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REVIEW ARTICLE

DEMETER-mediated DNA Demethylation in Gamete Companion Cells and the Endosperm, and its Possible Role in Embryo Development in *Arabidopsis*

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Abstract
Seed development begins upon double fertilization, producing the embryo and endosperm, which are genetically identical, except for their ploidy level. DEMETER (DME), a member of the DNA glycosylase family, functions as a DNA demethylase via the base excision repair pathway. DME is specifically expressed prior to fertilization in two gamete companion cells, central cell of the female gametophyte and vegetative cell of the male gametophyte, but not in the heritable gamete cells or embryo. Mutations in the *DME* gene cause hypermethylation in the endosperm, leading to endosperm overproliferation and seed abortion after fertilization. DME-mediated DNA demethylation preferentially targets euchromatic transposable elements (TEs), resulting in TE activation and initiation of de novo methylation through RNA-directed DNA methylation, and provides FERTILIZATION-INDEPENDENT SEED 2 (FIS2)-Polycomb Repressive Complex 2-binding sites, resulting in histone modifications and genomic imprinting during reproduction. The global demethylation of TEs in gamete companion cells and active de novo methylation in the embryo suggest a new role of sexual companion cells in reinforcing the genome integrity of the heritable tissue. In this review, we provide an overview of demethylation in sexual companion cells and the endosperm, and discuss its evolutionary effect on the heritable gamete cells and embryo.

Keywords Active DNA demethylation · DEMETER (DME) · Central cell · Vegetative cell · Gamete companion cells · Transposable elements (TEs)

Introduction

Double fertilization is an evolutionary hallmark of flowering plants, also known as angiosperms. In this process, one sperm cell fertilizes an egg cell to form a diploid zygote, which gives rise to the embryo, while the other sperm cell and the diploid central cell fuse to form a triploid endosperm, which provides nourishment to the developing embryo during embryogenesis. Although the origin of the endosperm remains unclear, the development of endosperm has been thoroughly investigated in plants, particularly in the model dicot, *Arabidopsis thaliana*. Endosperm has a distinct but interactive developmental process with the embryo. After the double fertilization event, while the diploid zygote divides and differentiates into an embryo, the triploid endosperm initially undergoes several nuclear divisions without cell division until the 8th mitotic cycle, forming the syncytium. The timing of the developmental transition from the endosperm proliferation stage to the cellularization stage is critical for embryo and seed development, which starts as the pattern formation of the embryo is being completed (Hehenberger et al. 2012; Lafon-Placette and Kohler 2014). The growth of the embryo using the accumulated storage reserves largely depends on endosperm cellularization. Interestingly, the endosperm shows global DEMETER (DME)-mediated DNA demethylation, especially in regions containing short euchromatic transposable elements (TEs) (Gehring et al. 2009; Hsieh et al. 2009; Ibarra et al. 2012; Frost et al. 2018), and the *DME* mutant shows endosperm overproliferation because of aberrant endosperm cellularization, eventually leading to seed abortion (Choi et al. 2002). Furthermore, *DME* is neither expressed in gametes, such as sperm and egg cells, nor in the embryo, which contributes to the next generation; however, it is expressed in gamete

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companion cells, such as the vegetative cell of the male gametophyte and central cell of the female gametophyte, which is a precursor of the endosperm (Choi et al. 2002; Park et al. 2017). Consistent with the expression patterns of *DME*, mutations in the *DME* gene lead to DNA hypermethylation in the central cell, vegetative cell, and endosperm. This suggests that abnormal DNA demethylation resulting from *DME* mutation in gamete companion cells (central cell and vegetative cell) or tissue (endosperm) blocks the development of the embryo, where *DME* is not expressed. This further suggests the possibility that DME-mediated hypomethylation in companion cells and tissues leads to methylation crosstalk between the gamete cells and embryo, which is essential for their development. In this review, we discuss DME-mediated DNA demethylation in gamete companion cells and its possible role in embryo development during sexual reproduction. We also provide suggestions for future experiments.

Highly Evolved DNA Methylation System of Seed Plants and Demethylase Family in *Arabidopsis*

Epigenetic modifications, such as DNA methylation (methylation at the 5th carbon of the cytosine base) and histone modifications, alter DNA accessibility and chromatin structure, thus regulating gene expression patterns. Although canonical histones, along with their variants and modifications, are found in the nuclei of all eukaryotic cells, DNA methylation is absent in some groups of eukaryotes. For example, DNA methylation and its associated enzymes are absent in some model organisms such as fruit fly (*Drosophila melanogaster*), roundworm (*Caenorhabditis elegans*), and fission yeast (*Saccharomyces pombe*). Like mammals, seed plants have one of the most comprehensive and versatile DNA methylation systems (Zemach et al. 2010; Zemach and Zilberman 2010; de Mendoza et al. 2019). Both mammals and plants have de novo DNA methyltransferase as well as maintenance methyltransferase to add a methyl group to all contexts of cytosine (CG, CHG, and CHH). Furthermore, the major DNA methylation profiles are similar to each other; for instance, the coexistence of preferential methylation on the TEs and gene body which is usually not seen (coexisted) in other species (de Mendoza et al. 2019). However, tissue specificity and molecular mechanisms of DNA methylation in each group are evolved differently. Indeed, non-CG (CHG, CHH) methylation is seen in all tissues of seed plants with various levels, whereas that in mammals is relatively rare except in stem cell and brain tissues with significant anti-correlation with transcription (Kinoshita et al. 2000; Laurent et al. 2010; Lister et al. 2013; Shirane et al. 2013; Guo et al. 2014; Patil et al. 2014). Furthermore, although both mammals and plants have an active DNA demethylation system, the way of DNA demethylation is evolved differently. Seed plants directly excise the methylcytosine base using *DME* family genes, whereas mammals exert stepwise demethylation through DNA oxidation, methylcytosine deamination, and base excision repair. Indeed, *DME* family genes, including *DME*, *Repressor of silencing 1 (ROS1)*, *DME-Like 2 (DML2)*, and *DML3*, are conserved only in the plant kingdom (Zhu 2009). Regardless of similaritis and differences of DNA methylation between these two groups, DNA methylation is widely and extensively used for their development; gamete and seed development, pattern formation, fruit ripening, responses to environmental stimuli in seed plants (Zhang et al. 2018a), gamete and embryo development, cancer, immunity, stem cell identification, and brain function in mammals (Deniz et al. 2019), respectively. Interestingly, global DME-mediated demethylation has been reported in *Arabidopsis*, especially in the short euchromatic TEs of gamete companion cells and the embryo-nourishing endosperm. It would be interesting to decipher the meaning of global reprogramming of DNA methylation in gamete cells and the endosperm, which do not contribute to the genome of the next generation.

Global Demethylation in the Endosperm and Gamete Companion Cells (in Chronological Order)

Endosperm

Because of the lack of a method for the isolation of gamete cells embedded in maternal tissues and the lack of a technique for library construction using a small number of cells, the endosperm methylome was reported before the central cell methylome (Gehring et al. 2009; Hsieh et al. 2009; Ibarra et al. 2012). Based on the endosperm methylome data, it was predicted that hypomethylation in the endosperm (relative to hypermethylation in the embryo) resulted from the action of DME in the central cell, the precursor cell of the endosperm, because global demethylation in the endosperm was specific only to the maternal genome, which originates from the central cell of the female gametophyte (Gehring et al. 2009; Hsieh et al. 2009, 2011; Gehring et al. 2011; Ibarra et al. 2012). Researchers showed that DME preferentially targets CG methylated euchromatic TEs (Gehring et al. 2009; Hsieh et al. 2009; Ibarra et al. 2012). Alongside global TE demethylation in the endosperm, the embryo showed the expression of genes responsible for CG, CHG, and CHH methylation, namely, *DNA METHYLTRANSFERASE 1* (*MET1*), *CHROMOMETHYLASE 3* (*CMT3*), and *DOMAINS REARRANGED METHYLTRANSFERASE 2* (*DRM2*), and increased levels of DNA methylation, especially CHH

methylation, with embryo development (Jullien et al. 2012; Lin et al. 2017). Interestingly, the level of CHH methylation in the embryo appears to be correlated with DME target regions in the endosperm (Ibarra et al. 2012).

Vegetative Cell

The male gametophyte vegetative cell, a companion cell of the gamete sperm cell, shows substantial similarity to the female gametophyte central cell, especially in terms of DNA methylation. The expression profiles of *DME*(on) and *MET1*(off), and global DNA demethylation of TEs are identical between the vegetative cell and central cell (Ibarra et al. 2012; Park et al. 2017). Furthermore, the maternal central cell and endosperm as well as the paternal vegetative cell share common DME-mediated demethylation of TE targets (Ibarra et al. 2012; Park et al. 2016b). Interestingly, the egg cell and sperm cell show slightly distinct expression patterns of genes involved in DNA methylation. Genes involved in the latter part of the RNA-directed DNA methylation (RdDM) pathway, such as those encoding non-CG methylases including *DRM1*, *DRM2*, and *CMT3*, are expressed in the egg cell (Jullien et al. 2012) and not in sperm cells (Borges et al. 2008). By contrast, genes involved in the former part of the RdDM pathway, including *ARGONAUTE 9* (*AGO9*), *RNA-dependent RNA polymerase 2* (*RDR2*), and *DICER 1* (*DCL1*), are expressed in sperm cells (Borges et al. 2008). The CHH methylation level of sperm cells is lower than that of the central cell, endosperm, embryo, and seedlings. Intriguingly, despite the low global CHH methylation level and the repression of non-CG methylases in sperm cells, the DME-targeted regions in the vegetative cell show CHH hypermethylation in wild-type sperm cells. Accordingly, *dme* heterozygous mutant sperm cells show CHH hypomethylation at loci that show hypermethylation in *dme* mutant vegetative cells (Ibarra et al. 2012; Kim et al. 2019).

Central Cell

Using the modified Isolation of Nuclei Tagged in specific Cell Types (INTACT) method (Park et al. 2016a), Park and colleagues purified central cells from the female gametophyte and showed global DNA methylation patterns in the central cell (Park et al. 2016b). Initiation of DME-mediated CG demethylation was detected in the central cell prior to fertilization in the same TE regions as those observed in the maternal endosperm genome after fertilization. Intriguingly, global CG methylation in the central cell is well maintained, despite the absence of the CG maintenance enzyme, MET1 (Jullien et al. 2008, 2012; Park et al. 2016b), possibly because MET1 homologs, MET2a and MET2b, which are specifically expressed in the central cell, compensate for the absence of MET1 (Jullien et al. 2012).

Chromatin Decondensation in Gametes and Companion Cells

In addition to DNA methylation patterns, chromatin condensation and histone modifications are also distinct between gamete companion cells and their gametes. The central cell shows a relaxed chromatin state (Pillot et al. 2010; Yelagandula et al. 2014; Park et al. 2016b) and does not show discernable chromocenters or relating spots (Ingouff et al. 2010). The vegetative cell also shows decondensed chromatin compared with the sperm cell (Slotkin et al. 2009; Calarco et al. 2012). Notably, *DME* is specifically expressed in the central cell and vegetative cell during reproduction (Choi et al. 2002; Park et al. 2017); therefore, decondensed chromatin is, at least partially, the structural base of DME function in both gamete companion cells.

Interplay Between Histone Methylation and DNA Methylation During Seed Development

Mutations in genes for components of the FIS2-PRC2 complex which is responsible for the deposition of the H3K27me3 mark and regulating Type I MADS-box genes (Kohler et al. 2003; Zhang et al. 2018b) result in seed abortion mainly due to a failure of endosperm cellularization, as observed in *dme* mutants (Lafon-Placette and Kohler 2014; Moreno-Romero et al. 2016; Batista et al. 2019). Furthermore, the FIS2-PRC2 complex is expressed in the central cell and endosperm. Therefore, it is plausible that DNA methylation and histone modifications are co-regulated in the central cell and endosperm. Indeed, the FIS2-PRC2-mediated H3K27me3 modification is preferentially enriched in DME target regions that are hypomethylated (Moreno-Romero et al. 2016). In contrast to the anti-correlation between H3K27me3 and DNA methylation, CHG methylation and H3K9 methylation display positive correlation (Jackson et al. 2002). Furthermore, SUVH4 which is a H3K9 methyltransferase is also involved in the maintenance of CHG DNA methylation (Malagnac et al. 2002). Another example of co-regulation of DNA and histone methylation is seen in imprinted genes, a phenomenon of monoallelic gene expression depending on a parent-of-origin. Both histone modifications and DNA methylation are strongly involved in gene imprinting. Many DME and FIS2-PRC2 target genes are imprinted in the endosperm (Hsieh et al. 2011). An allele can be silenced by DNA methylation or H3K27me3 such as in *FWA* paternal allele or *MEA* paternal allele, respectively

(Kinoshita et al. 1999; Kinoshita et al. 2004). Both maternal *FWA* and *MEA* alleles are expressed by DME-mediated demethylation (Choi et al. 2002; Kinoshita et al. 2004; Gehring et al. 2006). In case of *PHERES1*(*PHE1*) gene, a silenced maternal allele shows hypo-CG methylation and hyper-H3K27me3 marks, whereas an expressed paternal allele shows hyper DNA methylation (Kohler et al. 2005; Makarevich et al. 2008; Villar et al. 2009; Moreno-Romero et al. 2019). Therefore, future experiments should be designed to decipher an epigenetic regulation of gamete and seed development in a spatiotemporal manner with a comprehensive view of the relationship between histone modifications and DNA methylation.

Reinforcing the Genome Integrity of the Heritable Embryo by DNA Demethylation in the Non-heritable Gamete Companion Cells

CG demethylation in the endosperm is accompanied by CHH hypermethylation of the same TE regions in the embryo. DME-mediated demethylation is also involved in TE activation and de novo DNA methylation in the same TE regions in the endosperm (Ibarra et al. 2012). The *dme* mutant endosperm fails to undergo cellularization and exhibits simultaneous CG hypermethylation and CHH hypomethylation (Ibarra et al. 2012). Based on these two contrasting results, a new role of the endosperm was hypothesized (Fig. 1) (Hsieh et al. 2009; Ibarra et al. 2012). According to this hypothesis, global demethylation of TEs in the non-heritable endosperm tissue results in TE activation, generating short interfering RNAs (siRNAs), which cause de novo methylation, thus reinforcing the genome integrity of the heritable embryos. Therefore, TEs in the following

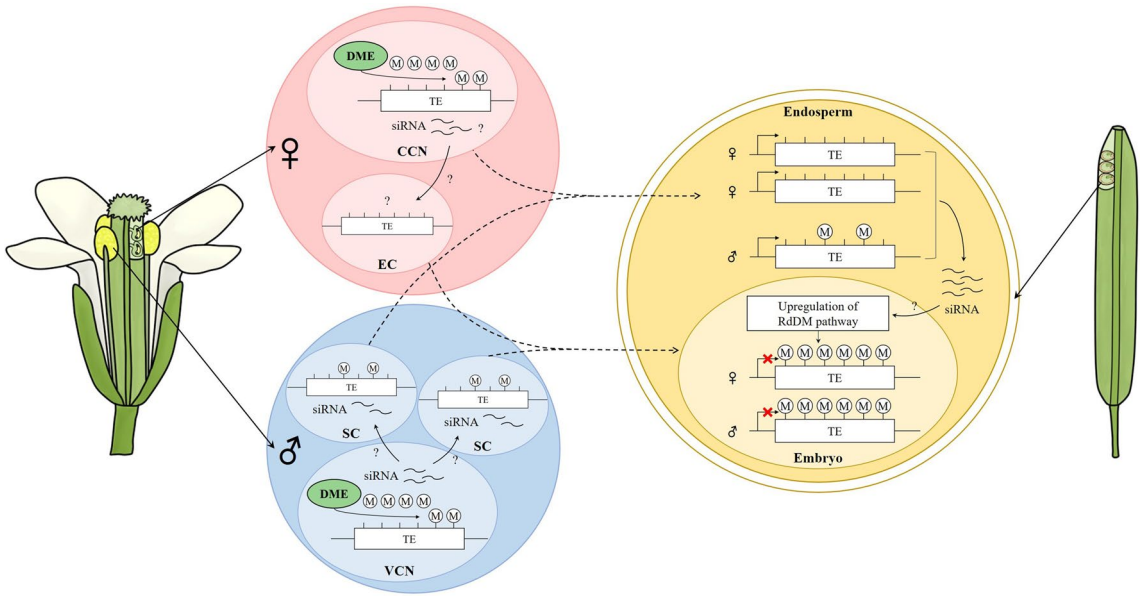

Fig. 1 Effect of DNA demethylation in gamete companion cells and the endosperm on embryo development in Arabidopsis. This figure summarizes what we know and need to know regarding the possible role (reinforcing TE silencing in the embryo during development) of DNA demethylation in gamete companion cells and the endosperm. DME expresses in both male and female gamete companion cells and preferentially targets TE regions (Calarco et al. 2012; Ibarra et al. 2012; Park et al. 2016b, 2017). In the pollen, siRNAs in both VCN and SC are detected (blue circle) (Slotkin et al. 2009; Calarco et al. 2012). However, the movement of siRNAs from VCN to sperm cells was confirmed only by the reporter experiment (Martinez et al. 2016). In the female gametophyte, only the CC methylome data was reported mainly due to its technical inaccessibility (Park et al. 2016b). DME-mediated DNA demethylation is initiated in the CCN (pink circle). However, the global methylation of EC, generation of siRNAs, movement of siRNAs between CCN and EC need to be confirmed in the future. After double fertilization, the hypomethylation of the maternal alleles that are frequently associated with euchromatic TE is a well-known feature in the endosperm (Hsieh et al. 2009; Ibarra et al. 2012). And a gradual increase of the non-CG methylation is observed in several genes (Jullien et al. 2012). However, the dynamics of spatiotemporal pure embryo methylome and the effect of the demethylation in companion cells on the embryos have not been verified with proper experiments. *DME* DEMETER, *TE* transposable elements, *siRNA* small interfering RNA, *CCN* central cell nucleus, *EC* egg cell, *VCN* vegetative cell nucleus, *SC* sperm cell, *RdDM* RNA dependent DNA methylation

generation would be stably silenced. In plants, this hypothesis is supported by three features: (1) silencing of TE activation by DNA methylation (Tsukahara et al. 2009; Stroud et al. 2014); (2) free RNA movement between plant cells (de Felippes et al. 2011; Dunoyer et al. 2013; Kehr and Kragler 2018); and (3) versatile RdDM pathway (Zhang et al. 2018a, b; Ashapkin et al. 2019; Han et al. 2019). This implies that DME-mediated CG demethylation in the endosperm activates target TEs, and TE transcripts or siRNAs generated from the activated TEs move into the embryo and signal the RdDM of the same TE regions. This process can be started by communication between the central cell and egg cell as well as between the vegetative cell and sperm cell, after which the combined communication from each sex cell in the zygote could serve as a signal for RdDM in the embryo.

Future Experiments to Confirm the Effect of DNA Demethylation in Non-heritable Companion Cells and the Endosperm on the Genomic Integrity of the Heritable Embryo

Interestingly non-heritable male and female companion cells and endosperm share many common features. However, the epigenetic crosstalk between heritable and non-heritable cells and tissues is still largely unknown. Here, we suggest a few experiments.

Mutant Experiment

The following experiments could be performed to test the above-mentioned hypothesis. Investigation of the methylome of mutant embryos defective in the DNA demethylation pathway genes expressed only in gamete companion cells or the endosperm can provide conclusive evidence. *dme* mutant embryo would provide the most direct evidence of the crosstalk hypothesis. However, a proper experimental design using *dme* mutants is required due to the lethality caused by inheritance of the maternal *dme* mutant allele even in heterozygous state. *dme* mutant embryos can be collected based on its distinct phenotype (aborted vs. normal) after crossing between maternal *dme* heterozygous plant and paternal wild type. Since *dme* mutants show seed abortion phenotype with hypermethylation that is solely depending on the maternal *dme* allele, it is possible to check the DME effect on the heritable embryo if we compare the embryo methylome from the above crosses. The other option would be to use companion cells and tissue-specific RNAi method (central cell or endosperm-specific promoter:: one of the RdDM genes). Companion cells and tissue-specific knock-down of the genes involved in the initial step of the RdDM pathway such as ARGONAUTE (AGO), RNA DEPENDENT RNA POLYMERASE (RDR), DICER-LIKE (DCL), and Pol IV genes would be the prior targets of that experiment. In addition, recent evidences shows that a non-canonical Pol II and RDR6-dependent RdDM pathway exists during sexual reproduction (Borges et al. 2018; Martinez et al. 2018). If there is a crosstalk between the central cell and egg cell or between the endosperm and embryo, we would see the effect of DNA methylation on the embryo by misregulating the RdDM-pathway genes in the central cell or endosperm.

Egg Cell Methylome

The methylome of the egg cell not only provides clues about its communication with its companion central cells but also completes the epigenetic map of gamete cells; please refer to the central cell and sperm cell methylomes published previously (Ibarra et al. 2012; Park et al. 2016b). However, because of technical difficulties in isolating a sufficient number of pure egg cells and constructing methylome libraries from a small number of egg cells, obtaining the egg cell methylome of *Arabidopsis* remains challenging. However, given the recent development of pure egg cell isolation methods in *Arabidopsis* (Park et al. 2016b; Palovaara et al. 2017; Shulse et al. 2019) and single-cell bisulfite sequencing (BS-seq) library construction protocol (Clark et al. 2017; Yu et al. 2017), it will be possible to determine the methylation landscape of the egg cell in the near future.

Methylome of *dme* Mutant Gametes

The previously reported central cell and vegetative cell methylome data were obtained from wild type and *dme* heterozygous mutants that is 50% cells carrying the wild-type *DME* allele and 50% cells carrying the *dme* mutant allele (Ibarra et al. 2012; Park et al. 2016b). However, to accurately determine the direct targets of DME in the central cell and vegetative cell, it will be necessary to separately isolate *dme* mutant gamete companion cells from wild-type cells. Although Zhang et al. (2019) developed a method to obtain *dme* homozygous mutants by plating premature *dme* mutant seeds on MS medium, the number of viable seeds produced is too small to maintain the transgenic lines containing cell-specific markers. Furthermore, *dme* homozygous mutants tend to have an accumulative effect via unknown mechanisms; consequently, the number of viable seeds increases in each generation, at least in the *Arabidopsis* ecotype Landsberg erecta (Ler) (unpublished data). Therefore, the methylation status of *dme* homozygous mutants may vary among different generations. This difference would be caused by the secondary or indirect effects of the *dme* mutations, possibly leading to different outcomes. Thus, it will be important to use cells from *dme* heterozygous mutants that have never been in the homozygous state.

Furthermore, since inheritance of the maternal *dme* mutant allele causes seed abortion (Choi et al. 2002), only *dme* heterozygous mutants are available for the *Arabidopsis* ecotype Columbia (Col-0). Although half of the *dme* heterozygous mutant egg cells inherit the *dme* mutant allele, there is no way to distinguish between wild-type and *dme* mutant egg cells in these mutants. However, *dme* mutant egg cells could be isolated from a *dme* heterozygous mutant generated by the CRISPR-Cas9 gene editing system (Miki et al. 2018). That *dme* heterozygous mutants should harbor a green fluorescent protein (GFP) reporter gene under the control of an egg cell-specific promoter, followed by a terminator sequence immediately downstream of the endogenous *DME* transcription start site (Fig. 2). If a donor DNA is properly inserted into the *DME* locus by the CRISPR-Cas9 system, only the donor DNA with *GFP* will be translated, not the functional endogenous *DME*. Therefore, two types of gametes will be produced from the *dme* heterozygous mutant: gametes expressing *GFP*, without endogenous *DME*, and gametes expressing the intact *DME* gene, without *GFP* expression. The fluorescent egg cells could then be selectively isolated by either picking the cells manually or using methods such as fluorescence-activated cell sorting (FACS) and INTACT. In this way, it should be possible to analyze the methylome of pure *dme* mutant gamete cells and elucidate the effect of *DME* expression in gamete companion cells on the heritable gamete cells. This strategy could be used to study the gametes of any species for which homozygous mutant lines are difficult to maintain because of their lethality.

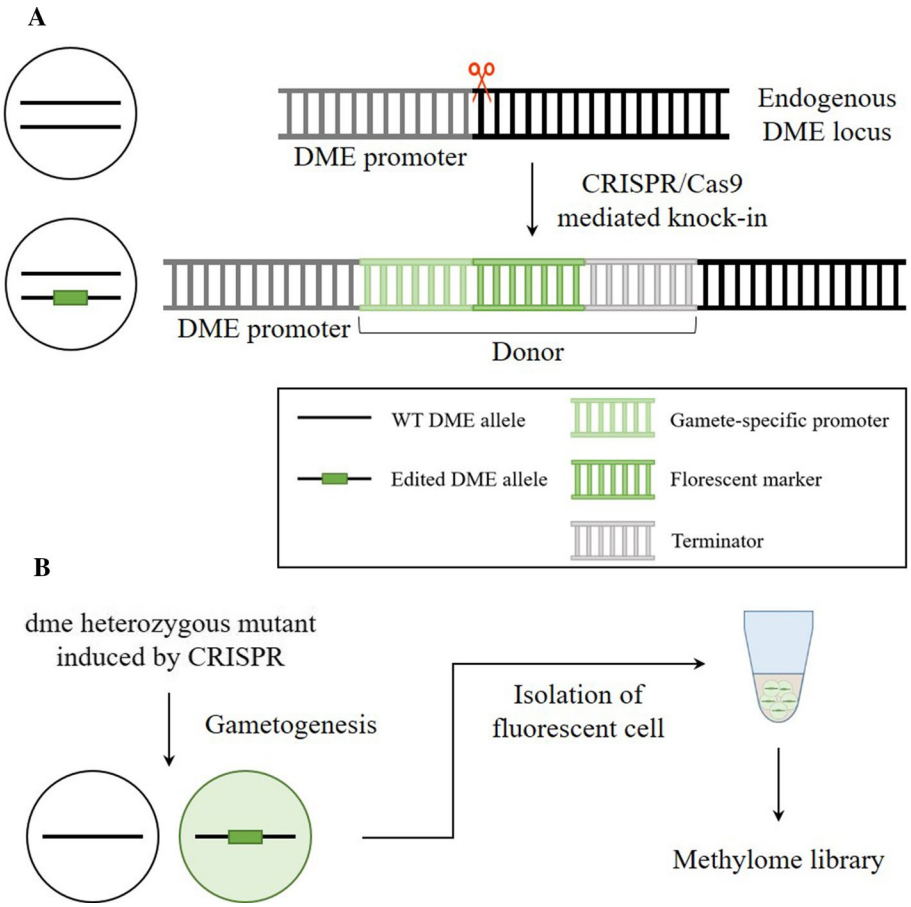

Fig. 2 Strategy for the isolation of pure *dme* mutant gamete cells. **a** To isolate pure *dme* mutant gamete cells, a donor DNA containing a florescent marker under the gamete-specific promoter followed by a terminator should be knocked-in to immediately downstream of the endogenous *DME* transcriptional start site. **b** If the donor DNA is properly inserted by CRISPR, the florescent marker will be translated instead of the endogenous DME. This will result in two types of gametes produced from the *DME* heterozygous mutant generated by CRISPR; one expressing fluorescent protein without *DME* expression and the other one expressing the intact *DME* without fluorescent protein expression. Isolating only fluorescent cells using methods such as FACS, INTACT or manual isolation should make it possible to collect pure *dme* mutant gamete cells and to obtain methylome data from them. *Green box* donor DNA, *green circle* fluorescent egg cell, *white circle* invisible cells without the fluorescent protein

Molecular Movement Between the Endosperm and Embryo

Small RNA movement from the central cell to egg cell has been shown by RNA interference (RNAi) and microinjection experiments (Ibarra et al. 2012; Erdmann et al. 2017). Additionally, protein-coding transcripts have been reported to move from the vegetative cell to sperm cells (Jiang et al. 2015). These data suggest that symplastic communication occurs between gamete companion cells and gametes. Thus, the movement of small RNAs followed by RdDM between the companion cells and their gametes is plausible. However, symplastic communication between the endosperm and embryo is not straightforward. For example, sucrose transport from the endosperm to the embryo is complicated in the spatiotemporal context (Lafon-Placette and Kohler 2014). Indeed, the embryo at the early developmental stage (before embryonic pattern formation) seems to block molecular movement from the endosperm (Erdmann et al. 2017). Therefore, simultaneous investigation of the dynamics of DNA methylation and symplastic small RNA movement between the endosperm and embryo is required.

Summary

Global demethylation has been reported in gamete companion cells (central cell and vegetative cell) where *DME* is expressed. After fertilization, DME-dependent global demethylation is observed in the endosperm, the descendant of the central cell (Gehring et al. 2009; Hsieh et al. 2009; Ibarra et al. 2012; Park et al. 2016b). DME-mediated DNA demethylation in the endosperm results in TE activation, which, in turn, initiates de novo DNA methylation, and provides the FIS2-PRC2 complex-binding site, resulting in histone modifications and genomic imprinting. Considering the expression patterns of epigenetic regulators, the egg cell seems to exhibit active RdDM without demethylation, and the resulting embryo shows active de novo methylation with robust CG maintenance (Jullien et al. 2012). The two distinct and significant observations of global demethylation of TEs in gamete companion cells and the endosperm, and active de novo methylation in the embryo suggest a new role of the sexual companion cells and tissue in reinforcing the genome integrity of the heritable tissue. Thus, conclusively, DME functions as the defender of TE activation in the heritable tissue by demethylation in gamete companion cells and the endosperm. To protect the embryo, flowering plants have evolved non-heritable cells and tissue, which exhibit global demethylation and dangerous TE activation. This might be one of the significant evolutionary advantages of angiosperms. It would be interesting to examine epigenetic communication between the companion cells and gametes, and between the endosperm and embryo, using experiments suggested in this review. Recently, Lafon-Placette et al. (Lafon-Placette and Kohler 2016) suggested another role for the endosperm, i.e., as a postzygotic hybridization barrier. Interestingly, both roles of the endosperm are engaged in epigenetics. Further studies will let us decipher the significance of the conservation of companion cells and the endosperm as well as the evolution of epigenetic regulation in angiosperms.

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