Report

Polycomb Group Complexes Self-Regulate Imprinting of the Polycomb Group Gene *MEDEA* in *Arabidopsis*

Pauline E. Jullien,[1] Aviva Katz,[2] Moran Oliva,[2] Nir Ohad,[2,*] and Frédéric Berger[1,*]
[1] Chromatin and Reproduction Group
Temasek LifeSciences Laboratory
1 Research Link
National University of Singapore
Singapore 117604
Republic of Singapore
[2] Department of Plant Sciences
Faculty of Life Sciences
Tel Aviv University
Tel Aviv, 69978
Israel

Summary

Fertilization in flowering plants initiates the development of the embryo and endosperm, which nurtures the embryo. A few genes subjected to imprinting are expressed in endosperm from their maternal allele, while their paternal allele remains silenced [1–3]. Imprinting of the *FWA* gene involves DNA methylation [4]. Mechanisms controlling imprinting of the Polycomb group (Pc-G) gene *MEDEA* (*MEA*) [5] are not yet fully understood [6–10]. Here we report that *MEA* imprinting is regulated by histone methylation. This epigenetic chromatin modification is mediated by several Pc-G activities during the entire plant life cycle. We show that Pc-G complexes maintain *MEA* transcription silenced throughout vegetative life and male gametogenesis. In endosperm, the maternal allele of *MEA* encodes an essential component of a Pc-G complex, which maintains silencing of the paternal *MEA* allele. Hence, we conclude that a feedback loop controls *MEA* imprinting. This feedback loop ensures a complete maternal control of *MEA* expression from both parental alleles and might have provided a template for evolution of imprinting in plants.

Results and Discussion

Silencing of the Imprinted *MEA* Gene during the Vegetative Phase Relies on Pc-G Activities

The mechanisms maintaining silencing of *MEA* during vegetative development remain unknown. Two major silencing machineries are likely to participate in the above process: the maintenance of DNA methylation [11] and the methylation of lysine residue 27 of histone 3 (H3K27me2) [12]. In *Arabidopsis*, DNA methylation involves the methyltransferase MET1 and affects cystosine residues of CpGs [13]. Repression of transcription by methylation of lysine 27 on histone 3 (H3K27) is mediated by a PRC2 Pc-G complex identified in *Drosophila* [14–16]. This complex contains a core of four proteins,

the WD40 proteins Extra Sex Combs (ESC) and Chromatin Assembly Factor 1 (CAF1/P55), the VEFS domain protein Suppressor of zeste 12 (SU(Z)12), and the SET domain protein Enhancer of zeste (E(Z)) [17]. In *Arabidopsis*, all putative Pc-G complexes are predicted to comprise FERTILIZATION INDEPENDENT ENDOSPERM (FIE) and MULTICOPYSUPRESSOR OF IRA 1 (MSI1), homologous to *Drosophila* ESC and CAF1/P55, respectively [18, 19]. Depending on the cell type and function, the other two *Arabidopsis* Pc-G subunits are one of the three VEFS proteins, EMBRYONIC FLOWER 2 (EMF2), VERNALIZATION 2 (VRN2), or FERTILIZATION INDEPENDENT SEED 2 (FIS2), and one of the three SET domains proteins, CURLY LEAF (CLF), SWINGER (SWN), and MEDEA (MEA) [20, 21].

We hypothesized that the silencing mechanism responsible for absence of expression of *MEA* in vegetative tissues and in pollen was also active in endosperm and responsible for maintenance of *MEA* imprinting. We investigated whether silencing in vegetative tissues results from DNA or H3K27 methylation. Reduced activity of the maintenance DNA methyltransferase MET1 was shown to cause ectopic expression of *FWA* in vegetative tissues [22] and loss of imprinting of *FWA* in endosperm [4]. However, here we show that loss of function of *MET1* does not result in ectopic expression of *MEA* (Figure 1A). By contrast, we detected ectopic expression of *MEA* in vegetative tissues when Pc-G activity was compromised due to cosuppression of *FIE* (Figure 1A and [21]). Further, loss-of-function mutants for other members of the vegetative Pc-G complexes *EMF2, VRN2, CLF*, and *SWN* express ectopically *MEA* in vegetative tissues (Figure 1A). Similarly, the translational reporter *MEA-GUS* [10] was ectopically expressed in vegetative tissues of *FIE* cosuppressed plants (see Figures S1A–S1C in the Supplemental Data available with this article online). Upregulation of *MEA* transcription in vegetative tissues lacking Pc-G activity resulted in protein accumulation (Figure S1E). These results indicate that the Pc-G complexes repress *MEA* expression in vegetative tissues.

Methylation of lysine residue 27 of Histone 3 (H3K27) by Pc-G activity is conserved in *Arabidopsis* [23]. To elucidate the mechanism by which *MEA* expression is silenced in leaves, we performed chromatin immunoprecipitation (ChIP) analysis for H3K27 di- and trimethylation on different regions of the *MEA* locus in wild-type and in mutants defective for activity of Pc-G genes during vegetative development (Figure 1B). In the wild type, regions enriched in H3K27 methylation were mostly detected in the 3′ end of *MEA* coding sequence, although a low signal was also present at the 5′ end. H3K27 methylation was strongly reduced both in *FIE* cosuppressed plants and in the *clf/clf* mutant. We thus concluded that a vegetative Pc-G complex containing at least FIE and CLF is responsible for mediating *MEA* silencing by methylation of H3K27 on specific parts of *MEA* genomic region. Loss of this epigenetic mark leads to ectopic

*Correspondence: niro@tauex.tau.ac.il (N.O.); fred@tll.org.sg (F.B.)
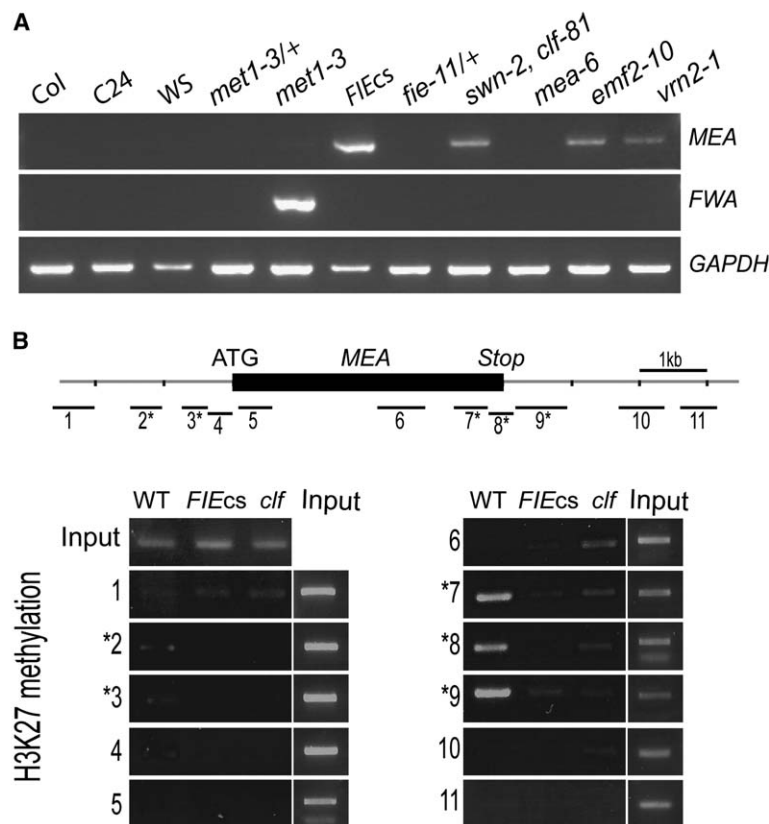
A

B

Figure 1. Silencing of *MEA* in Vegetative Tissues Depends on the Activity of Vegetative Pc-G Complexes

(A) RT-PCR analysis shows that imprinted genes *MEA* and *FWA* are not expressed in vegetative tissues of wild-type accessions Col, C24, and WS. Reduced methylation of DNA in null *met1-3* homozygous mutant plants causes ectopic expression of *FWA* in leaves. Reduction of the Pc-G activity in each of the following genetic backgrounds (*FIE* cosuppressed [*FIEcs*], *swn/swn,clf/clf* double mutant, and *emf2* and *vrn2* mutants) causes ectopic expression of *MEA* in leaves. As *MEA* is not expressed in vegetative tissue, we did not see any ectopic expression of *MEA* in *mea6* leaves, which served as an internal control. Equal amounts of template were calibrated according to the amount of GAPDH.

(B) ChIP analysis of H3K27 methylation on *MEA* genomic locus in wild-type, *FIEcs*, and *clf/clf* backgrounds. The equal amount of chromatin from the three genetic backgrounds was monitored by PCR ("Input," horizontal lane). The vertical "input" column demonstrates amplification conditions for each primer set on wild-type chromatin. H3K27me2, 3 is mostly detected in the promoter of *MEA* in the 3′ region of the coding sequence (fragments 7–9). H3K27me2, 3 is no longer detected in absence of Pc-G activity in *FIEcs* and *clf/clf* backgrounds.

expression of *MEA* in vegetative tissues (Figures 1A and 1B and Figure S1E). A regulatory role for the 3′ coding region of *MEA* is surprising. This region is mostly absent from the reporter *MEA-GUS*, which is not expressed in vegetative tissues and shows imprinted expression [10] but contains only the coding sequence up to the region corresponding to fragment 7 used for our ChIP analysis. Other *cis*-elements upstream of fragment 7 may dock Pc-G complexes, which could modify H3K27 in a larger area across the *MEA* coding sequence and regulate *MEA* transcriptional activity.

Pc-G Loss of Function in Paternal Tissues Prevents *MEA* Imprinting in Endosperm

We further tested whether Pc-G loss of function in vegetative paternal tissues causes not only ectopic expression of *MEA* but further prevents imprinting of *MEA* in endosperm. We used a polymorphic marker to distinguish between two wild-type accessions, RLD and Columbia (Col). This allowed us to detect the expression of each parental allele of *MEA* and to monitor *MEA* imprinting in endosperm resulting from crosses between RLD and Col genetic backgrounds [9]. Expression of *MEA* is detected mostly from the maternal allele, while that of the *MEA* paternal allele is detected at very low levels. However, the expression of the paternal allele of *MEA* increased dramatically when the paternal allele originated from *FIE* cosuppressed plants, leading to equal expression from both parents and loss of *MEA* imprinting (Figure 2A). This result was confirmed by the analysis of *MEA* paternal expression with the *MEA-GUS* reporter (Figures 2B and 2C). *MEA-GUS* provided paternally from a wild-type background was expressed at low levels

and at a low frequency. However, *FIE* cosuppression caused higher frequency of seeds expressing paternal *MEA-GUS* activity (Figures 2B and 2C). These results demonstrate that imprinting of *MEA* in endosperm depends on maintenance of H3K27 methylation by the PRC2 Pc-G complexes.

Plant gametes are produced after meiosis in stamen and ovules, which constitute the male and female reproductive structure, respectively. Stamens produce pollen, which delivers two male gametes to two female gametes in the ovule. The endosperm is produced by fertilization of one female gamete, the central cell. The other sperm cell fertilizes the egg cell, producing the embryo. During gametogenesis, the two parental alleles of *MEA* are subjected to distinct transcriptional regulations. *MEA* remains silent in pollen while its transcription is activated in the central cell [9, 10]. To investigate the role of Pc-G complexes in the maintenance of *MEA* silencing during pollen development and its impact on *MEA* imprinting, we have used heterozygous *fie/+* plants (Figure 1A). After meiosis, half of the haploid pollen produced by *fie/+* plants carry a *fie* allele. Since *FIE* is a single copy gene and encodes an essential function for Pc-G activity, pollen grains bearing a *fie* allele are expected to lack Pc-G function. In agreement with this hypothesis, the paternal *MEA* allele is expressed in endosperm resulting from fertilization of wild-type ovules with pollen from *fie/+* plants (Figure 2A). In addition, we observed ectopic expression of the reporter *MEA-GUS* in pollen grains of *FIEcs* plants (Figure S1D). These results indicate that the maintenance of *MEA* silencing during male gametogenesis depends on Pc-G activity in pollen and is essential for *MEA* imprinting in endosperm.
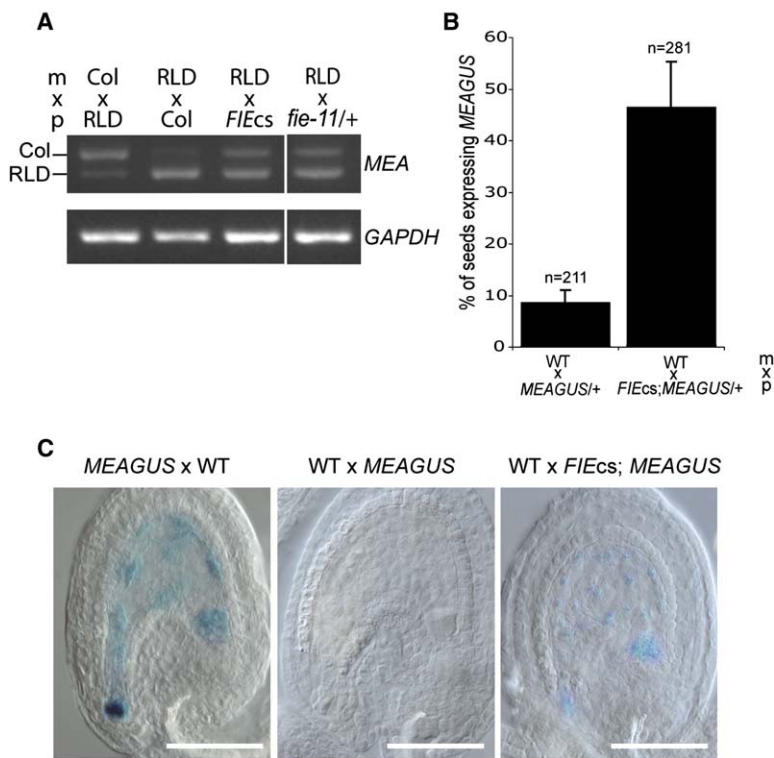
Figure 2. Imprinting of *MEA* in Endosperm Depends on the Paternal Activity of Pc-G Complexes

(A) *MEA* imprinting was analyzed by allele-specific RT-PCR at 2 days after pollination (DAP). Crosses between wild-type accessions RLD and Col show that *MEA* is imprinted with a predominant expression of the maternal allele. Removal of Pc-G activity in parental vegetative tissues of *FIE*cs plants causes expression of the paternal allele of *MEA*. Similarly, removal of Pc-G activity in half of pollen of *fie/+* plants causes expression of the paternal allele of *MEA*. Equal amounts of template were calibrated according to the amount of GAPDH. "p" and "m" represent paternal and maternal genotype, respectively.

(B and C) Removal of *FIE* activity in *FIE*cs plants causes expression of paternally provided *MEA-GUS* reporter. Wild-type female plants were crossed with male plants carrying the reporter *MEA-GUS* in wild-type or in *FIE*cs background. The genotype used as female is indicated first in all crosses throughout. Expression of *MEA-GUS* is detected in wild-type endosperm only when the reporter is provided maternally. Expression of paternally provided *MEA-GUS* is hardly detected. In contrast, paternal expression of *MEA-GUS* is strong when provided by a paternal *FIE*cs background. Crosses were observed at 1.5 DAP. Error bars indicate standard deviation, and the size of the sample (n) is indicated above each column (B). Scale bars correspond to 120 μm (C).

Silencing of the *MEA* Paternal Allele during Endosperm Development Depends on Self-Regulation by the Pc-G Complex Containing MEA

Genetic, molecular, and biochemical analyses have shown that the Pc-G complex active in endosperm comprises at least MEA, FIS2, FIE, and MSI1 [18, 19]. Maternal inheritance of loss-of-function alleles of any of these Pc-G genes causes abnormal development of endosperm [5, 19, 24–27]. We investigated the maternal requirement of Pc-G activity in maintaining *MEA* paternal allele silencing in endosperm. To this end, we tested the maternal effect of loss of function of *MEA*, *FIS2*, *FIE*, and *MSI1* on the expression of the paternally provided *MEA-GUS* reporter. The paternal *MEA-GUS* copy is expressed at very low levels and frequency in seeds resulting from crosses with wild-type plants used as females (Figures 2C and 3A). In contrast, high expression of the paternal *MEA-GUS* copy was observed in nearly half of the seeds resulting from crosses with *fie/+* plants used as females (Figure 3A). *MEA-GUS* reporter expression was also observed when *mea/+*, *fis2/+*, and *msi1/+* were used as females (Figure 3A), and the frequency of seeds with paternal *MEA-GUS* expression correlated with the penetrance of each mutation [19]. Similarly, the paternal allele of *MEA* became expressed when *fie* was maternally provided and Pc-G function was inactive during early endosperm development (Figure 3B, 2 DAP). We thus conclude that the MEA/FIS2/FIE/MSI1 Pc-G complex acting in the endosperm is necessary for maintaining silencing of the *MEA* paternal allele through a negative feedback regulation.

Removal of H3K27 methylation mediated by loss of Pc-G activity results either from an active loss of histone methylation or from a passive dilution of the epigenetic mark after DNA replication as shown for some histone methylation marks in budding yeast [28]. In support of the latter scenario, expression of *MEA* paternal allele in *fie* maternal background was detected only after 2 DAP and thus could be explained by a progressive loss of silencing (Figure 3B). Endosperm development is initiated by a series of synchronous cell cycles in the absence of cytokinesis leading to the formation of a syncytium [29]. We monitored expression of the paternally provided *MEA-GUS* after every round of cell cycle in *fie* background. Paternal *MEA-GUS* expression in endosperm was detected only after the third cycle of DNA replication and increased progressively (Figures 3C–3G). Thus, in the absence of Pc-G activity, a gradual dilution of the methylated H3K27 mark likely takes place through consecutive cycles of DNA replication.

In conclusion, the repressive H3K27 methylation causes silencing of *MEA* expression in vegetative tissues and pollen and plays a central role in the control of *MEA* imprinting in endosperm. The expression of *MEA* maternal allele in endosperm is the result of transcriptional activation of *MEA* in the central cell prior to fertilization [30]. A similar mechanism causes *FWA* imprinted status in endosperm, and it was shown that DNA methylation of *FWA* promoter is removed during the activation of its maternal allele in correlation with the activity of the DNA glycosylase DEMETER (DME) [4]. Similarly, *MEA* expression in the central cell depends
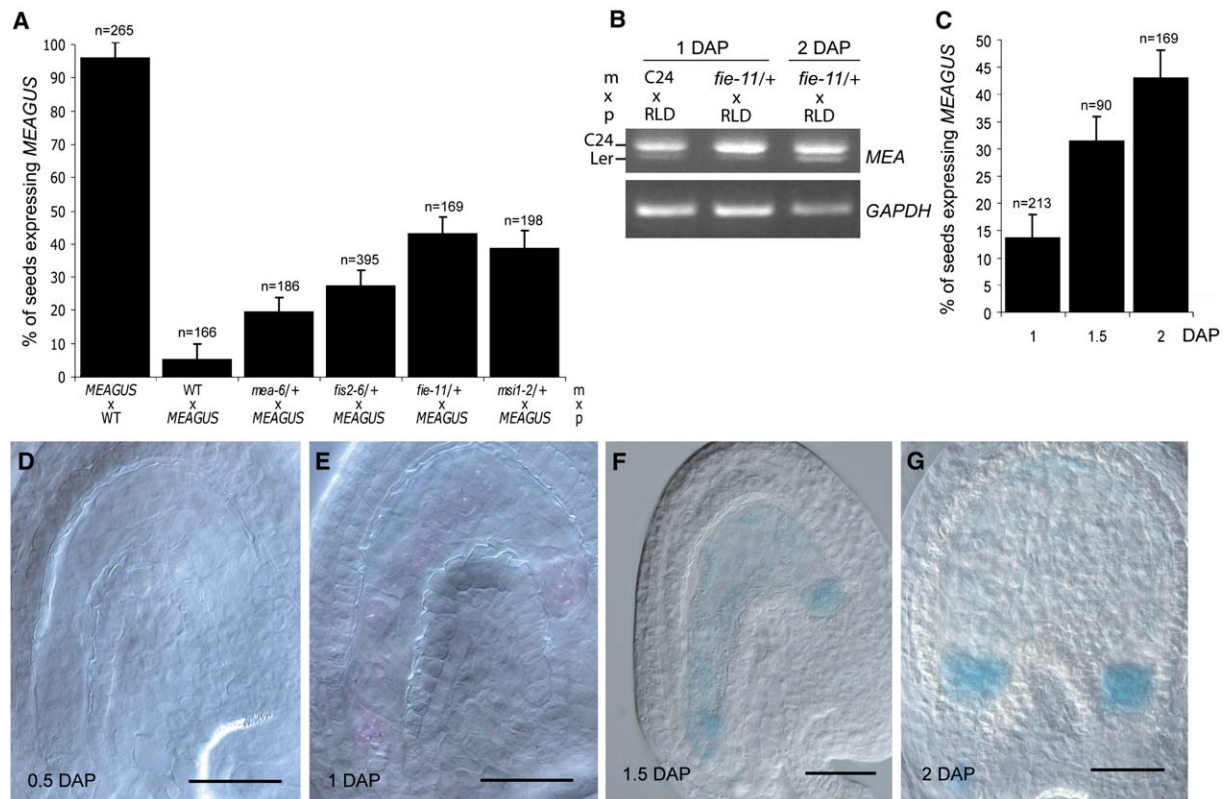
Figure 3. Maintenance of *MEA* Paternal Allele Silencing in Endosperm Requires the Activity of the Maternal Pc-G Complex

(A) By contrast to wild-type endosperm where *MEA-GUS* is not expressed from the paternal allele, *mea, fis2, fie,* and *msi1* mutants deficient for the activity of the Pc-G complex show expression of the paternal allele of *MEA-GUS* in endosperm at 2 DAP.

(B) Expression of the paternal allele of *MEA* in crosses between *fie-11/+* ovules and wild-type pollen. Allele-specific RT-PCR shows that crosses between ovules of *fie-11/+* plants (C24 accession) and wild-type RLD pollen express high amounts of the paternal *MEA* allele at 2 DAP but not at 1 DAP. The control for 2 DAP is shown in Figure 2A. This indicates a progressive loss of silencing of the *MEA* paternal allele in *fie* endosperm. GAPDH is used as a control.

(C–G) Progressive recovery of paternal *MEA-GUS* activity after pollination of *fie-11/+* ovules. (C) The percentage of seeds expressing MEA-GUS in crosses between ovules of *fie-11/+* plants and *MEA-GUS* pollen increases during endosperm development. Endosperm at 1, 1.5, and 2 DAP contains approximately 8, 16, and 28–50 nuclei, respectively. No expression of *MEA-GUS* is observed before the endosperm has undergone three rounds of nuclei division and contains 8 nuclei as shown in (D). When the endosperm contains 8 nuclei (E), a faint expression of paternally provided *MEA-GUS* is observed as red crystals in dark field. *MEA-GUS* expression observed in DIC microscopy (blue staining) increases during successive rounds of nuclei divisions ([F], 16 nuclei, and [G], 50 nuclei). Error bars indicate standard deviation and the size of the sample (n) is indicated in each column. Scale bars equal 100 μm (D–G).

on DME [31], and DNA methylation was suggested to regulate *MEA* imprinting [2, 3]. In support to the latter hypothesis, DDM1, which controls indirectly DNA methylation, behaves as a genetic modifier of *mea* [8] and the *MEA* promoter contains regions methylated by MET1 [7]. However, the degree of methylation varies depending on wild-type accessions [7], there was no demonstration of loss of *MEA* paternal allele silencing in *met1* background [6], and genetic analyses did not support a direct involvement of DNA methylation by MET1 in the control of *MEA* imprinting [10]. We conclude that MET1 probably plays a secondary role in the regulation of *MEA* imprinting, and we propose three hypotheses to explain the activation of *MEA* expression by DME. (1) DME removes both cytosine methylation from *FWA* promoter and histone methylation from the *MEA* locus as a result of DNA repair mechanisms after the endonuclease activity of DME. (2) Genetic evidence indicates antagonist controls exerted by *DME* and *MET1* on *MEA* expression in the central cell [7] and suggests that DME

acts indirectly on *MEA* via a DNA methylation-dependent imprinted gene, which controls histone methylation. (3) Loss of DNA methylation from *MEA* promoter may be a prerequisite for the action of another mechanism specific for the removal of methylated H3K27.

Our results lead to a model where a relay of Pc-G activities maintains epigenetic modifications of both alleles at the *MEA* locus during vegetative growth and male gametogenesis (Figure 4). After fertilization, *MEA* imprinting in endosperm depends on a negative self-regulation of silencing of *MEA* paternal allele mediated by the maternally expressed allele of *MEA*. Such a feedback loop also causes repression of both alleles of *MEA* later during endosperm development [32]. Similar controls could apply to the paternal expression of genes essential for the intake of maternal reserves as suggested for *PHERES1* [24, 33]. Flowering plants and mammals are characterized by a prominent maternal control of reserves delivery to the embryo [34]. According to the "kinship" theory, the maternal care provided to embryo
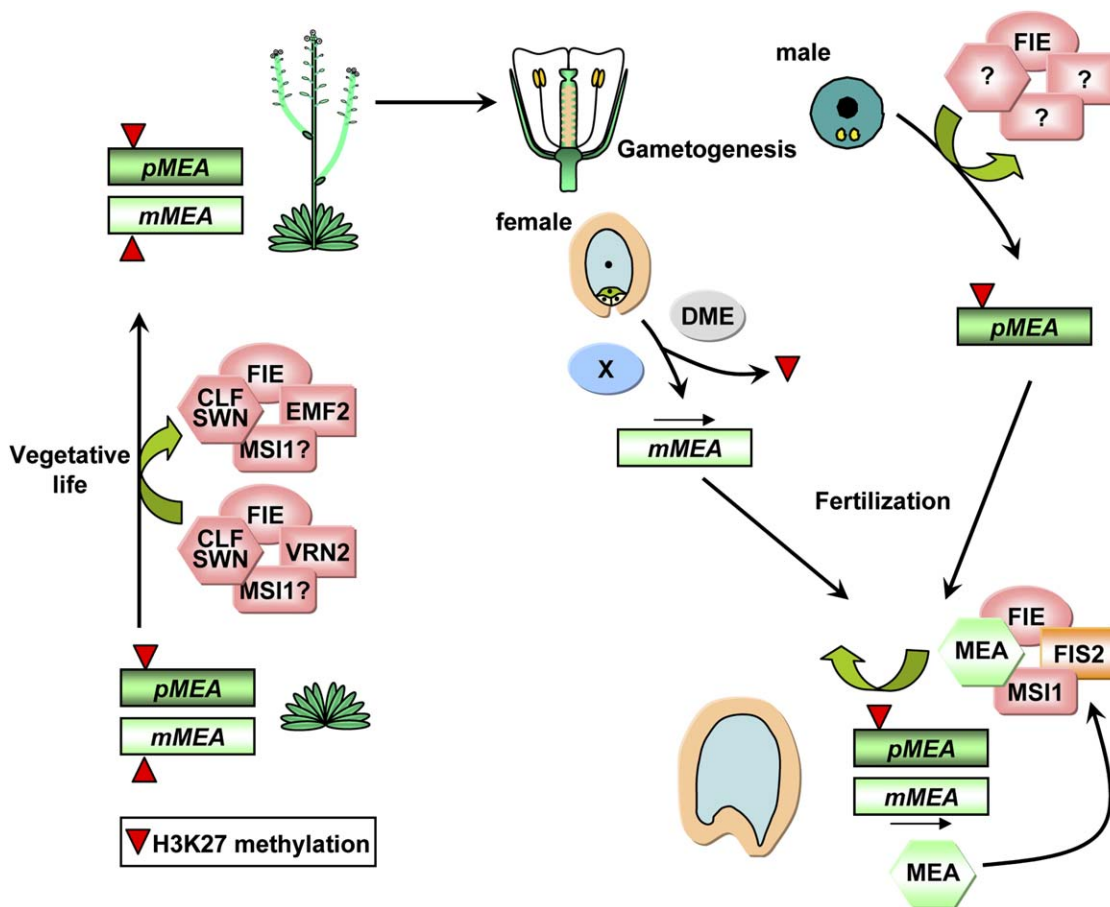
Figure 4. A Model for a Control of Parental Genomic Imprinting by Histone H3K27 Methylation in Plants

The Polycomb group (Pc-G) gene *MEA* is imprinted in endosperm and is subjected to silencing by sustained methylation of H3K27 by Pc-G complexes (red triangle). Pc-G complexes, which are active during the vegetative phase, silence *MEA* expression. *MEA* silencing is maintained during male gametogenesis by a Pc-G complex of unknown identity and passed on to endosperm. This phenomenon could also affect additional loci of Pc-G targets expressed in endosperm such as *PHERES1* [24, 33]. Such a memory of histone modification has been recently supported in mammals by evidence for semiconservative replication of histone methylation during DNA replication [40, 41]. Since the *MEA* Pc-G complex controls endosperm growth and proliferation [32, 42], we hypothesize that the *MEA* Pc-G activity may affect the capacity of endosperm to act as a sink for the maternal nutrients (source). Our results suggest that integrated epigenetic modifications of the *MEA* locus in endosperm may allow a balance between the maternal resources available with those requirements imposed by the developing seed. *MEA* expression is activated during female gametogenesis by the DNA glycosylase DEMETER (DME) either directly or by an unknown factor (X) capable of removal of the methyl group on H3K27me, which in turn leads to the expression of the maternal allele in endosperm after fertilization. The maintenance of silencing of the *MEA* paternal allele in endosperm is obtained by a negative feedback mechanism involving the Pc-G complex containing *MEA*. This mechanism facilitates a maternal control of the paternal expression of *MEA*.

development would favor selection of molecular mechanisms, which reflect the unequal involvement between the two parents in sexual reproduction. This would have caused imbalanced epigenetic regulation of genes essential for embryo nutrition and thus favored positive selection of parental genomic imprinting. Our observations provide direct support for this hypothesis.

Conclusions

We have shown that Pc-G activities regulate imprinting in flowering plants by silencing one of the parental allele throughout the plant life cycle while the other allele becomes activated by still undefined mechanisms. This regulation targets the *MEA* Pc-G gene via self-regulation. The feedback loop controlling *MEA* imprinting may represent direct evidence for a template from which imprinting evolved in flowering plants. Parental genomic imprinting has been initially characterized in mammals

[35, 36], and several imprinted loci are essential for the development of nonembryonic placenta supporting embryo growth [1, 3, 37]. Imprinted genes essential for mouse placental development are controlled by maintenance of DNA methylation or by H3K27 methylation [38, 39]. The selection of the same epigenetic mechanisms for the control of imprinting in endosperm provides direct evidence for a convergent evolution for parental genomic imprinting in both kingdoms.

References

1. Berger, F. (2004). Plant sciences. Imprinting—a green variation. Science *303*, 483–485.

2. Gehring, M., Choi, Y., and Fischer, R.L. (2004). Imprinting and seed development. Plant Cell *16* (Suppl), S203–S213.

3. Köhler, C., and Grossniklaus, U. (2005). Seed development and genomic imprinting in plants. Prog. Mol. Subcell. Biol. *38*, 237–262.

4. 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.

5. 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.

6. Spillane, C., Baroux, C., Escobar-Restrepo, J.M., Page, D.R., Laoueille, S., and Grossniklaus, U. (2004). Transposons and tandem repeats are not involved in the control of genomic imprinting at the *MEDEA* locus in *Arabidopsis*. Cold Spring Harb. Symp. Quant. Biol. *69*, 465–475.

7. 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.

8. 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.

9. 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.

10. 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.

11. Tariq, M., Saze, H., Probst, A.V., Lichota, J., Habu, Y., and Paszkowski, J. (2003). Erasure of CpG methylation in *Arabidopsis* alters patterns of histone H3 methylation in heterochromatin. Proc. Natl. Acad. Sci. USA *100*, 8823–8827.

12. Mathieu, O., Probst, A.V., and Paszkowski, J. (2005). Distinct regulation of histone H3 methylation at lysines 27 and 9 by CpG methylation in *Arabidopsis*. EMBO J. *24*, 2783–2791.

13. Finnegan, E.J., Peacock, W.J., and Dennis, E.S. (2000). DNA methylation, a key regulator of plant development and other processes. Curr. Opin. Genet. Dev. *10*, 217–223.

14. Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A., and Pirrotta, V. (2002). Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell *111*, 185–196.

15. Müller, J., Hart, C.M., Francis, N.J., Vargas, M.L., Sengupta, A., Wild, B., Miller, E.L., O'Connor, M.B., Kingston, R.E., and Simon, J.A. (2002). Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. Cell *111*, 197–208.

16. Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R.S., and Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science *298*, 1039–1043.

17. Tie, F., Prasad-Sinha, J., Birve, A., Rasmuson-Lestander, A., and Harte, P.J. (2003). A 1-megadalton ESC/E(Z) complex from *Drosophila* that contains polycomblike and RPD3. Mol. Cell. Biol. *23*, 3352–3362.

18. Köhler, C., Hennig, L., Bouveret, R., Gheyselinck, J., Grossniklaus, U., and Gruissem, W. (2003). *Arabidopsis* MSI1 is a component of the MEA/FIE Polycomb group complex and required for seed development. EMBO J. *22*, 4804–4814.

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

20. 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.

21. 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.

22. Saze, H., Scheid, O.M., and Paszkowski, J. (2003). Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. Nat. Genet. *34*, 65–69.

23. Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A., and Dean, C. (2004). Vernalization requires epigenetic silencing of *FLC* by histone methylation. Nature *427*, 164–167.

24. Köhler, C., Hennig, L., Spillane, C., Pien, S., Gruissem, W., and Grossniklaus, U. (2003). The Polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene *PHERES1*. Genes Dev. *17*, 1540–1553.

25. Hsieh, T.F., Hakim, O., Ohad, N., and Fischer, R.L. (2003). From flour to flower: how Polycomb group proteins influence multiple aspects of plant development. Trends Plant Sci. *8*, 439–445.

26. 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.

27. Chaudhury, A.M., Ming, L., 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.

28. Katan-Khaykovich, Y., and Struhl, K. (2005). Heterochromatin formation involves changes in histone modifications over multiple cell generations. EMBO J. *24*, 2138–2149.

29. Boisnard-Lorig, C., Colon-Carmona, A., Bauch, M., Hodge, S., Doerner, P., Bancharel, E., Dumas, C., Haseloff, J., and Berger, F. (2001). Dynamic analyses of the expression of the HISTONE::YFP fusion protein in *Arabidopsis* show that syncytial endosperm is divided in mitotic domains. Plant Cell *13*, 495–509.

30. 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.

31. 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.

32. Ingouff, M., Haseloff, J., and Berger, F. (2005). Polycomb group genes control developmental timing of endosperm. Plant J. *42*, 663–674.

33. Köhler, 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.

34. Haig, D. (2004). Genomic imprinting and kinship: how good is the evidence? Annu. Rev. Genet. *38*, 553–585.

35. Delaval, K., and Feil, R. (2004). Epigenetic regulation of mammalian genomic imprinting. Curr. Opin. Genet. Dev. *14*, 188–195.

36. Constancia, M., Kelsey, G., and Reik, W. (2004). Resourceful imprinting. Nature *432*, 53–57.

37. Reik, W., and Lewis, A. (2005). Co-evolution of X-chromosome inactivation and imprinting in mammals. Nat. Rev. Genet. *6*, 403–410.

38. Lewis, A., Mitsuya, K., Umlauf, D., Smith, P., Dean, W., Walter, J., Higgins, M., Feil, R., and Reik, W. (2004). Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. Nat. Genet. *36*, 1291–1295.

39. Umlauf, D., Goto, Y., Cao, R., Cerqueira, F., Wagschal, A., Zhang, Y., and Feil, R. (2004). Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. Nat. Genet. *36*, 1296–1300.

40. Tagami, H., Ray-Gallet, D., Almouzni, G., and Nakatani, Y. (2004). Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell *116*, 51–61.

41. Nakatani, Y., Ray-Gallet, D., Quivy, J.P., Tagami, H., and Almouzni, G. (2004). Two distinct nucleosome assembly pathways: dependent or independent of DNA synthesis promoted by histone H3.1 and H3.3 complexes. Cold Spring Harb. Symp. Quant. Biol. *69*, 273–280.

42. Kiyosue, T., Ohad, N., Yadegari, R., Hannon, M., Dinneny, J., Wells, D., Katz, A., Margossian, L., Harada, J.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.