FWA* and *FIS2*, the sporophytic silencing of the *MEDEA* (*MEA*) gene and the paternal allele in the endosperm is not released by *met1* mutations, suggesting that it is independent of CG methylation [3,6]. *MEA* silencing seems to rely on H3K27me2 (or me3) mediated by Polycomb group (PcG) protein(s) [6,7•]. Interestingly, the *MEA* gene itself encodes a PcG protein and the maternally expressed *MEA* allele is required for repression in the endosperm of its paternal allele, demonstrating that *MEA* transcription is auto-regulated [6,7•,8]. MEA is also required for repression by H3K27me3 of the maternal allele of *PHE1* in endosperm and the developing embryo [9–11]. In addition, both alleles of *PHE1* are silenced by H3K27me3 in vegetative tissues, where MEA is not expressed. This silencing requires the PcG proteins CURLY LEAF (CLF) and SWINGER (SWN) [11]. In addition, CLF mediates repression of the floral homeotic gene *AGAMOUS* and the homeobox *SHOOTMERISTEMLESS* (*STM*) in seedlings, also involving H3K27me3 [12].

These examples of PcG proteins mediating H3K27me3 demonstrate similarity between plant and animal PcG proteins in that both use H3K27 methylation as a guide for silencing [13]. A recent study that profiled H3K27me3 on a whole-genome tiling array broadened the spectrum of *Arabidopsis* genes associated with H3K27me3 [14]. These genes are localized mainly in euchromatin [14], confirming previous cytological observations [15,16•] and are predominantly activated in a tissue-specific manner. In general, they are expressed at levels lower than genes lacking this epigenetic mark [14]. These observations are consistent with the hypothesis that H3K27me3-mediated gene silencing is involved in developmental decisions.

Although the above results reveal a repressive function of H3K27me3 operating in euchromatin, H3K27me3 possibly acts differently in heterochromatic areas of chromosomes. Astonishingly, in *met1-3* mutant plants (*met1-3* is a complete loss-of-function allele of the *MET1* gene) a number of repeats related to transposons lose their DNA methylation and are transcribed; however, they display increased levels of H3K27me3 [16•]. In addition, a 180-bp repeat and the ribosomal 5S rDNA gene clusters also exhibit enhanced levels of H3K27me3 in *met1-3* [16•]. All these silencing targets have been shown to be transcriptionally activated in *met1* mutants [17–19]. Interestingly, a transposable element that remains silent in *met1-3* despite loss of CG methylation appears to retain the wild-type level of H3K27me3 [16•]. Therefore, it is conceivable that H3K27me3 has a dual function, serving as a repressive epigenetic mark for genes residing in euchromatin as well as marking transcriptional reactivation in heterochromatin. However, increase in H3K27me3 at heterochromatic targets upon their transcriptional reactivation may also reflect failed attempts to repress transcription following loss of CG methylation.

A further repressive histone mark in *Arabidopsis* is H3K9 methylation. The most abundant H3K9me2 mark is clearly enriched in heterochromatin (for a review, see for example [20]). The KRYPTONITE (KYP) protein, an *Arabidopsis* homologue of SU(VAR)3-9 histone K9 methyltransferase, has been considered to be the major H3K9 methylation activity in *Arabidopsis*. Although KYP has been implicated in gene and transposon silencing, it is surprising that the *kyp* mutation displays no morphological defects and that silencing release is rather marginal (for a review, see for example [20]). Moreover, KYP-mediated H3K9 methylation does not always determine transcriptional repression and decrease in H3K9me2 due to *kyp* mutation does not always release silencing of targets that can be activated by reduction of DNA methylation [16•]. Recently other *Arabidopsis* SU(VAR)3-9-like proteins have been shown to influence levels of H3K9me2 *in vivo* [21–23], making more apparent the redundancy in the regulation of H3K9 methylation. Ten SU(VAR)3-9 homologues are predicted in the *Arabidopsis* genome (SUVH1 to SUVH10, KYP being SUVH4) [24] and indeed overlapping function in the maintenance of the H3K9me1-me2 of SUVH4/KYP, SUVH5 and SUVH6 have been documented [21,22••]. Noticeably, their relative contributions seem to be target-specific and the triple mutant displays enhanced transcriptional reactivation of the silent *PAI2* locus and some transposon sequences but without showing any morphological defects. These changes are accompanied by reduction of DNA methylation but only outside CGs [21,22••]. Moreover, mutation or overexpression of SUVH2 coordinately reduce or elevate levels of all four heterochromatin-specific histone methylation marks (H3K9me1-me2 and H3K27me1-me2), respectively [23]. The involvement of histone methylation in the regulation of transcription has been recently documented also in rice. Mutations of rice histone H3K9-specific methyltransferase, SDG714, leads to activation of transcription and transposition of a transposable element [25].

Methylation levels of cytosines residing outside CG sequences seem to be tightly linked to the local histone methylation status. Several DNA methyltransferases are involved in securing appropriate levels of non-CG methylation. The DOMAINS REARRANGED METHYLTRANSFERASE 1 and 2 (DRM1 and 2) have been shown to target methylation to cytosines in any sequence

context of CG, CNG or CHH. DRM1 and 2 are considered to be the main *de novo* DNA methylation activities in *Arabidopsis*, required for example for the establishment but not subsequent maintenance of silencing at *FWA* and *SUPERMAN* (*SUP*) loci [26]. DRM1/2 are also required for non-CG methylation at other chromosomal targets [27,28]. The discovery of the involvement of small RNAs in DRM1/2-mediated *de novo* DNA methylation has provided a framework for an RNA-dependent DNA methylation process described in this volume by Marjori Matzke. Although DRM1/2 are clearly essential for perpetuation of non-CG methylation, it is remarkable that their combined mutation has not been shown so far to be sufficient to release transcriptional silencing from any silent chromosomal locus.

By contrast, although the CHROMOMETHYLASE3 (CMT3) is also essential for non-CG methylation (primarily CNG), its loss of function results in clear release of silencing at selected loci [29]. CMT3 and DRM1/2 seem to act in a redundant fashion since neither *drm1/2* nor *cmt3* mutants display developmental aberrations but the combination of all three mutations in a triple mutant leads to pleiotropic developmental abnormalities [29]. Importantly, developmental abnormalities seem to be non-autonomous and they are rapidly reversed upon restoration of DRM1/2 or CMT3 function [30].

Genome-wide methylation profiling has suggested that CMT3 and KYP are both involved in the targeting of CNG methylation into transposable elements [31,32]. However, in contrast to the massive transcriptional activation of transposons in mutants affecting CG methylation [33,34••], release of silencing in *cmt3* or *kyp* was observed at only a few transposons, despite a significant decrease in non-CG methylation [16•,17,18,35,36]. Similarly, only a few heterochromatin-associated transposon-related elements were transcriptionally activated in *drm1drm2cmt3* triple mutants. This suggests that non-CG methylation is more involved in transcriptional fine-tuning of genes residing in euchromatin than in the suppression of transcription in heterochromatin [34••]. Conversely, CG methylation is clearly essential for silencing in heterochromatin. However, such a functional distinction is an obvious simplification, since the double mutants *met1cmt3* have revealed synergistic effects on the transcriptional reactivation of some transposons [18,37] and also on their transposition [38]. Therefore, although CG hypermethylation is essential for controlling transposon silencing, non-CG methylation can in some cases provide an additional lock to retain transposons in an inactive state.

Transcriptional fine-tuning of gene expression by non-CG methylation requires dynamic regulation not only through *de novo* methylation but also through methylation removal. In *Arabidopsis*, DME and REPRESSOR OF SILENCING 1 (ROS1) possess activities that remove methylated cytosines via DNA-repair activity of glycosylase/lyase [5,39]. As mentioned above, DME has been implicated in the regulation of the parental imprinting phenomenon. By contrast, ROS1 seems to have much broader effects. The *ros1* mutation induces hypermethylation (mainly but not exclusively outside CGs) and transcriptional silencing of transgenes, endogenous genes, and transposon sequences [40,41•]. The overexpression of ROS1 reduces DNA methylation and upregulates transcription [39]. Thus, the regulation of DNA methylation seems to be very dynamic and the recent study of methylation profiling using genome-tiling microarrays has enlarged the number of loci known to be targeted by DNA demethylation [42]. Importantly, demethylated epialleles created in the *ros1* background cannot be propagated autonomously; they are reset to the previous wild-type status upon the return of ROS1 activity [40].

Autonomous epigenetic regulation of transcription

Massive release of transcriptional silencing is observed when activities required primarily for maintenance of DNA methylation at CG sites are compromised due to mutations in *MET1* and *DECREASE IN DNA METHYLATION1* (*DDM1*). Many heterochromatic transposable elements [16•,35,38,43,44] and repeats such as centromeric and pericentromeric multicopy sequences become transcribed [17,18,45]. In addition, transcriptional upregulation occurs at euchromatic loci such as *FWA*, *PAI*, Cyclophilin 40 [4,46–48]. Recent comparison of the genome-wide profiles of DNA methylation and transcript accumulation of wild-type and *met1-3* plants revealed a plethora of targets regulated by CG methylation [33,34••].

Mutant strains deficient in DDM1 function exhibit progressive decrease in DNA methylation during their inbreeding. This is accompanied by the aggravation of developmental defects in each consecutive generation [29]. Similar observations have been made for *met1-1*, a strain with partial loss of MET1 function [49,50]. Importantly, it has been found that CG methylation cannot be easily restored after its depletion in *ddm1* and *met1* mutants [49,51,52]. Therefore, stable epigenetic variants of loci devoid of CG methylation can be transmitted over many plant generations, even when all functions required for maintenance of CG methylation are provided. Moreover, studies of *met1* complete loss-of-function alleles (*met1-3* and *met1-4*) have clearly demonstrated that CG methylation is indispensable for gametophytic and, thus, transgenerational transmission of epigenetic information [52]. These studies also revealed the importance of CG methylation for plant gametogenesis and/or early embryogenesis, since only 2% of the expected number of plants homozygous for *met1-3* were recovered from self-pollinated *met1-3*/*MET1* heterozygous parents [52]. Recently, it has been confirmed using a different *met1* null allele

(*met1-6*) that MET1 is essential for embryogenesis and the formation of viable seeds [53]. Self-pollinated *met1-6* homozygous plants generated siliques containing an increased number of aborted seeds compared with wild type and approximately 30% of abnormal embryos, together with the deregulation of transcription of genes regulating cell identity during early embryogenesis. Self-pollination of heterozygous *met1-6/MET1* plants and their reciprocal crosses with wild type demonstrated that loss of CG methylation during female and male gametogenesis impairs embryogenesis and seed viability [53]. Moreover, a certain functional redundancy between MET1 and CMT3 was recorded, with both contributing unequally to seed viability and to later plant development [53].

Functional relationship between autonomous and non-autonomous epigenetic regulation of transcription

It has been demonstrated that the erasure of CG methylation in *met1* plants causes massive relocation H3K9me2 [16•,54,55] and it was postulated, therefore, that CG methylation is able to direct H3K9me2 [16•,20,54,55]. Moreover, it was also demonstrated that H3K9me2, mediated by KYP/SUVH4, directs non-CG methylation [56,57] and that SUVH4 acting redundantly with SUVH5 and SUVH6 is involved in the targeting of non-CG methylation, although this seems to involve H3K9me only indirectly [21]. In addition, the SUVH2 level also influences non-CG methylation [23]. Therefore, a 'two-step' regulation of transcriptional silencing has been proposed, in which CG methylation directs H3K9 methylation and H3K9 methylation recruits non-CG methylation. However, a number of cases do not conform to this model. In contrast to the 'two-step' regulation proposed for *Arabidopsis*, mutation of the H3K9me2 methyltransferase in rice (SDG714) decreases the level not only of CNG but also of CG methylation [25]. In addition, mutation of *CMT3* required for non-CG methylation causes some loss of H3K9me2 at the *Ta3* transposons *PAI2* and *PAI3* [21,37]. Noticeably, recent refinement of the functional relationship between DNA and H3K9 methylation has revealed locus-specific differences [58•]. Two sequences, one methylated mainly at cytosines in non-CG sequences (*AtSN1*) and the other mainly at CGs (*AtCOPIA4*), were examined for H3K9me2 in *met1* and *drm1drm2cmt3* mutant backgrounds. While depletion of CG methylation in *met1* led to the reduction of H3K9me2 at the *AtCOPIA4* element, CG depletion had no effect on H3K9me2 at *AtSN1*. Moreover, reduction of non-CG methylation levels in the *drm1drm2cmt3* triple mutant affected levels of H3K9me2 only at *AtSN1* and not at *AtCOPIA4*. The results of mobility-shift assays demonstrated that the SRA domains of KYP and SUVH6 bind methylated DNA with differing affinity for methylated CGs and non-CGs [58•]. This suggests that the targeting of H3K9me2 differentiates methylated targets with dominant CG or non-CG methylation. Recently, the two proteins TOUSLED and RPA2 have been shown to affect H3K9 methylation and gene silencing in a DNA methylation-independent manner [59–62]. This adds the further possibility that H3K9me2 and DNA methylation can be functionally disconnected altogether.

Similarly loose or flexible cooperation between H3K9me2 and any H3K27 methylation has been revealed by immunocytology and ChIP [15,16•]. Initially, Lindroth *et al.* proposed that cooperative binding to H3 methylated in K9 and K27 is required for the heterochromatic recruitment of CMT3 [15]. However, this attractive model may need further refinement, since the CMT3 binding assays of Lindroth *et al.* made use of H3K9me3 and H3K27me3 and these H3 modifications are very rare [63] or localized in euchromatin [15,16•], respectively. Moreover, the erasure of CG methylation in *met1-3* resulted in the relocation of H3K9me2 away from heterochromatin but the distribution of H3K27me1 or H3K27me2 remained unchanged. By contrast, H3K27me3, which is usually distributed in the nuclei of wild-type plants as speckles outside the heterochromatic chromocenters, was relocated to heterochromatic chromosomal regions, co-localizing with some (two to four) but not all chromocenters. Furthermore, in almost all *met1-3* nuclei analyzed at least one of the enriched H3K27me3 chromocenters corresponded to a 5S rDNA locus. Since *met1-3* releases silencing of 5S genes [18,19], Mathieu *et al.* speculated that some 5S rDNA locus in *met1* gain H3K27me3 but others not, which may reflect parental origin. However, this first suggestion of imprinting in the sporophyte needs further experimental support. Nevertheless, the results demonstrate that the relationship between CG methylation and H3K27me3 is rather complex and our view needs further refinement [16•].

Conclusions

CG methylation plays a key role in the transgenerational inheritance of epigenetic states of transcriptional activity. Patterns of CG methylation marks are relatively stable and once erased cannot be restored. On the other hand, histone and non-CG methylation marks are much more dynamic and this provides mechanisms for memorizing environmental cues and the ability to respond at the transcriptional level during much later developmental stages. Further studies of the intimate interplay between these two modes of transcriptional regulation are likely to provide fascinating insights into mechanisms of long-term adaptation by plants to changing environments.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lachner M, O'Sullivan RJ, Jenuwein T: **An epigenetic road map for histone lysine methylation**. *J Cell Sci* 2003, **116**:2117-2124.

2. Richards EJ, Elgin SC: **Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects**. *Cell* 2002, **108**:489-500.

3. Jullien PE, Kinoshita T, Ohad N, Berger F: **Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting**. *Plant Cell* 2006, **18**:1360-1372.

4. Kinoshita T, Miura A, Choi Y, Kinoshita Y, Cao X, Jacobsen SE, Fischer RL, Kakutani T: **One-way control of FWA imprinting in Arabidopsis endosperm by DNA methylation**. *Science* 2004, **303**:521-523.

5. Morales-Ruiz T, Ortega-Galisteo AP, Ponferrada-Marin MI, Martinez-Macias MI, Ariza RR, Roldan-Arjona T: **DEMETER and REPRESSOR OF SILENCING 1 encode 5-methylcytosine DNA glycosylases**. *Proc Natl Acad Sci U S A* 2006, **103**:6853-6858.

6. Gehring M, Huh JH, Hsieh TF, Penterman J, Choi Y, Harada JJ, Goldberg RB, Fischer RL: **DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation**. *Cell* 2006, **124**:495-506.

7. • Jullien PE, Katz A, Oliva M, Ohad N, Berger F: **Polycomb group complexes self-regulate imprinting of the Polycomb group gene MEDEA in Arabidopsis**. *Curr Biol* 2006, **16**:486-492.

The authors demonstrate that the imprinted *MEA* gene is silenced by PcG complexes independently of DNA methylation.

8. Baroux C, Gagliardini V, Page DR, Grossniklaus U: **Dynamic regulatory interactions of Polycomb group genes: MEDEA autoregulation is required for imprinted gene expression in Arabidopsis**. *Genes Dev* 2006, **20**:1081-1086.

9. Kohler C, Hennig L, Spillane C, Pien S, Gruissem W, Grossniklaus U: **The Polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene PHERES1**. *Genes Dev* 2003, **17**:1540-1553.

10. Kohler C, Page DR, Gagliardini V, Grossniklaus U: **The Arabidopsis thaliana MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting**. *Nat Genet* 2005, **37**:28-30.

11. Makarevich G, Leroy O, Akinci U, Schubert D, Clarenz O, Goodrich J, Grossniklaus U, Kohler C: **Different Polycomb group complexes regulate common target genes in Arabidopsis**. *EMBO Rep* 2006, **7**:947-952.

12. Schubert D, Primavesi L, Bishopp A, Roberts G, Doonan J, Jenuwein T, Goodrich J: **Silencing by plant Polycomb-group genes requires dispersed trimethylation of histone H3 at lysine 27**. *EMBO J* 2006, **25**:4638-4649.

13. Cao R, Zhang Y: **The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3**. *Curr Opin Genet Dev* 2004, **14**:155-164.

14. Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J, Jacobsen SE: **Whole-genome analysis of histone H3 lysine 27 trimethylation in Arabidopsis**. *PLoS Biol* 2007, **5**:e129.

15. Lindroth AM, Shultis D, Jasencakova Z, Fuchs J, Johnson L, Schubert D, Patnaik D, Pradhan S, Goodrich J, Schubert I *et al.*: **Dual histone H3 methylation marks at lysines 9 and 27 required for interaction with CHROMOMETHYLASE3**. *EMBO J* 2004, **23**:4286-4296.

16. • Mathieu O, Probst AV, Paszkowski J: **Distinct regulation of histone H3 methylation at lysines 27 and 9 by CpG methylation in Arabidopsis**. *EMBO J* 2005, **24**:2783-2791.

Through immunolocalization and chromatin immunoprecipitation experiments, this article demonstrates that the heterochromatic marks H3K27me1 and H3K27me2 are not influenced by DNA methylation, whereas loss of CG methylation allows the relocation of the euchromatic repressive mark H3K27me3 to heterochromatin.

17. May BP, Lippman ZB, Fang Y, Spector DL, Martienssen RA: **Differential regulation of strand-specific transcripts from Arabidopsis centromeric satellite repeats**. *PLoS Genet* 2005, **1**:e79.

18. Vaillant I, Schubert I, Tourmente S, Mathieu O: **MOM1 mediates DNA-methylation-independent silencing of repetitive sequences in Arabidopsis**. *EMBO Rep* 2006, **7**:1273-1278.

19. Vaillant I, Tutois S, Cuvillier C, Schubert I, Tourmente S: **Regulation of Arabidopsis thaliana 5S rRNA Genes**. *Plant Cell Physiol* 2007, **48**:745-752.

20. Tariq M, Paszkowski J: **DNA and histone methylation in plants**. *Trends Genet* 2004, **20**:244-251.

21. Ebbs ML, Bartee L, Bender J: **H3 lysine 9 methylation is maintained on a transcribed inverted repeat by combined action of SUVH6 and SUVH4 methyltransferases**. *Mol Cell Biol* 2005, **25**:10507-10515.

22. •• Ebbs ML, Bender J: **Locus-specific control of DNA methylation by the Arabidopsis SUVH5 histone methyltransferase**. *Plant Cell* 2006, **18**:1166-1176.

The authors demonstrate that SUVH4/KYP, SUVH5 and SUVH6 act redundantly in H3K9 methylation, which controls CMT3-mediated non-CG methylation in a target-specific manner.

23. Naumann K, Fischer A, Hofmann I, Krauss V, Phalke S, Irmler K, Hause G, Aurich AC, Dorn R, Jenuwein T *et al.*: **Pivotal role of AtSUVH2 in heterochromatic histone methylation and gene silencing in Arabidopsis**. *EMBO J* 2005, **24**:1418-1429.

24. Baumbusch LO, Thorstensen T, Krauss V, Fischer A, Naumann K, Assalkhou R, Schulz I, Reuter G, Aalen RB: **The Arabidopsis thaliana genome contains at least 29 active genes encoding SET domain proteins that can be assigned to four evolutionarily conserved classes**. *Nucleic Acids Res* 2001, **29**:4319-4333.

25. Ding Y, Wang X, Su L, Zhai J, Cao S, Zhang D, Liu C, Bi Y, Qian Q, Cheng Z *et al.*: **SDG714, a histone H3K9 methyltransferase, is involved in Tos17 DNA methylation and transposition in rice**. *Plant Cell* 2007, **19**:9-22.

26. Cao X, Jacobsen SE: **Role of the arabidopsis DRM methyltransferases in de novo DNA methylation and gene silencing**. *Curr Biol* 2002, **12**:1138-1144.

27. Cao X, Aufsatz W, Zilberman D, Mette MF, Huang MS, Matzke M, Jacobsen SE: **Role of the DRM and CMT3 methyltransferases in RNA-directed DNA methylation**. *Curr Biol* 2003, **13**:2212-2217.

28. Chan SW, Zilberman D, Xie Z, Johansen LK, Carrington JC, Jacobsen SE: **RNA silencing genes control de novo DNA methylation**. *Science* 2004, **303**:1336.

29. Chan SW, Henderson IR, Jacobsen SE: **Gardening the genome: DNA methylation in Arabidopsis thaliana**. *Nat Rev Genet* 2005, **6**:351-360.

30. Chan SW, Henderson IR, Zhang X, Shah G, Chien JS, Jacobsen SE: **RNAi, DRD1, and histone methylation actively target developmentally important non-CG DNA methylation in arabidopsis**. *PLoS Genet* 2006, **2**:e83.

31. Tompa R, McCallum CM, Delrow J, Henikoff JG, van Steensel B, Henikoff S: **Genome-wide profiling of DNA methylation reveals transposon targets of CHROMOMETHYLASE3**. *Curr Biol* 2002, **12**:65-68.

32. Tran RK, Zilberman D, de Bustos C, Ditt RF, Henikoff JG, Lindroth AM, Delrow J, Boyle T, Kwong S, Bryson TD *et al.*: **Chromatin and siRNA pathways cooperate to maintain DNA methylation of small transposable elements in Arabidopsis**. *Genome Biol* 2005, **6**:R90.

33. Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S: **Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription**. *Nat Genet* 2007, **39**:61-69.

34. Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, Chen H, •• Henderson IR, Shinn P, Pellegrini M, Jacobsen SE *et al.*: **Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis**. *Cell* 2006, **126**:1189-1201.
Using genome-wide tiling microarrays, the authors describe the distribution of DNA methylation in the *Arabidopsis* genome. Using different methylation mutants, the authors show that CG methylation mainly silences transposons and pseudogenes, whereas non-CG methylation associates and regulates genes.

35. Lippman Z, May B, Yordan C, Singer T, Martienssen R: **Distinct mechanisms determine transposon inheritance and methylation via small interfering RNA and histone modification**. *PLoS Biol* 2003, **1**:E67.

36. Rangwala SH, Richards EJ: **Differential epigenetics regulation within an arabidopsis retroposon family**. *Genetics* 2007, **176**:151-160.

37. Johnson L, Cao X, Jacobsen S: **Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation**. *Curr Biol* 2002, **12**:1360-1367.

38. Kato M, Miura A, Bender J, Jacobsen SE, Kakutani T: **Role of CG and non-CG methylation in immobilization of transposons in Arabidopsis**. *Curr Biol* 2003, **13**:421-426.

39. Agius F, Kapoor A, Zhu JK: **Role of the Arabidopsis DNA glycosylase/lyase ROS1 in active DNA demethylation**. *Proc Natl Acad Sci U S A* 2006, **103**:11796-11801.

40. Gong Z, Morales-Ruiz T, Ariza RR, Roldan-Arjona T, David L, Zhu JK: **ROS1, a repressor of transcriptional gene silencing in Arabidopsis, encodes a DNA glycosylase/lyase**. *Cell* 2002, **111**:803-814.

41. Zhu J, Kapoor A, Sridhar VV, Agius F, Zhu JK: **The DNA • glycosylase/lyase ROS1 functions in pruning DNA methylation patterns in Arabidopsis**. *Curr Biol* 2007, **17**:54-59.
This paper demonstrates the essential role of ROS1 demethylase activity in the maintenance of proper levels of non-CG methylation at many chromosomal targets.

42. Penterman J, Zilberman D, Huh JH, Ballinger T, Henikoff S, Fischer RL: **DNA demethylation in the Arabidopsis genome**. *Proc Natl Acad Sci U S A* 2007, **104**:6752-6757.

43. Lippman Z, Gendrel AV, Black M, Vaughn MW, Dedhia N, McCombie WR, Lavine K, Mittal V, May B, Kasschau KD *et al.*: **Role of transposable elements in heterochromatin and epigenetic control**. *Nature* 2004, **430**:471-476.

44. Rangwala SH, Elumalai R, Vanier C, Ozkan H, Galbraith DW, Richards EJ: **Meiotically stable natural epialleles of Sadhu, a novel Arabidopsis retroposon**. *PLoS Genet* 2006, **2**:e36.

45. Mathieu O, Jasencakova Z, Vaillant I, Gendrel AV, Colot V, Schubert I, Tourmente S: **Changes in 5S rDNA chromatin organization and transcription during heterochromatin establishment in Arabidopsis**. *Plant Cell* 2003, **15**:2929-2939.

46. Soppe WJ, Jacobsen SE, Alonso-Blanco C, Jackson JP, Kakutani T, Koornneef M, Peeters AJ: **The late flowering phenotype of fwa mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene**. *Mol Cell* 2000, **6**:791-802.

47. Bartee L, Bender J: **Two Arabidopsis methylation-deficiency mutations confer only partial effects on a methylated endogenous gene family**. *Nucleic Acids Res* 2001, **29**:2127-2134.

48. Habu Y, Mathieu O, Tariq M, Probst AV, Smathajitt C, Zhu T, Paszkowski J: **Epigenetic regulation of transcription in intermediate heterochromatin**. *EMBO Rep* 2006, **7**:1279-1284.

49. Kankel MW, Ramsey DE, Stokes TL, Flowers SK, Haag JR, Jeddeloh JA, Riddle NC, Verbsky ML, Richards EJ: **Arabidopsis MET1 cytosine methyltransferase mutants**. *Genetics* 2003, **163**:1109-1122.

50. Ikeda Y, Kobayashi Y, Yamaguchi A, Abe M, Araki T: **Molecular basis of late-flowering phenotype caused by dominant epi-alleles of the FWA locus in Arabidopsis**. *Plant Cell Physiol* 2007, **48**:205-220.

51. Kakutani T, Munakata K, Richards EJ, Hirochika H: **Meiotically and mitotically stable inheritance of DNA hypomethylation induced by ddm1 mutation of Arabidopsis thaliana**. *Genetics* 1999, **151**:831-838.

52. Saze H, Mittelsten Scheid O, Paszkowski J: **Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis**. *Nat Genet* 2003, **34**:65-69.

53. Xiao W, Custard KD, Brown RC, Lemmon BE, Harada JJ, Goldberg RB, Fischer RL: **DNA methylation is critical for Arabidopsis embryogenesis and seed viability**. *Plant Cell* 2006, **18**:805-814.

54. Soppe WJ, Jasencakova Z, Houben A, Kakutani T, Meister A, Huang MS, Jacobsen SE, Schubert I, Fransz PF: **DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in Arabidopsis**. *EMBO J* 2002, **21**:6549-6559.

55. Tariq M, Saze H, Probst AV, Lichota J, Habu Y, Paszkowski J: **Erasure of CpG methylation in Arabidopsis alters patterns of histone H3 methylation in heterochromatin**. *Proc Natl Acad Sci U S A* 2003, **100**:8823-8827.

56. Jackson JP, Lindroth AM, Cao X, Jacobsen SE: **Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase**. *Nature* 2002, **416**:556-560.

57. Malagnac F, Bartee L, Bender J: **An Arabidopsis SET domain protein required for maintenance but not establishment of DNA methylation**. *EMBO J* 2002, **21**:6842-6852.

58. Johnson LM, Bostick M, Zhang X, Kraft E, Henderson I, Callis J, • Jacobsen SE: **The SRA methyl-cytosine-binding domain links DNA and histone methylation**. *Curr Biol* 2007, **17**:379-384.
The authors demonstrate that CG methylation as well as non-CG methylation is able to differentially target H3K9 methylation via variant SRA domains.

59. Wang Y, Liu J, Xia R, Wang J, Shen J, Cao R, Hong X, Zhu JK, Gong Z: **The protein kinase TOUSLED is required for maintenance of transcriptional gene silencing in Arabidopsis**. *EMBO Rep* 2007, **8**:77-83.

60. Xia R, Wang J, Liu C, Wang Y, Zhai J, Liu J, Hong X, Cao X, Zhu JK, Gong Z: **ROR1/RPA2A, a putative replication protein A2, functions in epigenetic gene silencing and in regulation of meristem development in Arabidopsis**. *Plant Cell* 2006, **18**:85-103.

61. Elmayan T, Proux F, Vaucheret H: **Arabidopsis RPA2: a genetic link among transcriptional gene silencing, DNA repair, and DNA replication**. *Curr Biol* 2005, **15**:1919-1925.

62. Kapoor A, Agarwal M, Andreucci A, Zheng X, Gong Z, Hasegawa PM, Bressan RA, Zhu JK: **Mutations in a conserved replication protein suppress transcriptional gene silencing in a DNA-methylation-independent manner in Arabidopsis**. *Curr Biol* 2005, **15**:1912-1918.

63. Jackson JP, Johnson L, Jasencakova Z, Zhang X, PerezBurgos L, Singh PB, Cheng X, Schubert I, Jenuwein T, Jacobsen SE: **Dimethylation of histone H3 lysine 9 is a critical mark for DNA methylation and gene silencing in Arabidopsis thaliana**. *Chromosoma* 2004, **112**:308-315.