ACTA BIOLOGICA CRACOVIENSIA Series Botanica 47/1: 37–49, 2005

EPIGENETIC ASPECTS OF SEXUAL AND ASEXUAL SEED DEVELOPMENT

JULIO C.M. RODRIGUES[1,2,3] AND ANNA M.G. KOLTUNOW[2*]

[1]*Discipline of Wine and Horticulture, Waite Campus, University of Adelaide, Glen Osmond, South Australia 5064, Australia*
[2]*Commonwealth Scientific and Industrial Organisation Plant Industry, Horticultural Research, Glen Osmond, South Australia 5064, Australia*
[3]*Embrapa Genetic Resources and Biotechnology, 70770–900, Brasilia, Brazil*

Received February 1, 2005; revision accepted April 25, 2005

In angiosperms, seed development initiates after a double fertilization event in the female gametophyte, in which one male sperm cell fuses to the central cell to form the endosperm and the other to the egg cell to form the embryo. Sexually-derived seed is thus characterized by maternal and paternal contributions to the progeny. Some plant species have the capacity to form seeds asexually, a process known as apomixis. This mode of reproduction is characterized by a bypass of meiotic reduction and the absence of paternal contribution to the embryo, resulting in a seed with an embryo genetically identical to the mother. Little is known about the molecular events that regulate apomictic development. Recent findings show that the apomictic and sexual developmental programs share molecular components, suggesting that apomixis is a deregulated sexual program. Furthermore, the identification of apomictic developmental features in *fertilization-independent seed (fis)* mutants in the sexual model plant *Arabidopsis* has also shed light on the molecular events that control sexual seed development, and has opened new questions as to the molecular nature of autonomous seed development. *FIS*-class genes are homologues of the Polycomb Group (PcG) chromatin remodelling factors conserved in *Drosophila* and humans, where they have been implicated in gene repression and control of cell fate throughout development. *fis* phenotypes are affected by DNA methylation, a DNA alteration associated with heterochromatin formation and gene silencing. Thus, the chromatin environment can be manipulated to make certain regions of the genome more or less susceptible to transcription; this form of control, in which gene expression patterns are altered without a change in the DNA sequence itself, is defined as epigenetic regulation. Different aspects of plant development have been shown to be controlled by epigenetic regulation. This review will highlight recent advances in understanding the epigenetic control of seed development. They are discussed in light of a model whereby altered epigenetic mechanisms might lead to complete maternal control of reproductive development as seen in apomixis.

Key words: Apomixis, epigenetics, seed development.

INTRODUCTION

In angiosperms, the decision to reproduce is marked by a transition from vegetative to reproductive growth, leading to the formation of a flower, in which the gametes are formed. The multicellular male and female gametophytes of flowering plants are embedded in sporophytic tissue, and are formed after meiotic reduction and subsequent mitosis. Sexual reproduction is marked by a double fertilization event, leading to the discharge of two male sperm cells into the female gametophyte.

The female gametophyte is formed within the ovule located in the carpel. The development of the most common form of female gametophyte in angiosperms (Polygonum type) begins with the differentiation of an archesporial cell near the tip of the ovule primordium, enlarging to form the megaspore mother cell (MMC). The MMC undergoes a meiotic division, resulting in the formation of four megaspores. Three of these degenerate, and the selected megaspore divides mitotically. Following nuclear migration and cellularization, a mature 7-celled embryo sac (ES) is formed. During the double fertilization event, one male sperm cell fuses to the central cell nucleus, to give rise to the endosperm, while sperm cell fusion with the egg cell initiates embryo formation. Both endosperm and em-

*e-mail: Anna.Koltunow@csiro.au

PL ISSN 0001-5296

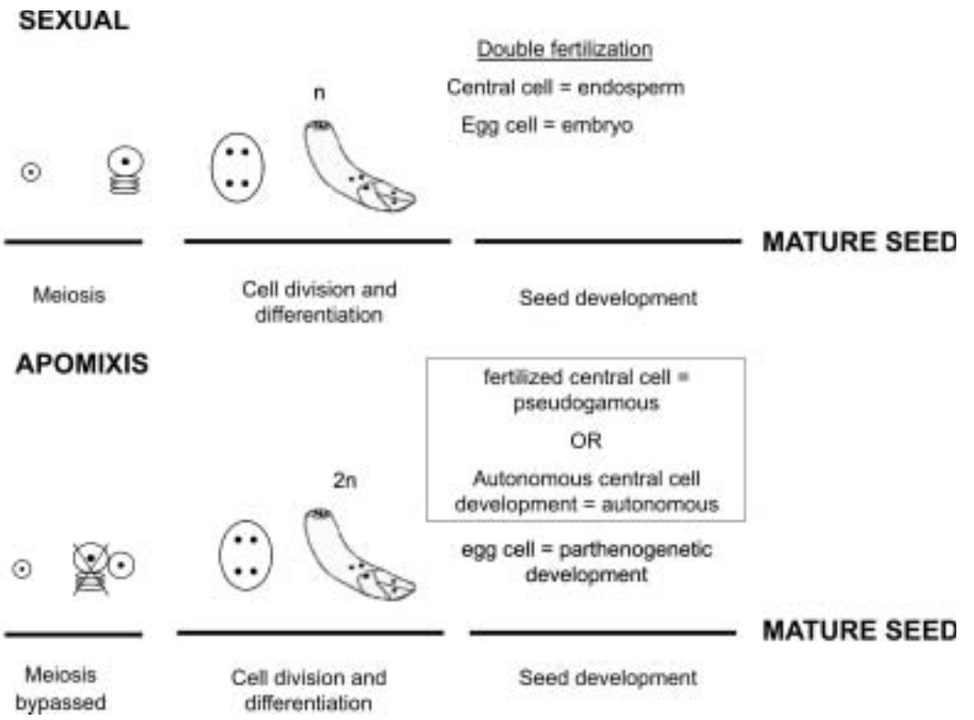

Fig. 1. Gametophytic apomixis is characterized by two major events that are altered relative to sexual reproduction. First, the cell that gives rise to the female gametophyte does not undergo meiotic reduction, producing an unreduced gametophyte. Second, the unreduced egg cell develops parthenogenetically to give rise to a seed with an embryo genetically identical to the mother plant. Variations in the mode of apomixis are encountered in different plant species. In diplospory, the progenitor cell of the unreduced embryo sac is the MMC, whereas in apospory, a somatic cell in the nucellus or even integument differentiates and initiates gametophytic development. In some plants termed pseudogamous, the central cell needs to be fertilized to produce endosperm, whereas in other plants, and less commonly, the central cell develops autonomously to produce the endosperm.

bryo develop in a coordinated way to form a mature seed.

Some plants form seed asexually by apomixis (Nogler, 1984; Asker and Jerling, 1992). The major events that characterize this mode of reproduction include the avoidance of meiotic reduction during embryo sac formation, fertilization-independent embryo development, and the formation of endosperm with or without fertilization (Koltunow and Grossniklaus, 2003). Apomixis results in seeds containing an embryo that is genetically identical to the female parent. Apomixis does not occur to the mutual exclusion of sexual reproduction in a plant, as the capacity to form a percentage of seeds via sexual reproduction is retained in most apomicts. Apomixis can occur by different pathways (Asker and Jerling, 1992). The most common form, gametophytic apomixis, occurs through the production of an unreduced female gametophyte (Fig. 1). The origin of the non-reduced gametophyte can be the MMC (diplospory); in others, a somatic nucellar cell enlarges and differentiates to form an aposporous initial (AI) which is the progenitor of the unreduced embryo sac (apospory). In both cases, the egg cell develops parthenogenetically. Endosperm development can occur autonomously in some species such as *Hieracium* (Koltunow et al., 1998), while in others, such as most grass species, fertilization of the central cell is required for appropriate seed development (pseudogamy).

The production of many agronomic crops depends largely on sexually derived hybrids, whose beneficial traits can be lost in successive generations as a result of the recombination and segregation events that define sexual reproduction. Apomixis is largely absent in agricultural crops, but harnessing apomixis in crops could be an important tool to fix genetically desired traits. This could allow the production of clonal seeds with embryos genetically identical to the maternal parent, enabling the maintenance of an elite genotype through seed (Bicknell and Koltunow, 2004). Understanding the molecular mechanisms that control apomixis is an essential step towards achieving this goal.

Although genetic studies show that apomixis is generally a dominant trait (Savidan, 2000; Bicknell and Koltunow, 2004), the molecular control of apomic-

tic development is still unknown. Recent advances in understanding the molecular relationship between sexual and apomictic reproduction have been made in the model plant *Hieracium*, which undergoes gametophytic apomixis. By analyzing the expression pattern of marker genes, it was shown that sexual and apomictic reproduction share molecular components (Tucker et al., 2003). Ovule initiation in apomictic *Hieracium* seems to progress within the same molecular framework as the sexual reproductive pathway, suggesting that apomixis may be a deregulated sexual program (Tucker et al., 2003).

Isolation of genes involved in apomixis has been approached in a number of ways: comparative screening of differentially expressed genes in related sex-ual and natural apomictic populations, analysis of induced mutants that have lost apomixis, and mutagenesis of sexual species to induce components of apomixis. In *Arabidopsis*, mutations in the *fertilization independent seed (fis)* genes cause central cell proliferation and initial events of endosperm development in the absence of fertilization, a component of apomixis. Such genes are related to the *Drosophila* chromatin modifying Polycomb group genes. However, their role during apomictic development is still unclear.

Given the demonstrated relationship between sexual and apomictic pathways, two models have been proposed to explain the molecular manifestation of apomixis. A genetic mutation model predicts that a key factor regulating sexual reproduction is mutated or has altered function, thereby leading to deregulation of the sexual pathway. The mutation is not fully penetrant, because the sexual process can also occur. The epimutation model predicts that reversible changes in chromatin configuration might alter the expression of key regulatory genes in both time and space, possibly triggering the apomictic pathway at different developmental time points or in different cell types (Koltunow and Grossniklaus, 2003). These models are not mutually exclusive, considering that a mutation in an epigenetic regulator could lead to altered heterochromatin formation. Some evidence supporting the view that apomixis is epigenetically regulated includes the observation that in most apomicts the developmental program is not tightly conserved, and that differences in the timing of initiation and variation in the structures formed occur in an individual plant in response to different environmental conditions and stresses (Koltunow, 1993; Koltunow et al., 2000). Such flexibilities are characteristic of epigenetic regulation. Epimutations in biology are also more frequent; they can be reversible and transmitted to progeny, thus making it more likely to generate multiple changes in gene expression to allow a complex developmental trait such as apomixis to co-evolve and occur simultaneously with sexual reproduction in an individual plant (Koltunow and Grossniklaus, 2003).

CHROMATIN MODIFICATIONS AND GENE EXPRESSION

Plant and animal growth and development depends on the coordinated expression of specific subsets of the total complement of genes found in the genome. The molecular events that integrate developmental signals and cell differentiation can be set early in development, defining specific gene expression patterns that must be precisely remembered throughout the subsequent divisions of cells comprising a particular tissue. These set states of gene expression programs can be established and maintained by controlling chromatin configurations in specific regions of the DNA and thus the expression of the genetic information of the DNA sequence itself.

In eukaryotes, DNA is packed into the nucleus through an association with histones, to form chromatin. The basic organization unit of chromatin is the nucleosome, consisting of approximately 165 bp of DNA wrapped around an octamer of histones. Histones are highly conserved basic proteins with a globular domain that interacts with DNA and a flexible amino-terminal region that remains somewhat protruded from the nucleosome (Jenuwein and Allis, 2001). Chromatin can exist in a more compact, condensed form (heterochromatin), inaccessible to transcription factors and RNA polymerase and thus transcriptionally silent; or in a less condensed state which is associated with transcriptional activity (euchromatin). The extent to which chromatin is condensed depends on DNA and histone modifications, and is of great biological significance. Chromatin condensation can be targeted to specific regions in the genome, leading to the formation of heterochromatin in telomeres, centromeres and transposable elements, ensuring genomic stability; or it can be targeted to specific genes, to control gene expression involved in different aspects of plant and animal development. In this context, cell differentiation is largely determined by what transcription factors are available, the accessibility of chromatin to these transcription factors, and how this interplay is controlled throughout a developmental process.

The condensation state of chromatin depends on chemical modifications that occur to both DNA and histones. The amino-terminal region of histones can be the target of several post-translational modifications, including phosphorylation, methylation and acetylation. To date, the most described modifications that control chromatin condensation are histone methylation and acetylation (Jenuwein and Allis, 2001). DNA can also be chemically modified by methylation of cytosine residues. Together these modifications interact to reinstate epigenetic marks, adding another layer of regulation on top of the DNA code itself, which is known as the histone code or epigenome (Fig. 2). According to the histone code hypothesis, histone modifications are

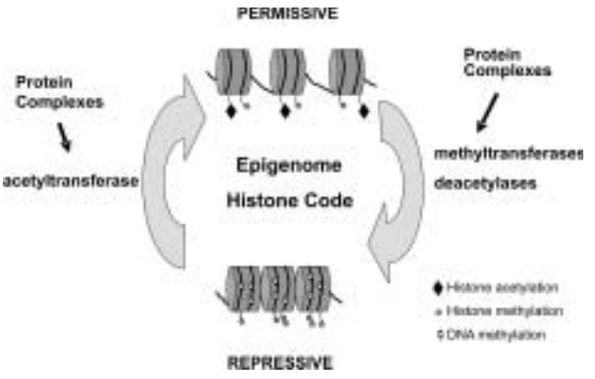

Fig. 2. Schematic diagram showing the dynamic chromatin modifications that make up a histone code, or epigenome. DNA is wrapped around histones to form nucleosomes, which are the basic structural unit of chromatin. Chromatin modifications can reinstate condensed chromatin, resulting in transcriptional silencing, or they can relax chromatin, ultimately resulting in transcriptional activity. The enzymes responsible for the histone and DNA modifications indicated can interact with different protein complexes to control gene expression.

interdependent and can be synergistic or antagonistic (Strahl and Allis, 2000; Jenuwein and Allis, 2001). Histone methylation is usually associated with DNA methylation and deacetylated histones. This particular combination of modifications trigger chromatin condensation associated with gene silencing. On the other hand, histone acetylation and histone methylation coexist to determine a more relaxed chromatin state, compatible with transcriptional activity (Fig. 2). The apparent contradiction of histone methylation being associated with both chromatin states can be explained by the fact that different amino acid residues are methylated in each case. Typically, condensed chromatin is marked by methylation of lysine residues 9 and 27 on histone 3 (H3K9 and H3K27), whereas active chromatin is associated with methylation of lysine residue 4 on histone 3 (H3K4).

Chromatin modifications are often carried out by chromatin remodelling complexes that have the ability to control gene expression and cell fate during development. These protein complexes may contain transcription factors that account for the specificity of the chromatin remodelling machinery. For instance, proteins of the Polycomb Group (PcG) can form complexes capable of maintaining chromatin states established early in development, defining a cellular memory of transcriptionally active or inactive genes (Pirrota et al., 2003). In *Drosophila*, PcG complexes recognize and bind to upstream responsive elements of homeotic genes to establish a predominantly repressive state and define segment identity. Recent findings in *Drosophila* and mammalian cells have shown that PcG complexes can also function as transcription activators (Pasini et al., 2004). Therefore, PcG complexes might be considered to be chromatin-remodelling machines that can function both to repress and activate transcription, depending on the protein interactions responsible for recruiting the complex. Trithorax complexes have an antagonistic action, preventing the establishment of repressed states on specific promoter sequences (Pirrota et al., 2003). The PcG complex comprised of the proteins EXTRA SEX COMBS (ESC) and ENHANCER OF ZESTE [E(z)] of *Drosophila* interacts with histone deacetylases, suggesting that histone modifications are important to establish and/or maintain a repressed state (Simon and Tamkun, 2002; Pirrota et al., 2003).

Small interfering RNA (siRNA) can also target chromatin modifications to regions of complementarity in the genome. Heterochromatin formation in centromeres and at transposable elements is established and maintained by siRNA-mediated silencing (Schramke and Allshire, 2003; Lippman et al., 2004). Furthermore, transcription factors can also be targeted by siRNA, leading to chromatin modifications in homologous regions and transcriptional repression, possibly having important functions in controlling plant and animal development (Steimer et al., 2004). Recent findings also point to a role for microRNAs in directing DNA methylation to specific regions to control the expression of genes involved in leaf morphology (Bao et al., 2004).

EPIGENETICS AND PLANT DEVELOPMENT

In plants, epigenetic regulation has been shown to be important for normal development. Epimutations, aberrant DNA methylation patterns and defects in chromatin remodelling genes all have been associated with different kinds of developmental abnormalities, such as homeotic transformations, sterility, transposon activation and defects in flowering response pathways (Finnegan et al., 1996; Ronemus et al., 1996; Soppe et al., 2000; Lippman et al., 2004).

Despite evidence supporting a role for epigenetic regulation in development, the molecular mechanisms that trigger such alterations and hence control development still need clarification. The next section summarizes recent findings showing epigenetic regulation to be involved in different aspects of plant development by integrating environmental and developmental signals to control gene expression.

DNA METHYLATION AND PLANT DEVELOPMENT

DNA methyltransferases

In *Arabidopsis*, the DNA methyltransferase family has been separated into classes differing in sequence, do-

main content and substrate specificity (Bestor, 2000; Finnegan and Kovac, 2000): class I, the MET class, which is closely related to the mammalian Dnmt1; chromodomain methyltransferases (chromomethylases, CMT) which are specific to plants; and finally, domain-rearranged methyltransferases (DRM), which are related to mammalian Dnmt3 (Bestor, 2000; Finnegan and Kovac, 2000). Class I is the main maintenance DNA methyltransferase, largely responsible for methylation at the symmetrical CpG site, whereas CMT is involved in methylation of cytosine residues in CpNpG sites and DRM for *de novo* (CpNpN) methylation (Henikoff and Comai, 1998; Genger et al., 1999; Finnegan and Kovac, 2000; Cao and Jacobsen, 2002; Cao and Jacobsen, 2002). The different classes of DNA methyltransferases in both mammals and plants probably operate cooperatively and redundantly to regulate DNA methylation in different regions (Bestor, 2000; Morel et al., 2000; Xiao et al., 2003). Previously established methylation marks can be copied to the daughter strand during DNA replication, and thus maintained indefinitely.

Methylated DNA is usually associated with chromatin condensation and transcriptional repression. A clear role for DNA methylation has been established in silencing repetitive DNA, transgenes and viral sequences, suggesting that it may have evolved as a mechanism to ensure genomic stability in larger genomes by controlling the activity of parasitic sequences (Matzke et al., 1999; Walsh and Bestor, 1999; Ehrlich, 2003). However, the extent to which it has been recruited to control tissue-specific expression during developmental processes is still an open issue.

Several lines of evidence show that DNA methylation is important for normal plant development, and more specifically, plant reproduction. DNA methylation is involved in the control of genomic imprinting (allele-specific gene expression) an important aspect of seed development (Grossniklaus, 2001; Jones and Takai, 2001). Ectopic down-regulation of a class I DNA methyltransferase leads to genome hypomethylation and alterations of leaf morphology and flowering time, and defects in gametophyte and seed development (Finnegan et al., 1996; Ronemus et al., 1996). Furthermore, reduced DNA methylation caused by a loss-of-function mutation in DDM (decreased DNA methylation), a chromatin remodelling gene of the SWI/SNF family, also leads to pleiotropic developmental defects which are initially weak but become more severe in subsequent generations (Jeddeloh et al., 1998; Jeddeloh et al., 1999).

The altered flowering time phenotype in *fwa* mutants has been shown to be caused by altered DNA methylation status of the *FWA* locus (Soppe et al., 2000), while locus-specific changes of DNA methylation patterns in flowering repressors, such as the early flowering locus *FWA* and *FLOWERING LOCUS C* (*FLC*) was reported to be the cause of altered flowering time observed in hypomethylated plants (Soppe et al., 2000; Genger et al., 2003). These findings provide evidence that DNA methylation has the potential to control the expression of specific genes involved in plant development. However, direct evidence of the relevance of these changes to the actual control of flowering in a wild type situation is still lacking.

DNA methylation and genomic imprinting

It has been shown genetically that DNA methyltransferase activity is necessary during gamete formation during plant reproduction (Saze et al., 2003), but little is known of how this epigenetic mark is recruited to specific regions of the genome to mediate gene expression. Nevertheless, DNA methylation has been shown to be involved in other aspects of seed development such as genomic imprinting, a phenomenon in which genes are differentially expressed depending on whether they are of maternal or paternal origin. In mammals, inheritance of aberrant DNA methylation marks in imprinted loci lead to abnormal development; imprinting marks are maintained mainly by the class I DNA methyltransferase DNMT1 (Fergunson-Smith and Surani, 2001). Similarly, DNA methylation is also involved in mediating "parent-of-origin" effects, in which a phenotype is transmitted only through one of the gametes regardless of the presence of a wild type allele in the other gamete. In *Arabidopsis*, distinct phenotypes are obtained in reciprocal crosses between hypomethylated and wild type plants (Vinkenoog et al., 2000). When crossed with wild type pollen, hypomethylated seed parents generate larger seeds in the offspring, phenocopying interploidy crosses in which a diploid seed parent is crossed with pollen from a tetraploid plant. Smaller seeds are produced in the reciprocal crosses (Adams et al., 2000; Vinkenoog et al., 2000). Furthermore, hypomethylated pollen is able to rescue maternal gametophytic mutations that affect seed development (Vielle-Calzada et al., 1999; Luo et al., 2000; Vielle-Calzada et al., 2000; Guitton et al., 2004). In sexual plants, the correct balance of maternal and paternal genomes is necessary for proper gene expression enabling normal development. In some plants, a 2:1 maternal-to-paternal genome ratio is essential for normal endosperm development, and if it is altered it affects embryo viability (Haig and Westoby, 1991; Adams et al., 2000). In sexual plants, genomic imprinting seems to be restricted to the central cell of the female gametophyte, thus associated with endosperm development (Gehring et al., 2004). Activation of the maternally expressed genes *MEDEA* (Choi et al., 2002; Xiao et al., 2003) and *FWA* (Kinoshita et al., 2004) occurs during female gametogenesis by the activity of a DNA glycosylase, DEMETER (*DME*). *DME* is activated in the central cell before fertilization. After fertilization it is down-regulated, but both *MEA* and *FWA*

expression continue even in the absence of *DME*. The absence of *DME* expression in stamens suggests that expression of *MEA* and *FWA* in the female gametophyte is largely due to the activation of *DME*; its absence in the male gametophyte would result in silencing of *MEA* and *FWA* (Gehring et al., 2004). Additionally, mutations in *MET1* supress the *dme* seed abortion phenotype restoring *MEA* expression, suggesting that DNA methylation has to be removed from *MEA* by DME (Xiao et al., 2003). Futhermore, *MEA* controls the expression of the MADS-box gene *PHERES1* (*PHE1*) by repressing transcription of the maternal allele, whereas the paternal allele of *PHE1* is normally expressed (Köhler et al., 2005).

Taken together, these results indicate that DNA methylation and chromatin modifications are actively modified in particular genes to control genomic imprinting. DNA methylation patterns are differentially established during male and female gametogenesis, and when the two gametes combine during sexual reproduction, these differences interact to control gene expression programs for proper seed development. In certain apomictic plants such as *Hieracium* sp., seed development is completely autonomous (Koltunow et al., 1998). It would be interesting to examine whether the bypass of the imprinting barrier is related to alterations of DNA methylation patterns and/or altered function of the epigenetic regulators that control endosperm development, such as DME.

DNA methylation and transposon silencing

DNA methylation has also been shown to be involved in silencing the activity of transposable elements in both plants and animals. Due to the widespread distribution of transposons in eukaryotic genomes and their potential to promote genomic rearrangements if activated, this function of DNA methylation has been attributed to maintaining genomic stability. In plants, transposon silencing can be reversed under certain types of stress, such as genomic stress imposed after hybridization between different genomes (Kashkush et al., 2003) or physiological stress, for example reduced temperature, in which an association with DNA methylation was established (Hashida et al., 2003). Transposon sequences can also attract epigenetic modifications that silence gene expression when inserted in the proximity of genes (Lippman et al., 2004). In this scenario, transposons can be a source of adaptive variability in response to different types of stress, mediating alterations in gene expression through epigenetic mechanisms. Furthermore, transposable elements can function as regulatory elements for host gene expression. Recent findings in mouse oocytes have shown that retrotransposons are expressed abundantly, and that this expression is developmentally regulated, apparently due to the presence of transcription sites within the retrotransposon sequence (Peaston et al., 2004). This leads to the expression of chimeric transcripts with altered exon composition, possibly encoding proteins with alternative functions (Peaston et al., 2004). However, the developmental significance of this control has yet to be demonstrated.

HISTONE MODIFICATIONS AND PLANT DEVELOPMENT

Histone acetylation

The most common sites for histone acetylation are lysine residues, and the overall effect of acetylation on local nucleosome structure is probably to reduce interaction with the negatively charged DNA molecule, making the DNA more accessible to the transcriptional machinery (Strahl and Allis, 2000). Conversely, histone deacetylation, promoted by histone deacetylases (HDs), would make chromatin more condensed (Strahl and Allis, 2000). Histone deacetylation is linked to DNA methylation. In mammalian cells, methylated DNA is recognized by methyl DNA-binding proteins (MBP), which in turn interact with HDs (Nan et al., 1998).

There are several classes of HDs in both plants and animals, suggesting divergence in function and/or substrate specificity (Pandey et al., 2002). In plants, functional analysis of a class I-type RPD3/HDA1 enzyme by an anti-sense strategy in *Arabidopsis* showed various developmental defects, including flower defects and male and female sterility (Tian and Chen, 2001). This particular HD class is common to both plant and animals. Plants have a unique family of histone deacetylases termed HD2 (Wu et al., 2000; Pandey et al., 2002; Lagace et al., 2003; Zhou et al., 2004). Down-regulation of the HD2 family member *HD2a* by an anti-sense strategy leads to seed abortion (Wu et al., 2000). Furthermore, somatic and zygotic embryo development induces the expression of *HD2a*, *Hd2b* and *HD2c*, and over-expression of *HD2a* leads to the repression of seed-specific genes (Lagace et al., 2003; Zhou et al., 2004). These data suggest that the HD2 family may have evolved functionally in plants to control gene expression during seed development.

Histone methylation

The major sites for histone methylation are lysine residues. Histone methyltransferase activity has been associated largely with the presence of a conserved SET domain, named after the *Drosophila* PcG proteins SU(VAR)3–9, ENHANCER OF ZESTE (E(Z)) and TRITHORAX (TRX), all of which mediate epigenetic processes during *Drosophila* development (Adams et al., 2000; Francis and Kingston, 2001). In *Arabidopsis* there are 29 expressed SET domain-containing proteins, divided into four classes according to their *Drosophila* counterparts E(z), Trx, Ash1 and Su(var)3–9,

suggesting functional diversity and preferences for certain lysine residues (Baumbusch et al., 2001).

A close relationship between histone methylation and DNA methylation has been reported in plants. Mutations in *KRYPTONITE* (*KYP*), a gene of the Su(var)3–9 family, lead to a loss of DNA methylation at CpNpG sites, consistent with loss of CHROMOMETHYLASE (CMT3) activity (Johnson et al., 2002). It was further shown that the chromodomain of CMT3 binds to histone 3 methylated at lysines 9 and 27, providing a mechanism by which histone methylation directs DNA methylation (Johnson et al., 2002; Jackson et al., 2004; Lindroth et al., 2004).

The large variety of histone methyltransferases in *Arabidopsis* suggests that they are involved in various aspects of plant development. *KYP* was originally identified as a suppressor of the *clark kent* alleles of *SUPERMAN*, a gene involved in defining floral homeotic gene expression boundaries (Sakai et al., 1995). The *clark kent* alleles are silenced by DNA methylation, leading to the production of more stamens and unfused carpels (Jacobsen and Meyerowitz, 1997). In *Arabidopsis* there are three E(Z) homologues, *CURLY LEAF* (*CLF*), *SWINGER* (*SWN*) and *MEDEA* (*MEA*), which are all involved in repressing gene expression of different processes of plant development. *CLF* and *SWN* are involved in epigenetic gene regulation of flower development, whereas *MEA* is involved in repressing endosperm development in the absence of fertilization (for review: Hsieh et al, 2003). Another PcG protein, the Su(z)12 homologue VERNALIZATION2 (VRN2), mediates FLC repression by promoting H3K27 methylation (Bastow et al., 2004; Sung and Amasino, 2004).

Present data on the activity of chromatin remodelling factors in plants are consistent with the histone code hypothesis, in which histone and DNA modifications interact to reinstate control of the local chromatin configuration in the genome. It is becoming clearer that epigenetic modification is an essential mechanism regulating gene expression patterns in plant development.

FIS-CLASS GENES AND SEED DEVELOPMENT

The importance of chromatin modifications in the control of gene expression during plant reproduction has been shown by the isolation of several mutants that have abnormal seed development phenotypes. Some of these mutations have been shown to affect the function of chromatin remodelling genes encoding homologues of the Polycomb Group-like proteins, called *FERTILIZATION INDEPENDENT SEED* (*FIS*) or *FIS*-class genes in *Arabidopsis* (Luo et al., 1999; Ohad et al., 1999). Maternally inherited mutations in *FIS*-class genes initiate endosperm development without the double fertilization event. If fertilization occurs, there is arrest of seed development characterized by overproliferation in endosperm development and an arrested heart-stage embryo (Ohad et al., 1996; Chaudhury et al., 1997; Grossniklaus et al., 1998; Luo et al., 2000). The *FIS*-class genes encode homologues of the Polycomb Group (PcG) proteins from *Drosophila*. FIS1 or MEA are closely related to the SET-domain protein E(Z), FIS2 is a zinc finger protein related to SUPPRESSOR OF ZESTE12 (SU(Z)12), and FIS3 (or FIE) is related to ESC (Grossniklaus and Schneitz, 1998; Luo et al., 1999; Ohad et al., 1999). The expression patterns for known *FIS*-class genes overlap. *FIS2* is expressed in both polar nuclei before they fuse, and continues until cellularization of the endosperm initiates; there it is restricted to the chalazal cyst (Luo et al., 2000). MEA expression also initiates in the female gametophyte before fertilization, where transcripts were detected in the nucleus of the egg cell and central cell, and, like FIS2, continued until cellularization of the endosperm where it was restricted to the chalazal cyst (Vielle-Calzada et al., 1999; Luo et al., 2000). *MEA* was also detected in the embryo until torpedo stage (Vielle-Calzada et al., 1999). *FIE* expression can be detected early in the ovule primordium, continuing throughout megagametogenesis, and at embryo sac maturity it is present in the endothelium and nucellus and all cells of the embryo sac (Spillane et al., 2000). After fertilization, *FIE* expression was observed in both the embryo until torpedo stage and in endosperm, where it persisted after cellularization (Luo et al., 2000; Spillane et al., 2000). The expression pattern for *MEA*, *FIS2* and *FIE* is consistent with a function in controlling early events of seed development (Spillane et al., 2000; Grossniklaus et al., 2001).

Other mutants with *fis*-like phenotypes have been identified recently (*medicis* and *borgia*; Guitton et al., 2004). MEDICIS was shown to be an orthologue of the yeast MULTICOPY SUPPRESSOR OF IRA (MSI1) and the retroblastoma-binding protein p55 of *Drosophila*. p55 interacts with ESC and E(Z) and histone deacetylase, thus providing a direct link between PcG function and chromatin modifications (Tie et al., 2001). This interaction has been conserved in plants, as MSI1 was shown to interact with FIE and MEA (Khler et al., 2003a). The molecular identity of *BORGIA* is unknown (Köhler et al., 2003a; Guitton et al., 2004).

The *Drosophila* and human orthologues of *PcG* genes interact with and are controlled by retinoblastoma proteins, a tumor suppressor involved in the G1/S transition of cell cycle progression (Pasini et al., 2004). Plant homologues of retinoblastoma-related proteins also control cell proliferation in the female gametophyte, and mutants show *fis*-related phenotypes, suggesting that retinoblastoma-mediated control of PcG activity also occurs in plants (Ebel et al., 2004). The biological function of these genes is consistent with their playing a role in proliferation and patterning

defects of the *fis* phenotypes seen in *Arabidopsis*. Possibly these proteins form a complex conserved throughout evolution because of their important roles in controlling development. Plant retinoblastoma-related proteins have been shown to interact with both MSI1 and FIE, further supporting a role in the control of cell proliferation and differentiation in response to fertilization (Ach et al., 1997; Mosquna et al., 2004).

In plants, a target gene of the *FIS* complex has been isolated, the class I MADS-box gene *PHERES* (*PHE1*) (Köhler et al., 2003b). In wild type plants, *PHE1* expression was detected after fertilization until the pre-globular stage of development and subsequently down-regulated by late globular stage, whereas in *mea* and *fie* mutants, *PHE1* expression persists until late globular stage. By silencing PHE1 expression in the mea mutant background, the seed abortion phenotype is rescued, thus suggesting that PHE1 is probably linked to this phenotype in the mutant (Köhler et al., 2003b). Both MEA and FIE are capable of binding to the promoter of *PHE1*, consistent with their role in controlling *PHE1* expression. It is still unclear how fertilization activates *PHE1* expression, as are the mechanisms by which MEA and FIE are recruited to *PHE1* promoter. Isolation of *PHE1* as a target of plant PcG complex, and its deregulation in the *fis* mutants associated with the seed abortion phenotype, suggest that epigenetic repression of gene expression is an essential component of sexual seed development (Köhler et al., 2003b).

FIS-CLASS GENES AND DNA METHYLATION

A genetic interaction between the *FIS* genes and DNA methylation has been shown by the observation that pollen from a hypomethylated plant can rescue the *fis* phenotype, independent of FIS function (Vielle-Calzada et al., 1999; Luo et al., 2000; Vinkenoog et al., 2000). Unlike other *fis* mutant backgrounds, hypomethylated pollen does not restore the expression of an endosperm-specific marker in *fie* and *msi1* mutants, although seed rescue still occurs, indicating that seed rescue can occur through alternate pathways (Guitton et al., 2004). Additionally, by combining a hypomethylated genomic background and the *fie* mutant, the phenotype is altered, and fertilization-independent endosperm develops further to produce cellularized and differentiated endosperm, in contrast to the phenotype of the *fie* mutation in a normally methylated background, when development arrests before cellularization (Vinkenoog et al., 2000). It has also been shown that repression of *PHERES* expression, which is necessary for normal seed development and lost in the *mea* mutant, can be restored when crossed to a hypomethylated DNA background (Köhler et al., 2003b). Together these findings indicate that DNA methylation interacts with PcG complex function to control seed development, and that alterations in gametophytic DNA methylation have profound effects on seed formation. Interestingly, in mammalian cells, the DNA methyltransferase DNMT1 is present in a complex with retinoblastoma protein and histone deacetylase (Robertson et al., 2000); this has not yet been determined in plants.

IS APOMIXIS EPIGENETICALLY REGULATED?

FIS GENES AND THE INITIATION OF APOMIXIS

Expression analysis of *Arabidopsis* FIS promoter::GUS fusions in sexual and apomictic *Hieracium* plants showed a conserved pattern in both plants during later events, suggesting that sexual and apomictic reproduction are closely related (Tucker et al., 2003). However, the AtFIS2::GUS expression pattern occurred earlier in ovule development in *Hieracium* than in *Arabidopsis*. Furthermore, the pattern of AtFIS2::GUS was also spatially shifted in two different apomictic species, compared to the sexual plant. In sexual plants, AtFIS2::GUS expression was restricted to the three megaspores destined to degenerate, and was absent in the selected spore and surrounding nucellar epidermis. In apomicts at the time of aposporous initial enlargement, AtFIS2::GUS expression was found in all four megaspores and in the surrounding nucellar epidermis enveloping them. These are cells that are destined to degenerate. Expression was absent in the aposporous initials as well as in the functional megaspore in the sexual plant until their nuclei divided (Tucker et al, 2003). *FIS2* belongs to a family of genes that includes *EMBRYONIC FLOWER2* (Yoshida et al., 2001) and *VERNALIZATION2* (Gendall et al., 2001), both of which are involved in epigenetic control of flowering. The earlier expression of AtFIS2:GUS in *Hieracium* may reflect a different function for this gene in *Hieracium* relative to that in *Arabidopsis*, which may have enabled the development of apomixis (Eckhardt, 2003). The pattern shift in AtFIS2:GUS expression at aposporous initial cell differentiation in *Hieracium* may be caused by signals emanating from the AI, or may be a consequence of AI differentiation. To investigate this further, it will be necessary to identify the cis-regulatory elements required for the spatial shift of AtFIS2:GUS expression, and to identify factors in *Hieracium* that interact with these sequences. Isolation of a *Hieracium* FIS2 orthologue would enable dissection of its role in apomictic development.

FIS complex and autonomous seed development

In sexually reproducing plants, the MEA/FIS2/FIE/MSI1/RBR complex appears to be involved in re-

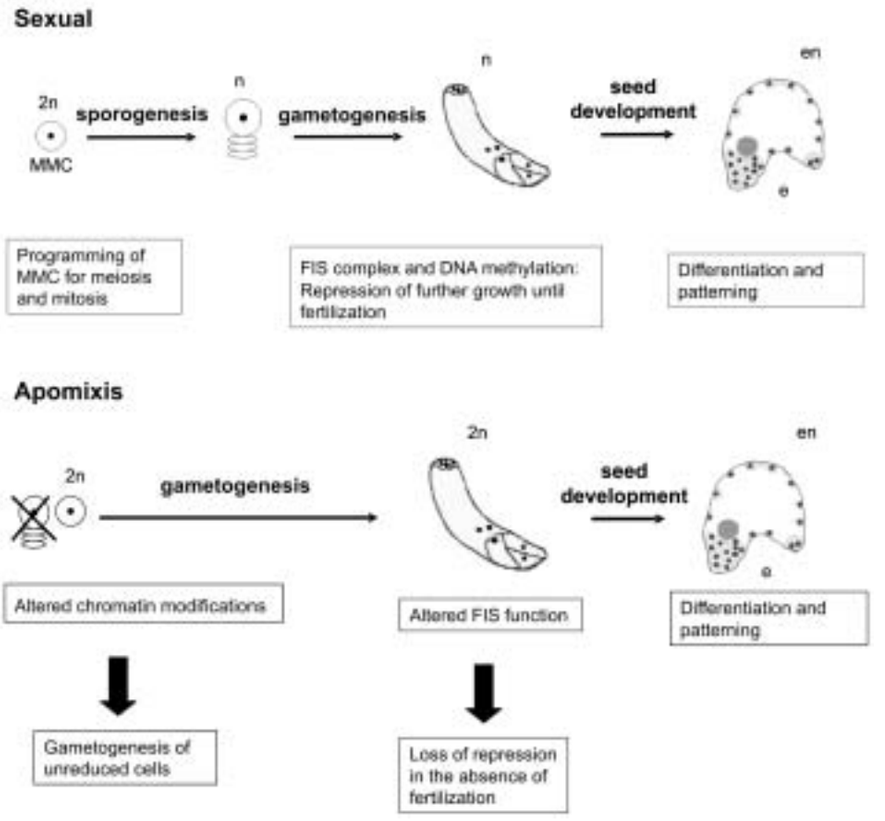

Fig. 3. Model for the role of epigenetic regulation in apomixis. A change in chromatin modifications controlling the genes required for female gametogenesis might cause ectopic induction of the mitotic events of female gametophyte development. In apomictic plants, these epigenetic changes might lead to the production of a functional, unreduced gametophyte with the capacity to enable autonomous development of the central cell and egg cell. Altered regulation of the FIS complex might be one way this is achieved. In apomicts, the "reprogrammed" complex might retain the cellular memory of the reproductive cell program, with additional features allowing autonomous seed development to occur. MMC – megaspore mother cell; e – embryo; en – endosperm (ploidy of endosperm in apomicts is dependent on fertilization of the central cell or the mode of apomixis).

pression of endosperm development in the absence of fertilization, probably by preventing transcription of *PHERES1* and other target genes (Köhler et al., 2003b). The molecular cues by which fertilization alters the function of the complex is still unknown. The observation that cellularized, differentiated endosperm can develop in the absence of fertilization when combining DNA hypomethylation and *fie* mutant background in sexual plants (Vinkenoog et al., 2000) leads to speculation that DNA methylation may be involved in autonomous endosperm development. The embryo-like phenotypes of *msi1* and *rbr* mutants futher point to their possible role in parthenogenetic embryo development in apomicts. An attractive model can be envisaged in which aberrant chromatin modification marks in key regulatory genes would alter their temporal and spatial expression patterns, leading to complete maternal control over reproduction, or autonomous development (Fig. 3). Due to the association of *fis* phenotypes with apomictic development, candidates for such key regulatory genes are chromatin-remodelling proteins. In this model, defects or untimely expression of these epigenetic regulators could lead to misexpression of the target genes that normally control seed development in response to fertilization.

EPIGENETIC ASPECTS OF APOMICTIC DEVELOPMENT

There are several lines of evidence that suggest a relationship between epigenetic regulation and apomictic development. Apomixis is not as tightly regulated as the sexual process. Variability in developmental timing and development exists even within a vegetatively propagated apomictic plant (Koltunow et al., 2000). The diploid apomictic *Hieracium piloselloides* shows an array of developmental variations,

producing an additional form of apomixis, adventitious embryony. Germinated seedlings vary widely in morphology and are often abnormal, yet as they grow they regain a normal growth pattern (Koltunow et al., 2000).

Autonomous endosperm development in the apomictic *Hieracium piloselloides* is initiated with irregular and rapid cell division patterns, with nuclei migrating in patterns that differ from the early divisions of fertilization-derived endosperm in the sexual plant. These aberrant early divisions resemble those seen in plants with defects in *FIS*-class genes. The later nuclear endosperm divisions in the apomict resemble those of the fertilization-induced sexual plant, indicating reversion to a normal growth pattern of endosperm development (Tucker, 2003). Developmental variation in a single genetic background and abnormal growth patterns that revert to normal are often hallmarks of epigenetically regulated processes, where epimutations deregulate the timing of expression of developmental genes.

If epigenetic modifications are involved in apomixis, how might altered epigenetic marks such as DNA methylation arise and be maintained? The genetic and molecular characteristics of DNA hypomethylation mutants suggest that disturbing the maintenance methylation machinery leads to the accumulation of altered methylation states that are not reset even after the genetic components are restored (Kakutani et al., 1996; Jedelloh et al., 1998; Saze et al., 2003). In these mutants, hypomethylated states at specific loci could coexist with methylated loci, thus generating epigenetic variation (Saze et al., 2003). Apomixis could have arisen through perturbation of the DNA methylation machinery, such as observed in *ddm* mutants (Jeddeloh et al., 1999), which would lead to the accumulation of epimutations, causing misexpression of key developmental genes. Alternatively, naturally occurring epimutations due to environmental stress or hybridization would cause altered expression of the genes that control the reproductive pathway, leading to apomixis. Key epimutations would be maintained through DNA replication by maintenance DNA methyltransferases.

The recent association of many transposable elements at a locus that confers apomixis in the grass species *Pennisetum* sp. (Labombarda et al., 2002; Akiyama et al., 2004) also may suggest that altered DNA methylation might be involved in controlling the chromatin configuration and the expression of genes leading to the manifestation of apomixis. Interestingly, a surge of transposon activity at the time of apomictic initiation is observed in *Hieracium*, and this activity decreases in mutant plants that have lost the capacity for apomixis (Ross Bicknell, personal communication). It remains unclear whether these levels of transposon activity are of functional significance for apomictic development or, indeed, what role alterations in transposon activity might have in regulating apomixis. In light of the information described above, it is tempting to speculate that transposons might be inserted in key epigenetic regulator(s) of sexual reproduction in apomictic plants; this could deregulate expression of the sexual developmental program in space and time. It has been shown that the expression of transposable elements can lead to inappropriate expression of neighboring genes (Lippman et al., 2004; Peaston et al., 2004). Alternatively, if a defect in the RNAi silencing machinery deregulates the expression of genes controlling sexual reproduction in apomictic plants, then the transposon activity observed would be mostly an effect rather than a cause of apomixis.

In spite of indirect evidence relating DNA methylation, chromatin remodelling genes and apomixis, molecular evidence that directly shows a significant association between epigenetic regulation and apomixis is lacking. This complex issue can be approached in many different ways. Isolation of *FIS* orthologues in apomictic plants would be a first step toward elucidating their expression and function in sexual and apomictic reproductive pathways. Moreover, if DNA methylation is indeed involved in controlling the expression of genes involved in apomictic development, altering methylation levels during key stages of apomictic reproduction could identify the stages in which this epigenetic mark is important. Patterns of DNA methylation in specific genes can also be investigated to identify possible differences between apomictic and sexual plants throughout ovule development.

CONCLUDING REMARKS

Epigenetic regulation provides a way to achieve variability and adaptive advantages without altering DNA sequences. The generation of epialleles can alter the timing of expression of key genes controlling cellular or physiological processes during plant development. Inheritance of such adaptive epialleles could provide increased fitness in certain conditions, and the potential reversibility of these changes could also be a source of adaptive flexibility. Identification and functional analysis of several epigenetic regulatory factors involved in gametophyte and seed development have confirmed a role for epigenetic regulation in the control of sexual plant reproduction. These factors can set heritable patterns that control gene activity over space and time, and thus cell fate. By isolating these factors and examining their function during apomictic development, it will be possible to verify whether epigenetic regulatory mechanisms are involved in apomictic reproduction.

ACKNOWLEDGEMENTS

We thank Paul K. Boss for critical comments on the manuscript. JCMR is supported by a Capes Foundation scholarship (Ministry of Education, Brazil). We are indebted to the late Professor Lesław Przywara for the example of his distinguished work in plant biology, and for many enjoyable discussions about the twists and turns of apomixis.

REFERENCES

ACH R, TARANTO P, and GRUISSEM W. 1997. A conserved family of WD–40 proteins binds to the retinoblastoma protein in both plants and animals. *The Plant Cell* 9: 1595–1606.

ADAMS S, VINKENOOG R, SPIELMAN M, DICKINSON HG, and SCOTT RJ. 2000. Parent-of-origin effects on seed development in *Arabidopsis thaliana* require DNA methylation. *Development* 127: 2493–2502.

AKIYAMA Y, CONNER J, GOEL S, MORISHIGE D, MULLET J, HANNA W, and OZIAS-AKINS P. 2004. High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. *Plant Physiology* 134: 1733–1741.

ASKER SE, and JERLING L. 1992. *Apomixis in plants*. Boca Raton, CRC Press.

BAO N, LYE K, and BARTON M. 2004. MicroRNA binding sites in Arabidopsis class III HD-ZIP mRNAs are required for methylation of the template chromosome. *Developmental Cell* 7: 653–662.

BASTOW R, MYINE JS, LISTER C, LIPPMAN Z, MARTIENSSEN RA, and DEAN C. 2004. Vernalization requires epigenetic silencing of FLC histone methylation. *Nature* 427: 164–167.

BAUMBUSCH A, THORSTENSEN T, KRAUS V, FISCHER A, NAUMANN K, ASSALKHOU R, SCHULZ I, REUTER G, and AALEN RB. 2001. The *Arabidopsis thaliana* genome contains at least 29 active genes encoding SET domain proteins tahta can be assigned to four evolutionarily conserved classes. *Nucleic Acids Research* 29: 4319–4333.

BESTOR T. 2000. The DNA methyltransferases of mammals. *Human Molecular Genetics* 9: 2395–2402.

BICKNELL R, and KOLTUNOW AM. 2004. Understanding apomixis: recent advances and remaining conundrums. *Plant Cell* 16: S228-S245.

CAO X, and JACOBSEN SE. 2002. Locus-specific control of asymmetric and CpNpG methylation by the DRM and CMT3 methyltransferase genes. *PNAS* 99: 16491–16498.

CAO X, and JACOBSEN SE. 2002. Role of *Arabdopsis* DRM methyltransferases in De Novo DNA methylation and gene silencing. *Current Biology* 12: 1138–1144.

CHAUDHURY AM, MING L, MILLER C, CRAIG S, DENNIS ES, and PEACOCK WJ. 1997. Fertilization-independent seed development in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the U.S.A.* 94: 4223–4228.

CHOI YH, GEHRING M, JOHNSON L, HANNON M, HARADA JJ, GOLDBERG RB, JACOBSEN SE, and FISCHER RL. 2002. DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in *Arabidopsis*. *Cell* 110: 33–42.

EBEL C, MARICONTI L, and GRUISSEM W. 2004. Plant retinoblastoma homologues control nuclear proliferation in the female gametophyte. *Nature* 429: 776–780.

EHRLICH M. 2003. Expression of various genes is controlled by DNA methylation during mammalian development. *Journal of Cellular Biochemistry* 88: 899–910.

FERGUNSON-SMITH AC, and SURANI M. 2001. Imprinting and the epigenetic asymmetry between parental genomes. *Science* 293: 1086–1089.

FINNEGAN EJ, and KOVAC KA. 2000. Plant DNA methyltransferases. *Plant Molecular Biology* 43: 189–201.

FINNEGAN EJ, PEACOCK WJ, and DENNIS ES. 1996. Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proceedings of the National Academy of Sciences of the U.S.A.* 93: 8449–8454.

FRANCIS NJ, and KINGSTON R. 2001. Mechanisms of transcriptional memory. *Nature Reviews Molecular Cell Biology* 2: 409–421.

GEHRING M, CHOI Y, and FISCHER RL. 2004. Imprinting and seed development. *Plant Cell* 16: S203-S213.

GENDALL AR, LEVY YY, WILSON A, and DEAN C. 2001. The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. *Cell* 107: 525–535.

GENGER RK, KOVAC KA, DENNIS ES, PEACOCK WJ, and FINNEGAN EJ. 1999. Multiple DNA methyltransferase genes in *Arabidopsis thaliana*. *Plant Molecular Biology* 41: 269–278.

GENGER RK, PEACOCK WJ, DENNIS ES, and FINNEGAN EJ. 2003. Opposing effects of reduced DNA methylation on flowering time in *Arabidopsis thaliana*. *Planta* 216: 461–466.

GROSSNIKLAUS U. 2001. From sexuality to apomixis: molecular and genetic approaches. In: Savidan Y, Carman JG, and Dresselhaus T [eds.], *The flowering of apomixis: From mechanisms to genetic engineering*, 168–211. Mexico, CIMMYT, IRS, Eur. Comm. DG VI.

GROSSNIKLAUS U, SPILLANE C, PAGE DR, and KÖHLER C. 2001. Genomic imprinting and seed development: endosperm formation with and without sex. *Current Opinion in Plant Biology* 4: 21–27.

GROSSNIKLAUS U, and SCHNEITZ K. 1998. The molecular and genetic basis of ovule and megagametophyte development. *Seminars in Cell and Developmental Biology* 9: 227–238.

GROSSNIKLAUS U, VIELLE-CALZADA JP, HOEPPNER MA, and GAGLIANO WB. 1998. Maternal control of embryogenesis by medea, a Polycomb group gene in *Arabidopsis*. *Science* 280(5362): 446–450.

GUITTON A, PAGE D, CHAMBRIER P, LIONNET C, FAURE J, GROSSNIKLAUS U, and BERGER F. 2004. Identification of new members of Fertilisation Independent Seed Polycomb Group pathway involved in the control os seed development in *Arabidopsis thaliana*. *Development* 131: 2971–2981.

HAIG D, and WESTOBY M. 1991. 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 of the Royal Society of London Series B-Biological Sciences* 333(1266): 1–13.

HASHIDA S, KITAMURA K, MIKAMI T, and KISHIMA Y. 2003. Temperature shift coordinately changes the activity and the methylation state of transposon Tam3 *Antirrhinum majus*. *Plant Physiology* 132: 1207–1216.

HE YM, MICHAELS SD, and AMASINO RM. 2003. Regulation of flowering time by histone acetylation in *Arabidopsis*. *Science* 302: 1751–1754.

HENIKOFF S, and COMAI L. 1998. A DNA methyltransferase homolog with a chromodomain exists in multiple polymorphic forms in *Arabidopsis*. *Genetics* 149: 307–318.

JACKSON JP, JOHNSON L, JASENCAKOVA Z, ZHANG X, PEREZBURGOS L, SINGH PB, CHENG X, SCHUBERT I, JENUWEIN T, and JACOBSEN SE. 2004. Demethylation of histone H3 lysine 9 is a critical mark for DNA methylation and gene silencing in *Arabidopsis thaliana*. *Chromosoma* 112: 308–315.

JACOBSEN SE, and MEYEROWITZ EM. 1997. Hypermethylated *SUPERMAN* epigenetic alleles in *Arabidopsis*. *Science* 277: 1100–1103.

JEDDELOH JA, BENDER J, and RICHARDS EJ. 1998. The DNA methylation locus DDM1 is required for maintenance of gene silencing in *Arabidopsis*. *Genes and Development* 12: 1714–1725.

JEDDELOH JA, STOKES TL, and RICHARDS EJ. 1999. Maintenance of genomic methylation requires a SW12/SNF2-like protein. *Nature Genetics* 22: 94–97.

JENUWEIN T, and ALLIS CD. 2001. Translating the histone code. *Science* 293: 1074–1080.

JOHNSON L, CAO X, and JACOBSEN S. 2002. Interplay between two epigenetic marks: DNA methylation and histone H3 lysine 9 methylation. *Current Biology* 12: 1360–1367.

JONES PA, and TAKAI D. 2001. The role of DNA methylation in mammalian epigenetics. *Science* 293: 1068–1070.

KASHKUSH K, FELDMAN M, and LEVY A. 2003. Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. *Nature Genetics* 33: 102–106.

KINOSHITA T, HARADA JJ, GOLDBERG RB, and FISCHER RL. 2001. Polycomb repression of flowering during early plant development. *Proceedings of the National Academy of Sciences of the U.S.A.* 98: 14156–14161.

KINOSHITA T, MIURA A, CHOI Y, KINOSHITA Y, CAO X, JACOBSEN SE, FISCHER RL, and KAKUTANI T. 2004. One-way control of FWA imprinting in *Arabidopsis* endosperm by DNA methylation. *Science* 303: 521–523.

KINOSHITA T, YADEGARI R, HARADA JJ, GOLDBERG RB, and FISCHER RL. 1999. Imprinting of the MEDEA polycomb gene in the *Arabidopsis* endosperm. *Plant Cell* 11: 1945–1952.

KÖHLER C, HENNING L, BOUVERET R, GHEYSELINCK J, GROSSNIKLAUS U, and GRUISSEM W. 2003a. *Arabidopsis* MSI1 is a component of the MEA/FIE polycomb group complex and required for seed development. *The EMBO Journal* 22: 4804–4814.

KÖHLER C, HENNING L, SPILLANE C, PIEN S, GRUISSEM W, and GROSSNIKLAUS U. 2003b. The polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene *PHERES1*. *Genes and Development* 17: 1540–1553.

KÖHLER C, PAGE DR, GAGLIARDINI V, and GROSSNIKLAUS U. 2005. The *Arabidopsis thaliana* MEDEA Polycomb group protein controls expression of *PHERES1* by parental imprinting. *Nature Genetics* 37: 28–30.

KOLTUNOW AM, JOHNSON SD, and BICKNELL RA. 1998. Sexual and apomictic development in *Hieracium*. *Sexual Plant Reproduction* 11: 213–230.

KOLTUNOW AM, JOHNSON SD, and BICKNELL RA. 2000. Apomixis is not developmentally conserved in related, genetically characterized *Hieracium* plants of varying ploidy. *Sexual Plant Reproduction* 12: 253–266.

KOLTUNOW AMG, and GROSSNIKLAUS U. 2003. Apomixis: a developmental perspective. *Annual Review of Plant Biology* 54: 547–574.

LABOMBARDA P, BUSTI A, CACERES M, PUPILLI F, and ARCIONI S. 2002. An AFLP marker tightly linked to apomixis reveals hemizygosity in a portion of the poamixis-controlling locus in *Paspalum simplex*. *Genome* 45: 513–519.

LAGACE M, CHANTHA S, MAJOR G, and MATTON DP. 2003. Fertilization induces strong accumulation of a histone deacetylase (HD2) and of the other chromatin-remodeling proteins in restricted areas of the ovules. *Plant Molecular Biology* 53: 759–769 .

LINDROTH A, SHULTIS D, JASENCAKOVA Z, FUCHS J, JOHNSON L, SCHUBERT D, PATNAIK D, PRADHAN S, GOODRICH J, SCHUBERT I, JENUWEIN T, KHORASANIZADEH S, and JACOBSEN SE. 2004. Dual histone H3 methylation marks at lysines 9 and 27 required for interaction with *CHROMOMETHYLASE3*. *The EMBO Journal* 3: 4286–4296.

LIPPMAN Z, GENDREL A, BLACK M, VAUGN M, DEDHIA N, MCCOMBLE R, LAVINE K, MITTAL V, MAY B, KASSCHAU K, CARRINGTON J, DOERGE R, COLOT V, and MARTIENSSEN R. 2004. Role of transposable elements in heterochromatin and epigenetic control. *Nature* 430: 471–476.

LUO, M, BILODEAU P, DENNIS ES, PEACOCK WJ, and CHAUDHURY A. 2000. Expression and parent-of-origin effects for *FIS2*, *MEA*, and *FIE* in the endosperm and embryo of developing *Arabidopsis* seeds. *Proceedings of the National Academy of Sciences of the U.S.A.* 97: 10637–10642.

LUO M, BILODEAU P, KOLTUNOW A, DENNIS ES, PEACOCK WJ, and CHAUDHURY AM. 1999. Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the U.S.A.* 96: 296–301.

MATZKE MA, METTE MF, AUFSATZ W, JAKOWITSCH J, and MATZKE AJM. 1999. Host defenses to parasitic sequences and the evolution of epigenetic control mechanism. *Genetica* 107: 271–287.

MOON Y, CHEN L, PAN R, CHANG H, ZHU T, MAFFEO D, and SUNG Z. 2003. *EMF* genes maintain vegetative development by repressing the flower program in *Arabidopsis*. *The Plant Cell* 15: 681–693.

MOREL J, MOURRAIN P, BECLIN C, and VAUCHERET H. 2000. DNA methylation and chromatin structure affect transcriptional and post-transcriptional transgene silencing in *Arabdopsis*. *Current Biology* 10: 1591–1594.

MOSQUNA A, KATZ A, SHOCHAT S, GRAFI G, and OHAD N. 2004. Interaction of FIE, a Polycomb protein, with pRb: a possible mechanism regulating endosperm development. *Molecular Genetics and Genomics* 271: 651–657.

NAN X, NG H, JOHNSON C, LAHERTY C, TURNER B, ELSENMAN R, and BIRD A. 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* 393: 386–389.

NOGLER G. 1984. Gametophytic apomixis. In: Johri BM [ed.], *Embryology of Angiosperms*, 475–518. Springer-Verlag, Berlin Heidelberg.

OHAD N, MARGOSSIAN L, HSU YC, WILLIAMS C, REPETTI P, and FISCHER RL. 1996. A mutation that allows endosperm development without fertilization. *Proceedings of the National Academy of Sciences of the U.S.A.* 93: 5319–5324.

OHAD N, YADEGARI R, MARGOSSIAN L, HANNON M, MICHAELI D, HARADA JJ, GOLDBERG RB, and FISCHER RL. 1999. Mutations in *FIE*, a WD polycomb group gene, allow endosperm development without fertilization. *Plant Cell* 11: 407–415.

PANDEY R, MULLER A, NAPOLI CA, SELINGER DA, PIKAARD CS, RICHARDS EJ, BENDER J, MOUNT DW, and JORGENSEN RA. 2002. Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucleic Acids Research* 30: 5036–5055.

PASINI D, BRACKEN A, and HELIN K. 2004. Polycomb Group Proteins in cell cycle progression and cancer. *Cell Cycle* 3: 396–400.

PEASTON A, EVSIKOV A, GRABER J, DE VRIES W, HOLBROOK A, SOLTER D, and KNOWLES B. 2004. Retrotransposons regulate host genes in mouse oocytes and preimplantation embryos. *Developmental Cell* 7: 597–606.

PIRROTA V, POUX S, MELFI R, and PILYUGIN M. 2003. Assembly of polycomb complexes and silencing mechanisms. *Genetica* 117: 191–197.

ROBERTSON K, AIT-SI-ALI S, YOKOCHI T, WADE P, JONES P, and WOLFFE A. 2000. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nature Genetics* 25: 338–342.

RONEMUS MJ, GALBIATI M, TICKNOR C, CHEN J, and DELLAPORTA SL. 1996. Demethylation-induced developmental pleiotropy in *Arabidopsis*. *Science* 273: 654–657.

SAKAI H, MEDRANO LJ, and MEYEROWITZ EM. 1995. Role of SUPERMAN in maintaining *Arabidopsis* floral whorl boundaries. *Nature* 378: 199–203.

SAVIDAN Y. 2000. Apomixis, the way of cloning seeds. *Biofutur* 2000(198): 38–43.

SAZE H, SCHEID OM, and PASZKOWSKI J. 2003. Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. *Nature Genetics* 34: 65–69.

SCHRAMKE V, and ALLSHIRE R. 2003. Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. *Science* 301: 1069–1074.

SIMON J, and TAMKUN J. 2002. Programming off and on states in chromatin: mechanisms of Polycomb and trithorax group complexes. *Current Opinion in Genetics and Development* 12: 210–218.

SOPPE WJJ, JACOBSEN SE, ALONSO-BLANCO C, JACKSON JP, KAKUTANI T, KOORNNEEF M, and PEETERS AJM. 2000. The late flowering phenotype of fwa mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. *Molecular Cell* 6: 791–802.

SPILLANE C, MACDOUGALL C, STOCK C, KÖHLER C, VIELLE-CALZADA JP, NUNES SM, GROSSNIKLAUS U, and GOODRICH J. 2000. Interaction of the *Arabidopsis* Polycomb group proteins FIE and MEA mediates their common phenotypes. *Current Biology* 10: 1535–1538.

STEIMER A, SCHOB H, and GROSSNIKLAUS U. 2004. Epigenetic control of plant development: new layers of complexity. *Current Opinion in Plant Biology* 7: 11–19.

STRAHL BD, and ALLIS CD. 2000. The language of covalent histone modifications. *Nature* 403: 41–45.

SUNG S, and AMASINO RM. 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* 427: 159–164.

TIAN L, and CHEN ZJ. 2001. Blocking histone deacetylation in *Arabidopsis* induces pleiotropic effects on plant gene regulation and development. *Proceedings of the National Academy of Sciences of the U.S.A.* 98: 200–205.

TIE F, FURUYAMA T, PRASAD-SINHA J, JANE E, and HARTE PJ. 2001. The *Drosophila* Polycomb Group proteins ESC and E(Z) are present in a complex containing the histone-binding protein p55 and the histone deacetylase RPD3. *Development* 128: 275–286.

TUCKER MR, ARAUJO AG, PAECH NA, HECHT V, SCHIMIDT EDL, ROSSEL J, VRIES SC, and KOLTUNOW AMG. 2003. Sexual and apomictic reproduction in *Hieracium subgenus pilosella* are closely interrelated developmental pathways. *Plant Cell* 15: 1524–1537.

VIELLE-CALZADA JP, BASKAR R, and GROSSNIKLAUS U. 2000. Delayed activation of the paternal genome during seed development. *Nature* 404(6773): 91–94.

VIELLE-CALZADA JP, THOMAS J, SPILLANE C, COLUCCIO A, HOEPPNER MA, and GROSSNIKLAUS U. 1999. Maintenance of genomic imprinting at the *Arabidopsis medea* locus requires zygotic *DDM1* activity. *Genes and Development* 13: 2971–2982.

VINKENOOG R, and SCOTT RJ. 2001. Autonomous endosperm development in flowering plants: how to overcome the imprinting problem? *Sexual Plant Reproduction* 14: 189–194.

VINKENOOG R, SPIELMAN M, ADAMS S, FISCHER RL, DICKINSON HG, and SCOTT RJ. 2000. Hypomethylation promotes autonomous endosperm development and rescues postfertilization lethality in fie mutants. *Plant Cell* 12: 2271–2282.

WALSH CP, and BESTOR TH. 1999. Cytosine methylation and mammalian development. *Genes and Development* 13: 26–34.

WU KQ, TIAN LN, MALIK K, BROWN D, and MIKI B. 2000. Functional analysis of HD2 histone deacetylase homologues in *Arabidopsis thaliana*. *Plant Journal* 22: 19–27.

XIAO W, GEHRING M, CHOI Y, MARGOSSIAN L, PU H, HARADA JJ, GOLDBERG RB, PENNELL RI, and FISCHER RL. 2003. Imprinting of the *MEA* polycomb gene is controlled by antagonism between MET1 methyltransferase and DME glycosylase. *Developmental Cell* 5: 891–901.

YOSHIDA N, YANAI Y, CHEN LJ, KATO Y, HIRATSUKA J, MIWA T, SUNG ZR, and TAKAHASHI S. 2001. EMBRYONIC

YOSHIDA N, YANAI Y, CHEN LJ, KATO Y, HIRATSUKA J, MIWA T, SUNG ZR, and TAKAHASHI S. 2001. EMBRYONIC FLOWER2, a novel polycomb group protein homolog, mediates shoot development and flowering in *Arabidopsis*. *Plant Cell* 13: 2471–2481.

ZHOU C, LABBE H, SRIDHA S, WANG L, TIAN L, LATOSZEK-GREEN M, YANG Z, BROWN D, MIKI B, and WU K. 2004. Expression and function of HD2-type histone deacetylases in *Arabidopsis* development. *The Plant Journal* 38: 715–724.