Plant chromatin: development and gene control

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Summary

It is increasingly clear that chromatin is not just a device for packing DNA within the nucleus but also a dynamic material that changes as cellular environments alter. The precise control of chromatin modification in response to developmental and environmental cues determines the correct spatial and temporal expression of genes. Here, we review exciting discoveries that reveal chromatin participation in many facets of plant development. These include: chromatin modification from embryonic and meristematic development to flowering and seed formation, the involvement of DNA methylation and chromatin in controlling invasive DNA and in maintenance of epigenetic states, and the function of chromatin modifying and remodeling complexes such as SWI/SNF and histone acetylases and deacetylases in gene control. Given the role chromatin structure plays in every facet of plant development, chromatin research will undoubtedly be integral in both basic and applied plant biology.

***BioEssays* 24:234–243, 2002.**

 DOI 10.1002/bies. 10055

Introduction

The basic unit of chromatin organization is the nucleosome, in which approximately 150 bp of DNA are wrapped around an octamer core of histone proteins (two copies each of H2A, H2B, H3, and H4). The linear arrays of nucleosomes are themselves packaged into more condensed chromosomal fiber structures. In the past, chromatin was generally thought to be a passive structure capable of repressing transcription.[1] It is now evident that chromatin is a dynamic fabric with an architecture that is constantly remodeled through the activities of various complexes. The precise control of chromatin modification by arrays of transcriptional regulators in response to changes in various cellular and environmental stimuli elicits the correct temporal and spatial development in eukaryotic organisms.

Recent advances suggest that the multiple changes in chromatin architecture during development are mediated by various histone modifications. It is well established that specific amino acids on the histone tails can be modified by acetylation, phosphorylation, ubiquitination and ADP-ribosylation.[2] While the contributions of histone acetylation to gene regulation have been recognized, however, an understanding of the crucial roles played by these various modifications in managing cellular processes is just emerging. A recently proposed "histone code" hypothesis suggests that the combination of covalent modifications of histones constitutes a specific "code" that interacts with specific domains of chromatin-associated proteins.[3–5] The chromatin-associated proteins containing these domains bind to chromatin regions with the specific histone code to alter its structure, provide additional enzymatic activity, or to target other regulatory proteins.[6] Thus, each combination of histone modifications interacts with specific set of proteins that define different epigenetic states, yielding various cellular consequences (Table 1). The histone code hypothesis can explain extremely well the findings that one type of histone modification can serve in several different, sometimes entirely opposite, biological functions. For example, histone H3 phosphorylation at Ser-10 (H3S10P) coupled with acetylation at Lys-9 (H3K9Ac) and/or Lys-14 (H3K14Ac) leads to activation of immediate-early genes, while H3 phosphorylation coupled with other signals such as the incorporation of the pericentric H3 analog Cenp-A would lead to mitotic chromosome condensation.[4] In addition, H3S10P can function as an epigenetic switch. It has been shown that in vitro H3S10P inhibits methylation of H3 at Lys-9 (H3K9Me) and vice versa. Since the H3K9Me leads to gene silencing and heterochromatin formation, H3S10P and H3K9Me could be used as a switch between the active and repressed state of chromatin configuration.[4]

Studies on plant chromatin started in the early 1970s.[7,8] However, difficulties inherent to the establishment of an in vitro transcription system contributed to the scarcity of studies on plant chromatin prior to revitalization of interest stimulated by studies of gene silencing and other plant epigenetic phenomena[9,10] and findings that chromatin-modifying homologs play crucial roles during plant development.[11–14] Plant chromatin

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Abbreviations: ABA, abscisic acid; CAF, chromatin assembly factor; 35S, Cauliflower Mosaic Virus 35S promoter; dsRNA, double stranded RNA; HAT, histone acetylase; Pc-G, polycomb-group; PTGS, post-transcriptional gene silencing; T-DNA, transfer DNA; TGS, transcriptional gene silencing.

Table 1. Histone codes and their cellular functions*

Combination of histone modification	Organism	Cellular function
H3K4me+H3K14Ac	Tetrahymena, mammals	Interact with chromo domain containing coactivators and lead to gene activation
H3K9me and/or H4K12Ac	Yeast, Drosophila, and mammals	Interact with chromo domain containing repressors eventually lead to gene inactivation and heterochromatin formation
H3K9me+H1K26me	Yeast, Drosophila, and mammals	Combinatorial signal to recruit Pc-G protein complexes and leads to repression
H3K9me+H4K12Ac	Mammals	Gene inactivation, chromatin condensation
H3S10P+CENP-A	Yeast, mammals	Chromatin condensation through mitosis and meiosis
H3S10P+H3K9Ac and/or H3K14Ac	Yeast, mammals	Transcriptional activation
H4R3me+H4K5Ac	Yeast, mammals	Interact with chromodomain containing coactivators and lead to gene activation

*The references that this table is based on are reviews by Tumer,[3] Jenuwein and Allis,[4] Stahl and Allis,[5] Berger[6] and references therein.

research is now making novel contributions to the understanding of roles played by chromatin factors during development. In contrast to the situation in animals where mutations of most chromatin-modifying factors cause embryonic lethality,[15–17] similar mutations in plant homologs result in plants that are detectably modified but viable. This makes it relatively easy to study the effects of chromatin components during plant development. Here, we first discuss mechanisms of chromatin action on gene expression. This is followed by an examination of the fundamental roles chromatin-related factors play in important plant developmental processes. Finally, we review the effects of DNA methylation and chromatin in controlling invasive DNA and in the maintenance of epigenetic states.

Plant chromatin: mechanisms of gene control

Although the histone code and the mechanism of chromatin control are less well studied in plants, it is becoming apparent that, like in animals and yeast, various chromatin-modifying or remodeling complexes, such as the SWI/SNF complex, histone acetyltransferases and histone deacetylases are equally crucial for plant gene control.[18,19]

The plant SWI/SNF complex

SWI/SNF is a highly conserved protein complex that has been proposed to function in transcription by remodeling repressive chromatin structures.[20] A recent model proposes that the SWI/SNF complex uses the energy from ATP hydrolysis to create changes in the DNA twist that diffuse throughout the nucleosome.[21] These changes cause the histone–DNA interaction to weaken and consequently the DNA is more accessible to transcription factors and other proteins.

SWI/SNF genes with striking similarity have been isolated from human, mouse, *Drosophila* and plants.[22] *BSH* (for bushy growth), a plant gene encoding one of the SWI/SNF components, was cloned by virtue of its homology to yeast *SNF5*.[23] The finding that *Arabidopsis BSH* partially complemented the *snf5* mutant in *S. cerevisiae* strongly indicates the functional homology between the two genes. *BSH* is expressed ubiquitously in *Arabidopsis*; however, a considerable reduction of its level was not lethal but yielded a distinctive morphological change resembling that of *aux1* mutants of *Arabidopsis*.[23] This result suggests a possible involvement of a plant SWI/SNF complex in the control of auxin-responsive genes.

The *Arabidopsis* gene *DDM1* (*DECREASE IN DNA METHYLATION 1*), which plays a role in maintenance of cytosine methylation patterns, is another SWI/SNF component. *DDM1* encodes a protein containing the eight signature motifs of *SNF2* family members and was originally identified by mutations that lead to a 70% reduction in genome-wide methylation.[12] This suggests that DDM1 functions in the DNA methylation system by affecting chromatin configuration. A very interesting possibility is that DDM1 is a part of a chromatin-remodeling complex that increases the accessibility of the hemimethylated DNA in newly replicated chromatin to the DNA methyltransferase. This would predict that *ddm1* mutations preferentially hypomethylate genomic sequences packaged in highly condensed chromatin. In support of this possibility, repeat sequences such as satellite DNA and ribosomal DNA lose methylation in the first *ddm1* generation while low-copy sequences lose their methylation following multiple generations of *ddm1* inbreeding.

A third SWI/SNF-like protein in *Arabidopsis*, MORPHEUS' MOLECULE (MOM), contains a region similar to domains IV, V, and VI of the helicase domain of the SWI2/SNF2 family.[24] It was speculated that the *MOM* encodes half of the SWI2/SNF2 helicase domain while a hypothetical binding partner supplies the other half. If MOM is indeed a component of a SWI/SNF complex, it should have a repressive role because mutations in MOM relieve transgene silencing. Recent whole genome microarray studies in yeast suggest that SWI/SNF not only activates but also represses transcription as only half of

the genes affected have increased mRNA levels.[25] It is possible that MOM and its helicase domain are required for the formation of inactive chromatin and, in its absence, genes maintain a relatively open chromatin structure even in the presence of heavy DNA methylation.

Histone acetyltransferase and deacetylase

As in animals and yeast, plants have two types of histone acetyltransferases: type A (HAT-A) and type B (HAT-B) .[26] HAT-B primarily acetylates histones in the cytoplasm while HAT-A is responsible for acetylating nucleosomal core histones to create codes that mostly signal transcriptional activation. Although more than ten HAT-A genes have been identified through sequence comparison in *Arabidopsis* (www.chromdb.org), only a couple have been studied. One HAT studied in *Arabidopsis* is AtGCN5, a homolog of yeast GCN5, the HAT component of yeast SAGA capable of acetylating histone H3 in the nucleosome.[27,28] AtGCN5 contains all domains essential for yeast GCN5 activity in vivo and in vitro and can effectively acetylate histone H3 in vitro.[28] Another HAT studied in *Arabidopsis* is PCAT2, a homolog of P300/CBP.[29] Although PCAT2 has histone acetyltransferase activity, it lacks the bromodomain of human P300/CBP. The bromodomain in HAT has been shown to interact with acetylated lysine in the context of H3 and H4 tail sequences and was suggested to have evolved to recognize histone acetylation.[4] Without the bromodomain, PCAT2 may be attracted to chromatin through other means, such as by interacting with a transcriptional activator like a plant homolog of the adenovirus E1A protein.

Direct evidence that hyperacetylation of histones is correlated with the active gene state in plants was obtained only recently. Gray and colleagues[30] showed that histone H3 and H4 hyperacetylation in *PetE* promoter region is associated with increased *PetE* transcription in green shoots. More specifically, hyperacetylation of both histones H3 and H4 was targeted to about 400 bp in the enhancer/promoter region of active *PetE*. It was speculated that a sequence-specific DNA-binding protein recruited HATs that in turn hyperacetylated a nearby region, making it more accessible to the binding of transcription factors. Similarly, *Arabidopsis* CBF1, a transcriptional activator of genes involved in cold tolerance and drought resistance, functions by recruiting HAT-containing adaptor complexes to the promoters of the genes that it activates. It was found that CBF1 interacts directly with the AtADA2 and AtGCN5 in vitro. In addition, CBF1 was unable to stimulate reporter gene expression in yeast strains that contained null mutations in the transcriptional adaptor proteins ADA2 or ADA3 or the HAT protein GCN5.[28]

Three types of histone deacetylases have been found in plants.[31] Among the three, two types are shared by all eukaryotes. One group is closely related to yeast RPD3 and the other group is more related to yeast HDA1. Plants have a third group of histone deacetylases (HD2 type) that has no ortholog in animals.[32] *Arabidopsis* has two HD2 type histone deacetylases, AtHD2A and AtHD2B. An AtHD2A fusion protein has been shown to repress gene expression when targeted to the promoter used to drive reporter gene expression. Antisense silencing of AtHD2A resulted in aborted seed development in transgenic *Arabidopsis*, suggesting its essential role in the reproduction process.[33] AtHD2B appears to have a more general role because its expression in *Arabidopsis* is constitutive. AtHD1, an *Arabidopsis* homolog of yeast RPD3, has been shown to play an important role in gene regulation. Downregulation of AtDH1 has been shown to induce developmental pleiotropy in *Arabidopsis*, including a delay of the phase transition from the vegetative to the reproductive stage.[34]

Chromatin and plant tissue-specific gene regulation

The first clear demonstration that chromatin structure is involved in plant tissue-specific regulation comes from studies on the phaseolin (*phas*) gene.[35] Although the *phas* promoter is not inducible by abscisic acid (ABA), a plant hormone in vegetative tissues, it is highly responsive to ABA in intact embryos.[36,37] Detailed studies showed that this differential response to ABA is caused by differential chromatin architecture in these two tissues. The *phas* promoter adopts a repressive chromatin configuration in leaf tissue; however, this configuration is disrupted concomitant to transcriptional activation in developing seeds.[38] This architectural change is mediated through PvALF, a seed-specific transcription factor.[39] Ectopic expression of PvALF can modify the chromatin structure of the *phas* promoter in vegetative tissue, making it much more accessible to DNase I.[40] Although this chromatin modification does not by itself activate the *phas* promoter, it appears to potentiate it, thereby permitting the binding of an ABA-activated transcription factor (or factors) to its recognition site (Fig. 1A). Interestingly, the promoter of *RAB28*, a non-tissue-specific ABA responsive gene, possesses an open chromatin structure before ABA induction.[41]

Studies on pea *PetE* transcription provide additional evidence for the involvement of chromatin structure in tissue-specific gene regulation. In pea, expression of the *PetE* gene is light-inducible in shoot tissue but no transcripts were detected in root tissues. Chromatin studies suggested that the contrast state of *PetE* expression was related to its chromatin states. The promoter and enhancer regions of the *PetE* gene were more sensitive to micrococcal nuclease and DNase I digestion than those in roots where *PetE* adopted an inactive chromatin configuration.[30] Although the open chromatin structure in shoot tissue is not sufficient for high levels of transcription, it makes the *PetE* promoter more accessible to the binding of transcription factors and inducible by light. These results suggest that, like the *phas* gene, the activation of

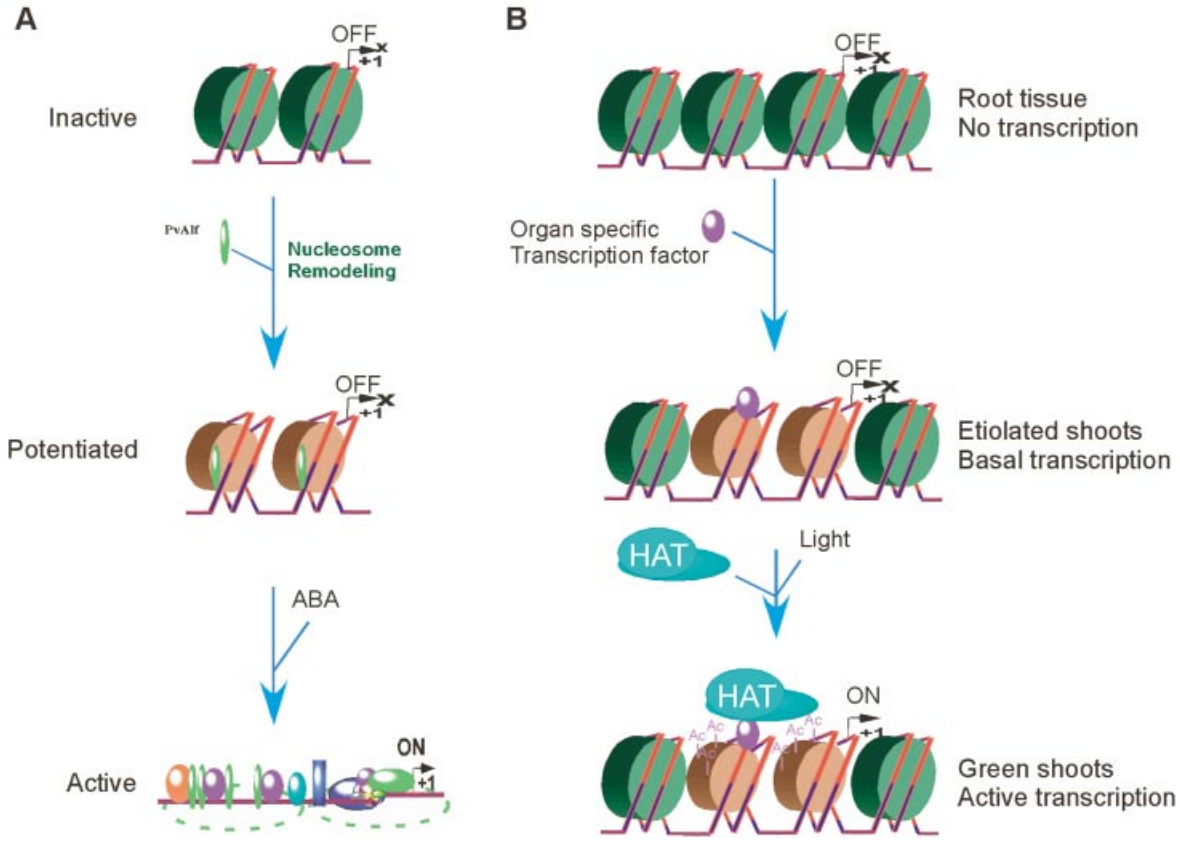

Figure 1. The two-step activation of tissue-specific genes. **A:** Activation of phaseolin promoter: chromatin remodeling by PvALF followed by ABA-mediated activation. **B:** Activation of pea plastocyanin gene: organ-specific chromatin remodeling is followed by light-induced activation, which is accompanied by histone acetylation in promoter and enhancer regions.

the *PetE* gene is a two-step process: chromatin modification followed by light-induced histone hyperacetylation during *PetE* activation (Fig. 1B).

Chromatin plays an important role in key developmental processes

During the entire life cycle of a plant, a wide variety of developmental decisions are carried out by selective activation or repression of different sets of genes. Advances in chromatin studies have made it clear that alteration of gene expression occurs in the context of chromosomes and several recently identified plant chromosomal factors clearly connect major plant developmental events with changes in chromatin structure (Fig. 2).

Polycomb group (Pc-G) proteins and maintenance of plant gene repression during development

Although cell fate is, in many cases, not determinate in plants,[11] the maintenance of spatially and temporally correct gene expression can still be crucial for normal plant development. Recent studies have shown that, like their animal counterparts, plant Pc-G proteins play very important roles in maintaining the repression of genes.[42,43] Mutations of Pc-G genes can result in the failure to maintain genes in a repressed state, leading to anomalies during plant development. CURLY LEAF, a Pc-G protein similar to Enhancer of zeste (E(z)), has been shown to be required to repress *AGAMOUS* transcription in leaves, pedicels, and flowers. Mutations in *CURLY LEAF* conferred pleiotropic effects on leaf and flower morphology, and on flowering time.[11] Another plant E(z) homolog, MEDEA, is required in maternal tissues to restrict cell proliferation in embryos. Its mutation promotes cell proliferation in the embryo but reduces cell proliferation in the endosperm, eventually leading to aborted seeds.[44] Other plant Pc-G proteins include FERTILIZATION-INDEPENDENT ENDOSPERM (FIE), a homolog of the WD motif-containing Pc-G proteins from *Drosophila* and mammals. One function of FIE is to suppress transcription of genes controlling the nuclear

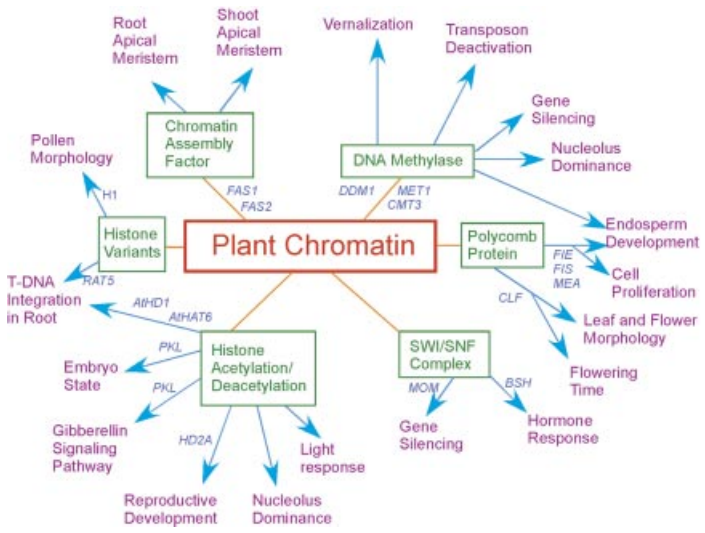

Figure 2. Panoply of plant chromatin-related factors and processes. The various developmental processes that chromatin participates are denoted by magenta type. Green boxes indicate various chromatin-related factors. Blue arrows connect chromatin-related factors with their respective developmental processes and blue italicized letters indicate genes involved in these processes.

replication of the central cell in the female gametophyte until fertilization occurs (Fig. 3A).[45]

The mechanism of Pc-G protein action is not clear but it may inhibit gene activity by forming chromatin structures that are inaccessible to transcriptional activators.[46] Interestingly, genetic analysis in *Drosophila* showed that the Mi-2 protein participates in Pc-G repression in vivo[47] and another study demonstrated that histone deacetylase (HDAC2) interacts with a human Pc-G protein both in vitro and in vivo,[48] suggesting that histone deacetylase function may be involved in the Pc-G-mediated repression. However, Strouboulis et al.[49] showed that repression by *Xenopus* Pc-G protein XPc1 is independent of histone deacetylase, indicating that an alternative repression mechanism exists for Pc-G-mediated gene repression.

Embryogenesis initiation: PICKLE represses the embryonic state

The PICKLE (PKL) is a developmental switch used by gibberellic acid (GA) to repress the embryonic state after germination and to allow transition to post-embryonic development. Mutation of *PKL* causes inappropriate expression of embryonic differentiation genes during postembryonic development. For example, *LEAFY COTYLEDON 1* (*LEC1*), a seed-specific gene encoding a transcription factor that promotes embryonic identity, is derepressed postembryonically in *pkl* mutants, as are genes for seed storage lipid and protein deposition.[13,50] *PKL*, isolated separately as *GYMNOS*, acts redundantly with *CRABS CLAW* to establish polarity.[14] These *pkl/gymnos* mutants displayed delayed maturation of several different tissue types, suggesting a role

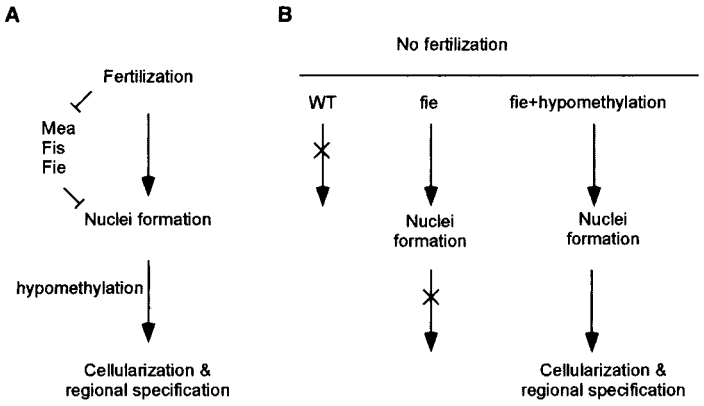

Figure 3. The role of Pc-G proteins and hypomethylation in endosperm development. **A:** A hypothetical model to explain the roles MEA, FIS, and FIE play in endosperm development. **B:** The combination of *fie* mutant and hypomethylation leads to autonomous endosperm development. Although fertilization is essential for the central cell to develop into endosperm in wild-type *Arabidopsis*, the mutation of FIE allows the central cell to proliferate autonomously without cellularization. Hypomethylation in *fie* mutants allows the endosperm to undergo cellularization and regional specification so that it resembles endosperm in sexually produced seeds.

for PKL/GYMNOS in repressing meristematic genes. Together, these studies suggest that transition to a determined cell type during *Arabidopsis* development requires repression of genes that promote a pluripotent character.

PKL/GYMNOS shows high similarity to human and *Drosophila* Mi2 (also called CHD4) and CHD3.[14] The CHD proteins derive their names from the possession of three domains: a chromo (chromatin organization modifier) domain, a SNF2-related helicase/ATPase domain, and a DNA-binding domain. It is possible that CHD proteins play a negative role in transcription. Genetic analysis in *Drosophila* indicates that dMi2 participates in both Hunchback and Pc-G-mediated repression in vivo.[47] In addition, *Xenopus* and human Mi2 have been shown to form complexes with histone deacetylase.[51,52] Hence, a plausible mechanism for repression of embryonic and meristematic genes by PKL in *Arabidopsis* is via histone deacetylation by a Mi2/NuRD complex.

Chromatin assembly factor and the postembryonic development of apical meristem

Chromatin assembly factor-1 (CAF-1) is a complex involved in chromatin assembly during DNA replication and DNA repair in vivo.[53] Recent studies have suggested that CAF-1 could serve to ensure the stable propagation of epigenetic states and maintenance of genome integrity.[54] Rapid reformation of nucleosomes onto newly replicated DNA by CAF-1 would prevent transcriptional regulators from being targeted to the DNA nonspecifically, thereby preventing random changes in gene expression patterns in daughter cells.

Like its animal counterpart, the plant CAF contributes to the establishment and maintenance of gene expression states. Mutations in either of the two CAF subunits (*fasciata-1* and *fasciata-2*) cause severely disturbed cellular and functional organization of both the shoot apical meristem (SAM) and the root apical meristem (RAM) in *Arabidopsis*.[55] This disturbed organization may result from a distorted expression pattern of both *WUSCHEL* and *SCARECROW*, which play key roles in the organization of the SAM and the RAM, respectively. In *fasciata* mutants, the *WUSCHEL* expression in SAM and the *SCARECROW* expression in RAM are not stably maintained. The pattern of ectopic expression is inconsistent, showing a wide range of variations among individual meristems, and the degree of ectopic expression tends to become more severe with time. It is likely that the *FASCIATA* genes facilitate the stable maintenance of gene expression states in plant apical meristems.[55]

Histone H1 and its role in plant development

H1 histones are abundant basic proteins that interact with nucleosomes and extend their contact into the linker DNA between nucleosomes.[56] Although considered to be a general repressor of transcription, recent studies support the notion that linker histones are involved in the selective regulation of specific classes of genes.[57] H1 and its variants have been shown to be responsible for the differential transcriptional regulation of the *5S rRNA* genes during early development in *Xenopus*.[57] H1 functions similarly in plants. Different H1 variants have been shown to be present in different growth condition and during various plant developmental stages.[58–60] Although the significance of these H1 variants is not clearly defined in plants,[58,60] the variability among the linker histones can be important in determining expression of some developmental stage-specific genes.

Using an antisense approach in transgenic tobacco, Prymakowska-Bosak et al.[59] demonstrated that the stoichiometry of linker histone variants is critical for pollen development. Although the change in stoichiometry of H1 variants in chromatin caused few morphological effects until flowering, the antisense plants showed distinctive morphological aberrations during flower development. The most characteristic of these aberrations was abnormally developed stamens and corollas. The stamens and petals were shortened, which resulted in styles that protruded from the corollas.[59] These phenotypic changes may have resulted from disturbances in correct pairing or segregation of homologous chromosomes, perhaps reflecting the abnormalities in heterochromatin regions that play a key role in establishing and maintaining the alignment of homologous chromosomes during meiosis.[59] In support of this speculation, plants with markedly altered proportions of H1 variants retained normal nucleosome spacing, but their chromosomes were less tightly packed than those of control plants.[59]

DNA methylation, chromatin and plant development

DNA methylation, the covalent addition of methyl groups to cytosine residues, is the most common form of DNA modification in higher eukaryotes. In *Arabidopsis*, methylation is established by the DOMAINS-REARRANGED METHYLTRANSFERASES[61] and is maintained by two types of maintenance methyltransferases, METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLTRANSFERASE 3 (CMT3). MET1 maintains methylation at CpG dinucleotides while CMT3 maintains methylation at CpNpG.[62,63] Recent studies showed that CpNpG and CpG methylation may operate in a partially redundant fashion to silence most plant genes.[62]

DNA methylation has been shown to play an important role in many processes during plant development. For example, DNA methylation is involved in preventing expression of genes related to initiation of flowers. Reduction in DNA methylation level was shown to be associated with the vernalization process that promotes early flowering in *Arabidopsis*. Treatment with the methylation inhibitor 5-azacytidine or debilitation of the *MET1* through antisense approaches leads to early flowering without cold treatment.[64] DNA methylation has also been shown to play a role in the inactivity of transposable

elements. Normally, transposons are heavily methylated and silenced. Reduction in DNA methylation can activate transposon activity. These activated transposons have been shown to be responsible for pleiotropic phenotypes observed in late generation *ddm1* mutants.[65,66]

DNA methylation may exert its effect on gene regulation via chromatin structure

It has been proposed that DNA methylation exerts its effect through the formation of inactive chromatin architecture. In support of this idea, DNA methylation has been shown to attract methyl-cytosine-binding protein (MeCP) complexes that recruit histone deacetylases to assist in the formation of an inactive chromatin structure.[67,68] Additional evidence comes from a recent finding that a mammalian DNMT1 is associated with histone deacetylase activity in vivo;[69] repression conveyed by a regulatory domain of DNMT1 can be partially alleviated by trichostatin A, an inhibitor of histone deacetylase.[70]

Because both DNA methyltransferases and histone deacetylases are evolutionarily conserved, a similar interaction should exist in plants. Although the work on plant MeCPs is limited, much evidence associates DNA methylation with histone deacetylation. For example, a strong correlation between DNA methylation and histone deacetylation was demonstrated by the fact that either 5-azacytidine or trichostatin A treatment leads to derepression of silent rRNA genes in *Brassica napus*.[71] Another link between DNA methylation and chromatin structure in plants comes from the existence of three CMTs (CMT1, CMT2, and CMT3).[72] These CMTs contain a chromodomain and a DNA methyltransferase domain. The chromodomain has been shown to be important in targeting Pc-G and Heterochromatin Protein1 (HP1) to the heterochromatic region. This suggests a possible targeting of methylation to the DNA in heterochromatic regions.

DNA methylation, imprinting and endosperm development

Genetic imprinting, the differential expression of a gene dependent on parental origin, has been shown to play an important role in endosperm development in plants.[73] There are two types of genetic imprinting in plants. One type is allelic-specific; for example, genetic imprinting of the *DZR1* gene[74] in maize endosperm. For this type of imprinting, only specific alleles are subject to epigenetic regulation by imprinting. The other type of genetic imprinting is locus-specific, for example, the genetic imprinting of *FIE-1, FIS,* and *MEA* genes involved in endosperm development. The regulation and function of the second type of imprinted gene is similar to that of imprinted loci in mammals.

DNA methylation is known to be an essential component of the imprinting mechanism in mammals[75] and evidence suggests that methylation is associated with allelic- and locus-specific genetic imprinting. Although the methylation patterns of *FIE, FIS,* and *MEA* have not been reported, a paternal *MEA* or *FIE* allele from methylation-deficient *ddm1* mutants can rescue seeds that carry a normally lethal maternal *mea* or *fie-1* mutation.[76,77] However, the reactivation of paternal *MEA* or *FIE* genes may not be related to hypomethylation. Unlike the effect of *ddm1* on other single-copy genes, the reactivation of the paternally inherited MEA allele occurs in the first generation when repeat, but not single copy DNA sequences, lose their methylation.[22] It is possible that imprinted genes, like repeat sequences, are assembled into heterochromatin and require chromatin remodeling to maintain their methylation status. It is also possible that reactivation of MEA is through DNA methylation-independent chromatin reconfiguration.

Although DNA methylation may not be involved in genetic imprinting of *MEA* or *FIE* genes, it certainly plays an important role in endosperm development. As discussed above, FIE, MEA, and FIS2 function to suppress proliferation of the central cell before fertilization (Fig. 3). Mutation of any of these genes would confer some degree of autonomous endosperm development without fertilization. However, none of these mutants appears to undergo full endosperm development, suggesting that an additional block must be present in these mutants. In an elegantly designed experiment, Scott and coworkers[77] showed that this additional block is related to DNA methylation (Fig. 3). Demethylating *fie-1/FIE* heterozygotes by using the *MET1* antisense construct allowed autonomous endosperm to develop much further. Therefore, demethylating the maternal genome appears to relieve the block on endosperm development in *fie-1* mutants, although hypomethylation did not by itself promote fertilization-independent seed development.

Chromatin and control of invasive DNA in plants

Mounting evidence suggests that chromatin is not only involved in regulation of endogenous genes but also plays a crucial role in silencing invasive foreign DNA.[78,79] In addition, recent evidence shows that chromatin participates in T-DNA integration events.[80]

Chromatin and transgene silencing

Both transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) have been shown to be associated with increased DNA methylation and inactive chromatin configuration.[81,82] TGS has been correlated with increased promoter methylation that is mitotically and meiotically heritable.[83] In PTGS, methylation occurs in the transcribed region. Although TGS and PTGS have been thought to be discrete processes, it is now clear that they have mechanistic similarities. Both PTGS and TGS can be induced by double-stranded RNA (dsRNA). In a carefully designed experiment, a truncated nopaline synthase (*nos*) promoter transcript driven

by the Cauliflower Mosaic Virus 35S promoter (*35S*) was found to trigger methylation and TGS of an unlinked homologous promoter (*nos* driving *nptII* that encodes resistance to kanamycin).[84] It was shown subsequently that the DNA methylation and TGS were accompanied by dsRNA.[85] The promoter dsRNA is partially cleaved into small RNAs ≈23 nucleotides in length, supporting the concept that dsRNA can trigger promoter DNA methylation and TGS.[85] It is envisioned that dsRNA can enter the nucleus, find homologous sequences and trigger DNA methylation that leads to the silencing of the corresponding gene(s).

Although there appears to be a close relationship between DNA methylation and silencing, recent results suggest that DNA methylation alone is not sufficient to maintain TGS or PTGS. Several mutations can reactivate TGS-silenced transgenes without altering their methylation status. For example, mutation of *MOM* resulted in transgene activation without affecting CG or CNG methylation of *35S/HPT*.[24] Similarly, transgene methylation was not affected when systemic PTGS was blocked.[86] Further evidence that methylation and silencing are not intrinsically linked is provided by the finding that antisense expression of *MET1* reduced methylation of a transgene without releasing silencing.[87] Therefore, it appears that some mechanism other than DNA methylation is the enforcer of gene silencing. One possibility is that DNA methylation-induced inactive chromatin structure maintains transgene gene silencing. Because *MOM* encodes half of the SWI/SNF helicase domain,[24] transgene reactivation in the *mom* mutant may result from alteration of chromatin architecture.

Chromatin and T-DNA integration

Although chromatin structure, in general, plays an important role in silencing intruding DNA, it can also act as a bridge for foreign DNA to enter the plant genome. In studying the mechanism of DNA transfer from bacteria to plants, Gelvin and colleagues found that one variant of histone H2A (RAT5) is essential for T-DNA integration in *Arabidopsis*.[80] They discovered that the *rat5* mutant was resistant to *Agrobacterium*-mediated root transformation; however, it is unclear how the transformation process is affected by this mutation. The original *rat5* mutation was generated by a T-DNA insertion into the 3′UTR; therefore, the effect of this mutation cannot result from an altered structure and function of the H2A variant.[80] It was speculated that the insertion of T-DNA into the 3′UTR may affect translation from RAT5 mRNA and therefore the level of RAT5 since the 3′UTR of histone H2A mRNA could be important for its stability.[80] It is possible that the structure or post-translational modification of RAT5 is different from other types of H2A variants and incorporation of RAT5 makes the chromatin more accessible to the T-DNA integration process. If less RAT5 were synthesized (as in *rat5* mutants), more of the other types of H2A variants would be integrated into the chromatin, therefore making the chromatin more compact and resistant to T-DNA integration. If the above hypothesis is correct, incorporation of more RAT5 into chromatin would make plants more susceptible to *Agrobacterium* transformation. In support of this concept, transgenic plants overexpressing *RAT5* were approximately twice as susceptible to *Agrobacterium* root transformation as were wild-type plants.[80]

The important role of chromatin structure in T-DNA integration is also supported by the observations that T-DNA insertion mutants of the *Arabidopsis* histone deacetylase *AtHD1* and histone acetyltransferase *AtHAT6* are resistant to *Agrobacterium*-mediated root transformation (S. Gelvin, personal communication). It is not clear why mutations in opposing enzymes both yield similar transformation-resistant phenotypes. The possibility exists that AtHD1 and AtHAT6 work on opposing histone variants and their respective products are required for efficient T-DNA integration. It is also possible that AtHD1 can repress a repressor of T-DNA integration and AtHAT6 activates an enhancer of T-DNA integration and that both are essential for *Agrobacterium*-mediated T-DNA integration.

Perspectives on chromatin research in plants

Tremendous progress has been made in the plant chromatin field in the past few years. Through identifying the modifiers of transgene silencing and other mutant screening procedures, many chromatin-related factors have been discovered and shown to participate in every aspect of plant development. Interestingly, some of these chromatin-related factors such as the HD2 type histone deacetylases and CMTs are proteins yet to be discovered in other systems. If these proteins prove to be unique to plants, further investigation would shed light on plant-specific chromatin control. For example, *Arabidopsis* CMTs combine DNA methylating activity with a chromodomain that targeting protein HP1 to histones methylated at Lys-9. Relevant questions to ask are: did CMT proteins evolve in plants to connect plants' predominant DNA methylation with the repressive histone code? If not, what roles do CMTs play?

Characterization of these mutants also illustrated the value of chromatin research towards applied plant study. For example, the histone H2A variant RAT5 has been shown to be involved in the T-DNA integration process[88] and its expression pattern appears to be a predictor of competent cells for *Agrobacterium*-mediated transformation (S. Gelvin, personal communication). Further research on this phenomenon may eventually lead to the ability to transform recalcitrant plant species with high efficiency. Likewise, studies on TGS and PTGS may guide us in devising ways to overcome unwanted transgene silencing.

Despite of the rapid advances described here, it is evident that we are just beginning to understand the intricate roles played by chromatin in plant development. Characterization of

plant SWI/SNF complexes, HATs and HDs has just started. Other modifying factors such as histone kinases and histone methyltransferases remain to be identified in plants. With more intensive mutant generating and screening efforts by a National Science Foundation-funded consortium (www.chromdb.org) and other plant chromatin researchers, it can be expected that these histone-modifying enzymes and many more chromatin-related genes will be discovered. Exciting novel insight into the way in which chromatin and the proposed histone code alter in response to changing cellular environments during plant development can be anticipated.

Acknowledgments

We thank Dr. Stanton Gelvin for allowing us to cite unpublished observations. We are particularly grateful to Drs. Casey Case, Chris Tse, Mahesh Chandrasekharan and Xiaosong Gong for critical reading of this review and to Jeff Chen and Philip Gregory for helpful discussion. We apologize to those whose work was not cited due to space restrictions.

References

1. Grunstein M. Histone function in transcription. Annu Rev Cell Biol 1990; 6:643–678.
2. Wolffe AP, Hayes JJ. Chromatin disruption and modification. Nucleic Acids Res 1999;27:711–720.
3. Turner BM. Histone acetylation and an epigenetic code. Bioessays 2000; 22:836–845.
4. Jenuwein T, Allis CD. Translating the histone code. Science 2001;293: 1074–1080.
5. Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403:41–45.
6. Berger SL. Molecular biology. The histone modification circus. Science 2001;292:64–65.
7. McGhee JD, Engel JD. Subunit structure of chromatin is the same in plants and animals. Nature 1975;254:449–450.
8. Spiker S, Krishnaswamy L. Constancy of wheat histones during development. Planta 1973;110:71–76.
9. Matzke AJM, Matzke MA. Position effects and epigenetic silencing of plant transgenes. Curr Opin Plant Biol 1998;1:142–148.
10. Wolffe AP, Matzke MA. Epigenetics: regulation through repression. Science 1999;286:481–486.
11. Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G. A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. Nature 1997;386:44–51.
12. Jeddeloh JA, Stokes TL, Richards EJ. Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat Genet 1999;22:94–97.
13. Ogas J, Kaufmann S, Henderson J, Somerville C. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. Proc Natl Acad Sci USA 1999;96:13839–13844.
14. Eshed Y, Baum SF, Bowman JL. Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis*. Cell 1999;99:199–209.
15. Roberts CW, Galusha SA, McMenamin ME, Fletcher CD, Orkin SH. Haploinsufficiency of Snf5 (integrase interactor 1) predisposes to malignant rhabdoid tumors in mice. Proc Natl Acad Sci USA 2000;97:13796–13800.
16. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999;99:247–257.
17. Quivy JP, Grandi P, Almouzni G. Dimerization of the largest subunit of chromatin assembly factor 1: importance in vitro and during *Xenopus* early development. EMBO J 2001;20:2015–2027.
18. Meyer P. Chromatin remodelling. Curr Opin Plant Biol 2001;4:457–462.
19. Verbsky ML, Richards EJ. Chromatin remodeling in plants. Curr Opin Plant Biol 2001;4:494–500.
20. Winston F, Carlson M. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet 1992;8:387–391.
21. Gavin I, Horn PJ, Peterson CL. SWI/SNF chromatin remodeling requires changes in DNA topology. Mol Cell 2001;7:97–104.
22. Martienssen R, Henikoff S. The House & Garden guide to chromatin remodelling. Nat Genet 1999;22:6–7.
23. Brzeski J, Podstolski W, Olczak K, Jerzmanowski A. Identification and analysis of the *Arabidopsis thaliana* BSH gene, a member of the SNF5 gene family. Nucleic Acids Res 1999;27:2393–2399.
24. Amedeo P, Habu Y, Afsar K, Scheid OM, Paszkowski J. Disruption of the plant gene MOM releases transcriptional silencing of methylated genes. Nature 2000;405:203–206.
25. Sudarsanam P, Iyer VR, Brown PO, Winston F. Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 2000;97:3364–3369.
26. Lusser A, Kolle D, Loidl P. Histone acetylation: lessons from the plant kingdom. Trends Plant Sci 2001;6:59–65.
27. Grant PA, Schieltz D, Pray-Grant MG, Steger DJ, Reese JC, Yates JR, 3rd, Workman JL. A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. Cell 1998;94:45–53.
28. Stockinger EJ, Mao Y, Regier MK, Triezenberg SJ, Thomashow MF. Transcriptional adaptor and histone acetyltransferase proteins in *Arabidopsis* and their interactions with CBF1, a transcriptional activator involved in cold-regulated gene expression. Nucleic Acids Res 2001;29: 1524–1533.
29. Bordoli L, Netsch M, Luthi U, Lutz W, Eckner R. Plant orthologs of p300/CBP: conservation of a core domain in metazoan p300/CBP acetyltransferase-related proteins. Nucleic Acids Res 2001;29:589–597.
30. Chua YL, Brown AP, Gray JC. Targeted histone acetylation and altered nuclease accessibility over short regions of the pea plastocyanin gene. Plant Cell 2001;13:599–612.
31. Kölle D, Brosch G, Lechner T, Pipal A, Helliger W, Taplick J, Loidl P. Different types of maize histone deacetylases are distinguished by a highly complex substrate and site specificity. Biochemistry 1999;38: 6769–6773.
32. Dangl M, Brosch G, Haas H, Loidl P, Lusser A. Comparative analysis of HD2 type histone deacetylases in higher plants. Planta 2001;213:280–285.
33. Wu K, Tian L, Malik K, Brown D, Miki B. Functional analysis of HD2 histone deacetylase homologues in *Arabidopsis thaliana*. Plant J 2000; 22:19–27.
34. Tian L, Chen ZJ. Blocking histone deacetylation in *Arabidopsis* induces pleiotropic effects on plant gene regulation and development. Proc Natl Acad Sci USA 2001;98:200–205.
35. Li G, Chandrasekharan MB, Wolffe AP, Hall TC. Chromatin structure and phaseolin gene regulation. Plant Mol Biol 2001;46:121–129.
36. Frisch DA, van der Geest AHM, Dias K, Hall TC. Chromosomal integration is required for spatial regulation of expression from the b-phaseolin promoter. Plant J 1995;7:503–512.
37. Bustos MM, Iyer M, Gagliardi SJ. Induction of a b-phaseolin promoter by exogenous abscisic acid in tobacco: developmental regulation and modulation by external sucrose and Ca^{2+} ions. Plant Mol Biol 1998;37: 265–274.
38. Li G, Chandler SP, Wolffe AP, Hall TC. Architectural specificity in chromatin structure at the TATA box in vivo: nucleosome displacement upon beta-phaseolin gene activation. Proc Natl Acad Sci USA 1998;95: 4772–4777.
39. Bobb AJ, Eiben HG, Bustos MM. PvAlf, an embryo-specific acidic transcriptional activator enhances gene expression from phaseolin and phytohemagglutinin promoters. Plant J 1995;8:331–343.
40. Li G, Bishop KJ, Chandrasekharan MB, Hall TC. b-Phaseolin gene activation is a two-step process: PvAlf- facilitated chromatin modification followed by abscisic acid-mediated gene activation. Proc Natl Acad Sci USA 1999;96:7104–7109.
41. Busk PK, Pujal J, Jessop A, Lumbreras V, Pages M. Constitutive protein-DNA interactions on the abscisic acid-responsive element before and after developmental activation of the rab28 gene. Plant Mol Biol 1999;41: 529–536.
42. Preuss D. Chromatin silencing and *Arabidopsis* development. A role for polycomb proteins. Plant Cell 1999;11:765–768.

43. Ingram R, Charrier B, Scollan C, Meyer P. Transgenic tobacco plants expressing the *Drosophila* Polycomb (Pc) chromodomain show developmental alterations: possible role of Pc chromodomain proteins in chromatin-mediated gene regulation in plants. Plant Cell 1999;11:1047–1060.
44. Grossniklaus U, Vielle-Calzada JP, Hoeppner MA, Gagliano WB. Maternal control of embryogenesis by MEDEA, a polycomb group gene in *Arabidopsis*. Science 1998;280:446–450.
45. Ohad N, Yadegari R, Margossian L, Hannon M, Michaeli D, Harada JJ, Goldberg RB, Fischer RL. Mutations in FIE, a WD polycomb group gene, allow endosperm development without fertilization. Plant Cell 1999;11: 407–416.
46. Pirrotta V. Polycombing the genome: PcG, trxG, and chromatin silencing. Cell 1998;93:333–336.
47. Kehle J, Beuchle D, Treuheit S, Christen B, Kennison JA, Bienz M, Muller J. dMi-2, a hunchback-interacting protein that functions in polycomb repression. Science 1998;282:1897–1900.
48. van der Vlag J, Otte AP. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. Nat Genet 1999;23:474–478.
49. Strouboulis J, Damjanovski S, Vermaak D, Meric F, Wolffe AP. Transcriptional repression by XPc1, a new Polycomb homolog in *Xenopus laevis* embryos, is independent of histone deacetylase. Mol Cell Biol 1999;19:3958–3968.
50. Ogas J, Cheng JC, Sung ZR, Somerville C. Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana* pickle mutant. Science 1997;277:91–94.
51. Wade PA, Gegonne A, Jones PL, Ballestar E, Aubry F, Wolffe AP. Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nature Genet 1999;23:62–66.
52. Zhang Y, LeRoy G, Seelig HP, Lane WS, Reinberg D. The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell 1998;95: 279–289.
53. Ridgway P, Almouzni G. CAF-1 and the inheritance of chromatin states: at the crossroads of DNA replication and repair. J Cell Sci 2000;113: 2647–2658.
54. Enomoto S, Berman J. Chromatin assembly factor I contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. Genes Dev 1998;12:219–232.
55. Kaya H, Shibahara KI, Taoka KI, Iwabuchi M, Stillman B, Araki T. FASCIATA genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems. Cell 2001;104:131–142.
56. Wolffe AP. Chromatin Structure and Function, 3rd edn. San Diego, CA: Academic Press; 1998.
57. Steinbach OC, Wolffe AP, Rupp RA. Somatic linker histones cause loss of mesodermal competence in *Xenopus*. Nature 1997;389:395–399.
58. Ascenzi R, Gantt JS. Molecular genetic analysis of the drought-inducible linker histone variant in *Arabidopsis thaliana*. Plant Mol Biol 1999;41: 159–169.
59. Prymakowska-Bosak M, Przewloka MR, Slusarczyk J, Kuras M, Lichota J, Kilianczyk B, Jerzmanowski A. Linker histones play a role in male meiosis and the development of pollen grains in tobacco. Plant Cell 1999;11: 2317–2330.
60. Scippa GS, Griffiths A, Chiatante D, Bray EA. The H1 histone variant of tomato, H1-S, is targeted to the nucleus and accumulates in chromatin in response to water-deficit stress. Planta 2000;211:173–181.
61. Cao X, Springer NM, Muszynski MG, Phillips RL, Kaeppler S, Jacobsen SE. Conserved plant genes with similarity to mammalian de novo DNA methyltransferases. Proc Natl Acad Sci USA 2000;97:4979–4984.
62. Lindroth AM, Cao X, Jackson JP, Zilberman D, McCallum CM, Henikoff S, Jacobsen SE. Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. Science 2001;292:2077–2080.
63. Bartee L, Malagnac F, Bender J. *Arabidopsis* cmt3 chromomethylase mutations block non-CG methylation and silencing of an endogenous gene. Genes Dev 2001;15:1753–1758.
64. Finnegan EJ, Genger RK, Kovac K, Peacock WJ, Dennis ES. DNA methylation and the promotion of flowering by vernalization. Proc Natl Acad Sci USA 1998;95:5824–5829.
65. Miura A, Yonebayashi S, Watanabe K, Toyama T, Shimada H, Kakutani T. Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*. Nature 2001;411:212–214.
66. Singer T, Yordan C, Martienssen RA. Robertson's Mutator transposons in A. thaliana are regulated by the chromatin-remodeling gene decrease in DNA Methylation (DDM1). Genes Dev 2001;15:591–602.
67. Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 1998;19:187–191.
68. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 1998;393:386–389.
69. Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. Nat Genet 2000;24:88–91.
70. Robertson KR, Wolffe AP. DNA methylation in health and disease. Nature Reviews Genetics 2000;1:11–19.
71. Chen ZJ, Pikaard CS. Epigenetic silencing of RNA polymerase I transcription: a role for DNA methylation and histone modification in nucleolar dominance. Genes Dev 1997;11:2124–2136.
72. Henikoff S, Comai L. A DNA methyltransferase homolog with a chromodomain exists in multiple polymorphic forms in *Arabidopsis*. Genetics 1998;149:307–318.
73. Haig D, Westoby M. Genomic imprinting in endosperm: its effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis. Philosophical Transactions. Royal Soc Lon 1991;333:1–13.
74. Chaudhuri S, Messing J. Allele-specific parental imprinting of dzr1, a posttranscriptional regulator of zein accumulation. Proc Natl Acad Sci USA 1994;91:4867–4871.
75. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science 2001;293:1089–1093.
76. Vielle-Calzada JP, Thomas J, Spillane C, Coluccio A, Hoeppner MA, Grossniklaus U. Maintenance of genomic imprinting at the *Arabidopsis* medea locus requires zygotic DDM1 activity. Genes Dev 1999;13:2971–2982.
77. Vinkenoog R, Spielman M, Adams S, Fischer RL, Dickinson HG, Scott RJ. Hypomethylation promotes autonomous endosperm development and rescues postfertilization lethality in fie mutants. Plant Cell 2000;12: 2271–2282.
78. Kumpatla SP, Chandrasekharan MB, Iyer LM, Li G, Hall TC. Genome intruder scanning and modulation systems and transgene silencing. Trends Plant Sci 1998;3:97–104.
79. Iyer LM, Kumpatla SP, Chandrasekharan MB, Hall TC. Transgene silencing in monocots. Plant Mol Biol 2000;43:323–346.
80. Mysore KS, Nam J, Gelvin SB. An *Arabidopsis* histone H2A mutant is deficient in *Agrobacterium* T-DNA integration. Proc Natl Acad Sci USA 2000;97:948–953.
81. Ye F, Signer ER. RIGS (repeat-induced gene silencing) in *Arabidopsis* is transcriptional and alters chromatin configuration. Proc Natl Acad Sci USA 1996;93:10881–10886.
82. van Blokland R, ten Lohuis M, Meyer P. Condensation of chromatin in transcriptional regions of an inactivated plant transgene: evidence for an active role of transcription in gene silencing. Mol Gen Genet 1997;257: 1–13.
83. Kooter JM, Matzke MA, Meyer P. Listening to the silent genes: transgene silencing, gene regulation and pathogen control. Trends Plant Sci 1999; 4:340–347.
84. Mette MF, van der Winden J, Matzke MA, Matzke AJ. Production of aberrant promoter transcripts contributes to methylation and silencing of unlinked homologous promoters in trans. EMBO J 1999;18:241–248.
85. Mette MF, Aufsatz W, van Der Winden J, Matzke MA, Matzke AJ. Transcriptional silencing and promoter methylation triggered by double-stranded RNA. EMBO J 2000;19:5194–5201.
86. Jones L, Hamilton AJ, Voinnet O, Thomas CL, Maule AJ, Baulcombe DC. RNA–DNA interactions and DNA methylation in post-transcriptional gene silencing. Plant Cell 1999;11:2291–2302.
87. Mittelsten Scheid O, Afsar K, Paszkowski J. Release of epigenetic gene silencing by trans-acting mutations in *Arabidopsis*. Proc Natl Acad Sci USA 1998;95:632–637.
88. Mysore KS, Kumar CT, Gelvin SB. *Arabidopsis* ecotypes and mutants that are recalcitrant to *Agrobacterium* root transformation are susceptible to germ-line transformation. Plant J 2000;21:9–16.