Epigenetic mechanisms governing seed development in plants

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Seed development in flowering plants is initiated by the fusion of two male gametes with two female gametes—the egg cell and the central cell—which leads to the formation of an embryo and an endosperm, respectively. Fertilization-independent seed formation is actively repressed by the FERTILIZATION-INDEPENDENT SEED (FIS) Polycomb group (PcG) proteins, an evolutionarily conserved class of proteins that ensures the stable transmission of developmental decisions. The FIS proteins act together in a complex and modify their target genes by applying repressive methylation on histone H3 lysine 27. In addition to its function before fertilization, the FIS complex restricts endosperm proliferation. This function is likely to be achieved by imprinting the maternal alleles of FIS target genes. However, imprinting in the endosperm is controlled not only by the FIS complex but also by DNA methylation, and the interconnections between these two processes are now being investigated.

Keywords: imprinting; seed development; Polycomb group proteins; MEDEA; PHERES1

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Introduction

The fusion of two male gametes with two female gametes—the haploid egg cell and the diploid central cell—marks the start of seed development in flowering plants, and results in the formation of the diploid embryo and the triploid endosperm, respectively. The embryo and the endosperm are surrounded by the maternally derived seed coat, which provides a protective shield for the developing seed. In most plant species, fertilization is required for seed development, and premature divisions of the egg cell and the central cell are actively suppressed. Insight into this suppressive mechanism has been provided by the identification of the *fertilization-independent seed (fis)* mutants that have the ability to form seed-like structures without fertilization (Ohad *et al*, 1996; Chaudhury *et al*, 1997). These fertilization-independent seed-like structures are characterized by ovule integuments that develop

into a seed coat, a central cell that starts nuclear divisions to form a diploid multi-nuclear endosperm, and an egg cell that occasionally starts to divide to form an embryo-like structure (Chaudhury *et al*, 1997; Guitton & Berger, 2005a). The FIS genes that have been identified in plants are *MEDEA* (MEA; Grossniklaus *et al*, 1998; Kiyosue *et al*, 1999), *FERTILIZATION-INDEPENDENT ENDOSPERM* (FIE; Ohad *et al*, 1999), *FIS2* (Luo *et al*, 1999) and *MULTI-COPY SUPPRESSOR OF IRA1* (MSI1; Köhler *et al*, 2003a; Guitton *et al*, 2004). The FIS genes encode Polycomb group (PcG) proteins that have close homologues in animals, such as insects and mammals, but not yeast. The corresponding homologues in *Drosophila* and mammals are E(z) (Ezh1, Ezh2), Esc (Eed), Su(z)12 and Nurf55 (RbAp46/48), respectively (reviewed by Cao & Zhang, 2004; Guitton & Berger, 2005b). In animals, PcG proteins ensure the stable propagation of established developmental decisions through mitotic cell cycles.

The FIS proteins MEA, FIE and MSI1 interact and form a protein complex of about 650 kDa (Köhler *et al*, 2003a), which probably contains FIS2 as well (Chanvivattana *et al*, 2004). Similarly, the PcG proteins in animals interact and are localized in large protein complexes that change their composition during development (reviewed by Lund & Lohuizen, 2004; Bantignies & Cavalli, 2006). One of the best-characterized PcG complexes in animals is the Polycomb repressive complex 2 (PRC2), which is similar in size and composition to the plant FIS complex (reviewed by Lund & Lohuizen, 2004). Insect and mammalian PRC2 complexes have intrinsic histone methyltransferase activity and target lysine (Lys) 27 of histone H3 (H3Lys27), suggesting that the maintenance of cellular memory involves the methylation of histones (reviewed by Cao & Zhang, 2004).

fis mutants are maternal gametophytic mutants

In addition to their ability to form seeds independently of fertilization, *fis* mutants have a striking maternally determined phenotype after fertilization. Every seed that inherits a *fis* mutant allele from the mother fails to complete development and eventually aborts, containing an embryo that has arrested at the late heart stage—5–6 days after fertilization—and an uncellularized endosperm that forms more nuclei than wild-type endosperm (Ohad *et al*, 1996; Chaudhury *et al*, 1997; Grossniklaus *et al*, 1998; Kiyosue *et al*, 1999; Ingouff *et al*, 2005). This phenotype can be interpreted in two ways: either the paternal alleles of FIS genes are not

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Fig 1 | Model of molecular mechanisms controlling maternal and paternal imprinting in *Arabidopsis*. The maternally imprinted gene *PHERES 1* (*PHE1*; red) is repressed in the female gametophyte by the FERTILIZATION INDEPENDENT SEED (FIS) Polycomb group (PcG) complex, which contains MEDEA (MEA), FERTILIZATION INDEPENDENT ENDOSPERM (FIE), FIS2 and MULTI-COPY SUPPRESSOR OF IRA1 (MSI1) and applies methylation on histone H3 lysine 27 (H3Lys27; 1). From the active paternal *PHE1* allele (blue) H3Lys27 methylation needs to be removed, but it is not known whether this is an active or passive process (2). Repression of the maternal *PHE1* allele in the endosperm is maintained by the FIS complex (3). In the central cell of the female gametophyte, DEMETER (DME) actively erases DNA methylation from the maternal allele (red) of the paternally imprinted gene *MEA* (4). It is not known whether repressive H3Lys27 methylation is actively erased from the maternal *MEA* allele as well. The paternal *MEA* allele (blue) is methylated by DNA METHYLTRANSFERASE 1 (MET1). Whether repressive histone methylation is maintained on the paternal allele is unknown (5). Repression of the paternal *MEA* allele in the endosperm is maintained by the FIS complex (6). In vegetative tissues, the silent state of both genes, *PHE1* and *MEA* is maintained by H3Lys27 methylation (7), which depends on PcG complexes containing CURLY LEAF (CLF), SWINGER (SWN) and FIE.

expressed and therefore cannot complement the mutant *fis* phenotype, or FIS genes act before fertilization in the female gametophyte and the phenotype observed after fertilization is a consequence of the missing expression in the female gametophyte. The first hypothesis has been tested and it has been found that only the maternal alleles of *MEA* and *FIS2* are expressed in the endosperm, whereas the paternal alleles are silenced throughout seed development; therefore, *MEA* and *FIS2* are regulated by genomic imprinting (Vielle-Calzada *et al*, 1999; Luo *et al*, 2000; Kinoshita *et al*, 1999; Jullien *et al*, 2006a).

Imprinting of *MEA* is established by two, probably independent, mechanisms. The paternal *MEA* allele is decorated with repressive histone methylation on H3Lys27 (Gehring *et al*, 2006) and becomes reactivated in the endosperm of *fis* mutants (Baroux *et al*, 2006; Gehring *et al*, 2006; Jullien *et al*, 2006b). The mechanism that prevents recruitment of the FIS complex to the maternal *MEA* allele is unknown. One additional distinctive feature of *MEA* endosperm alleles is DNA hypermethylation of the paternal *MEA* allele, mediated by DNA METHYLTRANSFERASE 1 (MET1; Gehring *et al*, 2006). MET1 targets methylation at CG residues in the *MEA* promoter as well as in the 3' untranslated region. The DNA glycosidase DEMETER (DME) is specifically expressed within the central cell and excises 5-methylcytosine from the maternal

MEA alleles, establishing a parent-of-origin specific methylation pattern (Choi *et al*, 2002; Gehring *et al*, 2006). DME activity in the central cell is necessary for *MEA* activation, which provides a link between DNA methylation and *MEA* imprinting (Choi *et al*, 2002). It is possible that the presence of DNA methylation at the paternal *MEA* allele is the distinctive mark that specifically recruits the FIS PcG complex to the paternal *MEA* endosperm allele. However, even the paternal *MEA* allele contributed by a *met1* mutant is not reactivated in the endosperm, although in this background DNA methylation is mostly lacking (Gehring *et al*, 2006). Thus, it is unlikely that DNA methylation of the paternal *MEA* allele provides the distinguishing marks for recruiting the FIS complex. Nonetheless, the importance of DNA methylation for the regulation of imprinted genes is supported by the observation that repression of the paternal alleles of *FWA* and *FIS2* is mediated by DNA methylation. In *met1* mutants the paternal alleles of *FWA* and *FIS2* are expressed, and expression of the maternal *FWA* and *FIS2* alleles depends on DME activity in the female gametophyte (Kinoshita *et al*, 2004; Jullien *et al*, 2006a). Thus, paternal imprinting of *MEA*, *FWA* and *FIS2* requires DME-mediated DNA demethylation to activate maternal alleles, whereas silencing of paternal alleles requires either DNA methylation (*FWA* and *FIS2*) or histone methylation on H3Lys27 (*MEA*; Fig 1).

***PHERES 1* is regulated by parental imprinting**

Microarray expression profiling of *fis* mutants and chromatin immunoprecipitation (ChIP) experiments identified the type I MADS-box gene *PHERES 1* (*PHE1*) as a direct target gene of the FIS PcG complex (Köhler *et al.*, 2003b). The paternal *PHE1* allele is readily expressed in the developing embryo and endosperm, whereas the maternal *PHE1* allele is silent or only weakly expressed. Mutations in *mea* cause increased expression of the maternal *PHE1* allele, but have little effect on the activity of the paternal *PHE1* allele. This suggests that the FIS complex specifically represses the maternal *PHE1* allele (Köhler *et al.*, 2005). The *PHE1* locus is decorated with repressive H3Lys27 tri-methylation marks, which accumulate most strongly before fertilization (Makarevich *et al.*, 2006). Therefore, it is likely that the FIS complex establishes *PHE1* imprinting by the methylation of histones at the maternal *PHE1* allele in the female gametophyte. Notably, the FIS genes continue to be expressed during early seed development (Luo *et al.*, 2000), indicating that a functional FIS complex is also present after fertilization. It is not yet known which mechanism prevents the FIS complex from silencing the paternal *PHE1* allele after fertilization.

Imprinting has evolved independently in mammals and flowering plants (reviewed by Haig & Westoby, 1989). It has been hypothesized that one of the main forces for the evolution of imprinted genes is the conflict over resource allocation from the mother to the many developing offspring. In both mammals and flowering plants, the mother provides all the nutrients to the offspring without substantial paternal contribution. Therefore, evolution would favour the expression of growth-promoting genes when paternally contributed and silencing of these genes when maternally contributed (reviewed by Haig & Westoby, 1989; Tilghman, 1999). *PHE1* is an imprinted gene, but whether the *PHE1* transcription factor has a function in resource allocation from the mother to the developing seed is unknown. Similarly, the imprinted genes *MEA* and *FIS2* have not been shown to regulate maternal resource allocation; however, the endosperm overproliferation in *mea* and *fis2* mutants would be consistent with this role. Interestingly, some autosomal imprinted genes and X-chromosome inactivation in mammals are also regulated by PcG proteins (Wang *et al.*, 2001; Mager *et al.*, 2003; Silva *et al.*, 2003). Therefore, although imprinting has evolved independently in mammals and flowering plants, evolutionarily conserved PcG complexes have been recruited for the regulation of imprinted genes in both taxa.

Impact of imprinted genes on seed development

In mammals, embryos containing only maternally or only paternally derived genomes do not complete development, suggesting that functions specific to each of the maternal and paternal genomes are required (reviewed by Haig & Westoby, 1989). By contrast, several angiosperm species can reproduce asexually and form viable maternal embryos without paternal contribution (reviewed by Köhler & Grossniklaus, 2005). However, many asexually derived embryos require a sexual endosperm to complete development (Koltunow, 1993), indicating that imprinted genes are acting mainly in the endosperm. Support for this hypothesis comes from studies in maize, in which gene expression dependent on the parent-of-origin has been shown to occur in the endosperm (reviewed by Köhler & Grossniklaus, 2005). Furthermore, interploidy crosses in *Arabidopsis* affect endosperm development more than embryo development (Scott *et al.*, 1998). Seeds from crosses of a diploid

mother plant with a tetraploid father contain an overproliferated endosperm with an increased number of nuclei. By contrast, seeds from the reciprocal cross contain endosperm with fewer nuclei (Scott *et al.*, 1998). Thus, an overdose of paternally derived genes (paternal excess) enhances growth, whereas an overdose of maternally derived genes (maternal excess) restricts growth, supporting the predictions proposed by Haig & Westoby (1989). Furthermore, imprinting of *MEA* occurs specifically in the endosperm and *MEA* is biparentally expressed in the embryo, at least from the torpedo stage onwards (Kinoshita *et al.*, 1999). The specific function of *MEA* and other FIS genes in the endosperm is evident from the phenotype of *fis* mutants, which develop highly overproliferated chalazal endosperm domains (Kiyosue *et al.*, 1999; Ingouff *et al.*, 2005), suggesting that the FIS complex represses the maternal allele of paternally expressed genes acting in the endosperm. This hypothesis is fully supported by the observed regulation of *PHE1*, which is a paternally expressed, maternally repressed FIS target gene. However, it is not yet known whether *PHE1* imprinting occurs specifically in the endosperm.

DNA methylation regulates imprinted genes in plants

As discussed above, it is likely that one of the main functions of the FIS PcG complex is the imprinting of paternally expressed genes in the endosperm. In *fis* mutants, loss of imprinting and increased expression of these genes confers the endosperm overgrowth phenotype. Similarly, at least one cluster of imprinted genes in the mammalian placenta is regulated by PRC2-mediated histone methylation, and it has been proposed that regulation of imprinting by PRC2 is an ancient mechanism acting in this organ (Lewis *et al.*, 2004; Umlauf *et al.*, 2004). Imprinting by PRC2 and histone methylation is not particularly stable, but as the placenta is short-lived, stable silencing might not be necessary. By contrast, stable imprinting of genes in the embryo might have been achieved by the additional recruitment of DNA methyltransferases (Lewis *et al.*, 2004).

Similar to the placenta, the endosperm in plants is short-lived. Therefore, imprinting in the endosperm does not need to be particularly stable. This is indeed the case for *PHE1*, for which the 'silent' maternal allele is weakly transcribed (Köhler *et al.*, 2005). However, there is evidence that DNA methylation has a substantial role in the regulation of imprinted genes in the endosperm. Seeds derived from a hypomethylated pollen donor have an underproliferated endosperm and resemble seeds with a maternal excess derived from a $4n \times 2n$ interploidy cross. The opposite phenotype is obtained when a hypomethylated mother plant is pollinated with wild-type pollen. Here, the endosperm overproliferates and seeds phenocopy paternal excess phenotypes obtained after $2n \times 4n$ crosses (Adams *et al.*, 2000). These results suggest that hypomethylation activates the expression of imprinted genes. In hypomethylated pollen, paternally silenced genes would be reactivated causing a maternal excess phenotype, whereas in hypomethylated female gametes maternally silenced genes would be reactivated causing a paternal excess phenotype (Adams *et al.*, 2000; Fig 2). Further support for this hypothesis comes from experiments showing that hypomethylated pollen can rescue heterozygous *fie* mutant seeds (Vinkenoog *et al.*, 2000). As discussed earlier, the phenotype of *fis* mutants might be caused by the de-repression of maternally silenced genes. If paternally silenced genes are reactivated in hypomethylated pollen, the

Fig 2 | Mutations in the DNA methylation maintenance system affect the maternal–paternal genome balance and seed size. **(A)** In female gametes (red), maternally expressed genes (red arrow), which often restrict seed growth, are hypomethylated and active whereas paternally expressed genes (blue), which often promote seed growth, are methylated and silenced. Reciprocally, in male gametes (blue), growth-promoting paternally expressed genes (blue arrow) are hypomethylated and active, whereas growth-restricting maternally expressed genes (red) are methylated and silenced. After fertilization, differential allele-specific gene expression persists in seeds (green), resulting in balanced maternal and paternal gene expression and normal-sized seeds. **(B)** If DNA methylation is impaired in male gametes, genes that are normally paternally silenced become active, causing excess expression of growth-restricting genes in seeds and decreased seed size. **(C)** If DNA methylation is impaired in female gametes, genes that are normally maternally silenced become active, causing excess expression of growth-promoting genes in seeds and increased seed size.

paternal excess phenotype in *flc* mutants might be alleviated. On the basis of these results, it is possible that imprinting in the endosperm involves DNA methylation. Whether the regulation of imprinted loci also involves histone methylation remains to be determined. In mammals, mutants deficient for EED—the mammalian FIE homologue—show alterations of the DNA methylation status at several imprinted loci that are associated with a loss of imprinting (Mager *et al*, 2003). Given that EZH2—the interaction partner of EED—interacts with DNA methyltransferases and is required for targeting methyltransferases to EZH2-regulated promoters (Vire *et al*, 2006), there might be a direct link between PcG-mediated imprinting and DNA methylation.

Concluding remarks and open questions

Results that have accumulated in recent years strongly support the idea that imprinted genes regulate endosperm development in flowering plants, and that imprinting in the endosperm is regulated by the FIS PcG complex and DNA methylation. How these processes are interconnected is still unknown and identifying the targeting information for PcG proteins and DNA methyltransferases will be the subject of future investigations. Finally, to understand why imprinting has evolved in flowering plants, it will be necessary to identify more genes that are regulated by imprinting and to uncover whether these genes regulate endosperm development.

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