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.

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 of a ‘histone code’ that, in association with information encoded by DNA methylation, determines the structure of chromatin and its transcriptional competence. 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 PHERESI (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 I (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 (SNW) [11]. In addition, CLF mediates repression of the floral homeotic gene AGAMOUS and the homeobox SHOOTMERISTEMSLESS (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 for silencing [13]. A recent study that profiled H3K27me3 on a whole-genome tiling array broadened the spectrum for silencing [13].

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The DOMAINS REARRANGED METHYLTRANSFERASE 1 and 2 (DRM1 and 2) have been shown to target methylation to cytosines in any sequence 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 (SUHV1 to SUHV10, KYP being SUHV4) [24] and indeed overlapping function in the maintenance of the H3K9me1-mc2 of SUHV4/KYP, SUHV5 and SUHV6 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 SUHV2 coordinately reduce or elevate levels of all four heterochromatin-specific histone methylation marks (H3K9me1-mc2 and H3K27mc1-mc2), 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-tilling 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 (DDMI). 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 [44,46–48]. 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 normal 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


