Published in final edited form as:
Curr Opin Genet Dev. 2007 December ; 17(6): 480–485.

Endosperm Gene Imprinting and Seed Development

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Summary

Imprinting occurs in the endosperm of flowering plants. Endosperm, produced by fertilization of the central cell in the female gametophyte, is essential for embryo and seed development. Several imprinted genes play an important role in endosperm development. The mechanism of gene imprinting involves DNA methylation and histone modification. DNA methylation is actively removed at the imprinted alleles to be activated. Histone methylation mediated by the Polycomb group complex provides another layer of epigenetic regulation at the silenced alleles. Endosperm gene imprinting can be uncoupled from seed development when fertilization of the central cell is prevented. Imprinting may be a mechanism to ensure fertilization of the central cell thereby preventing parthenogenic development of the endosperm.

Introduction

Genomic imprinting is the phenomenon in which a set of genes is expressed according to their parent of origin. Imprinting occurs primarily in the placenta of mammals and in the endosperm of flowering plants. Both structures support the developing embryo, and according to the parental conflict theory [1], imprinting is implemented to allocate limited resources to the offspring over which both paternal and maternal parents are competing. A decade ago, a specific class of mutants was identified that shows fertilization-independent seed development (for review, see [2]). Later studies revealed that these mutants are impaired in the Polycomb group (PcG) complex in the endosperm [2]. This PcG complex plays a crucial role in genomic imprinting in the endosperm, and interestingly, several of its components are imprinted.

Several years ago, DNA methylation was found to be involved in the regulation of endosperm gene imprinting [3,4]. DNA methylation is a well-known epigenetic mark often associated with gene silencing. DNA methylation is an essential factor, regulating imprinting in both plants and mammals. Recent studies revealed that imprinting is a consequence of dynamic processes of DNA methylation and demethylation, and histone modification [5••,6•,7•]. In this review, we discuss the recent findings towards the understanding of imprinting mechanisms at the molecular level in Arabidopsis.

Seeds – Where Imprinting Occurs

A seed is the ripened ovule in gymnosperms and angiosperms that contains the embryo. A new plant grows from the embryo under proper conditions, which again endeavors to produce seeds for the next generation. The life cycle of Spermatophyta (seed-bearing plants) therefore begins with and ends up with seeds. Gymnosperms and angiosperms differ in seed structure and fertilization processes. In gymnosperms such as cycads or conifers, seeds are not enclosed within the ovary (thus they are called naked seeds) and the fertilization process is relatively simple. The gymnosperm female gametophyte has several archegonia in the ovule. Upon

fertilization, sperm cells are released from the growing pollen tube penetrating the archegonium in which the egg cell is located. The resulting zygotic embryo absorbs nutrients from the surrounding female gametophyte tissue for growth and maturation. In contrast, the angiosperm seeds contain the endosperm, a product of double fertilization that is a distinguishing feature of flowering plants. Upon double fertilization, two sperm are released from the pollen tube into the embryo sac - a female gametophyte in angiosperms. One sperm fertilizes the egg cell and the other fertilizes the central cell. The resulting embryo and endosperm are genetically identical except for their ploidy level: the embryo is diploid and the endosperm is triploid. The endosperm, analogous to the mammalian placenta, supports and nurtures the growing embryo as does the gymnosperm female gametophyte. In the endosperm, specialized transfer cells facilitate nutrient uptake [2]. And, surprisingly, the endosperm in a developing seed is the only place where imprinting is known to occur.

Polycomb Group Genes Control Endosperm Development and are Imprinted

In angiosperms, double fertilization initiates two organs – embryo and endosperm – and their development is highly coordinated. Crosstalk between these two organs and fertilization signals appear to ensure synchronized development of each organ residing in the same ovule. However, mutations in a specific class of genes disrupt such developmental synchrony and seeds eventually abort. The Arabidopsis *FIS* class genes – *MEDEA* (*MEA*), *FERTILIZATION-INDEPENDENT SEED2* (*FIS2*), *FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*) – encode PcG components and their mutations allow the unfertilized central cell to proliferate autonomously without fertilization forming an endosperm-like structure [8,9,10,11]. The characteristic seed abortion phenotype is observed only when the mutation is maternally inherited. Paternal mutations do not affect seed development.

Several imprinted genes have been identified in maize and Arabidopsis (Table 1). We discuss here the mechanisms and imprinted genes revealed in Arabidopsis and recommend to readers the following reviews [12] and [13] on maize genomic imprinting.

DNA Methylation and Demethylation in Gene Imprinting

Arabidopsis *METHYLTRANSFERASE 1* (*MET1*), the homolog of mammalian *Dnmt1*, is the primary DNA methyltransferase that maintains cytosine methylation at CG sites [14]. *met1* mutants display a global reduction of cytosine methylation accompanied with developmental abnormalities [15]. From genetic studies, it was shown that imprinting of *MEA*, *FIS2*, and the *FWA* transcription factor gene, involves MET1-mediated DNA methylation [4,16•,17]. Further studies revealed that there exists differential DNA methylation between the paternal and maternal alleles of *MEA*, *FIS2*, and *FWA* [5••,16•]. Maternal alleles of these imprinted genes are hypomethylated, whereas the paternal alleles are hypermethylated in the endosperm. Therefore, it was hypothesized that the differential expression activity between the two parental alleles was determined by the status of DNA methylation that has been epigenetically inherited from the gametes. Unlike mammals, however, the maternal-specific expression of imprinted genes is not achieved by paternal-specific *de novo* methylation during gametogenesis. Rather, the default state of these imprinted genes is more likely to be MET1-dependent methylation and transcriptional silencing. Thus, a maternal-specific activator(s) releases the default silencing and activates maternal expression only in the female gametophyte. In the male gametophyte, by contrast, the paternal allele would remain silent due to an absence of a maternal-specific activator(s).

What is the maternal-specific activator(s) in the female gametophyte? Does DNA methylation serve as a silencing mark and is it removed directly or indirectly by the activator(s)? *DEMETER* (*DME*) has been identified as a transcriptional activator positively regulating *MEA* in the central cell [3]. *DME* is a parent-of-origin effect gene because only the maternal

DME is important for seed viability. *DME* expression is confined to the central cell and its expression disappears after fertilization, whereas maternal *MEA* allele expression persists in the endosperm. Ovules carrying mutant *dme* do not express *MEA* and as a result the seeds eventually abort. The finding that the *met1* mutation suppresses *dme* seed abortion by restoring *MEA* expression suggests *DME* and *MET1* antagonistically regulate *MEA* [4]. It was thus hypothesized that DME removes DNA methylation at the maternal *MEA* allele in the central cell and the hypomethylated maternal *MEA* is exclusively expressed in the early endosperm while the methylated paternal *MEA* is transcriptionally silenced (Figure 1).

A recent study demonstrated that *DME* is necessary for demethylation and transcriptional activation of the maternal *MEA* in the endosperm [5••]. *DME* encodes a DNA glycosylase that specifically removes 5-methylcytosine from DNA [5••,18]. DNA glycosylases are repair enzymes that initiate the base excision repair by removing damaged or mismatched bases [19]. DNA glycosylase activity of DME is required for removal of cytosine methylation both *in vivo* and *in vitro* [5••,20]. Only the paternal *MEA* is methylated and silenced in the wild type endosperm, whereas, both parental alleles are methylated in the *dme* mutant endosperm, indicating maternal allele-specific hypomethylation [5••]. This finding suggests a mechanism of active DNA demethylation because expression of *DME* and its demethylation take place in the mature central cell after cell divisions within the female gametophyte have ceased. Thus, DME demethylation does not likely involve a passive demethylation process via a series of cell division without maintenance of DNA methylation. *In vitro*, DME removes 5-methylcytosine at any sequence contexts, whereas *in vivo* DME demethylation occurs in a locus-specific manner [5••]. Moreover, such DNA demethylation activity is observed at specific sites even within the same locus. For example, regulation of DNA methylation and demethylation at the maternal *MEA* only takes place in the 5’ and 3’ of the coding region [5••]. How DME is targeted to a specific region is still unknown. DME is also required for maternal activation of two other imprinted genes *FIS2* and *FWA*, and their DNA methylation/demethylation pattern in both parental alleles is very similar to that of *MEA* [16•,17].

Therefore, imprinting of *MEA*, *FIS2*, and *FWA* in the endosperm is initiated and established by DME-mediated active DNA demethylation in the central cell, while the paternal alleles remain methylated and silenced (Figure 1). The methylation state of each allele is likely to persist via epigenetic mechanisms throughout nuclear divisions during early endosperm development. The on/off switch of DNA methylation is sufficient for the establishment and maintenance of both *FIS2* and *FWA* imprinting [16•]. By contrast, *MEA* imprinting requires an additional regulatory mechanism, which is discussed below.

Maintenance of Gene Imprinting by PcG Silencing

Both *MEA* and *FIS2* are imprinted in the endosperm. MEA is homologous to Drosophila E(z) whose SET domain has methyltransferase activity on lysine 27 of histone 3 (H3K27) [9,11]. FIS2 is a zinc-finger transcription factor homologous to *Drosophila* Suppressor of Zeste12 [Su(z)12] [21]. The *FIS* class gene products, MEA, FIS2, and FIE appear to function in a large PcG complex along with additional components such as MULTI-COPY SUPPRESSOR OF IRA1 (MSI1) and retinoblastoma-related protein RBR1[22,23,24]. This multimeric PcG complex is predicted to repress gene transcription via histone modification and chromatin remodeling, and the established patterns are stably propagated through mitotic cell cycles [25]. This PcG complex is thought to negatively regulate endosperm cell proliferation because autonomous central cell divisions occur in *mea*, *fis2*, or *fie* mutants in the absence of fertilization [8,9,10,11].

Activation by demethylation of the maternal *MEA* allele is accomplished by DME in the central cell [3,5••], while the paternal allele is methylated and silenced. The differential methylation

patterns are inherited in the endosperm after fertilization. However, DNA methylation is not directly involved in the maintenance of paternal *MEA* silencing because even the unmethylated paternal *MEA* allele contributed by *met1* mutants is not expressed in the endosperm [5••]. Rather, the FIS-PcG complex containing MEA itself seems to keep the silenced paternal *MEA* repressed [5••,6•,7•]. Disruption of the FIS-PcG complex causes loss of *MEA* imprinting as silencing of the paternal allele is released [5••,6•,7•]. In addition, MEA is physically associated with the *MEA* promoter sequence [7•]. These findings propose a self-imprinting mechanism of *MEA*, in which maternally expressed *MEA* replenishes the FIS-PcG complex, and in turn, the complex keeps repressing the silenced paternal *MEA* allele (Figure 1) [5••].

PHERES1 (*PHE1*) is another imprinted gene in the Arabidopsis endosperm [26]. Whereas *MEA*, *FIS2*, and *FWA* are maternally expressed, paternal *PHE1* expression predominates in the endosperm, while the maternal *PHE1* is silent or very weakly expressed [27]. The silenced maternal *PHE1* allele is a direct target of the FIS-PcG complex [27]. In *mea* mutant seeds, for example, silencing of the maternal *PHE1* is released leading to biallelic expression [27]. Unlike other imprinted genes, however, the role of DNA methylation in *PHE1* imprinting is not reported. Rather, histone modification via the FIS-PcG complex likely both establishes and maintains the silencing of the paternal *PHE1* (Figure 1).

Notably, MEA is required for H3K27 methylation, one of the epigenetic silencing marks, at the silenced paternal *MEA* and the maternal *PHE1* alleles [5••,6•,28•]. Silencing of the paternal *MEA* is released in the *mea* mutant endosperm accompanied with loss of H3K27 methylation [5••]. Repression of the *PHE1* allele is also associated with H3K27 methylation [28•]. A mutation in the catalytic center of the MEA SET domain abolishes *PHE1* repression, suggesting that histone methyltransferase activity of MEA is necessary for its function in PcG silencing and gene imprinting [28•].

Imprinting Bypass and Seed Development

Genomic imprinting in the Arabidopsis endosperm is regulated by both DNA methylation and PcG silencing. Is imprinting an integral feature of seed development that cannot be uncoupled? A recent study demonstrated that seeds are produced without double fertilization by bypassing genomic imprinting [29••]. Mutants for *CDKA;1* which encodes a Cdc2/Cdc28 homologue produce pollen with only one sperm [30•,31•]. This mutant pollen preferentially fertilizes the egg cell while the binucleate central cell remains unfertilized. Embryos from eggs fertilized with *cdka;1* mutant pollen abort about 3 days after pollination and only a few divisions of the unfertilized central cell occurs [31•]. This suggests that a positive signal is generated from a developing embryo to initiate central cell proliferation independent of second fertilization. Strikingly, disruption of PcG inhibition in the female gametophyte allows single-fertilized seeds with unfertilized homodiploid endosperm [29••]. When PcG mutants such as *mea*, *fis2*, and *fie* are pollinated with *cdka;1* pollen, viable seeds form albeit the seed size is smaller than wild type [29••]. This implies that genomic imprinting in the endosperm is not necessary for seed development under certain circumstances and that an unfertilized diploid central cell in the female gametophyte has the full potential to develop functional endosperm without a paternal contribution. These results support the hypothesis that during the evolution of plants, the multicellular gymnosperm female gametophyte was reduced to the central cell in the angiosperm female gametophyte, and that fertilization of the central cell is the trigger that activates development of the multicellular endosperm. [29••].

Conclusions

Two epigenetic mechanisms, DNA methylation and histone modification involving PcG proteins, regulate gene imprinting in seed development. Initiation of gene imprinting requires

DME in the female gametophyte for allele-specific DNA demethylation. Differential methylation distinguishes the two parental alleles in the endosperm after fertilization and results in parent-of-origin patterns of gene expression. These allele-specific epigenetic marks are maintained and fortified by the PcG complex, which, in turn, auto-regulates its own components. Such epigenetic regulation and imprinting are vital to proper endosperm development and seed viability since mutations in the components of this regulatory circuit produce unviable seeds.

Nevertheless, loss of imprinting (i.e., gain of biallelic expression) does not always compromise seed development. When the paternal genome is derived from *met1* mutants, *FIS2* and *FWA* are biparentally expressed in the endosperm producing viable seeds [16•]. Fertilization of a *fis* mutant ovule with *cdka;1* pollen produces viable seeds with homodiploid endosperm in the absence of paternal genome contribution, thus bypassing the requirement of gene imprinting [29••]. That the diploid condition is sufficient for a viable seed is evident by the presence of biparental diploid endosperm in *Nuphar polysepalum*, a basal angiosperm [32]. Therefore it is reasonable to speculate that endosperms of most flowering plants might have evolved a unique imprinting mechanism to ensure that fertilization of the central cell takes place, and that the male contributes to the production of healthy endosperm for the next generation. Thus, in flowering plants and mammals, imprinting prevents parthenogenic development of the endosperm and embryo, respectively [33].

Acknowledgements

We acknowledge support from the National Institutes of Health (GM069415), United States Department of Agriculture (2005-02355), and the United States - Israel Binational Research Fund (IS-3604-04CR) to Robert L. Fischer.

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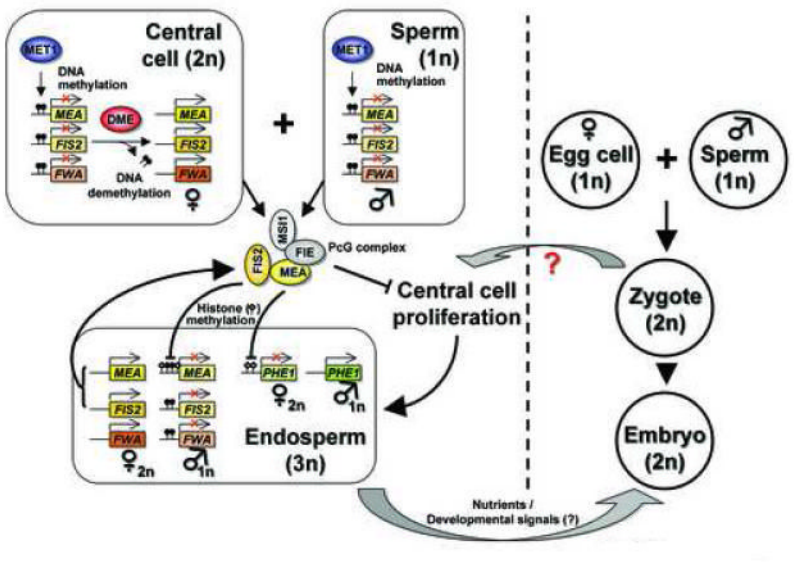

Figure 1. Gene imprinting during Arabidopsis seed development

Both maternal and paternal alleles of imprinted genes are methylated by MET1 as a default state in the central cell and sperm, respectively. DME, 5-methylcytosine DNA glycosylase in the central cell, demethylates and activates *MEA*, *FIS2*, and *FWA* alleles [5••,16•]. Upon fertilization, maternally expressed but paternally silenced *MEA* and *FIS2* participate in a PcG complex. In turn, the PcG complex represses its targets such as the paternal *MEA* and the maternal *PHE1* through histone modifications involving H3K27 methylation [5••,28•]. Maternal *MEA* and *FIS2*, which are not repressed by the PcG complex, are continuously expressed replenishing the PcG complex, which forms an auto-regulatory feedback loop [5••]. Silencing of the paternal *FIS2* and *FWA* appears to be solely dependent upon DNA methylation, which is inherited from the gametes [16•]. Consequently, *MEA*, *FIS2*, and *FWA* are maternally expressed and *PHE1* paternally expressed in the early endosperm.

Table 1

List of imprinted genes in flowering plants.

Gene	Product	Function[a]	Allelic expression[b]	References
Arabidopsis thaliana				
FERTILIZATION-INDEPENDENT SEED2 (*FIS2*)	Zinc-finger transcription factor	PcG silencing	Maternal	8,16•,21,34
FWA	Homeodomain transcription factor	Unknown	Maternal	16•,17,35
MEDEA (*MEA*)	PcG SET-domain protein	PcG silencing/ H3K27 methylation	Maternal	4,5••,6•,7•,9, 11,21,22,26, 28•,34,36,37
PHERES1 (*PHE1*)	MADS-box transcription factor	Unknown	Paternal	26,27,28•
Zea mays				
fertilization-independent endosperm1 (*fie1*)	WD-40 repeat protein	Unknown	Maternal	38,39,40•,41
fertilization-independent endosperm2 (*fie2*)	WD-40 repeat protein	Unknown	Maternal	38,39,40•
maternally expressed gene1 (*meg1*)	Cys-rich glycosylated protein	Structural role in basal endosperm transfer region (?)	Maternal	42
maize E(z)-like gene1 (mez1)	PcG SET-domain protein	Unknown	Maternal	43
no-apical-meristem-related protein (*nrp*)	NAM family transcription factor	Unknown	Maternal	44
Paternally expressed gene1 (*peg1*)	Unknown	Unknown	Paternal	39

[a] Function known in the endosperm.

[b] Allelic expression pattern observed in the endosperm.