New Phytologist

TANSLEY INSIGHTS

Tansley insight

Epigenetic dynamics during flowering plant reproduction: evidence for reprogramming?

Author for correspondence:
Mary Gehring
Tel: +1 617 324 0343
Email: mgehring@wi.mit.edu

Received: *17 February 2019*
Accepted: *5 April 2019*

Mary Gehring[1,2] iD

[1]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA; [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Contents

Summary

Over the last 10 yr there have been major advances in documenting and understanding dynamic changes to DNA methylation, small RNAs, chromatin modifications and chromatin structure that accompany reproductive development in flowering plants, from germline specification to seed maturation. Here I highlight recent advances in the field, mostly made possible by microscopic analysis of epigenetic states or by the ability to isolate specific cell types or tissues and apply omics approaches. I consider in which contexts there is potentially reprogramming vs maintenance or reinforcement of epigenetic states.
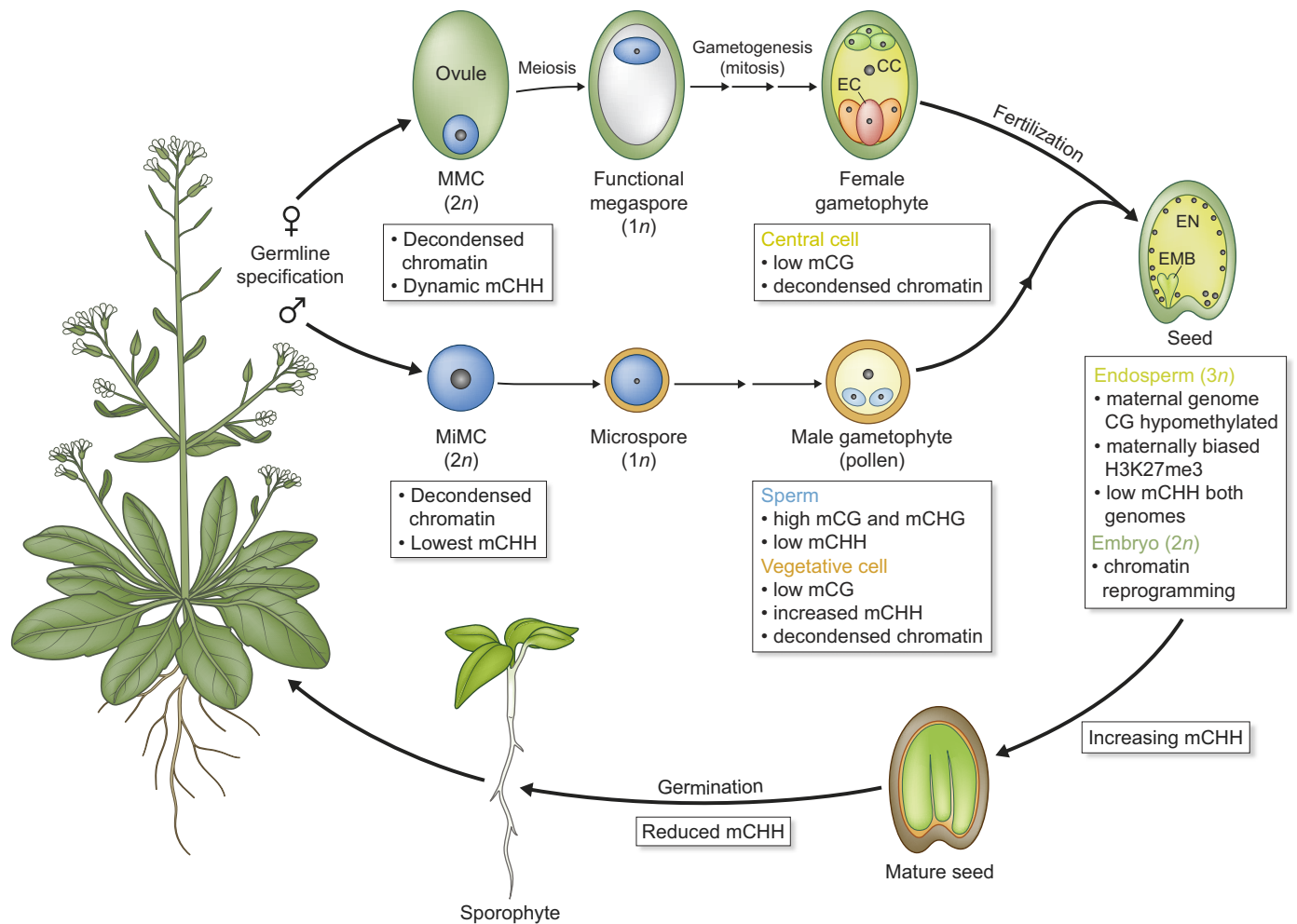
I. Introduction

Reproduction is the most fundamental task of all organisms. In flowering plants, which alternate between haploid and diploid forms, successful reproduction occurs when egg and sperm unite to form a diploid embryo and, ultimately, a viable seed. Reproduction begins when cells in the diploid sporophyte undergo meiosis, creating haploid spores. Spores divide mitotically to form the haploid gametophyte phase of the life cycle. Gametes are specified in the male and female gametophytes (Fig. 1).

Current evidence suggests that the most dynamic and conse-quential alterations to the epigenome occur during reproductive development (Fig. 1). This is sometimes referred to as epigenetic reprogramming. The term reprogramming is most frequently used in the context of animal stem cell biology, where it is taken to mean the erasure of information and the establishment of new informa-tion that allows cells to undergo a fate change over multiple cell divisions. For plants, this definition is perhaps useful with regards to regeneration from callus tissue, but appears less applicable to processes that occur during reproduction, in which differences in cell identity can be established over a single cell division. The key questions addressed here are: (1) How much molecular repro-gramming takes places during reproduction? and (2) What are the functional consequences of these dynamics?

Chromatin structure and modification, small noncoding RNAs and DNA methylation will be considered here as reprogrammable substrates (Matzke & Mosher, 2014; Mozgova *et al.*, 2015). These are not the only potential mediators of epigenetic inheritance, but

Fig. 1 Epigenetic dynamics during flowering plant reproduction. Summary of key steps in the flowering plant life cycle using Arabidopsis as an example. Associated epigenetic changes are described in the text. Functional megaspore and microspore cells are shown in dark blue. CC, central cell; EC, egg cell; EMB, embryo; EN, endosperm; mCG, CG methylation; mCHG, CHG methylation; mCHH, CHH methylation; MiMC, microspore mother cell; MMC, megaspore mother cell.

they can be reasonably measured and the pathways that control them are understood genetically or biochemically. A major challenge in evaluating the extent of reproductive reprogramming is that the epigenetic status of the cells that become the next generation (the starting point for reprogramming) are few in number and inaccessible. All cells arise from meristems, with reproductive cells formed specifically from the L2 layer of apical meristems. The epigenetic state of the L2 cells is largely unknown, although features of meristematic regions are beginning to be probed (Gutzat *et al.*, 2018; Sijacic *et al.*, 2018). Thus, the molecular epigenetic state of reproductive cells is often compared to leaf or seedling tissue, and it is unknown how reflective these tissues are of the relevant cells in meristems.

II. Meiosis

In flowering plants, the cells that undergo meiosis are chosen based on their position within the developing flower. Lack of a segregated germline is a fundamental distinction between plant and animal reproduction. A subepidermal cell in the ovule primordium is chosen as the megaspore mother cell (MMC), which undergoes female meiosis. Both the MMC and its male analog, the microspore mother cell (MiMC), experience large-scale chromatin changes during cell specification, including heterochromatin decondensation and increased nuclear volume, perhaps indicative of a highly active transcriptional state (She *et al.*, 2013; She & Baroux, 2015). In maize and Arabidopsis, mutations in different *AGO* (*ARGONAUTE*) genes involved in small RNA-mediated heterochromatin silencing, and in the maize *de novo* DNA methyltransferases *DMT102/103*, either cause division of the MMC without meiosis (germ cells act like somatic cells) or result in the formation of additional cells that display physical and gene expression signatures similar to MMCs (somatic cells act like germ cells) (Garcia-Aguilar *et al.*, 2010; Olmedo-Monfil *et al.*, 2010; Singh *et al.*, 2011). The *AGO* genes are expressed in the cells surrounding the MMC, indicating a cell nonautonomous effect on meiosis, which could be mediated by mobile small RNAs. The targets of these processes are unknown and the epigenetic state of the MMC has not been profiled at molecular resolution. Recently, fusions between DNA methylation-binding proteins and

fluorescent reporters allowed visualization of methylation dynamics at a broad scale (Ingouff *et al.*, 2017). CG methylation remains steady but CHH methylation decreases transiently and is then restored during MMC specification. Typically, CG and CHG methylation are maintained by maintenance methyltransferases *MET1* and *CMT3*. CHH methylation is maintained by CMT2 or by the RNA-directed DNA methylation (RdDM) pathway, in which 24 nt small RNAs guide *de novo* methyltransferases to matching sites, usually in repetitive DNA, such as transposable elements (TEs) (Matzke & Mosher, 2014). Thus, low CHH methylation is often indicative of low *de novo* methylation activity. The cause and function of CHH methylation dynamics in the MMC is unknown.

DNA methylation recently has been profiled in the diploid Arabidopsis MiMC (Walker *et al.*, 2018). The MiMC has high levels of CG and CHG methylation but low levels of CHH methylation. Interestingly, despite global CHH hypomethylation, Walker *et al.* (2018) identified several hundred genic regions of RdDM-dependent CHH hypermethylation compared to leaves. This is unusual because genes are not typical targets of RdDM. In some instances, this hypermethylation has functional consequences – for the *MPS1* gene, loss of reproduction-specific RdDM led to incorrect splicing and a mild meiosis defect (Walker *et al.*, 2018). Why and how RdDM is directed to genic regions should be an interesting area of future research.

III. Male gametogenesis

Mitosis of the haploid meiotic products generates the gametophytes. The ability to isolate pure populations of cells at different stages of male gametogenesis has illuminated developmental DNA methylation dynamics in Arabidopsis (Calarco *et al.*, 2012; Ibarra *et al.*, 2012; Walker *et al.*, 2018). The male gametophyte, or pollen grain, consists of two gametes (sperm) and a vegetative cell, which functions in pollen tube growth. The vegetative cell and sperm are in distinct epigenetic states, despite a separation of only two mitotic divisions (Fig. 1). Sperm DNA is highly methylated in the CG and CHG context but has low CHH methylation in retrotransposons. By contrast, the vegetative cell has low CG and CHG methylation and high CHH methylation in heterochromatic TEs, chromatin is decondensed, and hypomethylated retrotransposons are mobile (Slotkin *et al.*, 2009). How do these differences arise? CG and CHG methylation levels are quite similar from the MiMC, to the microspores, and then sperm (Walker *et al.*, 2018). CHH methylation progressively increases, although it remains lower in sperm than in somatic cells (Calarco *et al.*, 2012; Ibarra *et al.*, 2012; Walker *et al.*, 2018). The functional consequences of these dynamics are unknown. By contrast, in the vegetative cell RdDM restores CHH methylation from the lower levels present in the microspore. RdDM in heterochromatin might be important to counteract the loss of *DDM1* expression in the vegetative cell because *DDM1* is normally required to maintain heterochromatic DNA methylation (Schoft *et al.*, 2009; Slotkin *et al.*, 2009). Loss of CG methylation in the vegetative cell is at least partially due to active DNA demethylation by 5-methylcytosine DNA glycosylases (Calarco *et al.*, 2012; Ibarra *et al.*, 2012). There is increasing

evidence that the epigenetic state of the vegetative cell might influence mRNA or translation profiles of the sperm (Slotkin *et al.*, 2009; Grant-Downton *et al.*, 2013; Martinez *et al.*, 2016). In vegetative cells, hypomethylated, expressed retrotransposons give rise to abundant small RNAs that have been termed epigenetically activated siRNAs (easiRNAs). In a series of elegant experiments, Martinez *et al.* (2016) demonstrated that small RNAs expressed in the vegetative cell can target a miRNA site or an Athila retrotransposon siRNA site on reporter constructs in sperm. This process is dependent on *AGO* and Dicer genes typically involved in post-transcriptional gene silencing (Martinez *et al.*, 2016). It has been speculated that small RNAs loaded into sperm from the vegetative cell might have functions after fertilization in the zygote or endosperm, but direct evidence is lacking (Slotkin *et al.*, 2009; Calarco *et al.*, 2012; Martinez *et al.*, 2016).

IV. Female gametogenesis

The epigenetic dynamics associated with female gametogenesis are less clear, largely due to its relative inaccessibility. Female gametogenesis occurs when the haploid functional megaspore (the surviving product of meiosis) divides mitotically three times to yield the eight-celled female gametophyte, which is surrounded by maternal integument tissue (Fig. 1). As part of a recurring theme, *AGO5* acts in a cell nonautonomous manner to promote the mitotic division of the functional megaspore (Tucker *et al.*, 2012). Fluorescent reporters suggest that CG and CHH methylation remain fairly steady throughout female gametogenesis (Ingouff *et al.*, 2017). The mature female gametophyte contains two gametes, a haploid egg cell and a diploid central cell. Fertilization of the egg by sperm makes the embryo, whereas fertilization of the central cell generates the triploid endosperm, which supports embryo growth. The female gametes, separated by one to three mitoses, are cytologically distinct, with the central cell exhibiting decondensed chromatin and reduced repressive histone modifications compared to the egg (Pillot *et al.*, 2010). Like the pollen vegetative cell, the central cell expresses the 5-methylcytosine DNA glycosylase DME. It also has reduced expression of the maintenance and *de novo* methyltransferases (Jullien *et al.*, 2012). Methylation analysis has been performed on egg and central cells isolated from rice, Arabidopsis and maize using manual microdissection or tagging approaches such as INTACT. There is lower CG methylation in genes and TEs in Arabidopsis central cells compared to sperm, which is at least partly due to the activity of DME (Park *et al.*, 2016). Similar to the vegetative cell and sperm, it has been proposed that small RNAs move from the central cell to the egg to promote TE silencing (Ibarra *et al.*, 2012). Direct evidence for such a process occurring is scarcer than for vegetative cell–sperm cell transmission of siRNAs. In some instances, passive movement of exogenously supplied small RNAs has been observed. In *Torenia fournieri*, 25-nt small RNAs injected into the central cell acted as morpholinos in the synergid cells, which flank the egg (Okuda *et al.*, 2009). In Arabidopsis, labeled double-stranded 24-nt small RNAs injected into the central cell were later detected as both double- and single-stranded RNAs in the egg cell, indicating movement (Erdmann *et al.*, 2017a). Additionally, expression of an

artificial miRNA against GFP from a central cell promoter reduced green fluorescent protein (GFP) fluorescence in the Arabidopsis egg cell (Ibarra *et al.*, 2012).

V. Heritability and reinforcement of DNA methylation in the embryo

The frequency with which stable epialleles have been discovered in plants and direct measurements of methylation heritability between generations indicate that most DNA methylation is inherited through sexual reproduction (Becker *et al.*, 2011; Schmitz *et al.*, 2011). Existing methylation is reinforced during embryogenesis. When methylation is lost in hypomethylated recombinant inbred lines, it is partially replaced by RdDM upon passage through reproduction (Teixeira *et al.*, 2009). Wild-type sperm has low CHH methylation (Calarco *et al.*, 2012), indicating that the paternal genome must gain methylation after fertilization. Recent studies from soybean and Arabidopsis have shown that CHH methylation increases during embryogenesis (Kawakatsu *et al.*, 2017; Lin *et al.*, 2017; Narsai *et al.*, 2017). In mature Arabidopsis embryos, CHH methylation approaches 100% at individual cytosines, whereas in other tissues, including younger embryos, individual CHH sites are *c.* 20% methylated (Bouyer *et al.*, 2017). Methylation decreases upon seed germination, likely through a passive mechanism (Bouyer *et al.*, 2017; Kawakatsu *et al.*, 2017; Lin *et al.*, 2017; Narsai *et al.*, 2017). It is unknown whether the increased CHH methylation in developing embryos is functional: mutation of the *de novo* methyltransferases DRM2 and CMT2 prevents CHH methylation but without obvious effects on seed development or germination, although it is unclear how comprehensively phenotypes have been assessed.

VI. Chromatin-based epigenetic reprogramming in the embryo

Despite the heritability of DNA methylation between generations, there is clear evidence that chromatin-based epigenetic memory established during vegetative growth and development is erased during embryogenesis. In Arabidopsis, histone H3 from the egg and sperm are removed in the zygote and replaced by newly synthesized histones (Ingouf *et al.*, 2010). The *FLC* locus represents a specific case of reprogramming. Levels of *FLC* expression control flowering time in vernalization-sensitive plants. Stable mitotic epigenetic silencing of *FLC* after prolonged cold stimulus is mediated by spreading of H3K27me3, mediated by PRC2 complexes (Yang *et al.*, 2017). Vernalization memory is not transmitted to the next generation, indicating the occurrence of resetting. In vernalized plants, *FLC* is silenced in gametes but expression is reactivated during early embryogenesis (Sheldon *et al.*, 2008; Choi *et al.*, 2009; Tao *et al.*, 2017). Two mechanisms have been indicated: activation by the NF-YB transcription factor *LEC1* (Tao *et al.*, 2017) and regulation by the histone demethylase *ELF6* (Crevillén *et al.*, 2014), which appears to play a more minor role. Reprogramming of chromatin by pioneer transcription factors (defined as transcription factors that can bind to their DNA motif even in the context of a nucleosome) during embryonic

development is well-documented in animals (Zaret & Mango, 2016). It has been suggested that LEC1 acts as a pioneer factor, but this has not been specifically demonstrated biochemically (Tao *et al.*, 2017).

VII. Modulation of epigenetic information in the endosperm

Endosperm is epigenetically divergent from other plant tissues, as has been reviewed extensively elsewhere (Satyaki & Gehring, 2017), with the added twist that some effects are specific to maternally or paternally inherited genomes. Consistent with DNA demethylation in the central cell, endosperm DNA is maternally hypomethylated. The endosperm maternal genome is even less CG-methylated than the central cell, suggesting that additional loss of DNA methylation occurs either passively or actively after fertilization (Park *et al.*, 2016). CHH methylation also appears to be dynamic, with low expression of RdDM genes early in development (Jullien *et al.*, 2012; Belmonte *et al.*, 2013) and low CHH methylation at 4 d after pollination (DAP) (Moreno-Romero *et al.*, 2016). By 6–8 DAP when the endosperm has cellularized and differentiated, typical levels of CHH methylation are observed on the paternal endosperm genome (Ibarra *et al.*, 2012; Pignatta *et al.*, 2014). Histone modifications also are asymmetrical between maternal and paternal genomes, with the PRC2 mark H3K27me3 favoring maternal alleles in euchromatin, particularly at regions that were demethylated in the central cell, and paternal alleles in pericentromeric heterochromatin (Moreno-Romero *et al.*, 2016). Small RNAs have been profiled in Arabidopsis, rice and maize endosperm. In Arabidopsis, the endosperm 24-nt small RNA population is paternally biased, consistent with higher methylation of the paternal genome (Erdmann *et al.*, 2017b). Endosperm also has a population of genic small RNAs, another instance of increased genic small RNAs observed during reproduction. Discrete regions of maternal and paternal sRNA bias have been identified, but it is unknown how small RNAs are preferentially produced from one allele or whether this is connected to other asymmetric epigenetic modifications.

There are several possible consequences of endosperm epigenetic dynamics. Methylation state can impact the expression of key epigenetic regulators, thus potentially creating cascading effects caused by the expression of alternative epigenetic pathways in tissues that undergo dynamic DNA methylation changes, like endosperm. Epigenetic regulators regulated by DNA methylation include the histone demethylase *IBM1*, the 5-methylcytosine DNA glycosylase *ROS1* and the Dicer enzyme *DCL4* (Rigal *et al.*, 2012; Williams *et al.*, 2015; Pumplin *et al.*, 2016). For these genes, DNA methylation alters splicing, promotes expression, or alters transcriptional start site selection. Another consequence of endosperm methylation dynamics is the establishment of gene imprinting, the preferential expression of one allele of a gene dependent on its parent-of-origin. For example, the majority of paternally expressed imprinted genes are associated with methylated paternal alleles and repressed maternal alleles that are DNA hypomethylated, modified by H3K27me3 and associated with genic small RNAs (Pignatta *et al.*, 2014; Moreno-Romero *et al.*,

2016; Erdmann *et al.*, 2017b; Satyaki & Gehring, 2017). Imprinting dynamics can intersect with natural epigenetic polymorphisms or environmentally induced methylation changes to generate diverse seed phenotypes (Pignatta *et al.*, 2018; Iwasaki *et al.*, 2019). For example, the maternally expressed imprinted gene *ALN* negatively regulates seed dormancy (Iwasaki *et al.*, 2019). Seeds that develop at 10°C have increased DNA methylation at the *ALN* locus, reduced *ALN* expression, and higher levels of dormancy, consistent with an increase in seed dormancy associated with development in cold temperatures (Iwasaki *et al.*, 2019). Differences in endosperm DNA methylation and gene expression programs induced by environmental conditions are largely unexplored (and exciting) territory.

VIII. Conclusions

Recent studies with improved tissue- or cell-type specificity have expanded understanding of the extent of epigenetic dynamics that occur during flowering plant reproductive development. Unlike in mammals, there is no genome-wide erasure and re-establishment of epigenetic information during reproduction. Rather, chromatin-based epigenetic information appears to be either passively lost or actively reprogrammed in the embryonic lineage, at least based on a limited number of examples, and DNA methylation-based epigenetic information is maintained, reinforced and inherited. Although CHH methylation dynamics have been discovered at multiple stages of reproductive development, it is not yet clear whether any of these instances represent reprogramming or are important for change from one cell state to another. Integrating concepts and language from other disciplines is important for bridging scientific fields, especially those with many true commonalities, like the study of epigenetics in plant, animal and fungal systems. When we talk about epigenetic reprogramming in plants, it is important to be clear where this concept might differ from other organisms, and how this is integrated with differences in development.

ORCID

Mary Gehring 🔗 https://orcid.org/0000-0003-2280-1522

References

Becker C, Hagmann J, Müller J, Koenig D, Stegle O, Borgwardt K, Weigel D. 2011. Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature* 480: 245–249.

Belmonte MF, Kirkbride RC, Stone SL, Pelletier JM, Bui AQ, Yeung EC, Hashimoto M, Fei J, Harada CM, Munoz MD *et al.* 2013. Comprehensive developmental profiles of gene activity in regions and subregions of the Arabidopsis seed. *Proceedings of the National Academy of Sciences, USA* 110: E435–E444.

Bouyer D, Kramdi A, Kassam M, Heese M, Schnittger A, Roudier F, Colot V. 2017. DNA methylation dynamics during early plant life. *Genome Biology* 18: 179.

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

Choi J, Hyun Y, Kang MJ, In Yun H, Yun JY, Lister C, Dean C, Amasino RM, Noh B, Noh YS *et al.* 2009. Resetting and regulation of *Flowering Locus C* expression during Arabidopsis reproductive development. *The Plant Journal* 57: 918–931.

Crevillén P, Yang H, Cui X, Greeff C, Trick M, Qiu Q, Cao X, Dean C. 2014. Epigenetic reprogramming that prevents transgenerational inheritance of the vernalized state. *Nature* 515: 587–590.

Erdmann RM, Hoffmann A, Walter H-K, Wagenknecht H-A, Grob-Hardt R, Gehring M. 2017a. Molecular movement in the *Arabidopsis thaliana* female gametophyte. *Plant Reproduction* 30: 141–146.

Erdmann RM, Satyaki PR, Klosinska M, Gehring M. 2017b. A small RNA pathway mediates allelic dosage in endosperm. *Cell Reports* 21: 3364–3372.

Garcia-Aguilar M, Michaud C, Leblanc O, Grimanelli D. 2010. Inactivation of a DNA methylation pathway in maize reproductive organs results in apomixis-like phenotypes. *Plant Cell* 22: 3249–3267.

Grant-Downton R, Kourmpetli S, Hafidh S, Khatab H, Le Trionnaire G, Dickinson H, Twell D. 2013. Artificial microRNAs reveal cell-specific differences in small RNA activity in pollen. *Current Biology* 23: R599–R601.

Gutzat R, Rembart K, Nussbaumer T, Pisupati R, Hofmann F, Bradamante G, Daubel N, Gaidora A, Lettner N, Dona M *et al.* 2018. Stage-specific transcriptomes and DNA methylomes indicate an early and transient loss of transposon control in Arabidopsis shoot stem cells. *bioRxiv*: doi: 10.1101/430447.

Ibarra CA, Feng X, Schoft VK, Hsieh TF, Uzawa R, Rodrigues JA, Zemach A, Chumak N, Machlicova A, Nishimura T *et al.* 2012. Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science* 337: 1360–1364.

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

Ingouff M, Selles B, Michaud C, Vu TM, Berger F, Schorn AJ, Autran D, Nowack MK, Martienssen RA, Grimanelli D. 2017. Live-cell analysis of DNA methylation during sexual reproduction in *Arabidopsis* reveals context and sex-specific dynamics controlled by noncanonical RdDM. *Genes & Development* 31: 72–83.

Iwasaki M, Hyvärinen L, Piskurewicz U, Lopez-Molina L. 2019. Non-canonical RNA-directed DNA methylation participates in maternal and environmental control of seed dormancy. *eLife* 8: e37434.

Jullien PE, Susaki D, Yelagandula R, Higashiyama T, Berger F. 2012. DNA methylation dynamics during sexual reproduction in *Arabidopsis thaliana*. *Current Biology* 22: 1825–1830.

Kawakatsu T, Nery JR, Castanon R, Ecker JR. 2017. Dynamic DNA methylation reconfiguration during seed development. *Genome Biology* 18: 171.

Lin JY, Le BH, Chen M, Henry KF, Hur J, Hsieh TF, Chen PY, Pelletier JM, Pellegrini M, Fischer RL *et al.* 2017. Similarity between soybean and *Arabidopsis* seed methylomes and loss of non-CG methylation does not affect seed development. *Proceedings of the National Academy of Sciences, USA* 114: E9730–E9739.

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

Matzke MA, Mosher RA. 2014. RNA-directed DNA methylation: an epigenetic pathways of increasing complexity. *Nature Reviews Genetics* 15: 394–408.

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

Mozgova I, Kohler C, Hennig L. 2015. Keeping the gate closed: functions of the polycomb repressive complex PRC2 in development. *The Plant Journal* 83: 121–132.

Narsai R, Gouil Q, Secco D, Srivastava A, Karpievitch YV, Liew LC, Lister R, Lewsey MG, Whelan J. 2017. Extensive transcriptomic and epigenomic

remodeling occurs during *Arabidopsis thaliana* germination. *Genome Biology* **18**: 172.

Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, Kasahara RD, Hamamura Y, Mizukami A, Susaki D *et al.* **2009.** Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. *Nature* **458**: 357–361.

Olmedo-Monfil V, Durán-Figueroa N, Arteaga-Vázquez M, Demesa-Arévalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada JP. 2010. Control of female gamete formation by a small RNA pathway in Arabidopsis. *Nature* **464**: 628–632.

Park K, Kim MY, Vickers M, Park JS, Hyun Y, Okamoto T, Zilberman D, Fischer RL, Feng X, Choi Y *et al.* **2016.** DNA demethylation is initiated in the central cells of Arabidopsis and rice. *Proceedings of the National Academy of Sciences, USA* **113**: 15138–15143.

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

Pignatta D, Novitzky K, Satyaki PRV, Gehring M. 2018. A variably imprinted epiallele impacts seed development. *PLoS Genetics* **14**: e1007469.

Pillot M, Baroux C, Vazquez MA, Autran D, Leblanc O, Vielle-Calzada JP, Grossniklaus U, Grimanelli D. 2010. Embryo and endosperm inherit distinct chromatin and transcriptional states from the female gamete in Arabidopsis. *Plant Cell* **22**: 307–320.

Pumplin N, Sarazin A, Jullien PE, Bologna NG, Oberlin S, Voinnet O. 2016. DNA methylation influences the expression of *DICER-LIKE4* isoforms, which encode proteins of alternative localization and function. *Plant Cell* **28**: 2786–2804.

Rigal M, Kevei Z, Pélissier T, Mathieu O. 2012. DNA methylation in an intron of the *IBM1* histone demethylase gene stabilizes chromatin modification patterns. *EMBO Journal* **31**: 2981–2993.

Satyaki PR, Gehring M. 2017. DNA methylation and imprinting in plants: machinery and mechanisms. *Critical Reviews in Biochemistry and Molecular Biology* **52**: 163–175.

Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA, Libiger O, Schork NJ, Ecker JR. 2011. Transgenerational epigenetic instability is a source of novel methylation variants. *Science* **334**: 369–373.

Schoft VK, Chumak N, Mosiolek M, Slusarz L, Komnenovic V, Brownfield L, Twell D, Kakutani T, Tamaru H. 2009. Induction of RNA-directed DNA methylation upon decondensation of constitutive heterochromatin. *EMBO Reports* **10**: 1015–1021.

She W, Baroux C. 2015. Chromatin dynamics in pollen mother cells underpin a common scenario at the somatic-to-reproductive fate transition of both the male and female lineages in *Arabidopsis*. *Frontiers in Plant Science* **6**: 294.

She W, Grimanelli D, Rutowicz K, Whitehead MW, Puzio M, Kotlinski M, Jerzmanowski A, Baroux C. 2013. Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. *Development* **140**: 4008–4019.

Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ. 2008. Resetting of *FLOWERING LOCUS C* expression after epigenetic repression by vernalization. *Proceedings of the National Academy of Sciences, USA* **105**: 2214–2219.

Sijacic P, Bajic M, McKinney EC, Meagher RB, Deal RB. 2018. Changes in chromatin accessibility between Arabidopsis stem cells and mesophyll cells illuminate cell type-specific transcription factor networks. *The Plant Journal* **94**: 215–231.

Singh M, Goel S, Meeley RB, Dantec C, Parrinello H, Michaud C, Leblanc O, Grimanelli D. 2011. Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein. *Plant Cell* **23**: 443–458.

Slotkin RK, Vaughn M, Borges F, Tanurdžić M, Becker JD, Feijó JA, Martienssen RA. 2009. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* **136**: 461–472.

Tao Z, Shen L, Gu X, Wang Y, Yu H, He Y. 2017. Embryonic epigenetic reprogramming by a pioneer transcription factor in plants. *Nature* **551**: 124–128.

Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, Ciaudo C, Cruaud C, Poulain J, Berdasco M, Fraga MF *et al.* **2009.** A role for RNAi in the selective correction of DNA methylation defects. *Science* **323**: 1600–1604.

Tucker MR, Okada T, Hu Y, Scholefield A, Taylor JM, Koltunow AM. 2012. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis. *Development* **139**: 1399–1404.

Walker J, Gao H, Zhang J, Aldridge B, Vickers M, Higgins JD, Feng X. 2018. Sexual-lineage-specific DNA methylation regulates meiosis in *Arabidopsis*. *Nature Genetics* **50**: 130–137.

Williams BP, Pignatta D, Henikoff S, Gehring M. 2015. Methylation-sensitive expression of a DNA demethylase gene serves as an epigenetic rheostat. *PLoS Genetics* **11**: e1005142.

Yang H, Berry S, Olsson TSG, Hartley M, Howard M, Dean C. 2017. Distinct phases of Polycomb silencing to hold epigenetic memory of cold in *Arabidopsis*. *Science* **357**: 1142–1145.

Zaret KS, Mango SE. 2016. Pioneer transcription factors, chromatin dynamics, and cell fate control. *Current Opinion in Genetics & Development* **37**: 76–81.