Leading Edge
Review
Cell

Cellular Programming of Plant Gene Imprinting

Jin Hoe Huh,[1,2] Matthew J. Bauer,[1,2] Tzung-Fu Hsieh,[1] and Robert L. Fischer[1,*]
[1]Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA 94720, USA
[2]These authors contributed equally to this work.
*Correspondence: rfischer@berkeley.edu
DOI 10.1016/j.cell.2008.02.018

Gene imprinting, the differential expression of maternal and paternal alleles, independently evolved in mammals and in flowering plants. A unique feature of flowering plants is a double-fertilization event in which the sperm fertilize not only the egg, which forms the embryo, but also the central cell, which develops into the endosperm (an embryo-supporting tissue). The distinctive mechanisms of gene imprinting in the endosperm, which involve DNA demethylation and histone methylation, begin in the central cell and sperm prior to fertilization. Flowering plants might have coevolved double fertilization and imprinting to prevent parthenogenetic development of the endosperm.

Introduction

Gene imprinting primarily occurs in the placenta of mammals and the endosperm of flowering plants, structures that nourish the developing embryo (Fowden et al., 2006; Gehring et al., 2004). According to the parental conflict theory, it is this nourishing function that led to the evolution of imprinting in mammals and plants (Haig and Westoby, 1991; Moore and Haig, 1991). Recent reviews discuss the possible convergent mechanisms leading to imprinting in plants and mammals (Feil and Berger, 2007; Scott and Spielman, 2006). There are over 80 imprinted genes identified in mammals, and many of these genes are crucial for placental development (Fowden et al., 2006). Imprinted genes in mammals are usually found in clusters that are regulated by an imprinting control region whose DNA methylation state determines the parent-of-origin expression of genes in the cluster. For each generation, DNA methylation at imprinted genes is removed and reset in a gender-specific way by de novo DNA methylation and/or protection against DNA demethylation. Repressive histone modifications reinforce the silencing of alleles by DNA methylation.

In this review, we focus on the cellular programming that establishes imprinting in flowering plants. Imprinting has been detected in the endosperm and not the embryo. The sexual process that forms the endosperm, a product of fertilization of a specialized central cell by a sperm cell, is unique to flowering plants and underlies the distinctive cellular programming of plant gene imprinting. DNA demethylation and histone modifications catalyzed by DNA glycosylases and Polycomb group proteins in the central and sperm cells are key regulatory elements in plant gene imprinting. These epigenetic marks that distinguish paternal and maternal imprinted alleles need not be reset for each generation in plants, because the endosperm, formed by a distinct fertilization event, does not transmit its genome to the next generation. Recent data suggest that imprinting and double fertilization may have evolved together to prevent parthenogenetic development of the endosperm.

Epigenetic Regulators that Program Plant Imprinting

Research over the last decade has shown that epigenetic marks (DNA methylation and histone modification) and their regulators (Polycomb group proteins, DNA methyltransferases, DNA demethylating DNA glycosylases) establish and maintain plant gene imprinting.

Histone Modifications by Polycomb Group Proteins

In animals, Polycomb group proteins silence gene expression by directing the posttranslational modification of histones (Schuettengruber et al., 2007). Polycomb group genes, discovered in the fruit fly *Drosophila melanogaster* as repressors of homeotic genes, play an important role in the control of cell proliferation, stem cell identity, cancer, gene imprinting, and X chromosome inactivation. Three Polycomb complexes—PRC1, PRC2, and PhoRC—work together to silence genes. A simple step-wise model for Polycomb group proteins has a component of the PhoRC complex binding to DNA motifs and recruiting a PRC2 complex to the locus. The PRC1 complex is then recruited to the methylated histone 3 lysine 27 (H3K27) mark placed by PRC2. There has been marked conservation of the structure and function of the PRC2 complex during evolution. In *Drosophila*, E(Z) (enhancer of zeste; a SET-domain polypeptide) methylates H3K27, a histone modification associated with gene silencing. E(Z) functions in a complex with SU(Z)12 (supressor of zeste 12; a C2H2 zinc-finger protein) and two WD-40 proteins, Extra sex comb (ESC) and p55.

Flowering plants have genes encoding proteins in the PRC2 complex that regulate developmental processes, including the response of the shoot apical meristem to environmental cues that promote the generation of a reproductive floral meristem, regulation of homeotic genes that control flower organ identity, the maternal control of seed viability, and gene imprinting (Pien and Grossniklaus, 2007). However, flowering plants lack genes encoding proteins in the PRC1 complex, and it is either thought that H3K27 methylation represses transcription directly, or that other proteins replace PRC1. One candidate is LIKE HETEROCHROMATIN PROTEIN 1 (LHP1; also called TERMINAL

FLOWER 2), the only protein in the model plant *Arabidopsis* with overall sequence similarity to the HETEROCHROMATIN PROTEIN 1 (HP1) family, which is required for the formation of heterochromatin in metazoans and the fission yeast *Schizosaccharomyces pombe* (Grewal and Jia, 2007). LHP1 specifically recognizes H3K27 in vivo and maintains repression of certain genes targeted by the *Arabidopsis* PRC2 complex (Sung et al., 2006; Turck et al., 2007).

The homologs in *Arabidopsis* that have a profound effect on cellular programming of gene imprinting are the SET-domain Polycomb group protein MEDEA (MEA) (Grossniklaus et al., 1998; Kiyosue et al., 1999), the C2H2 zinc-finger protein FERTILIZATION INDEPENDENT SEED2 (FIS2) (Luo et al., 1999), and two WD-40 proteins, FERTILIZATON INDEPENDENT ENDOSPERM (FIE) (Ohad et al., 1999) and MULTICOPY SUPPRESSOR OF IRA1 (MSI1) (Guitton et al., 2004; Kohler et al., 2003a). These plant PRC2 components, like their animal counterparts, form a 600 kDa complex (Chanvivattana et al., 2004; Kohler et al., 2003a) and are necessary for H3K27 methylation at their target loci (Gehring et al., 2006; Makarevich et al., 2006).

DNA Methylation by DNA Methyltransferases

In mammals, DNA methylation (5-methylcytosine) in the symmetric CG sequence context is an abundant epigenetic modification (Klose and Bird, 2006). DNA methylation regulates gene imprinting, X chromosome inactivation, and silences transposons and retrotransposons. Aberrant promoter DNA methylation is associated with gene silencing and plays a critical role in disease. The de novo DNA methyltransferases, DNMT3a and DNMT3b, methylate cytosines at previously unmethylated CG sites. Patterns of symmetric CG methylation are maintained after DNA replication by the maintenance DNA methyltransferase DNMT1, which methylates cytosines in the newly synthesized DNA strands. In mammals, the DNA methylation marks are erased and reset each generation during gametogenesis and embryogenesis (Reik, 2007). DNA methylation prevents gene transcription by multiple mechanisms: blocking the access of transcription factors to DNA, recruiting methyl-CG binding proteins that form complexes with histone deacetylases, histone methyltransferases or chromatin-remodeling proteins, and promoting repressive chromatin structure.

As in mammals, DNA methylation in flowering plants regulates gene imprinting and silences transposons, retrotransposons, and repeated sequences (Gehring and Henikoff, 2007; Henderson and Jacobsen, 2007; Matzke et al., 2007). In *Arabidopsis*, orthologs of DNMT1 and DNMT3 DNA methyltransferases, METHYLTRANSFERASE1 (MET1) and DOMAINS REARRANGED METHYLTRANSFERASE (DRM) family enzymes, maintain and establish de novo DNA methylation, respectively. However, several properties of plant DNA methylation are distinct. In addition to methylation in the CG sequence, plant DNA methylation is present at CNG (where N is any nucleotide) and CHH (where H is A, C, or T) sequences that are maintained, in part, by plant-specific DNA methyltransferases. Also, to a great extent, small RNAs generated by RNA-interference pathways guide the placement of non-CG DNA methylation in plants. Finally, unlike mammals, genome DNA methylation is not reset each generation, which, as described below, has profound implications for the mechanisms plants use to regulate gene imprinting.

DNA Demethylation Catalyzed by DNA-Repair Pathways

DNA demethylation can occur by passive or active mechanisms. Passive DNA demethylation is when 5-methylcytosine is replaced with cytosine during DNA replication. Active DNA demethylation is when the methyl group is directly removed from 5-methylcytosine, a mechanism that has yet to be substantiated (Reik, 2007), or when 5-methylcytosine is enzymatically removed and replaced with cytosine. In mammals, active DNA demethylation is thought to be responsible for the widespread loss of DNA methylation that occurs in the epigenetic reprogramming of primordial germ cells. DNA glycosylases that excise 5-methylcytosine and initiate the base excision DNA-repair pathway (see below) might be involved (Jost et al., 2001). More recently, it was shown that the growth arrest and DNA-damage-inducible protein 45 alpha (Gadd45a) promotes nucleotide excision DNA repair, which replaces 5-methylcytosine with cytosine (Barreto et al., 2007).

In animals, plants, and microbes, DNA glycosylase enzymes initiate the base excision DNA-repair pathway by excising modified, damaged, or mispaired bases from DNA (David et al., 2007). The DNA glycosylase removes a base by cleaving the N-glycosylic bond, creating an abasic site. An AP endonuclease generates a 3′ hydroxyl, which is used by a DNA-repair polymerase that inserts the proper nucleotide. A DNA ligase seals the nick to complete the repair process. The *Arabidopsis* DEMETER (DME) family of DNA glycosylases has been shown to excise 5-methylcytosine in vitro, in *E. coli*, and *in planta*, creating an abasic site (Agius et al., 2006; Gehring et al., 2006; Gong et al., 2002; Morales-Ruiz et al., 2006). Downstream enzymes in the base excision pathway then repair the abasic site by inserting cytosine. This active DNA demethylation pathway regulates gene imprinting (Choi et al., 2002; Gehring et al., 2006) and protects the *Arabidopsis* genome from accumulating inappropriate DNA methylation (Penterman et al., 2007a, 2007b; Zhu et al., 2007).

Gene Imprinting and the Unique Reproductive Strategies of Flowering Plants

Plant gene imprinting occurs in the endosperm, a product of fertilization, which nourishes the developing embryo. The evolution and mechanisms of plant gene imprinting are intimately tied to this distinct reproductive strategy described below (Haig and Westoby, 1991).

Germ Cells

Unlike animal germ cells that directly give rise to male and female gametes, plant germ cells are committed first to producing multicellular haploid gametophytes. Thus, the life cycle of the flowering plant has alternating multicellular diploid (sporophyte) and haploid (gametophyte) generations.

Female Gametogenesis

Plant reproduction occurs within the ovule (Skinner et al., 2004; Yadegari and Drews, 2004). An archesporial cell, the plant version of a female primordial germ cell, is formed inside the ovule. The archesporial cell differentiates to form the megaspore mother cell, which undergoes meiosis result-

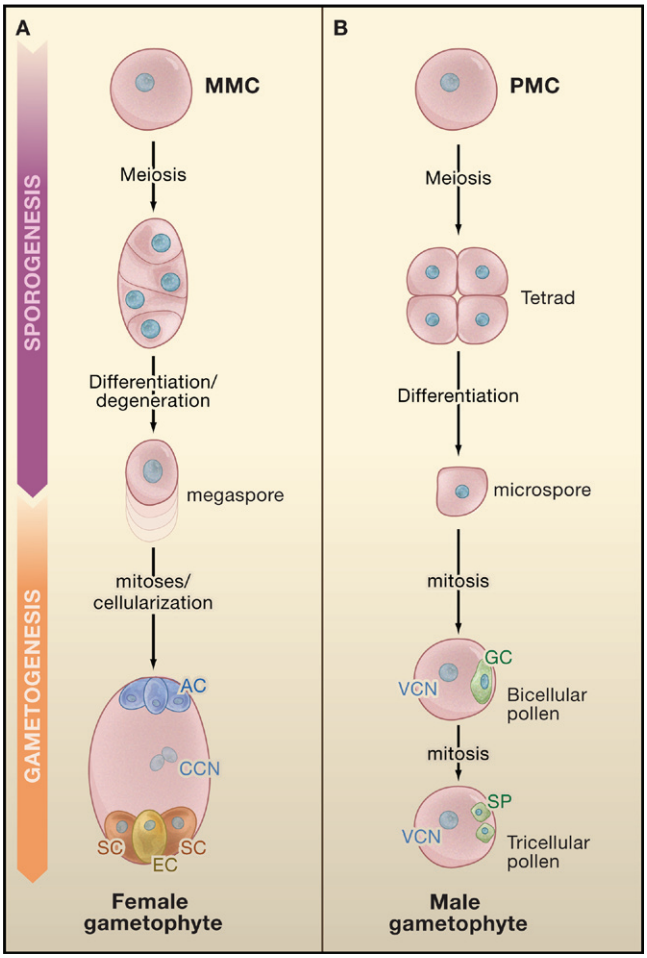

Figure 1. Male and Female Sporogenesis and Gametogenesis in Flowering Plants

(A) Megaspore and female gametophyte development. AC, antipodal cell; CCN, central cell nucleus; EC, egg cell; MMC, megaspore mother cell; SC, synergid cell.

(B) Microspore and male gametophyte development. GC, generative cell; PMC, pollen mother cell; SP, sperm cell; VCN, vegetative cell nucleus.

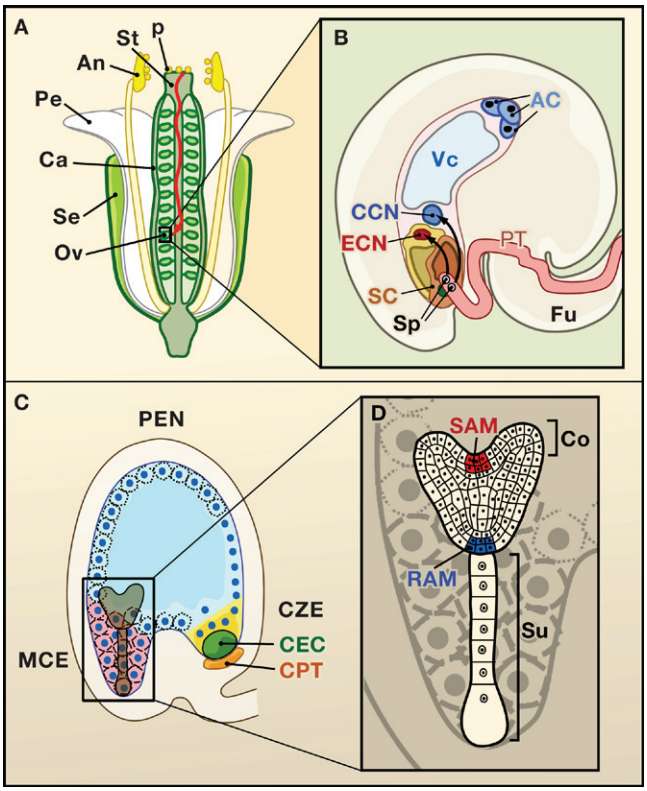

Figure 2. Plant Reproduction

(A) Structure of the mature flower. An, anther; Ca, Carpel; Ov, ovule; p, pollen; Pe, petal; Se, sepal; St, stigma.

(B) Structure of the female gametophyte in the ovule at fertilization. A growing pollen tube releases two sperm, which fuse with the egg cell and the central cell, respectively (arrows). AC, antipodal cell; CCN, central cell nucleus; ECN, egg cell nucleus; Fu, funiculus; PT, pollen tube; SC, synergid cell; Sp, sperm; Vc, vacuole.

(C) Endosperm with a heart stage embryo. Cellularization of the endosperm surrounding the developing embryo at the micropylar end (pink) is completed. A wave of cellularization spreads into the peripheral zone (blue), whereas the chalazal endosperm (yellow) remains syncitial. A chalazal endosperm cyst (green), a specialized structure at the chalazal end, makes direct contact with the chalazal proliferating tissue, which is sporophytic. CEC, chalazal endosperm cyst; CPT, chalazal proliferating tissue; CZE, chalazal endosperm; MCE, micropylar endosperm; PEN, peripheral endosperm.

(D) Structure of a heart stage embryo. Co, cotyledon; RAM, root apical meristem; SAM, shoot apical meristem; Su, suspensor.

ing in the formation of four haploid megaspores (Figure 1A). In the majority of flowering plants, only one megaspore survives, whereas the other three go through programmed cell death. In most flowering plants (Friedman, 2006), the functional haploid megaspore undergoes three mitoses to form the multicellular haploid female gametophyte. In a coenocyte with eight nuclei, cell walls partition the female gametophyte into cells: egg, central, synergid, and antipodal. During cellularization, two nuclei migrate to the center of the female gametophyte, fuse, and are enclosed by cell walls to form a diploid central cell. The central cell is adjacent to the haploid egg cell, which is flanked by two haploid synergid cells (Figures 1A and 2B).

Molecular processes controlling specification and differentiation of the central cell during female gametophyte development are especially important for understanding gene imprinting in the endosperm. Recently it was shown that a MADS-box transcription factor, AGL80, is required for central cell and endosperm development in *Arabidopsis* (Portereiko et al., 2006).

Male Gametogenesis

The male germ cells are generated in the stamen (Singh and Bhalla, 2007). During early stamen development, archesporial cells are initiated and differentiate into pollen mother cells, which undergo meiosis to produce a tetrad of haploid microspores (Figure 1B). All microspores survive and undergo two mitoses to form the three-cell haploid male gametophyte, pollen (Figure 1B). A vegetative cell produces the pollen tube that carries two sperm cells to the ovule and female gametophyte. Transcriptional repression of male germline-specific genes in nongerm cells is crucial for spatial and temporal control of male germline development (Haerizadeh et al., 2006).

Double Fertilization

Pollen is released from stamen and germinates a pollen tube on specialized stigma cells of the carpel (Figure 2A). The pollen tube grows within a transmitting tract to the ovules, penetrates

a synergid cell in the female gametophyte, and releases the two sperm cells that migrate and fertilize the egg and central cells to form the diploid embryo and triploid endosperm, respectively (Figure 2B).

The formation of endosperm by double fertilization is a defining characteristic of the more than 250,000 species of flowering plants, called angiosperms, which have seeds that are covered and protected from the environment. Nonflowering seed plants, called gymnosperms (e.g., conifers with naked seeds exposed to the environment) have a single fertilization event, and a large multicellular female gametophyte acquires nutrients from the parent plant and nutritionally supports the embryo. By contrast, in angiosperms, nutritional support of the embryo is primarily provided by the endosperm. Imprinting occurs in the angiosperm endosperm and has not been detected in angiosperm or gymnosperm embryos.

The origin and rapid evolution of the dominant angiosperms with their species richness, distribution, and complexity of plant forms, has been called Darwin's "abominable mystery" (Charles Darwin's letter to Joseph Dalton Hooker, July 23, 1879, cited in Friedman, 2006). Over 100 years ago, it was discovered that the endosperm is a product of double fertilization. However, the evolutionary origin of double fertilization and endosperm is still debated, and recent examination of the female gametophytes of basal angiosperms reveals an extensive degree of developmental and structural lability (Friedman, 2006). Endosperm might be derived from a supernumerary embryo that acquired an embryo-nourishing function. Alternatively, the female gametophyte, greatly reduced in cell number in modern angiosperms, may have been sexualized by fertilization of the central cell (Friedman, 2001). As described below, recent molecular data are shedding new light on this mystery and appear to support the latter hypothesis (Nowack et al., 2007).

Embryogenesis

After fertilization, the embryo establishes a basic body plan (Le et al., 2007). The zygote undergoes an asymmetric division to form a small apical cell that acquires an embryonic fate, and a larger basal cell that primarily produces a suspensor, which is a conduit for nutrients during the very early stages of embryogenesis. Along the apical-basal axis the embryo generates a shoot apical meristem, cotyledon leaves that function in nutrient storage, a hypocotyl, root, and root apical meristem (Figures 2C and 2D). The meristems, similar to animal stem cells, are undifferentiated cells that have the properties of self-renewal and multiple differentiation potential, and are responsible for generating all of the organs of the adult plant.

A Sexual Endosperm Supports Embryo Development

Although the endosperm and embryo are genetically identical with the exception that the endosperm has an extra maternal genome, they have dramatically different patterns of development (Brown and Lemmon, 2007). Distinct developmental pathways of the embryo and the endosperm are likely due to differential genetic and epigenetic programming of the egg and central cells.

Fertilization of the central cell produces a primary endosperm nucleus surrounded by cytoplasm, which proliferates rapidly to form a syncytium of nuclei that are positioned by nuclear-based radial microtubules. Three-developmental domains are formed along the anterior/posterior axis of the endosperm; a micropylar domain that surrounds the embryo, a central domain composed of a thin layer of cells, and a chalazal domain located above maternal tissue sitting atop a vascular system (Figure 2C). These domains are distinguished by expression of different genes (Berger et al., 2007). Further proliferation accompanied by cellularization occurs in a wave along the anterior/posterior axis, yet the chalazal endosperm remains syncytial, and forms a basal haustorial portion that penetrates the maternal tissue (Nguyen et al., 2000). The highly differentiated ultrastructure of the chalazal endosperm, intimately associated with specialized maternal cells above a vascular system, suggests an important role in transporting maternal resources into the developing endosperm (Figure 2C) (Brown et al., 2003; Nguyen et al., 2000).

Besides importing nutrients from maternal tissue, the endosperm synthesizes copious reserves of starch, protein, and lipids. In dicotyledon seeds (e.g., *Arabidopsis*, legumes), the developing embryo absorbs the nutritive endosperm. In monocotyledon seeds (e.g., maize, rice, wheat), the endosperm persists, comprises the bulk of the seed, and is broken down and absorbed by the embryo soon after germination. It is not only the embryo that depends heavily upon resources provided by endosperm—two-thirds of human caloric intake is derived from the endosperm in angiosperm seeds.

Endosperm in Parthenogenic Plants

Over 400 flowering plant species are capable of producing seed asexually, leading to parthenogenic embryo development, by a process termed apomixis (Bicknell and Koltunow, 2004). Although multiple developmental mechanisms exist, in all cases a cell is generated that undergoes embryogenesis without meiosis or fertilization, and an endosperm is produced that supports the development of the parthenogenic embryo. It is notable that the central cell is fertilized to form a sexual endosperm in most apomictic species. That is, there is a single fertilization event to produce an endosperm with maternal and paternal genomes. This underscores the importance of biparental endosperm in angiosperm reproduction. As described below, gene imprinting may be a reason why a sexually derived endosperm is nearly indispensable.

Cellular Programming of Imprinting

Plant endosperm imprinting is usually due to the differences in epigenetic marks, DNA, and histone methylation, on alleles in the central and sperm cells. Analysis of imprinted Polycomb group genes (*Arabidopsis MEA* and *FIS2*, maize *fie1* and *fie2*) and transcription factor genes (*FLOWERING WAGENINGEN* [*FWA*] and *PHERES1* [*PHE1*]) reveals the different paradigms for plant gene imprinting.

Programming the Arabidopsis *Central Cell for Endosperm Imprinting*

Identification of mutations that allow spontaneous proliferation of the *Arabidopsis* central cell to form an endosperm without fertilization provided early clues about the central cell epigenetic programming (Chaudhury et al., 1997; Ohad et al., 1996). Mutations in any of the four components of the PRC2 Polycomb group complex (*MEA*, *FIS2*, *FIE*, and *MSI1*) all produce autonomous endosperm in the absence of fertilization, and all four genes are expressed in the central

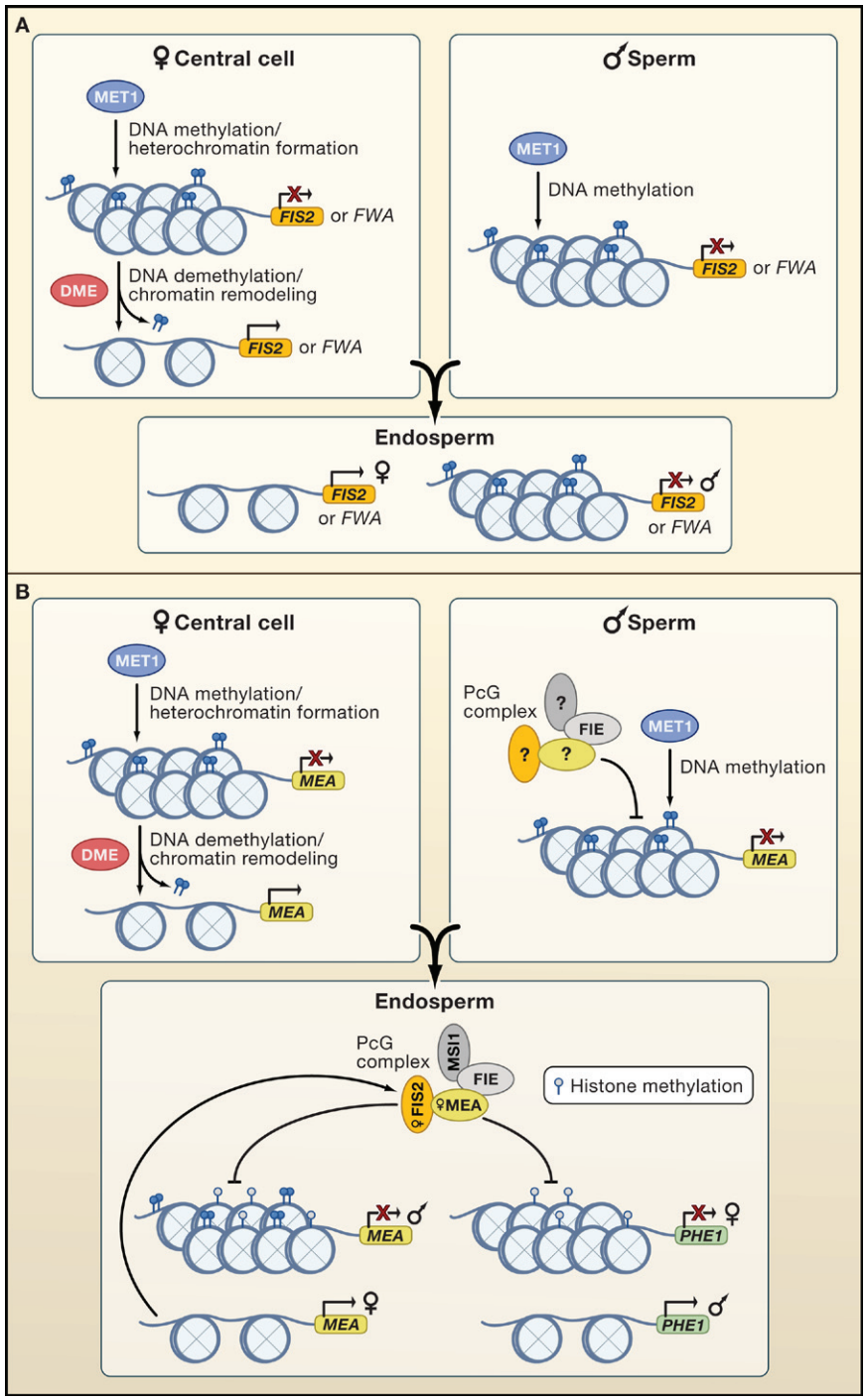

Figure 3. Imprinting in Endosperm Development

(A) Model for the Imprinting of *FWA* and *FIS2*. Both maternal and paternal *FWA* and *FIS2* alleles are methylated and silenced by MET1 as a default state in the central cell and sperm, respectively. DME DNA glycosylase is expressed in the central cell and demethylates and activates *FWA* and *FIS2* alleles. Expression of the demethylated maternal alleles and silencing of the methylated paternal alleles persist in the endosperm. Only one of the two maternal alleles is shown in the central cell and endosperm.

(B) Model for the Imprinting of *MEA* and *PHE1*. The maternal *MEA* allele is demethylated and activated by DME DNA glycosylase in the central cell. Silencing of the paternal *MEA* allele in sperm may be due to methylation by MET1, as well as histone H3K27 methylation by a PRC2 complex that includes the FIE Polycomb group protein. Upon fertilization, maternally expressed *MEA* participates in a PRC2 complex that represses targets such as the paternal *MEA* allele and the maternal *PHE1* allele via histone H3K27 methylation. Maternal *MEA* that is not repressed is continuously expressed in the early endosperm, replenishing the PRC2 complex. Consequently, *MEA* is maternally expressed while *PHE1* is paternally expressed in the endosperm. Only one of the two maternal alleles is shown in the central cell and endosperm.

cell (Grossniklaus et al., 1998; Guitton et al., 2004; Kiyosue et al., 1999; Kohler et al., 2003a; Luo et al., 1999; Ohad et al., 1999). These data support the model that a PRC2 complex composed of MEA, FIS2, FIE, and MSI1 is active and represses central cell proliferation until fertilization (Pien and Grossniklaus, 2007).

Mutations in *Arabidopsis* Polycomb group genes also cause parent-of-origin effects on seed viability (Grossniklaus et al., 1998; Guitton et al., 2004; Kiyosue et al., 1999; Kohler et al., 2003a; Luo et al., 1999; Ohad et al., 1999). Seeds with a mutant maternal allele display embryo abortion, as well as defects in endosperm development, including failure to cellularize, loss of anterior/posterior polarity, and excessive nuclear proliferation (Berger et al., 2007). These phenotypes are observed regardless of the genotype of the paternal allele. It is likely that initial defects in the central cell are responsible, at least in part, for the loss of seed viability. This is supported by findings showing that expression of *FIE* transgene in the central cell, or an *MSI1* transgene in the central and synergid cells, is sufficient to complement their respective maternal mutant alleles and restore seed viability (Kinoshita et al., 2001; Leroy et al., 2007).

The *FIS2* and *MEA* Polycomb group genes are imprinted in the *Arabidopsis* endosperm (Figure 3A). In both cases, maternal alleles are expressed and the paternal alleles are silenced (Gehring et al., 2006; Jullien et al., 2006b; Kinoshita et al., 1999; Luo et al., 2000; Vielle-Calzada et al., 1999). The imprinting of the *MEA* and *FIS2* genes is in accord with what is observed in mammalian genes imprinted in the placenta, as well as predictions of the parental conflict theory (see below), where maternally expressed imprinted genes suppress growth (Fowden et al., 2006; Haig and Westoby, 1991; Moore and Haig, 1991).

FWA encodes a homeodomain transcription factor and is imprinted in the *Arabidopsis* endosperm (Kinoshita et al., 2004). In wild-type plants, *FWA* is silenced throughout plant development except in the central cell and the endosperm, where the maternal allele is expressed. Although gain-of-expression mutations cause dramatic phenotypes that delay the onset of flowering (Soppe et al., 2000), loss-of-function mutations show no overt phenotype, and the function of FWA in the central cell and endosperm is not known.

DNA methylation plays an important role in the regulation of *FWA*, *FIS2,* and *MEA* imprinting (Figure 3A). DNA methylation maintained by the MET1 DNA methyltransferase is present on all three genes. *FWA* is methylated at SINE-related direct repeats at its promoter. This methylation is significantly lower in the endosperm compared to other plant organs, loss of methylation is associated with biallelic *FWA* expression in the endosperm, and targeting methylation back to the repeats reestablishes *FWA* imprinting (Kinoshita et al., 2004, 2007). *MEA* is methylated 500 base pairs upstream of the start of transcription and at 3′ tandem direct repeats in most *Arabidopsis* strains (Gehring et al., 2006). Bisulfite-sequencing experiments show that in the endosperm, the maternal *MEA* allele is hypomethylated in these two regions compared to the paternal *MEA* allele (Gehring et al., 2006). DNA methylation was detected in the *FIS2* promoter in vegetative tissues, and silencing of the paternal *FIS2* allele depends on MET1-mediated DNA methylation (Jullien et al., 2006b).

The DME DNA glycosylase activates *FWA*, *FIS2*, and *MEA* maternal allele expression in the central cell (Figures 3A and 3B) (Choi et al., 2002; Jullien et al., 2006b; Kinoshita et al., 2004). A *dme* mutation, like *mea* and *fis2*, has a parent-of-origin effect on seed viability (Choi et al., 2002). Seeds with a maternal *dme* allele abort even when the paternal allele is wild-type. *DME* is primarily expressed in the central cell; its expression decreases after fertilization and is not detected in the male reproductive organ, stamen, or the male gametophyte, pollen (Choi et al., 2002, 2004; Gehring et al., 2006). The loss of seed viability associated with the *dme* mutation is suppressed by mutations in the *MET1* DNA methyltransferase, consistent with DME's ability to excise 5-methylcytosine in vitro and in *E. coli* (Gehring et al., 2006; Xiao et al., 2003). DME is necessary for hypomethylation of the maternal *MEA* allele in the endosperm. That is, in endosperm with a maternal *dme* mutant allele, both the maternal and paternal *MEA* alleles are highly methylated. Taken together, these results suggest that DME demethylates the maternal *MEA*, *FIS2*, and *FWA* alleles, but not their respective paternal alleles, which establishes parent-of-origin epigenetic marks essential for imprinted expression in the endosperm.

Programming the Arabidopsis *Sperm Cells for Endosperm Imprinting*

In contrast to maternal-allele regulation, the epigenetic mechanisms that regulate the paternal *MEA* versus *FIS2* and *FWA* alleles are distinct. For *FWA* and *FIS2*, hypomethylation of the paternal allele by a mutation in *MET1* is sufficient to cause biparental gene expression in the endosperm. Thus, hypermethylation of the paternal alleles is sufficient for paternal allele silencing in the endosperm (Figure 3A) (Jullien et al., 2006b; Kinoshita et al., 2004).

Hypomethylation of the paternal *MEA* allele by mutations in different DNA methyltransferase genes did not result in paternal-allele expression in the endosperm (Gehring et al., 2006; Jullien et al., 2006a). Thus, another epigenetic mechanism silences the paternal *MEA* allele, and several lines of evidence suggest that Polycomb group proteins play this role. Polycomb group-dependent H3K27 methylation is observed at the 5′ and 3′ ends of the *MEA* gene, suggesting Polycomb group proteins directly regulate *MEA* expression (Gehring et al., 2006; Jullien et al., 2006a). Also, the endosperm generated from pollen with a mutation in the *FIE* gene displays biparental *MEA* expression, suggesting paternal *MEA* allele silencing requires functional Polycomb group complexes in the male gametophyte (Jullien et al., 2006a). Polycomb group proteins reduce the level of biparental *MEA* expression in vegetative tissues (Katz et al., 2004; Kinoshita et al., 1999). Finally, endosperm with maternal mutant *fie* or *mea* alleles shows biparental *MEA* expression (Baroux et al., 2006; Gehring et al., 2006; Jullien et al., 2006a), and loss of H3K27 methylation at the paternal *MEA* allele (Gehring et al., 2006). Taken together, these results show that *MEA* is a self-imprinted gene, where both maternally and paternally derived Polycomb group proteins silence the paternal *MEA* allele in the endosperm (Figure 3B). It is not known how the maternal *MEA* allele escapes self-imprinting. It is possible that activation by DME puts the *MEA* maternal allele into a chromatin state that is resistant to silencing by Polycomb group proteins.

How H3K27 methylation silences paternal *MEA* expression is not known. LHP1 was shown by chromatin immunoprecipitation and hybridization to a tiling array to be associated with *MEA* in vegetative cells (Turck et al., 2007). However, defects in seed development and reproduction are not detected in *lhp1* mutant plants. Although LHP1 is required to maintain repression of certain PRC2 targets (Sung et al., 2006), its possible repressive function of paternal *MEA* allele silencing may be redundant with other factors.

Programming Maize Central and Sperm Cells for Gene Imprinting

The maize Polycomb group gene, *fie1*, is related to the *Arabidopsis FIE* gene and is imprinted in the endosperm (Danilevskaya et al., 2003; Hermon et al., 2007). Thus, endosperm imprinting of Polycomb group components is conserved in plants separated by a large evolutionary distance. *fie1* expression is restricted to the maize endosperm and is derived exclusively from the maternal allele. The analysis of DNA methylation in isolated maize gametes has shown that the maternal *fie1* in the central cell is hypomethylated, whereas the maternal *fie1* allele in the egg cell and the paternal *fie1* allele in the sperm cells are significantly methylated (Gutierrez-Marcos et al., 2006). These results are in accord with the model for *FWA* imprinting in *Arabidopsis* (Figure 3A), in which the default state is silence, and DNA demethylation activates maternal allele expression in the central cell, which persists in the endosperm. The two maize nuclei in the central cell are sisters of the egg and antipodal cells. Thus, it is tempting to speculate that an active DNA demethylation process, which is independent of DNA replication, is responsible for maternal hypomethylation in the central cell.

Programming in the Endosperm

PHE1 encodes a MADS box transcription factor that is imprinted in the endosperm (Kohler et al., 2003b, 2005), where the paternal *PHE1* allele is preferentially expressed. *PHE1* expression in seeds is increased by *mea*, *fie*, and *fis2* mutations, the maternal *PHE1* allele is markedly upregulated in seeds that inherit a maternal *mea* mutation, and chromatin immunoprecipitation experiments demonstrate that MEA and FIE bind to the *PHE1* promoter. These results suggest that the Polycomb group complex (MEA, FIE, FIS2) silences the maternal *PHE1* allele in the endosperm after fertilization (Figure 3B).

The maize *fie2* Polycomb group gene, related to *fie1*, is also imprinted in the maize endosperm. In early endosperm development, *fie2* shows monoallelic maternal expression, whereas later biallelic expression is detected (Danilevskaya et al., 2003). Methylation is not detected in the central or sperm cells; however, the paternal allele is hypermethylated in the endosperm (Gutierrez-Marcos et al., 2006). Thus, the differential DNA methylation was not programmed in the central or sperm cells, but rather appears to be due to de novo methylation of the paternal allele in the endosperm after fertilization.

Paradigms and Unanswered Questions for Imprinting

Several paradigms for imprinting have emerged from the above studies. For *FWA* and *FIS2*, DNA methylation plays the major role in imprinting. For these genes, DME-mediated DNA demethylation is essential for establishing their imprinting in the endosperm (Figure 3A). How DME identifies its targets to demethylate in the central cell, and what role they play in seed development, remain to be determined. Although DME is necessary to activate expression of the maternal *MEA* allele, Polycomb group proteins are necessary to silence the paternal *MEA* allele. Expression of maternal Polycomb group alleles in the central cell and endosperm, and paternal Polycomb group alleles in the male gametophyte, are required for stable silencing. It is likely that the same Polycomb group complex (MEA, FIE, FIS2) that silences the paternal *MEA* allele also silences the maternal *PHE1* allele in the endosperm (Figure 3B). This complex may silence and imprint other genes in the endosperm, as well. The number of targets for silencing, their function, and how alleles are distinguished, are not yet known. Finally, the maize *fie2* gene, which may be regulated by allele-specific acquisition of DNA methylation, suggests that additional mechanisms may regulate imprinting in plants.

Origins of Endosperm and Endosperm Imprinting

The endosperm is an unusual tissue. It is a product of fertilization and could be considered a separate organism from the embryo. However, it does not transmit any genetic information to the next generation. Its single purpose appears to be altruistic, working and sacrificing itself to ensure the success of its embryo sibling. As mentioned previously, the evolutionary origin of endosperm is a mystery. Understanding its evolutionary origin could provide valuable insights into the mechanism of female gametophyte and seed development. Because imprinting in plants appears to be confined to the endosperm, understanding the evolutionary forces that drive imprinting will ultimately provide insight into endosperm origins. Below are hypotheses for the origin of imprinting and recent experiments that attempt to test their validity.

Parental Conflict Hypothesis

In angiosperms, the developing seeds all have the same maternal origin but potentially have different pollen donors. The parental conflict theory for the evolution of imprinting is based on the idea that the inherited maternal and paternal genomes have a different interest in the allocation of resources (Haig and Westoby, 1991). For example, the maternal plant contributes genetic information to all seeds and would evolve to distribute resources to all progeny equally, whereas the paternal genome would evolve to maximize resource allocation by taking away resources from seeds resulting from less-fit pollen parents. This theoretical framework, along with the parent-of-origin effects observed in both mammals and plants, predicts that an intergenomic conflict between the maternal and paternal genomes for the allocation of resources is the evolutionary driving force for the origin of imprinting. Alleles in the maternal genome that would increase resource acquisition would be silenced, while the paternal genome would express them. Alleles in the paternal genome that would inhibit nutrient acquisition would be silenced while the maternal genomes would express them (Haig and Westoby, 1991).

The parental conflict hypothesis is supported by the results from interploidy crosses: crossing a diploid (2×) with a tetraploid (4×). In *A. thaliana*, crossing a 2× with a 4× pollen donor (creating a 2m:2p endosperm) results in viable seeds that are slightly larger than normal (Scott et al., 1998). This result appears to be consistent with the parental conflict hypothesis suggesting that the extra paternal genome would cause an overabundance of paternal imprinted genes, acquiring more resources than normal. Seed abortion in *A. thaliana* occurs if a 6× pollen donor is used, suggesting that an increase in dosage of the paternal genome also disrupts normal endosperm development (Scott et al., 1998). The reciprocal cross, a maternal 4× crossed with a paternal 2× (a 4m:1p endosperm) also produces viable but smaller than wild-type seeds, again consistent with the parental conflict theory predicting that the extra maternal copies would further inhibit nutrient acquisition (Scott et al., 1998). Crossing wild-type and DNA methylation mutant plants creates a similar phenotype, further supporting the link between phenotypes of interploidy crosses and the number and origin of imprinted genes (Adams et al., 2000).

Evolution of the Imprinted Gene MEA

Whether the parental conflict theory predicts that imprinted genes evolve under positive selection in an outcrossing species (e.g., *Arabidopsis lyrata*) is a matter of debate (McVean and Hurst, 1997; Spillane et al., 2007). *MEA* genes were analyzed in the closely related species *A. lyrata* and *A. thaliana* (a selfing species) to test this hypothesis. Positive selection of *MEA* was measured by comparing nucleotide variations in both species. Spillane et al. (2007) reported that the *MEA* ortholog in *A. lyrata* had proceeded through a greater positive selection than that of *A. thaliana*. A closely related gene (*SWINGER*), which is not imprinted, had not gone through positive selection in either species (Spillane et al., 2007). These results would support the parental conflict hypothesis for the evolution of gene imprinting. By contrast, Kawabe et al. (2007) only detected positive selection in the *MEA* promoter, and not in the *MEA* gene, suggesting that the MEA protein function is conserved while there

is a positive selection on the regulation of *MEA* expression. The latter study suggests another possible pathway of endosperm evolution, which can be explained by the differential dosage hypothesis, described below.

Differential Dosage Hypothesis

The differential dosage hypothesis predicts that imprinting evolved to control the relative dosage of the regulatory factors in the endosperm (Birchler, 1993; Dilkes and Comai, 2004). According to the parental conflict theory, when double fertilization arose, an intergenomic conflict between the maternal and paternal alleles for the allocation of resources was created. By contrast, according to the differential dosage hypothesis, double fertilization created an imbalance, and imprinting mechanisms were used to adjust the dosage of regulators participating in multiprotein complexes. This might exert positive selection on elements, such as promoters, that influence the dosage of regulators as described above.

The differential dosage hypothesis is supported by the loss of *PHE1* imprinting in interspecific crosses (Josefsson et al., 2006). *PHE1* is normally paternally expressed and maternally silenced in the *A. thaliana* endosperm (Kohler et al., 2005). In both intra- and interploidy crosses involving *A. thaliana* and *A. arenosa* as a pollen parent, imprinting of *PHE1* was lost with biallelic expression of paternal and maternal alleles in the endosperm. Imprinting of *PHE1* is due to the repressive effects of a maternal Polycomb group complex. It was interpreted that the loss of *PHE1* imprinting was due to an overabundance of Polycomb group complex target sites in the *A. arenosa* paternal genome as compared to the normally inherited *A. thaliana* paternal genome. Thus, the overabundance of target sites in the *A. arenosa* paternal genome could overwhelm the dosage of maternal Polycomb group complexes, allowing the maternal *PHE1* allele to escape complete silencing. Consistent with that hypothesis, 4× *A thaliana*, containing a higher dosage of PcG complex, crossed with 2× *A. arenosa* was able to rescue seed abortion and maintain *A. thaliana* maternal *PHE1* repression (Josefsson et al., 2006).

Endosperm Development that Bypasses Both Imprinting and Double Fertilization

Double fertilization, which occurs in the vast number of angiosperms, emphasizes the importance of the paternal contributed genome in the endosperm. However, a recent study using a combination of specific mutations revealed that this requirement can be bypassed (Nowack et al., 2007). Pollen carrying a mutation in the *CDKA*;*1* gene, a Cdc2/Cdc28 homolog, produces only one sperm nucleus that predominately fertilizes the egg leaving the diploid central cell unfertilized (Iwakawa et al., 2006; Nowack et al., 2006). The fertilized eggs from a *cdka*;*1* pollen abort. The unfertilized central cell goes through a few rounds of division before seed abortion, suggesting that the paternal genome is required to complete endosperm development, and that a signal is sent from the fertilized egg to the central cell, triggering its proliferation. Surprisingly, disruptions in the maternal Polycomb group complex (*mea*, *fis2*, and *fie* mutations) can rescue seed abortion due to *cdka*;*1* pollen, albeit the seeds are smaller than wild-type (Nowack et al., 2007). This suggests that a developing homodiploid central cell will form a functional endosperm tissue in the absence of maternal Polycomb-mediated imprinting. Development of a homodiploid endosperm with a loss of imprinting supports the hypothesis that the triploid endosperm may have originated from a diploid origin. Thus, the evolutionary origin of endosperm may have been the sexualization of the female gametophyte, rather than the acquisition of an embryo-nourishing function by a supernumerary embryo.

With the loss of Polycomb-mediated imprinting, the diploid central cell apparently has necessary molecular factors that regulate gene expression appropriately, resulting in a functional endosperm that supports embryo development. These results support the idea that one function of imprinting may be to prevent parthenogenic endosperm development. As shown above, seeds can develop when both double fertilization and components of imprinting are abolished or when both are present. This highly suggests that the two processes are intimately linked and possibly coevolved.

ACKNOWLEDGMENTS

Work in our lab is supported by grants from the National Institutes of Health (R01-GM069415) and the United States Department of Agriculture (2005-02355).

REFERENCES

Adams, S., Vinkenoog, R., Spielman, M., Dickinson, H.G., and Scott, R.J. (2000). Parent-of-origin effects on seed development in Arabidopsis thaliana require DNA methylation. Development *127*, 2493–2502.

Agius, F., Kapoor, A., and Zhu, J.K. (2006). Role of the Arabidopsis DNA glycosylase/lyase ROS1 in active DNA demethylation. Proc. Natl. Acad. Sci. USA *103*, 1796–1801.

Baroux, C., Gagliardini, V., Page, D.R., and Grossniklaus, U. (2006). Dynamic regulatory interactions of Polycomb group genes: MEDEA autoregulation is required for imprinted gene expression in Arabidopsis. Genes Dev. *20*, 1081–1086.

Barreto, G., Schafer, A., Marhold, J., Stach, D., Swaminathan, S.K., Handa, V., Doderlein, G., Maltry, N., Wu, W., Lyko, F., et al. (2007). Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature *445*, 671–675.

Berger, F., Fitz Gerald, J.N., and Ingouff, M. (2007). Arabidopsis as a model for undertanding the basics of endosperm development. In Endosperm: Developmental and Molecular Biology, O.-A. Olsen, ed. (Berlin: Springer-Verlag), pp. 91–110.

Bicknell, R.A., and Koltunow, A.M. (2004). Understanding apomixis: Recent advances and remaining conundrums. Plant Cell *16*, S228–S245.

Birchler, J.A. (1993). Dosage analysis of maize endosperm development. Annu. Rev. Genet. *27*, 181–204.

Brown, R.C., and Lemmon, B.E. (2007). The developmental biology of cereal endosperm. In Endosperm: Developmental and Molecular Biology, O.-A. Olsen, ed. (Berlin: Springer-Verlag), pp. 1–20.

Brown, R.C., Lemmon, B.E., and Nguyen, H. (2003). Events during the first four rounds of mitosis establish three developmental domains in the syncytial endosperm of *Arabidopsis thaliana.* Protoplasma *222*, 167–174.

Chanvivattana, Y., Bishopp, A., Schubert, D., Stock, C., Moon, Y.H., Sung, Z.R., and Goodrich, J. (2004). Interaction of Polycomb-group proteins controlling flowering in Arabidopsis. Development *131*, 5263–5276.

Chaudhury, A.M., Luo, M., Miller, C., Craig, S., Dennis, E.S., and Peacock, W.J. (1997). Fertilization-independent seed development in *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. USA *94*, 4223–4228.

Choi, Y., Gehring, M., Johnson, L., Hannon, M., Harada, J.J., Goldberg, R.B., Jacobsen, S.E., and Fischer, R.L. (2002). DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in *Arabidopsis.* Cell *110*, 33–42.

Choi, Y., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (2004). An invariant aspartic acid in the DNA glycosylase domain of DEMETER is necessary for transcriptional activation of the imprinted MEDEA gene. Proc. Natl. Acad. Sci. USA *101*, 7481–7486.

Danilevskaya, O.N., Hermon, P., Hantke, S., Muszynski, M.G., Kollipara, K., and Ananiev, E.V. (2003). Duplicated fie genes in maize: expression pattern and imprinting suggest distinct functions. Plant Cell *15*, 425–438.

David, S.S., O'Shea, V.L., and Kundu, S. (2007). Base-excision repair of oxidative DNA damage. Nature *447*, 941–950.

Dilkes, B.P., and Comai, L. (2004). A differential dosage hypothesis for parental effects in seed development. Plant Cell *16*, 3174–3180.

Feil, R., and Berger, F. (2007). Convergent evolution of genomic imprinting in plants and mammals. Trends Genet. *23*, 192–199.

Fowden, A.L., Sibley, C., Reik, W., and Constancia, M. (2006). Imprinted genes, placental development and fetal growth. Horm. Res. *65* (*Suppl 3*), 50–58.

Friedman, W.E. (2001). Developmental and evolutionary hypotheses for the origin of double fertilization and endosperm. C.R. Acad. Sci. III. *324*, 559–567.

Friedman, W.E. (2006). Embryological evidence for developmental lability during early angiosperm evolution. Nature *441*, 337–340.

Gehring, M., Choi, Y., and Fischer, R.L. (2004). Imprinting and seed development. Plant Cell *16*, 10.1105/tpc.017988.

Gehring, M., and Henikoff, S. (2007). DNA methylation dynamics in plant genomes. Biochim. Biophys. Acta *1769*, 276–286.

Gehring, M., Huh, J.H., Hsieh, T.F., Penterman, J., Choi, Y., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (2006). DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. Cell *124*, 495–506.

Gong, Z., Morales-Ruiz, T., Ariza, R.R., Roldan-Arjona, T., David, L., and Zhu, J.-K. (2002). ROS1, a repressor of transcriptional gene silencing in Arabidopsis, encodes a DNA glycosylase/lyase. Cell *111*, 803–814.

Grewal, S.I.S., and Jia, S. (2007). Heterochromatin revisited. Nat. Rev. Genet. *8*, 35–46.

Grossniklaus, U., Vielle-Calzada, J.-P., Hoeppner, M.A., and Gagliano, W.B. (1998). Maternal control of embryogenesis by *MEDEA*, a polycomb-group gene in *Arabidopsis.* Science *280*, 446–450.

Guitton, A.-E., Page, D.R., Chambrier, P., Lionnet, C., Faure, J.-E., Grossniklaus, U., and Berger, F. (2004). Identification of new members of Fertilization Independent Seed Polycomb Group pathway involved in the control of seed development in Arabidopsis thaliana. Development *131*, 2971–2981.

Gutierrez-Marcos, J.F., Costa, L.M., Dal Pra, M., Scholten, S., Kranz, E., Perez, P., and Dickinson, H.G. (2006). Epigenetic asymmetry of imprinted genes in plant gametes. Nat. Genet. *38*, 876–878.

Haerizadeh, F., Singh, M.B., and Bhalla, P.L. (2006). Transcriptional repression distinguishes somatic from germ cell lineages in a plant. Science *313*, 496–499.

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. Philos. Trans. R. Soc. Lond. B Biol. Sci. *333*, 1–14.

Henderson, I.R., and Jacobsen, S.E. (2007). Epigenetic inheritance in plants. Nature *447*, 418–424.

Hermon, P., Srilunchang, K.O., Zou, J., Dresselhaus, T., and Danilevskaya, O.N. (2007). Activation of the imprinted Polycomb Group Fie1 gene in maize endosperm requires demethylation of the maternal allele. Plant Mol. Biol. *64*, 387–395.

Iwakawa, H., Shinmyo, A., and Sekine, M. (2006). Arabidopsis CDKA;1, a cdc2 homologue, controls proliferation of generative cells in male gametogenesis. Plant J. *45*, 819–831.

Josefsson, C., Dilkes, B., and Comai, L. (2006). Parent-dependent loss of gene silencing during interspecies hybridization. Curr. Biol. *16*, 1322–1328.

Jost, J.-P., Oakeley, E.J., Zhu, B., Benjamin, D., Thiry, S., Siegmann, M., and Jost, Y.-C. (2001). 5-Methylcytosine DNA glycosylase participates in the genome-wide loss of DNA methylation occurring during mouse myoblast differentiation. Nucleic Acids Res. *29*, 4452–4461.

Jullien, P.E., Katz, A., Oliva, M., Ohad, N., and Berger, F. (2006a). Polycomb group complexes self-regulate imprinting of the Polycomb group gene MEDEA in Arabidopsis. Curr. Biol. *16*, 486–492.

Jullien, P.E., Kinoshita, T., Ohad, N., and Berger, F. (2006b). Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting. Plant Cell *18*, 1360–1372.

Katz, A., Oliva, M., Mosquna, A., Hakim, O., and Ohad, N. (2004). FIE and CURLY LEAF polycomb proteins interact in the regulation of homeobox gene expression during sporophyte development. Plant J. *37*, 707–719.

Kawabe, A., Fujimoto, R., and Charlesworth, D. (2007). High diversity due to balancing selection in the promoter region of the Medea gene in Arabidopsis lyrata. Curr. Biol. *17*, 1885–1889.

Kinoshita, T., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (2001). Polycomb repression of flowering during early plant development. Proc. Natl. Acad. Sci. USA *98*, 14156–14161.

Kinoshita, T., Yadegari, R., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (1999). Imprinting of the *MEDEA* polycomb gene in the *Arabidopsis* endosperm. Plant Cell *11*, 1945–1952.

Kinoshita, T., Miura, A., Choi, Y., Kinoshita, Y., Cao, X., Jacobsen, S.E., Fischer, R.L., and Kakutani, T. (2004). One-way control of FWA imprinting in Arabidopsis endosperm by DNA methylation. Science *303*, 521–523.

Kinoshita, Y., Saze, H., Kinoshita, T., Miura, A., Soppe, W.J., Koornneef, M., and Kakutani, T. (2007). Control of FWA gene silencing in Arabidopsis thaliana by SINE-related direct repeats. Plant J. *49*, 38–45.

Kiyosue, T., Ohad, N., Yadegari, R., Hannon, M., Dinneny, J., Wells, D., Katz, A., Margossian, L., Harada, J., Goldberg, R.B., et al. (1999). Control of fertilization-independent endosperm development by the *MEDEA* polycomb gene in *Arabidopsis.* Proc. Natl. Acad. Sci. USA *96*, 4186–4191.

Klose, R.J., and Bird, A.P. (2006). Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. *31*, 89–97.

Kohler, C., Hennig, 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. EMBO J. *22*, 4804–4814.

Kohler, C., Hennig, 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 Dev. *17*, 1540–1553.

Kohler, C., Page, D.R., Gagliardini, V., and Grossniklaus, U. (2005). The Arabidopsis thaliana MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. Nat. Genet. *37*, 28–30.

Le, B.H., Wagmaister, J.A., Kawashima, T., Bui, A.Q., Harada, J.J., and Goldberg, R.B. (2007). Using genomics to study legume seed development. Plant Physiol. *144*, 562–574.

Leroy, O., Hennig, L., Breuninger, H., Laux, T., and Kohler, C. (2007). Polycomb group proteins function in the female gametophyte to determine seed development in plants. Development *134*, 3639–3648.

Luo, M., Bilodeau, P., Dennis, E.S., Peacock, W.J., and Chaudhury, A. (2000). Expression and parent-of-origin effects for *FIS2*, *MEA*, and *FIE* in the endosperm and embryo of developing *Arabidopsis* seeds. Proc. Natl. Acad. Sci. USA *97*, 10637–10642.

Luo, M., Bilodeau, P., Koltunow, A., Dennis, E.S., Peacock, W.J., and Chaudhury, A.M. (1999). Genes controlling fertilization-independent seed development in *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. USA *96*, 296–301.

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

Matzke, M.A., Kanno, T., Huettel, B., Daxinger, L., and Matzke, A.J.M. (2007). Targets of RNA-directed DNA methylatoin. Curr. Opin. Plant Biol. *10*, 512–519.

McVean, G.T., and Hurst, L.D. (1997). Molecular evolution of imprinted genes: no evidence for antagonistic coevolution. Proc. Biol. Sci. *264*, 739–746.

Moore, T., and Haig, D. (1991). Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet. *7*, 45–48.

Morales-Ruiz, T., Ortega-Galisteo, A.P., Ponferrada-Marin, M.I., Martinez-Macias, M.I., Ariza, R.R., and Roldan-Arjona, T. (2006). DEMETER and REPRESSOR OF SILENCING 1 encode 5-methylcytosine DNA glycosylases. Proc. Natl. Acad. Sci. USA *103*, 6853–6858.

Nguyen, H., Brown, R.C., and Lemmon, B.E. (2000). The specialized chalazal endosperm in *Arabidopsis thaliana* and *Lepidium virginicum* (Brassicaceae). Protoplasma *212*, 99–110.

Nowack, M.K., Grini, P.E., Jakoby, M.J., Lafos, M., Koncz, C., and Schnittger, A. (2006). A positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis. Nat. Genet. *38*, 63–67.

Nowack, M.K., Shirzadi, R., Dissmeyer, N., Dolf, A., Endl, E., Grini, P.E., and Schnittger, A. (2007). Bypassing genomic imprinting allows seed development. Nature *447*, 312–315.

Ohad, N., Margossian, L., Hsu, Y.C., Williams, C., Repetti, P., and Fischer, R.L. (1996). A mutation that allows endosperm development without fertilization. Proc. Natl. Acad. Sci. USA *93*, 5319–5324.

Ohad, N., Yadegari, R., Margossian, L., Hannon, M., Michaeli, D., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (1999). Mutations in *FIE*, a WD polycomb group gene, allow endosperm development without fertilization. Plant Cell *11*, 407–415.

Penterman, J., Uzawa, R., and Fischer, R.L. (2007a). Genetic interactions between DNA demethylation and methylation in Arabidopsis. Plant Physiol. *145*, 1549–1557.

Penterman, J., Zilberman, D., Huh, J.H., Ballinger, T., Henikoff, S., and Fischer, R.L. (2007b). DNA demethylation in the Arabidopsis genome. Proc. Natl. Acad. Sci. USA *104*, 6752–6757.

Pien, S., and Grossniklaus, U. (2007). Polycomb group and trithorax group proteins in Arabidopsis. Biochim. Biophys. Acta *1769*, 375–382.

Portereiko, M.F., Lloyd, A., Steffen, J.G., Punwani, J.A., Otsuga, D., and Drews, G.N. (2006). AGL80 is required for central cell and endosperm development in Arabidopsis. Plant Cell *18*, 1862–1872.

Reik, W. (2007). Stability and flexibility of epigenetic gene regulation in mammalian development. Nature *447*, 425–432.

Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B., and Cavalli, G. (2007). Genome regulation by Polycomb and Trithorax Proteins. Cell *128*, 735–745.

Scott, R.J., and Spielman, M. (2006). Genomic imprinting in plants and mammals: how life history constrains convergence. Cytogenet. Genome Res. *113*, 53–67.

Scott, R.J., Spielman, M., Bailey, J., and Dickinson, H.G. (1998). Parent-of-origin effects on seed development in *Arabidopsis thaliana.* Development *125*, 3329–3341.

Singh, M.B., and Bhalla, P.L. (2007). Control of male germ-cell development in flowering plants. Bioessays *29*, 1124–1132.

Skinner, D.J., Hill, T.A., and Gasser, C.S. (2004). Regulation of ovule development. Plant Cell *16*, S32–S45.

Soppe, W.J., Jacobsen, S.E., Alonso-Blanco, C., Jackson, J.P., Kakutani, T., Koorneef, M., and Peeters, A.J. (2000). The late flowering phenotype of fwa mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. Mol. Cell *6*, 791–802.

Spillane, C., Schmid, K.J., Laoueille-Duprat, S., Pien, S., Escobar-Restrepo, J.M., Baroux, C., Gagliardini, V., Page, D.R., Wolfe, K.H., and Grossniklaus, U. (2007). Positive Darwinian selection at the imprinted MEDEA locus in plants. Nature *448*, 349–352.

Sung, S., He, Y., Eshoo, T.W., Tamada, Y., Johnson, L., Nakahigashi, K., Goto, K., Jacobsen, S.E., and Amasino, R.M. (2006). Epigenetic maintenance of the vernalized state in Arabidopsis thaliana requires LIKE HETEROCHROMATIN PROTEIN 1. Nat. Genet. *38*, 706–710.

Turck, F., Roudier, F., Farrona, S., Martin-Magniette, M.-L., Guillaume, E., Buisine, N., Gagnot, S., Martienssen, R.A., Coupland, G., and Colot, V. (2007). Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 Lysine 27. PLoS Genet. *3*, e86.

Vielle-Calzada, J.-P., Thomas, J., Spillane, C., Coluccio, A., Hoeppner, M.A., and Grossniklaus, U. (1999). Maintenance of genomic imprinting at the *Arabidopsis medea* locus requires zygotic DDM1 activity. Genes Dev. *13*, 2971–2982.

Xiao, W., Gehring, M., Choi, Y., Margossian, L., Pu, H., Harada, J.J., Goldberg, R.B., Pennell, R.I., and Fischer, R.L. (2003). Imprinting of the MEA Polycomb gene is controlled by antagonism between MET1 methyltransferase and DME glycosylase. Dev. Cell *5*, 891–901.

Yadegari, R., and Drews, G.N. (2004). Female gametophyte development. Plant Cell *16*, S133–S141.

Zhu, J., Kapoor, A., Sridhar, V.V., Agius, F., and Zhu, J.K. (2007). The DNA glycosylas/lyase ROS1 functions in pruning DNA methylation patternsn in Arabidopsis. Curr. Biol. *17*, 54–59.