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REVIEW

Mechanisms and evolution of genomic imprinting in plants

C Köhler and I Weinhofer-Molisch
Department of Biology and Zurich-Basel Plant Science Center, Swiss Federal Institute of Technology, ETH Centre, Zurich, Switzerland

Genomic imprinting, the allele-specific expression of a gene dependent on its parent-of-origin, has independently evolved in flowering plants and mammals. In mammals and flowering plants, imprinting occurs in the embryo as well as in embryo-nourishing tissues, the placenta and the endosperm, respectively, and it has been suggested that imprinted genes control the nutrient flow from the mother to the offspring ('kinship theory'). Alternatively, imprinting might have evolved as a by-product of a defense mechanism destined to control transposon activity in gametes ('defense hypothesis'). Recent studies provide substantial evidence for the 'defense hypothesis' by showing that imprinted genes in plants are located in the vicinity of transposon or repeat sequences, suggesting that the insertion of transposon or repeat sequences was a prerequisite for imprinting evolution. Transposons or repeat sequences are silenced by DNA methylation, causing silencing of neighboring genes in vegetative tissues. However, because of genome-wide DNA demethylation in the central cell, genes located in the vicinity of transposon or repeat sequences will be active in the central cell and the maternal alleles will remain unmethylated and active in the descendent endosperm, assuming an imprinted expression. Consequently, many imprinted genes are likely to have an endosperm-restricted function, or, alternatively, they have no functional role in the endosperm and are on the trajectory to convert to pseudogenes. Thus, the 'defense hypothesis' as well as 'kinship theory' together can explain the origin of genomic imprinting; whereas the first hypothesis explains how imprinting originates, the latter explains how imprinting is manifested and maintained.
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Introduction

Genomic imprinting is an epigenetic phenomenon present in mammals and flowering plants that leads to differential expression of maternal and paternal alleles, depending on their parent-of-origin (Feil and Berger, 2007). Imprinted genes are differentially marked in the gametes before fertilization, rendering maternal and paternal chromosomes functionally different. It has been suggested that imprinting serves to control the nutrient flow from the mother to the progeny with maternally and paternally imprinted genes having different roles in nutrient allocation (Haig and Westoby, 1989). Whereas maternally expressed imprinted genes are suggested to reduce nutrient flow to the embryo, paternally expressed imprinted genes rather promote nutrient flow to the embryo (Haig and Westoby, 1989). This theory, known as the 'parental conflict theory' (Haig and Westoby, 1989) or 'kinship theory' (Trivers and Burt, 1999) has been supported by results of interploidy crosses in plants; while an increased dosage of paternal chromosomes promotes endosperm development, an increased dosage of maternal chromosomes represses endosperm development (Birchler, 1993; Scott *et al*., 1998). Importantly, these experiments revealed that the endosperm is particularly sensitive to changes in the parental chromosome dosage, suggesting that imprinting has a predominant role in the endosperm. Dramatic progress in our understanding of the imprinting mechanism has recently been achieved with the elucidation of the endosperm DNA methylation profile and the discovery of several novel imprinted genes that will allow to test the role of imprinted genes in the endosperm (Gehring *et al*., 2009; Hsieh *et al*., 2009). This review will focus on these recent new developments in the plant imprinting field and will discuss mechanisms underlying genomic imprinting in flowering plants as well as the evolution and effect of genomic imprinting for seed development.

Correspondence: Dr C Köhler, ETH Zurich, Department of Biology and Zurich-Basel Plant Science Center, Universitätsstrasse 2, CH-8092 Zurich, Switzerland.
E-mail: koehlerc@ethz.ch
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Imprinting mechanisms

On double fertilization, two sperm cells are released from the pollen tube into the embryo sac, with one of them fertilizing the egg cell and the other one fertilizing the homodiploid central cell, resulting in the formation of a diploid embryo and a triploid endosperm, respectively. The endosperm is a functional analog of the mammalian placenta and serves to support and nurture the growing embryo (Berger, 2003). Imprinting in plants has long been believed to be restricted to the ephemeral endosperm that is not transmitted to the next generation. However, based on recent results showing that the maize imprinted gene *maternally expressed in embryo 1* (*mee1*) is as well imprinted in the endosperm and during early

embryo development this dogma has to be revised (Jahnke and Scholten, 2009). Thus, similar to mammals imprinting in plants is not restricted to ephemeral tissues but extends to tissues contributing to the next generation, suggesting that plants as well as mammals had to develop strategies that allowed the resetting of epigenetic marks in gametic cells to restore totipotency (Feil and Berger, 2007; Jahnke and Scholten, 2009). However, as there are no data yet available on the mechanism leading to establishment and resetting of imprinting marks in plant embryos, the emphasis of this review will be on novel findings illuminating mechanisms of imprinting establishment in the endosperm. Parent-of-origin-specific expression of genetically identical alleles is achieved by the application of specific epigenetic modifications in the gametes. In particular, DNA methylation and Polycomb group (PcG)-mediated trimethylation of histone H3 at lysine 27 (H3K27me3) have been widely recognized as important epigenetic marks distinguishing maternally and paternally inherited alleles in mammals (Umlauf *et al*., 2004; Edwards and Ferguson-Smith, 2007) as well as in plants (Kinoshita *et al*., 2004; Baroux *et al*., 2006; Gehring *et al*., 2006; Xiao *et al*., 2006; Makarevich *et al*., 2008; Jullien *et al*., 2006a, b).

One-way control of imprinted genes by DNA methylation in the endosperm

In mammals, differential DNA methylation of maternal and paternal alleles occurs during gametogenesis after DNA methylation imprints of the previous generation have been erased in primordial germ cells (Sasaki and Matsui, 2008). Establishment of novel imprints requires the *de novo* DNA methyltransferases DNMT3A and DNMT3L (Bourc'his *et al*., 2001; Kaneda *et al*., 2004). In contrast, *de novo* methyltransferases of the DOMAINS REARRANGED METHYLTRANSFERASE (DRM) gene family are not recognized to have an important role for genomic imprinting in plants (Cao and Jacobsen, 2002), suggesting major mechanistic differences in the establishment of imprinting marks in flowering plants and mammals. In support of this view, differential DNA methylation of maternal and paternal alleles in the Arabidopsis endosperm requires the 5-methylcytosine excising activity of the DNA glycosylase DEMETER (DME) (Kinoshita *et al*., 2004; Gehring *et al*., 2006). DME is primarily expressed in the central cell of the female gametophyte (Choi *et al*., 2002), leading to specific removal of DNA methylation marks on the maternal alleles of genes, such as *MEDEA (MEA), FWA* and *FERTILIZATION INDEPENDENT SEED2 (FIS2).* Consequently, the maternal alleles of *MEA, FWA* and *FIS2* are expressed in the endosperm, whereas the paternal alleles are silenced by DNA methylation because of lack of DME in sperm cells (Choi *et al*., 2002; Kinoshita *et al*., 2004; Jullien *et al*., 2006a). The Retinoblastoma pathway imposes an additional control layer by repressing the DNA methyltransferase *MET1* during female gametogenesis (Jullien *et al*., 2008), leading to the formation of hemimethylated DNA, which is preferentially targeted by DME (Gehring *et al*., 2006; Morales-Ruiz *et al*., 2006). Thus, imprinting in the endosperm of flowering plants is not established by acquisition of DNA methylation but rather through specific demethylation in the female gametophyte. In contrast, based on data of the imprinted *mee1* gene (Jahnke and Scholten, 2009), active remethylation of imprinted genes might occur in plant embryos by an as yet unknown mechanism.

Genome-wide demethylation of repeat sequences in the endosperm

A significant advance in our understanding of the relationship between DNA methylation and genomic imprinting has recently been achieved by two independent studies reporting the genome-wide DNA methylation profile in the endosperm (Gehring *et al*., 2009; Hsieh *et al*., 2009). Both studies revealed a genome-wide hypomethylation of transposon and repeat sequences in the endosperm, with virtually all CG sequences being methylated in the embryo having reduced methylation levels in the endosperm (Hsieh *et al*., 2009). Methylation levels are partially restored in *dme* mutant endosperm (Hsieh *et al*., 2009), implying a functional requirement of DME for genome-wide CG demethylation in the endosperm. Therefore, imprinted gene expression will arise whenever transposon insertions or local sequence duplications occur close to gene regulatory sequences that will induce methylation and gene silencing in vegetative tissues as well as in paternally inherited alleles in the endosperm. DME-mediated demethylation of maternal alleles in the central cell will cause these genes to be predominantly maternally expressed in the endosperm. Thus, genomic imprinting in plants is largely a consequence of a genome-wide DME-mediated demethylation activity in the central cell.

Using the finding that many known imprinted genes are hypomethylated at the 5′ end and show endosperm-specific expression, Gehring *et al.* (2009) identified five earlier unknown imprinted genes, with all of them encoding putative transcription factors. Three of these genes, *HDG3*, *HDG6* and *HDG8* are members of the homeodomain-leucine zipper (HD-ZIP) family that constitutes a large family of transcription factors unique to plants and includes the known imprinted gene *FWA* (Nakamura *et al*., 2006). Thus, 4 of the 10 so far known imprinted genes are related homeodomain transcription factors. Whereas *HDG8* and *HDG9* are predominantly maternally expressed, *HDG3* is expressed from the paternal allele. Therefore, in agreement with previous findings (Makarevich *et al*., 2008, Villar *et al*., 2009), demethylation of transposons or repeat sequences of the maternally inherited alleles can result in imprinted expression with the paternal allele being expressed.

Gehring *et al.* (2009) suggest that the best candidates for imprinted genes are those that are less methylated in the endosperm than in the embryo, show endosperm-preferred expression and are transcribed at low levels in other parts of the plant. On the basis of these criteria they estimate that there are around 50 imprinted genes in *Arabidopsis,* with many of them encoding transcription factors and proteins with chromatin-related functions. It will be important to elucidate whether the suggested candidates are indeed regulated by genomic imprinting and to determine their functional role during endosperm development.

Maternal-specific expression of small interfering RNAs

A possible connection between small interfering RNAs (siRNAs) and genomic imprinting was recently discov-

ered by Mosher *et al.* (2009), who showed a predominant maternal origin of siRNAs in the endosperm, thus greatly expanding the number of known imprinted loci in the *Arabidopsis* genome. siRNAs consist of a complex population of more than 100 000 different small RNAs that regulate gene expression at the transcriptional and post-transcriptional level and are required to establish epigenetic modifications on DNA and chromatin (Gendrel and Colot, 2005; Ramachandran and Chen, 2008). siRNAs in plants target *de novo* methylation at CHG (H is A, C or T) and CHH sites by the RNA interference machinery, involving the *de novo* methyltransferase DRM2 (Henderson and Jacobsen, 2007). However, also DNA demethylation might be targeted by siRNAs, based on the recent discovery of the siRNA binding protein ROS3, which acts in the DNA demethylation pathway involving the DME homolog ROS1 (Zheng *et al*., 2008). These findings raise the interesting possibility that maternal-specific siRNAs guide genome-wide hypomethylation in the endosperm. This might generate a self-enforcing loop, as DNA hypomethylation results in massive reactivation of transposable elements, pseudogenes and intergenic noncoding RNAs (Lippman *et al*., 2004; Zhang *et al*., 2006), causing a further increased production of siRNAs (Onodera *et al*., 2005; Mathieu *et al*., 2007). If this scenario was true, then maternal-specific siRNA accumulation would be cause as well as the consequence of genome-wide hypomethylation of the maternal genome in the endosperm.

Polycomb group proteins control imprinted gene expression

Although DNA methylation is widely recognized as the major mechanism responsible for imprinted gene expression, there are examples that DNA methylation alone is not sufficient for imprinted gene expression. Thus, silencing of the maternal alleles of *PHERES1* (*PHE1*) and the paternal alleles of *MEDEA* (*MEA*) and *ARABIDOPSIS FORMIN HOMOLOGUE 5* depend on repressive activity of PcG proteins (Köhler *et al*., 2005; Baroux *et al*., 2006; Gehring *et al*., 2006; Jullien *et al*., 2006b; Makarevich *et al*., 2008; Fitz Gerald *et al*., 2009). PcG proteins act in complexes that apply H3K27me3 on their target genes, causing gene repression by not well understood mechanisms (Köhler and Villar, 2008). Although activity of the maternal *MEA* allele depends on DME-mediated DNA demethylation (Choi *et al*., 2002; Xiao *et al*., 2003), the DNA methylation status of the paternal *MEA* allele seems to be irrelevant for its expression (Gehring *et al*., 2006). Rather, repression of the paternal *MEA* allele requires the activity of the FERTILIZATION INDEPENDENT SEED (FIS) PcG complex with MEA itself being a subunit of this complex (Baroux *et al*., 2006; Gehring *et al*., 2006; Jullien *et al*., 2006b). Similarly, imprinted expression of *PHE1* depends on both, the FIS PcG complex and DME-mediated DNA demethylation (Makarevich *et al*., 2008; Hsieh *et al*., 2009). Demethylation of a distantly located repeat region at the 3′ end of the *PHE1* locus as well as binding of the FIS PcG complex to the *PHE1* promoter region are required for silencing of the maternal *PHE1* alleles, suggesting long-range interactions between the repeat region and PcG proteins (Villar *et al*., 2009). The complex transcriptional regulation of the *PHE1* locus is reminiscent of the suggested imprinting mechanism at the *IGF2/H19* locus in mammals that involves long-range intrachromosomal loop formation and PcG-mediated allele-specific H3K27me3 as well (Murrell *et al*., 2004; Kurukuti *et al*., 2006; Li *et al*., 2008), suggesting a notable convergent evolution of PcG-mediated imprinting mechanisms between flowering plants and mammals.

Apart from *PHE1* there are additional examples of paternally expressed imprinted genes in the endosperm (Gehring *et al*., 2009) and it will be interesting to learn whether repression of the maternal alleles depends on FIS PcG activity and whether demethylation of maternal alleles and/or methylation of the paternal alleles are required for imprinted expression.

Regulation of dosage sensitive gene expression in the endosperm

The FIS PcG complex is essential for seed development, and lack of any known component of this complex causes abnormal embryo and endosperm development leading to seed abortion (Ohad *et al*., 1996; Chaudhury *et al*., 1997; Grossniklaus *et al*., 1998; Kiyosue *et al*., 1999; Köhler *et al*., 2003a; Guitton *et al*., 2004). Developmental defects are associated with increased expression of *PHE1* and many other genes in the endosperm (Kang *et al*., 2008; Köhler *et al*., 2003b; Erilova *et al*., 2009), suggesting that the main function of the FIS PcG complex is to suppress dosage sensitive genes in the endosperm. Support for this idea stems from experiments showing that *fis* mutants can form viable seeds if a sexually derived *fis* embryo is supported by an autonomously developing diploid endosperm (Nowack *et al*., 2007). This can be achieved by fertilizing *fis* mutants with single sperm cell containing pollen of the *cdka;1* mutant that preferentially fertilizes the egg cell (Nowack *et al*., 2006). Lack of FIS function causes autonomous endosperm development and the formation of seed-like structures without viable embryos (Chaudhury *et al*., 1997). However, sexually derived embryos surrounded by a diploid autonomous *fis* endosperm develop into viable seeds (Nowack *et al*., 2007), suggesting that developmental aberrations in *fis* mutant endosperm are caused by increased expression of dosage sensitive genes and reducing genome dose by bypassing fertilization can restore viable seed formation.

The importance of balanced maternal to paternal genome dose for viable seed formation has also been shown by interploidy crosses resulting in opposing phenotypes depending on the direction of the cross (Scott *et al*., 1998). Importantly, interploidy crosses of diploid maternal plants with pollen donors of increased ploidy result in the formation of seeds with striking phenotypic similarities to *fis* mutant seeds; endosperm mitotic activity is prolonged, cellularization is delayed and the chalazal endosperm is highly overproliferated, resulting in seed abortion at accession-dependent variable frequency (Scott *et al*., 1998; Dilkes *et al*., 2008). Results from our group reveal a mechanistic connection between interploidy paternal-excess and *fis* mutant phenotypes by showing an important role of imprinted expression of the FIS subunit *MEA* in sensing increased paternal genome dose in the endosperm (Erilova *et al*.,

2009). As *MEA* is only maternally expressed in the endosperm, *MEA* transcript levels are relatively reduced in endosperm with increased paternal genome contribution, causing reduced FIS PcG activity and increased expression of dosage sensitive FIS target genes (Erilova *et al*., 2009). Together, the FIS PcG complex regulates dosage sensitive genes in the endosperm that have an important role for endosperm development. Whether dosage sensitive genes are necessarily regulated by genomic imprinting will be an important issue to clarify in the near future.

Evolution of the imprinting mechanism in the endosperm

With the recent discovery of extensive demethylation of transposable elements and repeat sequences in the endosperm, a model has been suggested whereby imprinting arose as a by-product of a silencing mechanism targeting invading foreign DNA (Gehring *et al*., 2009; Hsieh *et al*., 2009). According to this hypothesis, DNA methylation-dependent parent-of-origin-specific gene expression could potentially arise whenever a transposon insertion or sequence duplication occurs close to a gene regulatory region, as these regions will be targeted by DME-dependent demethylation (Gehring *et al*., 2009; Hsieh *et al*., 2009).

Interestingly, genome-wide hypomethylation of CG sequences in the endosperm is accompanied by an extensive CHH (H is A, C or T) hypermethylation in the embryo (Hsieh *et al*., 2009). Asymmetric CHH methylation requires active targeting through the RNA interference machinery (Henderson and Jacobsen, 2007), suggesting enhanced siRNA-mediated DNA methylation activity in the embryo. Hsieh *et al*. (2009) suggest an intriguing connection between enhanced siRNA-mediated DNA methylation activity in the embryo and reduced DNA methylation levels in the central cell. According to their hypothesis, hypomethylation in the central cell will cause an accumulation of siRNAs that will be transported to the egg cell, leading to DNA hypermethylation in the egg cell and later on in the embryo to ensure proper silencing of transposons and repetitive elements. This mechanism has striking parallels to a recently suggested mechanism operating between sperm cells and the vegetative cell in pollen (Slotkin *et al*., 2009). Slotkin *et al*. (2009) suggest that hypomethylation in the vegetative pollen nucleus generates siRNAs that migrate to the sperm cells, inducing hypermethylation of transposable and repetitive elements in sperm cells. Thus, hypomethylation in germ cell accompanying cells and their descendents that do not contribute to the next generation could drive silencing of transposons and repetitive elements in male and female gametes and the descendent zygote (Figure 1). If so, genomic imprinting in the endosperm is likely a consequence of a mechanism destined to silence invading foreign DNA in the embryo. Similarly, the host defense hypothesis suggested that genomic imprinting in mammals evolved from existing mechanisms destined to silence foreign DNA elements (Barlow, 1993) and substantial supportive evidence for this hypothesis has been obtained recently (Suzuki *et al*., 2007; Pask *et al*., 2009).

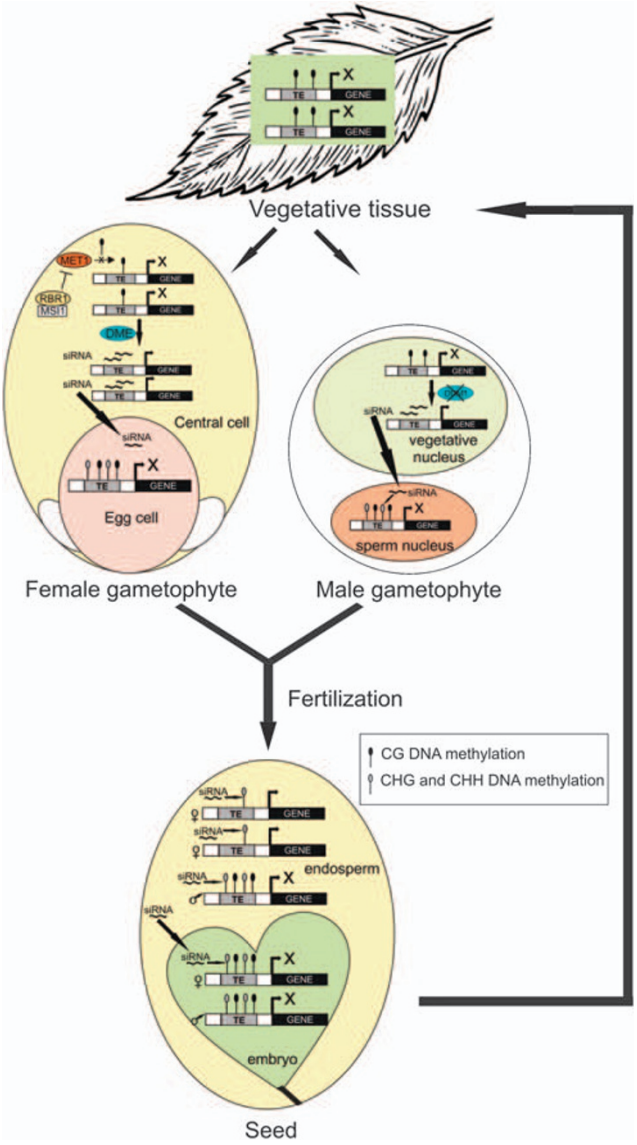

Figure 1 Reprogramming of epigenetic marks in gametes. During female gametogenesis, expression of the DNA methyltransferase *MET1* is repressed by the Retinoblastoma pathway involving RBR1 and its interacting partner MSI1, causing passively reduced DNA methylation. In the mature central cell, the DNA glycosylase DEMETER (*DME*) is expressed and actively erases DNA methylation on the maternal alleles. *DME* is not expressed in egg or sperm cells, therefore, maternal and paternal alleles remain methylated. In the embryo, an initial demethylation of maternal alleles can occur by an as yet unknown mechanism (not shown in model), however, maternal alleles are then remethylated and silenced in the embryo. In the vegetative nucleus of the male gamethophyte, lack of the chromatin remodelling factor DDM1 causes a reduction in DNA methylation levels and transposon reactivation that is accompanied by production of small interfering RNAs (siRNAs). These siRNAs are possibly transported into sperm cells to induce asymmetric CHG and CHH (H is A, C or T) DNA methylation preventing transposon reactivation. Similarly, in the central cell and in the endosperm, the widespread loss of DNA methylation is likely to cause genome-wide transcriptional reactivation of transposons and repeat sequences, resulting in massive production of siRNAs that trigger asymmetric DNA methylation in egg cell and endosperm. As suggested for pollen grains, an attractive hypothesis could be a transport of siRNAs from the central cell and the endosperm to egg cell and embryo, respectively, reinforcing transposon silencing by the addition of asymmetric DNA methylation.

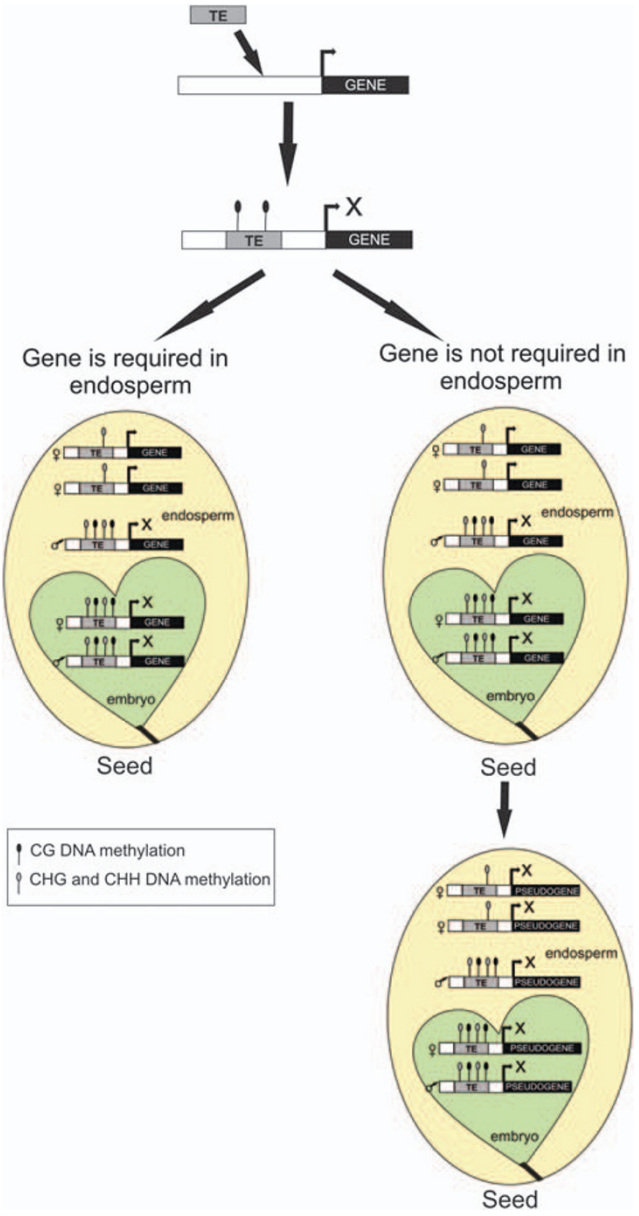

Figure 2 Imprinting in the endosperm evolved as a by-product of silencing of invading foreign DNA. Whenever a transposon insertion or sequence duplication occurs in the vicinity of a gene, this region will be targeted by DNA methylation causing silencing of the invading foreign DNA and the neighboring gene. As a result, this gene will be silenced in sporophytic organs and will loose its functional role during the vegetative life phase. Owing to active and passive DNA demethylation in the central cell, transposon or repeat sequences are hypomethylated in the central cell and will remain hypomethylated in the endosperm, thus assuming an imprinted expression. Consequently, many imprinted genes are likely to have an endosperm-restricted function, or, alternatively, they have no functional role in the endosperm and are on the trajectory to convert to pseudogenes.

Selective advantage of genomic imprinting in the endosperm

How does the host defense hypothesis with all its supportive evidence reconcile with the widely accepted 'parental conflict' (Haig and Westoby, 1989) or 'kinship' theory (Trivers and burt, 1999), stating that imprinting arose as a consequence of a conflict over the distribution of resources from the mother to the offspring? According to this theory, there will be a selection of paternally active genes that maximize the transfer of nutrients to the developing embryo, whereas the mother protects herself against the demands of the embryo by suppressing the growth induced by the paternally active genes. In agreement with the predictions of this theory, imprinting occurs in placental mammals and flowering plants, both contributing maternal resources to the progeny (Feil and Berger, 2007). Furthermore, many imprinted genes in mammals affect both the demand and supply of nutrients across the placenta, adding additional support to this theory (Reik *et al*., 2003). In flowering plants, imprinting occurs in embryo and endosperm; with the latter constituting a separate organism that similar to the placenta is dedicated to nourish the developing embryo. Although there are only few imprinted genes and their functions identified in plants, at least some of the known genes affect endosperm growth (Chaudhury *et al*., 1997; Kiyosue *et al*., 1999; Tiwari *et al*., 2008). On the basis of the recent findings that repeat and transposon insertions might be the driving force for genomic imprinting in the endosperm (Gehring *et al*., 2009; Hsieh *et al*., 2009), any gene that by chance was located in the vicinity of a repeat or transposon insertion is destined to become imprinted and will, in most instances, be silenced in sporophytic organs. As a consequence, these genes will loose their functional role during the vegetative life phase and could assume an endosperm-constrained function. After this logic, many imprinted genes are likely to have an endosperm-constrained function, or, alternatively, they have no functional role in the endosperm and are on the trajectory to convert to pseudogenes (Figure 2). Although there are exceptions (for example, *PHE1* and *HDG3* (Köhler *et al*., 2005; Gehring *et al*., 2009)), the majority of imprinted genes is likely to be maternally active and paternally silenced, imposing a strong maternal control over endosperm development, as it could be predicted based on the hypothesis that the endosperm is an extension of the maternal gametophytic life phase (Nowack *et al*., 2007). To conclude, both hypotheses, the 'defense hypothesis' as well as 'kinship theory' together can explain the origin of genomic imprinting in the endosperm; whereas the first hypothesis explains how imprinting originates, the latter explains how imprinting will be manifested and maintained.

Conflict of interest

The authors declare no conflict of interest.

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