Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Genomic imprinting regulates establishment and release of seed dormancy

Hikaru Sato[1,a] and Claudia Köhler[1,2]

Abstract

Seed dormancy enables plant seeds to time germination until environmental conditions become favorable for seedling survival. This trait has high adaptive value and is of great agricultural relevance. The endosperm is a reproductive tissue formed after fertilization that in addition to support embryo growth has major roles in establishing seed dormancy. Many genes adopt parent-of-origin specific expression patterns in the endosperm, a phenomenon that has been termed genomic imprinting. Imprinted genes are targeted by epigenetic mechanisms acting before and after fertilization. Recent studies revealed that imprinted genes are involved in establishing seed dormancy, highlighting a new mechanism of parental control over this adaptive trait. Here, we review the regulatory mechanisms establishing genomic imprinting and their effect on seed dormancy.

Addresses

[1] Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Centre for Plant Biology, Uppsala, Sweden

[2] Department of Plant Reproductive Biology and Epigenetics, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

Corresponding author: Köhler, Claudia (Koehler@mpimp-golm.mpg.de)

[a] Current address: Department of Integrated Biosciences, The University of Tokyo, Chiba, Japan.

Current Opinion in Plant Biology 2022, **69**:102264

This review comes from a themed issue on **Epigenetics and gene regulation (2022)**

Edited by **Dr. Bob Schmitz** and **Dr. Ortrun Mittelsten Scheid**

For complete overview of the section, please refer the article collection - Epigenetics and gene regulation (2022)

Available online 21 July 2022

https://doi.org/10.1016/j.pbi.2022.102264

Keywords

Endosperm, Seed, Dormancy, Imprinting, Histone modifications, DNA methylation.

Introduction

Seed dormancy is defined as the inability of a viable seed to germinate under favorable conditions [1,2]. This trait prevents seed germination under short periods of favorable conditions within unfavorable seasons and is therefore of high adaptive value and great agricultural relevance [3,4]. Seed dormancy is established during seed maturation, in which seeds obtain dehydration tolerance and longevity through the accumulation of seed storage proteins and abscisic acid (ABA), the major hormone connected with seed dormancy establishment [5,6]. After seed maturation is completed, seed dormancy is gradually released by after-ripening (dry storage conditions) or stratification (incubation under cold and moist conditions) [7,8]. The regulation of the balance between establishment and release of seed dormancy is critically important to maximize the adaptive capacity of plants and is thus a major factor controlling yield. Low dormancy may cause precocious germination of seeds on the maternal plant (vivipary, or pre-harvest sprouting), resulting in reduced seed quality and storage capacity [8]. Conversely, high dormancy may lead to non-uniform germination, which in an agricultural context is problematic by its negative effect on harvesting schedules [9].

Seeds of flowering plants are composed of three major tissues; the embryo, the endosperm and the seed coat. While the seed coat is a sporophytic tissue derived from the maternal integuments, the embryo and the endosperm are formed in the process of double fertilization, whereby one of the two sperm cells fertilizes the haploid egg cell, giving rise to the diploid embryo and the other sperm cell fertilizes the predominantly diploid central cell, giving rise to the triploid endosperm [10]. The endosperm has critical roles in supporting embryo and seed coat growth, dormancy establishment and germination [11–13]. These functions are in part governed by epigenetically modified genes with parental-specific expression in the endosperm [14–16]. Here, we discuss our current understanding of how parental-specific gene expression is established and overview recently discovered epigenetic mechanisms that control seed dormancy in a parental-specific manner.

Establishment of genomic imprinting in the endosperm through epigenetic mechanisms in the gametes

Parental-specific gene expression patterns after fertilization are a consequence of epigenetic mechanisms

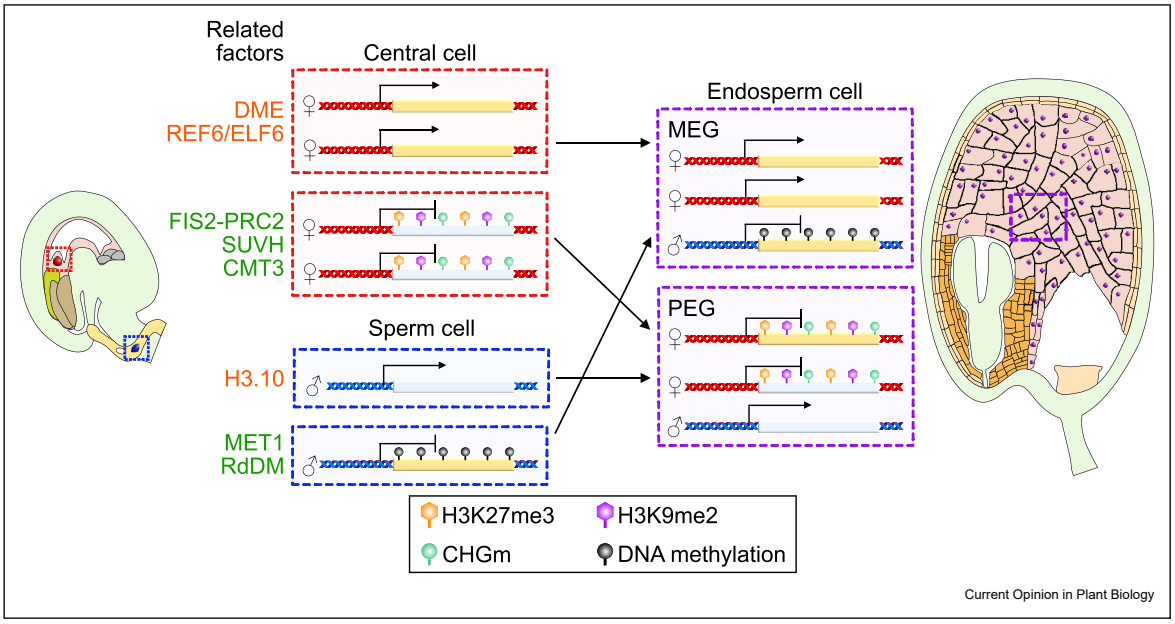
acting before fertilization during male and female gametogenesis (Figure 1) [17,18]. The resulting asymmetric epigenetic modifications are referred to as imprints [19] that cause alleles to be either maternally active and paternally silenced (referred to as maternally expressed genes (MEGs)), or paternally active and maternally silenced (referred to a paternally expressed genes (PEGs)). Below we discuss the currently known main mechanisms establishing imprinted gene expression.

Establishment of MEGs

Methylation of DNA at the C-5 position of cytosine is a typical silencing mark that in plants occurs in three different sequence contexts; CG, CHG, and CHH, whereby H corresponds to A, T or G [20]. Removal of 5-methylcytosine via base excision repair-dependent DNA demethylation is mediated by DEMETER-like DNA glycosylases [21]. The name-giving DNA glycosylase DEMETER (DME) is active in the central cell of the female gametophyte but not in sperm cells [22], resulting in maternally-biased gene expression due to local hypomethylation of the maternal alleles of MEGs in the endosperm [23,24]. Importantly, DME activates two genes encoding components of the central cell and endosperm-specific FERTILIZATION INDEPENDENT SEED (FIS)-Polycomb Repressive Complex 2 (PRC2), *FIS2* and *MEDEA* (*MEA*) [25,26]. The FIS-PRC2 establishes trimethylation marks on lysine 27 of histone H3 (H3K27me3) on the maternal alleles of PEGs [17], causing their repression (details are described below). Demethylation of H3K27me3 is mediated by Jumonji-type (JMJ) histone demethylases that act antagonistically to the PRC2 [27]. Recent work revealed that the JMJ histone demethylases RELATIVE OF EARLY FLOWERING 6 (REF6) and EARLY FLOWERING 6 (ELF6) control seed dormancy by activating the maternal alleles of MEGs [28–30].

Suppression of the paternal alleles of MEGs is mediated by the methyltransferase MET1 that maintains methylation in CG context during replication. Loss of paternal MET1 function is sufficient to activate the paternal alleles of MEGs and thus abolishes their maternally-biased expression [16]. In addition to MET1-mediated CGm, also the RNA-directed DNA methylation (RdDM) pathway in the vegetative and sperm cells is important to suppress paternal alleles of MEGs; a process which likely involves cell-to-cell movement of small interfering RNAs (siRNAs) [31,32]. In this pathway, the activity of DME and REPRESSOR OF SILENCING 1 (ROS1) in the pollen vegetative cell leads to excision of methylated cytosines on transposable elements (TEs), causing activation of TE expression and production of small

Figure 1



Model depicting how parental-specific gene expression is established. To establish MEGs, DME and REF6/ELF6 activate maternal alleles by removing DNA methylation and H3K27me3, respectively, in the central cell, while the paternal alleles are silenced by DNA methylation established by MET1 and the non-canonical RdDM pathway in the sperm cell. To establish PEGs, FIS2-PRC2, SUVH family proteins and CMT3 silence maternal alleles by applying endosperm-specific triple repressive marks (H3K27me3, H3K9me2, and CHGm) in the central cell, while the sperm cell-specific histone variant H3.10 is incorporated into the paternal genome and inhibits deposition of H3K27me3. The amino acid divergence at lysine27 in H3.10 prevents PRC2 targeting and keeps the paternal alleles active. Activating and repressing epigenetic factors establishing asymmetric epigenetic marks before fertilization are shown in orange and green letters, respectively.

interfering RNAs (siRNAs) that move from the vegetative cell to the sperm cells to establish CHHm on target regions [33,34]. These siRNAs preferentially accumulate at MEG loci in sperm cells [33].

Establishment of PEGs

In addition to the function of DME to directly activate genes encoding PRC2 components in the central cell, the removal of DNA methylation by DME contributes to the spread of the PRC2-mediated deposition of H3K27me3 since DNA methylation and H3K27me3 are mutually antagonistic [23,35,36]. Maternal-specific H3K27me3 was found to associate with paternally-biased expression [23], which was extended by recent work revealing that in addition to H3K27me3, the presence of dimethylation on lysine 9 of histone H3 (H3K9me2) and CHGm on the maternal alleles are hallmarks for stable paternally-biased expression [14,23,37]. Loss of the PRC2 component FERTILIZATION INDEPENDENT ENDOSPERM (FIE) causes depletion of CHGm in the endosperm, indicating that the establishment of CHGm on PEGs depends on FIS-PRC2 activity [14]. In vegetative tissues, CHGm is established by the chromo-methyltransferase CMT3 that acts in a feedback loop with SU(VAR)3−9 homologous proteins (SUVHs) catalyzing H3K9me2 [38,39]. Nevertheless, by which mechanism CMT3 and SUVHs are recruited to PEGs in the endosperm remains to be established.

The active status of the paternal alleles of PEGs is connected to an epigenetic resetting mechanism acting in sperm, causing removal of H3K27me3. One key component of this epigenetic resetting is the sperm cell-specific histone variant H3.10 that is replaces the histone variants H3.1 and H3.3. H3.10 is highly divergent around lysine 27 and therefore not targeted by PRC2 [40]. This, together with the activity of H3K27me3 demethylases in sperm, causes removal of H3K27me3 at most positions of the sperm genome [41]. After fertilization, the MADS-box transcription factor PHERES1 (PHE1) binds and activates the paternal PEG alleles, likely preventing them to be silenced by PRC2 activity after fertilization [42]. Whether or not resetting of H3K27me3 is required to allow PHE1 to activate its targets remains to be shown.

Genomic imprinting controlling seed dormancy

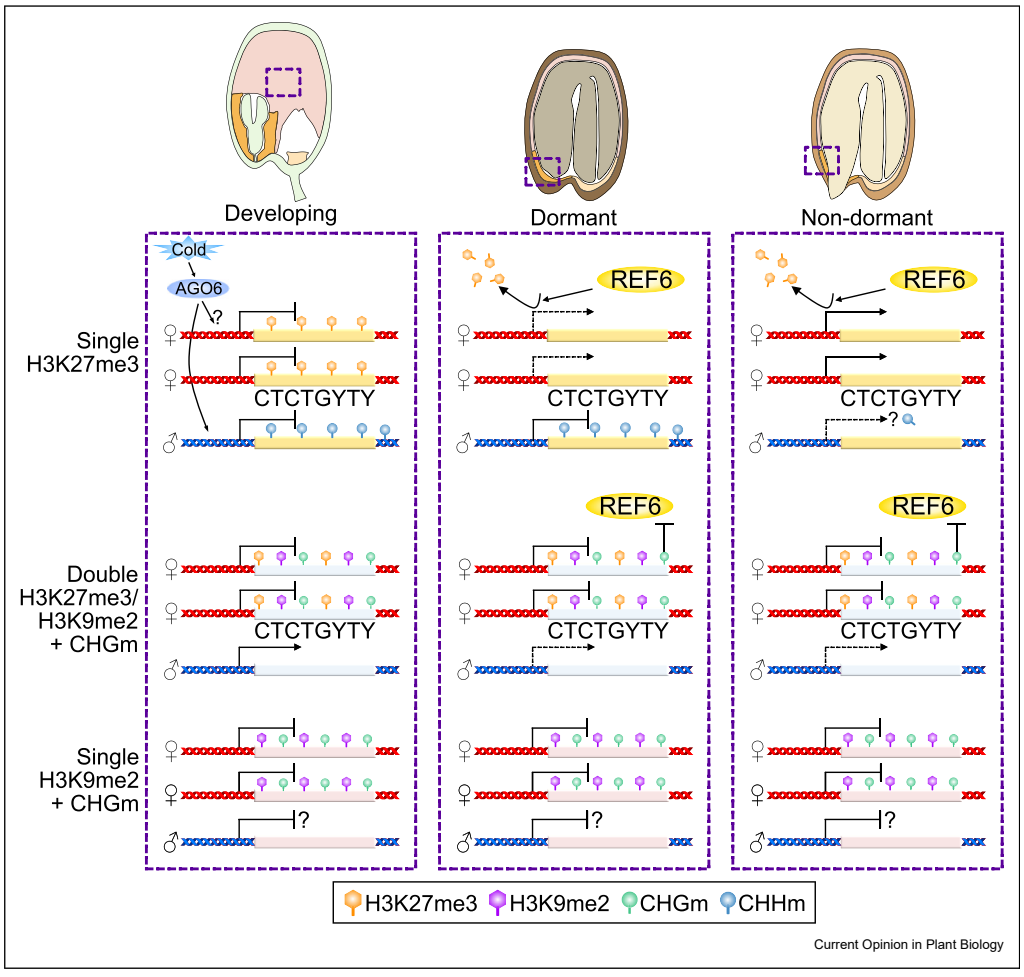
As outlined above, stable paternally-biased expression is more strongly associated with the presence of triple repressive marks H3K27me3, H3K9me2, and CHGm (referred to as H3K27me3/H3K9me2/CHGm) on the maternal alleles compared to single H3K27me3 [14], suggesting that there are molecular mechanisms distinguishing both patterns of epigenetic marks. Indeed, it was found that different combinations of epigenetic marks determine the activity status of the maternal alleles in the developing endosperm of Arabidopsis. While maternal alleles marked by triple repressive marks tend to remain silenced during endosperm development, alleles marked by H3K27me3 alone are likely to become activated and play important roles in controlling seed dormancy [28]. In the following, we will discuss the regulation of genes marked by parental-specific epigenetic modifications in the endosperm and their role in controlling seed dormancy in a parental-specific manner (Figure 2).

Genes with single H3K27me3 on maternal alleles control seed dormancy

Many genes marked by single H3K27me3 were found to be induced during germination and to be enriched for functions related to ethylene responses [28]. Ethylene is known to accelerate germination through cell-loosening [13], suggesting that removal of H3K27me3 has a functional role in promoting germination by activating ethylene responses. In support of this notion, mutants in the H3K27me3 demethylases REF6/ELF6 exhibit increased seed dormancy and decreased expression of many genes marked by single H3K27me3 [28,29]. Interestingly, genes with single H3K27me3 show maternally-biased expression patterns in dormant, but not in non-dormant seeds [28,30], which is connected with the accumulation of CHHm on the paternal alleles [43]. Cold-induced CHHm is mediated by the non-canonical RdDM pathway [44] and cold sensitivity correlates with cold-induced ARGONAUTE 6 (AGO6) accumulation [43]. This data suggests that maternal-specific control of seed dormancy is mediated by two distinct epigenetic mechanisms; the removal of H3K27me3 causing activation of maternal alleles and the cold-induced silencing of the maternal alleles by CHHm establishment. Thus, parental-specific epigenetic marks can give rise to parental-specific expression patterns not just after fertilization, but also at later stages of seed development to exert parental-specific effects on seed development (Figure 2).

The time when REF6/ELF6 act inthe central cell and endosperm to remove H3K27me3 remains to be resolved. The REF6 targets *CYP707A1* and *CYP707A3* encode cytochrome P450 monooxygenases that induce germination through catabolizing abscisic acid (ABA) [29,45]. Both genes are significantly downregulated in the *ref6* mutant endosperm during germination [28] and *CYP707A3* shows maternally-biased expression in the developing endosperm [23,24]. However, both genes have no detectable H3K27me3 on the maternal alleles in 4 DAP endosperm [23], indicating that REF6 acts before 4 DAP, possibly in the central cell before fertilization. Similarly, two MEGs with known roles in seed dormancy, *CYSTEINE PROTEASE1* (*CP1*) and *ALLANTOINASE* (*ALN*) [30,43] have no detectable H3K27me3 on the

Figure 2



Model depicting how different combinations of epigenetic modifications on parental alleles affect gene expression in the endosperm and control seed dormancy. Genes whose maternal alleles are suppressed by FIS-PRC2-mediated H3K27me3 encode ethylene-pathway genes that are necessary to induce germination. The paternal alleles of those genes are likely suppressed by CHHm established by the non-canonical RdDM pathway. Cold stress-inducible AGO6 may also target maternal alleles and apply cold stress-responsive CHHm by the non-canonical RdDM pathway. REF6 binds to the CTCTGYTY motif (Y = C or T) to activate the target genes. In dormant seeds, the maternal alleles are activated by the H3K27me3 demethylase REF6 and the genes show maternally biased expression patterns. In non-dormant seeds, the genes are biallelically expressed, likely because the RdDM pathway is specifically active under dormancy-inducing conditions. FIS-PRC2, SUVH family proteins and CMT3 establish H3K27me3, H3K9me2, and CHGm, respectively, on maternal alleles of PEGs. Because CHGm on the REF6-binding site inhibits binding activity of REF6, the maternal alleles are continuously silenced, and the genes show paternally biased gene expression patterns throughout endosperm development including germination. ABI3, an important transcription factor establishing seed dormancy belongs to this category. Genes with single H3K9me2 on the maternal alleles are fully silenced throughout development. These maternal-specific epigenetic modifications are detected in developing endosperm at 4 DAP; the epigenetic status before 4 DAP remains to be explored.

maternal alleles in 4 DAP endosperm [23]. Like *CYP707A1* and *CYP707A3*, both genes are downregulated in *ref6* mutant endosperm during germination [28], suggesting their REF6-mediated activation occurs before 4 DAP. A possible role of REF6 in the central cell of the female gametophyte is also supported by genetic data showing that maternal homo- and heterozygous *ref6* mutants have increased seed dormancy [28]. Nevertheless, since REF6 targets marked by H3K27me3 are detected at 4 DAP indicates that there are at least two different timepoints of REF6 action; one before fertilization and one at later stages of endosperm development. Further analyses are required to determine the time of REF6 action and to understand the difference between early and late REF6 targets.

Genes with triple H3K27me3/H3K9me2/CHGm repressive marks remain stably imprinted

In contrast to genes with single H3K27me3 that become activated during germination by REF6/ELF6 activity, the presence of triple repressive marks H3K27me3/H3K9me2/CHGm likely prevents REF6/ELF6 to target

and activate those genes [28], consistent with the known role of CHGm to prevent binding activity of REF6 [46]. In support of this view, loss of H3K9me2 and CHGm in mutants of *SUVH4*, *SUVH5*, and *SUVH6* (*suvh456*) causes upregulation of genes with triple repressive marks in the endosperm during germination. Among those upregulated genes is the critical dormancy regulator *ABA INSENSITIVE 3* (*ABI3*) [28,47], which is likely responsible for increased dormancy in *suvh456* and *suvh45* mutants [28,48]. Together, this data suggests that there is an endosperm-specific double control layer established by H3K27me3/H3K9me2/CHGm that prevents gene activation during germination. The maternal alleles of genes containing triple modifications will remain repressed during germination; in contrast, genes marked by single H3K27me3 can become activated during germination and have important roles in controlling this process (Figure 2). This mechanism ensures maternal control over seed dormancy and thus contributes to maximize reproductive success. The key difference between genes that become activated by REF6/ELF6 and those that do not is the presence of H3K9me2/CHGm on H3K27me3 marked alleles, pointing at a key role of SUVH456 and CMT3 in controlling seed dormancy. Furthermore, it is also worth noting that a small ratio of genes with triple marks is activated during germination, suggesting an unknown molecular mechanism removing this epigenetic repression. Nevertheless, genome-wide epigenetic profiles of the endosperm during germination are required to fully understand the dynamics of epigenetic modifications and their consequences on seed dormancy.

Conclusions and future perspectives

Seed dormancy is a critical trait for both wild plants and agricultural crops, and epigenetic regulators take a central position in establishing and releasing seed dormancy [49,50]. Epigenetic effects regulating seed dormancy take place in the endosperm, a unique seed tissue with parental-specific gene expression patterns throughout its development [16,30]. This contrasts with the largely biallelic expression in the embryo [51], suggesting that the central cell/endosperm is the critical place where maternally determined dormancy control is established. The endosperm differs from other tissues by the co-occurrence of repressive epigenetic modifications, H3K27me3 and H3K9me2/CHGm that in sporophytic tissues generally do not co-occur [14], raising the questions of the underlying mechanism leading to the establishment of this particular epigenetic signature. Since seed dormancy is regulated by environmental conditions [2], another important open question is whether and how parental-specific epigenetic modifications and genomic imprinting are affected by different environmental conditions. Future research unveiling these relationships will provide exiting new insights into dormancy control and its underlying epigenetic mechanisms.

Disclosure statement

Given her role as Editor-in-Cheif, Claudia Kohler had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Ortrun Mittelsten Scheid.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Acknowledgments

This review was supported by grants from the Swedish Research Council VR (2017−04119, to CK), a grant from the Knut and Alice Wallenberg Foundation (2018−0206, to CK), a grant from the Göran Gustafsson Foundation for Research in Natural Sciences and Medicine (to CK), and a fellowship grant from the Human Frontier Scientific Program (LT000162/2018-L, to HS).

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Bewley JD: **Seed germination and dormancy**. *Plant Cell* 1997, **9**:1055.
2. Koornneef M, Bentsink L, Hilhorst H: **Seed dormancy and germination**. *Curr Opin Plant Biol* 2002, **5**:33−36.
3. Finch-Savage WE, Leubner-Metzger G: **Seed dormancy and the control of germination**. *New Phytol* 2006, **171**:501−523.
4. Holdsworth MJ, Bentsink L, Soppe WJJ: **Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination**. *New Phytol* 2008, **179**:33−54.
5. Nonogaki H: **ABA responses during seed development and germination**. *Adv Bot Res* 2019, **92**:171−217.
6. Née G, Xiang Y, Soppe WJ: **The release of dormancy, a wake-up call for seeds to germinate**. *Curr Opin Plant Biol* 2017, **35**:8−14.
7. Gubler F, Millar AA, Jacobsen JV: **Dormancy release, ABA and pre-harvest sprouting**. *Curr Opin Plant Biol* 2005, **8**:183−187.
8. Fang J, Chu C: **Abscisic acid and the pre-harvest sprouting in cereals**. *Plant Signal Behav* 2008, **3**:1046−1048.
9. Tai L, Wang HJ, Xu XJ, Sun WH, Ju L, Liu WT, Li WQ, Sun J, Chen KM: **Pre-harvest sprouting in cereals: genetic and biochemical mechanisms**. *J Exp Bot* 2021, **72**:2857−2876.
10. Zhou LZ, Dresselhaus T: **Friend or foe: signaling mechanisms during double fertilization in flowering seed plants**. *Curr Top Dev Biol* 2019, **131**:453−496.
11. Costa LM, Yuan J, Rouster J, Paul W, Dickinson H, Gutierrez-Marcos JF: **Maternal control of nutrient allocation in plant seeds by genomic imprinting**. *Curr Biol* 2012, **22**:160−165.
12. Lee KP, Piskurewicz U, Turecková V, Strnad M, Lopez-Molina L: **A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds**. *Proc Natl Acad Sci U S A* 2010, **107**:19108−19113.

13. Linkies A, Müller K, Morris K, Turecková V, Wenk M, Cadman CS, Corbineau F, Strnad M, Lynn JR, Finch-Savage WE, *et al.*: **Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using Lepidium sativum and Arabidopsis thaliana**. *Plant Cell* 2009, **21**:3803–3822.

14. • Moreno-Romero J, Del Toro-De León G, Yadav VK, Santos-González J, Köhler C: **Epigenetic signatures associated with imprinted paternally expressed genes in the Arabidopsis endosperm**. *Genome Biol* 2019, **20**:41.

This paper reveals the correlation between the combination of H3K27me3/H3K9me2/CHGm and paternally-biased gene expression in the endosperm.

15. Gehring M, Missirian V, Henikoff S: **Genomic analysis of parent-of-origin allelic expression in Arabidopsis thaliana seeds**. *PLoS One* 2011, **6**, e23687.

16. Hsieh TF, Shin J, Uzawa R, Silva P, Cohen S, Bauer MJ, Hashimoto M, Kirkbride RC, Harada JJ, Zilberman D, *et al.*: **Regulation of imprinted gene expression in Arabidopsis endosperm**. *Proc Natl Acad Sci U S A* 2011, **108**:1755–1762.

17. Batista RA, Köhler C: **Genomic imprinting in plants-revisiting existing models**. *Genes Dev* 2020, **34**:24–36.

18. Rodrigues JA, Zilberman D: **Evolution and function of genomic imprinting in plants**. *Genes Dev* 2015, **29**:2517–2531.

19. Barlow DP: **Gametic imprinting in mammals**. *Science* 1995, **270**:1610–1613.

20. Zhang H, Lang Z, Zhu JK: **Dynamics and function of DNA methylation in plants**. *Nat Rev Mol Cell Biol* 2018, **19**:489–506.

21. Penterman J, Zilberman D, Huh JH, Ballinger T, Henikoff S, Fischer RL: **DNA demethylation in the Arabidopsis genome**. *Proc Natl Acad Sci U S A* 2007, **104**:6752–6757.

22. Park JS, Frost JM, Park K, Ohr H, Park GT, Kim S, Eom H, Lee I, Brooks JS, Fischer RL, *et al.*: **Control of DEMETER DNA demethylase gene transcription in male and female gamete companion cells in**. *Proc Natl Acad Sci U S A* 2017, **114**:2078–2083.

23. Moreno-Romero J, Jiang H, Santos-González J, Köhler C: **Parental epigenetic asymmetry of PRC2-mediated histone modifications in the Arabidopsis endosperm**. *EMBO J* 2016, **35**:1298–1311.

24. Pignatta D, Erdmann RM, Scheer E, Picard CL, Bell GW, Gehring M: **Natural epigenetic polymorphisms lead to intraspecific variation in Arabidopsis gene imprinting**. *Elife* 2014, **3**, e03198.

25. Choi Y, Gehring M, Johnson L, Hannon M, Harada JJ, Goldberg RB, Jacobsen SE, Fischer RL: **DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in arabidopsis**. *Cell* 2002, **110**:33–42.

26. Jullien PE, Kinoshita T, Ohad N, Berger F: **Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting**. *Plant Cell* 2006, **18**:1360–1372.

27. Yan W, Chen D, Smaczniak C, Engelhorn J, Liu H, Yang W, Graf A, Carles CC, Zhou DX, Kaufmann K: **Dynamic and spatial restriction of Polycomb activity by plant histone demethylases**. *Nat Plants* 2018, **4**:681–689.

28. •• Sato H, Santos-González J, Köhler C: **Combinations of maternal-specific repressive epigenetic marks in the endosperm control seed dormancy**. *Elife* 2021, **10**.

This paper reveals the relationships between maternal-specific epigenetic modifications and gene expression patterns during germination in the endosperm. The authors show that genomic imprinting in the endosperm is involved in establishing and releasing seed dormancy.

29. • Chen H, Tong J, Fu W, Liang Z, Ruan J, Yu Y, Song X, Yuan L, Xiao L, Liu J, *et al.*: **The H3K27me3 demethylase RELATIVE of early FLOWERING6 Suppresses seed dormancy by inducing abscisic acid catabolism**. *Plant Physiol* 2020, **184**:1969–1978.

This paper reveals that REF6 directly targets *CYP707A1* and *CYP707A3*, encoding catabolizing enzymes of ABA, and contributes to suppress seed dormancy.

30. •• Piskurewicz U, Iwasaki M, Susaki D, Megies C, Kinoshita T, Lopez-Molina L: **Dormancy-specific imprinting underlies maternal inheritance of seed dormancy in Arabidopsis thaliana**. *Elife* 2016, **5**.

This paper reveals parental-specific gene expression patterns in the endosperm during germination. The authors identified the MEGs *CP1* and *ALN* that functions to release dormancy.

31. Vu TM, Nakamura M, Calarco JP, Susaki D, Lim PQ, Kinoshita T, Higashiyama T, Martienssen RA, Berger F: **RNA-directed DNA methylation regulates parental genomic imprinting at several loci in Arabidopsis**. *Development* 2013, **140**:2953–2960.

32. Martínez G, Panda K, Köhler C, Slotkin RK: **Silencing in sperm cells is directed by RNA movement from the surrounding nurse cell**. *Nat Plants* 2016, **2**, 16030.

33. Calarco JP, Borges F, Donoghue MT, Van Ex F, Jullien PE, Lopes T, Gardner R, Berger F, Feijó JA, Becker JD, *et al.*: **Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA**. *Cell* 2012, **151**:194–205.

34. • Kim MY, Ono A, Scholten S, Kinoshita T, Zilberman D, Okamoto T, Fischer RL: **DNA demethylation by ROS1a in rice vegetative cells promotes methylation in sperm**. *Proc Natl Acad Sci U S A* 2019, **116**:9652–9657.

This paper reveals that rice and *Arabidopsis thaliana* share a common mechanism by which DNA demethylation by ROS1a in vegetative cells promotes non-CG methylation in sperm cells through the RdDM pathway.

35. Brinkman AB, Gu H, Bartels SJ, Zhang Y, Matarese F, Simmer F, Marks H, Bock C, Gnirke A, Meissner A, *et al.*: **Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk**. *Genome Res* 2012, **22**:1128–1138.

36. Weinhofer I, Hehenberger E, Roszak P, Hennig L, Köhler C: **H3K27me3 profiling of the endosperm implies exclusion of polycomb group protein targeting by DNA methylation**. *PLoS Genet* 2010, **6**.

37. Zhang M, Xie S, Dong X, Zhao X, Zeng B, Chen J, Li H, Yang W, Zhao H, Wang G, *et al.*: **Genome-wide high resolution parental-specific DNA and histone methylation maps uncover patterns of imprinting regulation in maize**. *Genome Res* 2014, **24**:167–176.

38. Du J, Zhong X, Bernatavichute YV, Stroud H, Feng S, Caro E, Vashisht AA, Terragni J, Chin HG, Tu A, *et al.*: **Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants**. *Cell* 2012, **151**:167–180.

39. Jackson JP, Lindroth AM, Cao X, Jacobsen SE: **Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase**. *Nature* 2002, **416**:556–560.

40. Ingouff M, Rademacher S, Holec S, Soljić L, Xin N, Readshaw A, Foo SH, Lahouze B, Sprunck S, Berger F: **Zygotic resetting of the HISTONE 3 variant repertoire participates in epigenetic reprogramming in Arabidopsis**. *Curr Biol* 2010, **20**:2137–2143.

41. •• Borg M, Jacob Y, Susaki D, LeBlanc C, Buendía D, Axelsson E, Kawashima T, Voigt P, Boavida L, Becker J, *et al.*: **Targeted reprogramming of H3K27me3 resets epigenetic memory in plant paternal chromatin**. *Nat Cell Biol* 2020, **22**:621–629.

This paper reveals that the sperm cell-specific H3 variant, H3.10 is not targeted by the PRC2 due to amino acid divergence at lysine 27. The authors further show that incorporation of H3.10 is associated with decreased levels of H3K27me3 on PEGs in sperm cells.

42. Batista RA, Moreno-Romero J, Qiu Y, van Boven J, Santos-González J, Figueiredo DD, Köhler C: **The MADS-box transcription factor PHERES1 controls imprinting in the endosperm by binding to domesticated transposons**. *Elife* 2019, **8**.

43. •• Iwasaki M, Hyvärinen L, Piskurewicz U, Lopez-Molina L: **Non-canonical RNA-directed DNA methylation participates in maternal and environmental control of seed dormancy**. *Elife* 2019, **8**.

This paper reveals the molecular mechanisms how each parental allele of *ALN* is suppressed by non-canonical RdDM pathway under control and cold stress conditions to regulate seed dormancy in a parental-specific manner.

44. Cuerda-Gil D, Slotkin RK: **Non-canonical RNA-directed DNA methylation**. *Nat Plants* 2016, **2**, 16163.

45. Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E: **The Arabidopsis cytochrome P450 CYP707A encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism**. *EMBO J* 2004, **23**:1647–1656.

46. • Qiu Q, Mei H, Deng X, He K, Wu B, Yao Q, Zhang J, Lu F, Ma J, Cao X: **DNA methylation repels targeting of Arabidopsis REF6**. *Nat Commun* 2019, **10**:2063.
This paper reveals that DNA methylation on the REF6 binding motif inhibits binding activity of REF6 *in vitro*. The authors further show that REF6 exhibits increased levels of binding activity at several loci in a quadruple mutant of *CMT2*, *CMT3*, *DRM1* and *DRM2* in which genome-wide DNA methylation is abolished.

47. Santos-Mendoza M, Dubreucq B, Baud S, Parcy F, Caboche M, Lepiniec L: **Deciphering gene regulatory networks that control seed development and maturation in Arabidopsis**. *Plant J* 2008, **54**:608–620.

48. Zheng J, Chen F, Wang Z, Cao H, Li X, Deng X, Soppe WJJ, Li Y, Liu Y: **A novel role for histone methyltransferase KYP/SUVH4 in the control of Arabidopsis primary seed dormancy**. *New Phytol* 2012, **193**:605–616.

49. Alizadeh M, Hoy R, Lu B, Song L: **Team effort: combinatorial control of seed maturation by transcription factors**. *Curr Opin Plant Biol* 2021, **63**, 102091.

50. Xie Y, Chen L: **Epigenetic regulation of gibberellin metabolism and signaling**. *Plant Cell Physiol* 2020, **61**:1912–1918.

51. Zhao P, Zhou X, Shen K, Liu Z, Cheng T, Liu D, Cheng Y, Peng X, Sun MX: **Two-step maternal-to-zygotic transition with two-phase parental genome contributions**. *Dev Cell* 2019, **49**: 882–893. e885.