Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Genetics & Development

Epigenetic regulation and reprogramming during gamete formation in plants

Célia Baroux, Michael T Raissig and Ueli Grossniklaus

Plants and animals reproduce sexually via specialized, highly differentiated gametes. Yet, gamete formation drastically differs between the two kingdoms. In flowering plants, the specification of cells destined to enter meiosis occurs late in development, gametic and accessory cells are usually derived from the same meiotic product, and two distinct female gametes involved in double fertilization differentiate. This poses fascinating questions in terms of gamete development and the associated epigenetic processes. Although studies in this area remain at their infancy, it becomes clear that large-scale epigenetic reprograming, involving RNA-directed DNA methylation, chromatin modifications, and nucleosome remodeling, contributes to the establishment of transcriptionally repressive or permissive epigenetic landscapes. Furthermore, a role for small RNAs in the regulation of transposable elements during gametogenesis is emerging.

Address
Institute of Plant Biology, Zürich-Basel Plant Science Center, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

Corresponding author: Baroux, Célia (cbaroux@botinst.uzh.ch)

Current Opinion in Genetics & Development 2011, **21**:124–133

This review comes from a themed issue on
Chromosomes and expression mechanisms
Edited by Peter Verrijzer and Ali Shilatifard

Available online 14th February 2011

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2011.01.017

Introduction

In multicellular organisms, sexual reproduction involves the union of highly specialized, haploid gametes. Between plants and animals the ontology of the gametes as well as the fate of the fertilization products differ [1,2,3•]. In particular, plants do not set aside a germ line lineage early in development as animals do. Instead spore mother cells (SMCs), the cells destined to undergo meiosis, are specified late during the development of the diploid generation of the plant life cycle, the sporophyte. In flowering plants this occurs in specialized male and female floral organs, the anthers and ovules, respectively. Moreover, the meiotic products in plants, the spores, do not differentiate directly into gametes as in animals; rather, they divide mitotically to produce multicellular, haploid gametophytes. These can be autonomous, free living organism as for instance in mosses and ferns [4] or highly reduced to a few cells as in flowering plants [5,6]. In the majority of flowering plants, the male gametophyte (pollen) consists of three cells: two gametic sperm cells harbored within an accessory, vegetative cell responsible to deliver the sperm cells to the female gametes. The female gametophyte (embryo sac) produces two gametes, the egg and central cell, and five accessory cells: two synergids assisting fertilization and three antipodals [5–7]. Importantly, double fertilization generates two fertilization products with distinct developmental fates: while the fertilized egg gives rise to the embryo, the fertilized central cell generates an extra-embryonic nurturing tissue, the endosperm [7]. This complex scheme of gamete formation has implications in terms of developmental strategies governing first, sporogenesis in sporophytic ('somatic') floral tissues, second the specification of gametic versus accessory fate in the gametophytes, and third the distinct developmental fates of the egg and central cells. Not surprisingly, a role for epigenetic processes in these different events has been recognized [3•,8] and is supported by recent studies [9••,10••,11•,12••] which we will discuss here.

Epigenetic patterns in sporogenic fate control and meiotic progression

In flowering plants, on which our review will focus, undifferentiated stem cells reside in apically localized meristems. These meristems produce cells contributing to vegetative (root, shoot) and floral tissues [13] in which SMCs will be specified. Yet, there is no germ line lineage with a traceable sporogenic fate [14]. Instead, SMCs are newly specified in a subepidermal position within multicellular sporangia, the ovule primordium and anther locule, respectively [1]. The highly regular, predictable position of SMCs suggests a specification process dependent on the cell's position [3•,15,16] rather than its lineage as in animals, a developmental strategy commonly used in plants [17]. Consistent with this idea, maize, rice and *Arabidopsis* mutants lacking specific Leu-rich repeat receptor-like protein kinases, potentially involved in cellular signaling, produce supernumerary SMCs, the microspore and/or megaspore mother cells, respectively [16,18,19]. The observed phenotypes suggest that subepidermal cells surrounding the SMCs share a sporogenic potential [3•,20•,21]. Recent findings indicate that the sporogenic fate is epigenetically suppressed in cells other than the SMCs. *Arabidopsis* mutants lacking *AGO9* function develop multiple megaspore mother cells (MMCs) in the nucellus

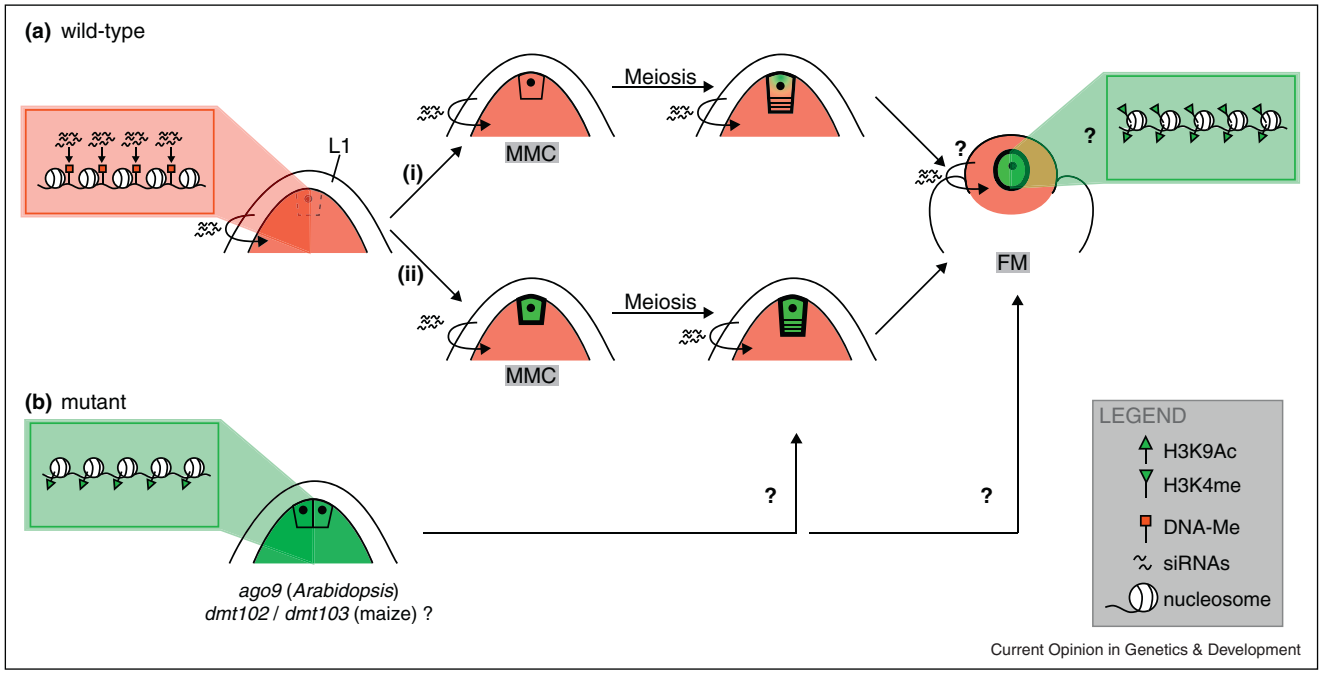
of the ovule [10••,22]. AGO9 belongs to the ARGONAUTE protein family involved in the processing of RNAs into microRNAs (mi-RNAs) and small-interfering RNAs (siRNAs) directing post-transcriptional gene silencing (PTGS) and RNA-dependent DNA methylation (RdDM) [22]. The detection of the AGO9 protein in the epidermal (L1) cell layer specifically suggests that female sporogenic cell fate may be restricted to a single cell via a non-cell autonomous, small RNA-dependent mechanism possibly involving RdDM [10••]. Consistent with this hypothesis, maize nucellar cells express high levels of *Dmt102* transcripts encoding a homologue of the *Arabidopsis* CHROMOMETHYLTRANSFERASE3 (CMT3) [12••], which is responsible for DNA methylation at non-CG sites and acting downstream of siRNA targeting [23]. At the same time and in contrast to adjacent somatic cells, their chromatin is strongly depleted in H3K9Ac [12••], a transcriptionally permissive mark antagonistic to H3K9me2 and DNA methylation, at least in *Arabidopsis* [24]. Down-regulation of *Dmt102*, while accompanied by hyperacetylation of H3K9 in subepidermal cells, was not sufficient to produce supernumerary MMCs as observable at the cytological level, suggesting additional components inhibiting MMC formation [12••]. However, the combined down-regulation of *Dmt102* and *Dmt103*, a close homologue of the *de novo* DNA methyltransferase DOMAIN REARRANGED METHYLTRANSFERASE2 (DRM2), resulted in the formation of ectopic embryo sacs. Some of these may have arisen from supernumerary MMCs [12••], although this awaits confirmation. *Dmt103* is specifically expressed in the epidermal cells of the nucellus [12••]; whether its function is mechanistically connected to an AGO9-related pathway in maize remains to be investigated. This study also revealed the involvement of non-CG methylation (via *Dmt102* and *Dmt103*) rather than CG methylation [12••]. It will be of particular interest to identify the genomic loci affected by siRNA-mediated silencing and non-CG methylation in target cells during sporogenesis.

Profiling at later stages of ovule development revealed that AGO9 preferentially associates with 24nt siRNA targeting transposable elements (TEs), which they regulate in the mature embryo sac, but also with some 20–21nt mi-RNAs species [10••,25]; whether the same small RNAs also act during sporogenesis is yet unknown. To accommodate the observed chromatin patterns in the maize nucellus, one might expect the involvement of small RNAs in targeting — or at least influencing — the silencing in genic regions (not only TEs) to repress sporogenesis and possibly gametogenesis in these cells. The emerging parallel roles of non-coding RNAs controlling germ cell development in animals and plants are of outstanding interest [3•,26]. Whether a similar mechanism is in place during microspore mother cell development should be addressed in the near future. The observation that anther tapetal cells — surrounding the male SMCs and their meiotic daughter cells — produce trans-acting siRNAs targeting the male gametophyte makes this a plausible scenario [27].

Strikingly, the global transcriptionally repressive landscape in the nucellus is reminiscent of the transcriptional quiescence established in the animal germ line. There, it is required to repress the somatic fate but also to reset and establish novel epigenetic profiles in the gametes compatible with totipotency in the zygote [12••,28]. The question is thus which fate is repressed by this apparent transcriptional quiescence in plants? One possibility is the supression of the gametophytic fate (Figure 1, panel (i)). Consistent with this model, the lack of epigenetic repression in the nucellar cells of *Arabidopdis* or maize mutants promotes gametophytic development with the differentiation of additional, albeit abnormal or incomplete, embryo sacs [10••,12••]. Possibly meiosis was bypassed, although more detailed investigations are necessary. If confirmed, this would be reminiscent of apospory, which generates unreduced gametes in some plant species that reproduce through apomixis, the asexual reproduction through seeds [29]. An alternative model invokes the temporal and spatial regulation of sporogenic fate repression (Figure 1, panel (ii)). There, global epigenetic transcriptional repression may first be established in the entire nucellus; later, the selected MMC may escape this repression while it is retained in neighboring cells. In this scenario, siRNA-mediated repression might become less effective in the MMC, possibly as a result of callose deposition in the cell wall. This may constrain intercellular cytoplasmic connections (plasmodesmata) and, thus, transport of siRNAs [30,31]. Consequently, the MMC could recover a transcriptionally permissive state allowing meiosis to take place. Consistent with this hypothesis, meiotic progression — but not entry — is critically dependant on a complex set of histone modifications (reviewed in [3•,32]), including transcriptionally permissive marks established by histone lysine methyltransferases of the SET Domain Group (SDG) [33]. However, the nature of the trigger promoting the mitosis-to-meiosis switch remains unknown. Nonetheless, the prediction that siRNAs were to play an important role in the regulation of meiosis [34] was recently supported by the identification of *MEL1* in rice. *MEL1* encodes an ARGONAUTE protein specifically expressed in SMCs and its loss-of-function causes an arrest at leptotene, the earliest stage where meiosis is distinguished from mitosis [20•]. While the MEL1 clade is closest to the Arabidopsis AGO1-containing clade, its closest homolog in *Arabidopsis*, At2g27880 [20•] was recently annotated as AGO5. The identification of MEL1 targets and their function promises exciting findings with regard to the epigenetic processes controlling the meiotic switch.

Altogether, it becomes clear that sporogenesis is epigenetically controlled both during SMC selection and

Figure 1



Large-scale epigenetic reprogramming during megasporogenesis. **(a)** In ovule primordia, nucellar cells (except for the L1 layer) display a transcriptionally repressive landscape associated with genome-wide histone hypoacetylation, which seems to rely on the RdDM pathway involving AGO9-associated small RNAs originating from L1 cells as well as CMT3-like and DMR2-like activities in nucellar cells. To accommodate mutant phenotypes and expression patterns of epigenetic components as described in the text, two models are proposed that involve dynamic changes of epigenetic landscapes during megasporogenesis. (I) Transcriptional repression inhibits gametophyte development in nucellar cells and the MMC until meiosis is completed. During meiosis permissive histone methylation marks are established that are required for meiotic progression (red-green background). The functional megaspore (FM) has a transcriptionally permissive landscape promoting gametophyte development. (II) Transcriptional repression suppresses the sporogenic fate in nucellar cells. The selected MMC escapes this repression, potentially following isolation from mobile small RNAs. A transcriptionnally permissive landscape is required for completion of meiosis and the promotion of gametophytic development. **(b)** In *Arabidopsis* or maize mutants lacking the function of one epigenetic component mediating transcriptional repression, nucellar cells have a transcriptionally permissive landscape leading to the selection of multiple MMCs and the differentiation of a gametophyte, although abnormal or incomplete.

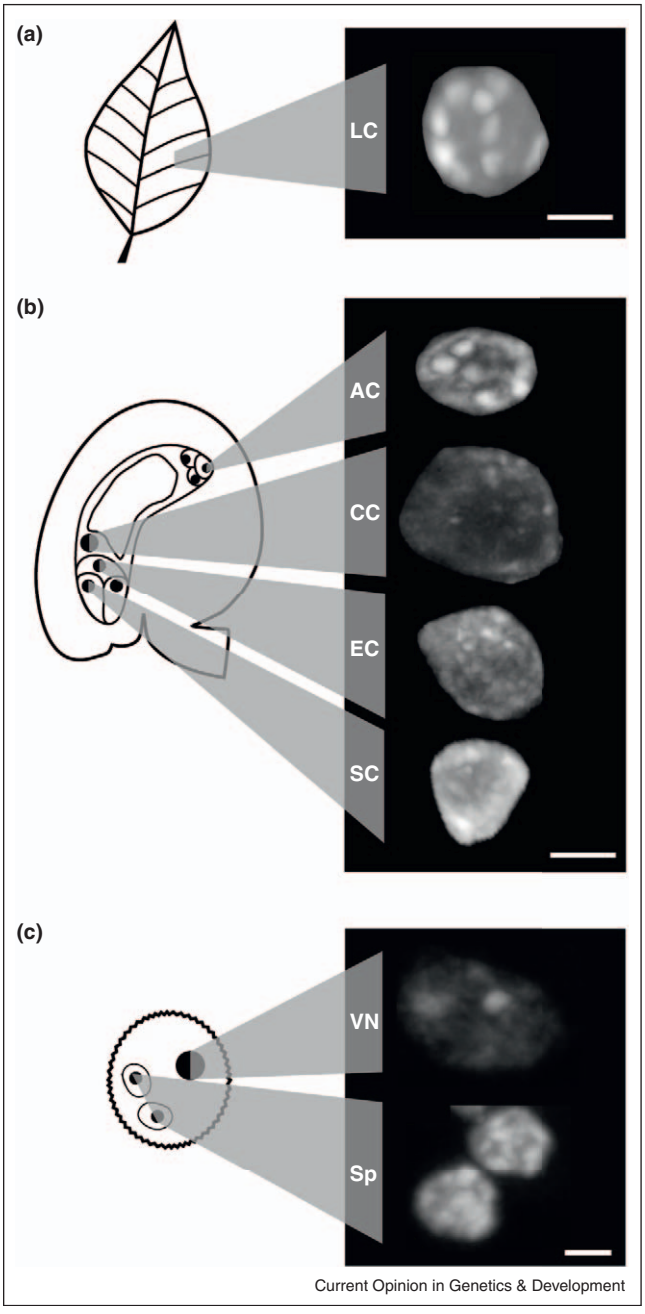
during meiotic progression. Transcriptional quiescence mediated by mobile siRNAs in particular seems instrumental in controlling the sporogenic and possibly the gametophytic fate. Future investigations should aim at revealing the targets of this transcriptional repression, possibly by profiling small RNAs specifically in nucellar cells and MMCs (e.g. using laser-assisted microdissection [35]), but also at elucidating the precise epigenetic chromatin landscape (using immunostaining for different informative histone marks) during sporogenesis.

Epigenetic patterns and reprogramming during gametophyte development

In plants, the meiotic products (spores) enter mitosis and produce a multicelllar, haploid gametophyte, which differentiates gametic as well as accessory cells. Despite the stark reduction and thus simplification of the gametophytes in flowering plants, little is known about the specification of the distinct cell fates [36]. A distinct organization of the chromatin among gametophytic nuclei has been documented early ([1] and Figure 2) but it is only recently that inferences are drawn in terms of epigenetic processes. Several studies now suggest large-scale chromatin modifications associated with the epigenetic differentiation of gametophytic cells.

On the male side, the first asymmetric mitosis generates a small generative cell and a large vegetative cell (Figure 3). The generative cell further divides to produce two sperm cells. Several studies, although fragmented across different species, clearly show that the chromatin composition drastically differs between the sperm and vegetative nucleus. The sperm chromatin, in contrast to the chromatin of the vegetative nucleus, contains gamete-specific core nucleosome variants (gH2A, H2B, gH3) including a gamete-specific H3.3 variant [37–40] (Figure 3). The compact appearance of sperm chromatin (Figure 2) is often interpreted as a transcriptionally inactive state. But, in fact, immunostaining for chromatin modifications reveal a bivalent status: transcriptionally permissive H3K4me3 and H3K36me2/3 marks [41] are below detection level, while euchromatic regions are enriched in

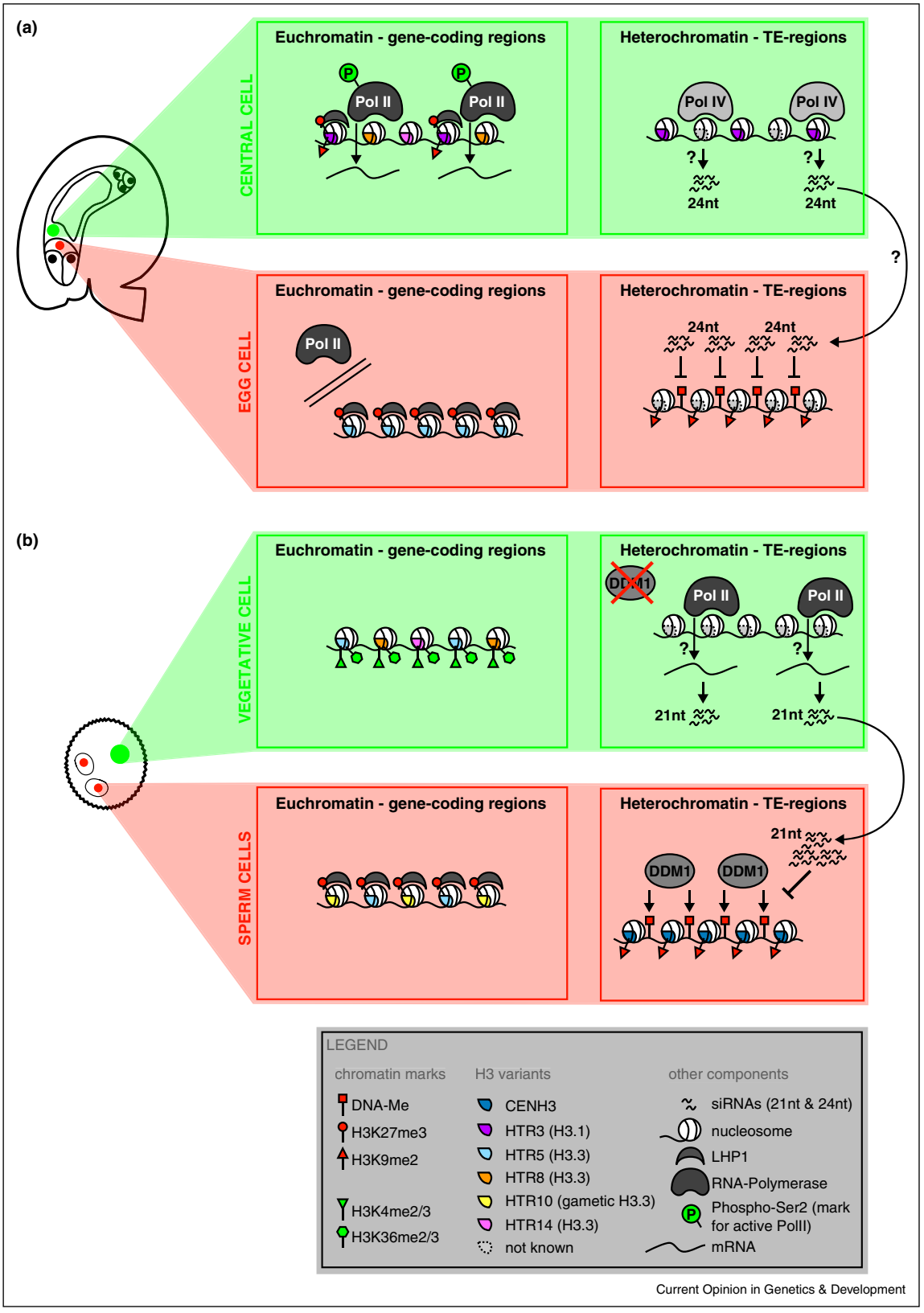
Figure 2



The male and female gametophyte differentiate gametic and accessory cell types with distinct chromatin organization. A distinct organization of the nuclear chromatin in gametophytic nuclei is revealed by whole-mount, non-denaturing DNA staining [77], suggesting that large-scale epigenetic modifications differentiate the mitotic sister cells of the gametophyte (see also Figure 3 and text). **(a)** In sporophytic nuclei, heterochromatin is microscopically visible as well defined, brightly stained foci. **(b)** In the female gametophyte, the gametic nuclei show small, dispersed heterochromatin foci, a feature that is more pronounced in the central cell than the egg cell nucleus. By contrast nuclei of the accessory cells (antipodals and synergids) show higher heterochromatin condensation, with a chromatin organization in antipodal nuclei close to that of a sporophytic cell. **(c)** In the male gametophyte, the vegetative nucleus has largely decondensed chromatin with no apparent heterochromatin organization albeit for a single focus; this organization contrasts with the highly condensed sperm chromatin as inferred by the nuclear size and the discernable heterochromatic foci. This illustrative figure relates to the model presented in Figure 3. All pictures are maximum projections from 3-dimensional reconstructions. Pictures in panel (b) are from C.B. and were reproduced with permission from [78]. Scale bar is 2 μm. AC, antipodal cell; CC, central cell; EC, egg cell; SC, synergid cell; VN, vegetative nucleus; Sp, sperm cells.

LIKE HETEROCHROMATIN PROTEIN1 (LHP1), a protein associated with the transcriptionally repressive H3K27me3 mark [42•,43]. At the same time, hyperacetylation on H3 and H4 residues, together with low levels of DNA methylation [42•,44], suggest that the sperm chromatin retains — to a certain degree — a transcriptionally permissive environment. In stark contrast, the vegetative chromatin appears less condensed, is composed of distinct H3 isoforms, and harbors transcriptionally permissive histone modifications [37,41,42•] (Figures 2 and 3). It also differs in heterochromatin organization and centromeres identity with, particularly, the absence of the centromere-specific CENH3 variant [45] and the heterochromatin-specific somatic H3.1 variants [46•]. This corroborates the absence of the SWI/SNF chromatin remodeler DECREASE DNA METHYLATION1 (DDM1) [9••], normally required for centromeric heterochromatin organization [47]. Thus, clearly, the gametic and accessory chromatin displays an opposite chromatin organization, likely in relation to the resetting of epigenetic marks in the germ line as proposed earlier [3•], but also to the distinct transcriptional programs [48]. The key question remains, however, as to when these differences are established and whether they are cause or consequence of gametic versus accessory cell fate differentiation. The observation that sperm-specific H3 variants are already present in the generative cell [49] suggests that chromatin differentiation is coupled, at least in part, with the first mitosis. Generative and vegetative nuclei clearly show distinct transcriptional patterns supporting their distinct function [48,50]. Misexpression of specific chromatin constituents or modifiers in one mitotic product but not the other may help to understand their influence on the transcriptional programs and downstream differentiation events. Finally, the specific chromatin organization in the vegetative nucleus seems to have a role in the regulation of TEs in the germ line. This was inferred from the observation that the loss of heterochromatin organization results in massive derepression of TEs and the production of 21nt siRNAs, in a DDM1-dependent fashion [9••]. The siRNAs are thought to be transported into the sperm cells where they likely reinforce silencing of TEs. Hence, the vegetative cell may function as a safeguard of genome integrity in the sperm cells in a manner reminiscent of the role of piwiRNAs, 24–31 single-stranded non-coding RNAs principally targeting TEs in the animal germ line [9••,51]. The discovery of a vast and complex population of additional

Figure 3



Distinct epigenetic landscapes regions are established in gametic and accessory nuclei in the gametophytes. This figures combines the findings from several studies on different species but is representative of the situation in *Arabidopsis*. The repertoire of epigenetic modifications shown is not

small RNAs in the male gametophyte, as well as the genetic components producing and targeting them, predicts additional roles in regulating male gametophyte development and repressing somatic functions [52–54].

On the female side, the situation may appear a bit more complex, and certainly less well studied. More complex, because there are three syncytial mitoses followed by nuclear migration and cellularization (Figure 3 represents the most frequent, *Polygonum*-type female gametophyte [1]). Less is known partly because of the difficulty in investigating these cells, which are deeply embedded in maternal tissues. The embryo sac is highly polarized and cellularization establishes distinct cell types (Figure 3). The egg apparatus constituted of the egg cell and two flanking synergids is formed at the micropylar pole, antipodal cells — of yet unknown function — are formed at the chalazal pole, while the central cell in the middle contains two polar nuclei. These migrate towards the center of the embryo sac before cellularization and fuse before or at fertilization depending on the species [7]. These cell types are distinct not only at the cellular, but also at the level of microscopic chromatin organization. Typically, the central and egg cells show a low compaction level together with dispersed heterochromatin foci compared to antipodal and synergid cells (Figure 2, [46•] and C.B., unpublished), suggesting that the gametic versus accessory cell fate may be accompanied by a distinct epigenetic status of the gametophytic cells. It is not yet known whether these distinct features are established before cellularization, in the syncytium, or later. The epigenetic status of these nuclei, defined at the level of the transcriptional pattern, chromatin modifications, and nucleosome constitution, still remains to be analyzed in detail, especially on a temporal scale along with mitotic progression, polarity establishment, and cellularization. Chromatin changes affecting nucleosome constitution and biochemical modifications appear to occur dynamically following cellularization [11•,46•] and contribute to establish a distinct transcriptional status in egg and central cell (see below). Conversely, DNA methylation patterns of gametophytic nuclei may regulate cell identity, cell number and embryo sac polarity as shown in the *dmt102* and *dmt103* maize mutants [12••]. To date, no such phenotypes have been documented in the corresponding *Arabidopsis* mutants, which are fully fertile [55,56] suggesting species-dependent epigenetic control during gametophyte development.

Epigenetic dimorphism between the female gametes

Double fertilization in flowering plants poses the intriguing problem to (epigenetically) distinguish the developmental fates of the fertilization products. In most species each pair of male or female gametes arise from a single male or female spore, respectively. In most species the two sperm are cytologically indistinguishable and can fertilize either the central or egg cell [57,58]. Thus, it was proposed that the female gametes must have different epigenetic states that contribute to the distinct post-fertilization fates [21,8]. But only recently detailed chromatin investigations revealed a stark epigenetic dimorphism between the two female gametes, in both *Arabidopsis* and maize [11•,12••,46•]. The egg cell displays a global transcriptionally quiescent chromatin state with undetectable levels of active RNA Polymerase II (PolII) concomitant with enrichment in LHP1. By contrast the central cell chromatin displays a transcriptionally active and permissive chromatin organization [10••,11•], Figure 3). At the same time, microscopically visible heterochromatin regions, mostly composed of TEs [59], are well defined and enriched in H3K9me2 in the egg as compared to the central cell where heterochromatic foci are less pronounced (Figure 2 and [46•]). In *Arabidopsis*, this dimorphism requires the activity of CMT3 in the egg cell, and DEMETER-LIKE enzymes, catalyzing the removal of methylcytosine residues, in the central cell [11•,42•]. Importantly, the distinct global epigenetic

(Figure 3 Legend Continued) exhaustive and only represents studied marks. **(a)** In the female gametophyte the two female gametes show an epigenetic dimorphism. In comparison to the egg cell, the central cell is abundant for the active form of RNA Pol II, features a transcriptionally permissive environment in euchromatic regions with quantitatively little repressive marks, as inferred from LHP1 levels (LHP1 is a H3K27me3-associated protein [43]). By contrast the egg's euchromatin displays a transcriptionally quiescent state with opposite active Pol II and LHP1 abundance (note that the inactive form of Pol II is present in the egg nucleus). Distinct variants of H3.3 histones are found in the egg and central cell but their role in transcriptional control is not known. At heterochromatin regions, the scenario is hypothetical and based on observations made at later stage of seed development (see text), mirroring a similar scenario in the male gametophyte **(b)**. The central cell is proposed to produce small RNAs potentially targeting TEs in the egg cell. The lack of heterochromatin at TE regions linked to a lack of centromeric identity (dispersed heterochromatin foci [Figure 2] and absence of detectable CENH3 (see text) may favor reactivation of TEs. This hypothetically involves RNA Pol IV to produce 24nt-siRNAs as they were detected at late stages of endosperm development. Potentially, these siRNA are transported into the egg cell to ensure TE silencing via RdDM as was proposed for the embryo at later stages, and they might be linked to high levels of H3K9me2 as observed in the egg cell. (b) In the male gametophyte, epigenetic dimorphism is not found between the gametes but rather between the sperm cells and the vegetative cell. Euchromatin regions show histone modifications linked with a transcriptionally permissive landscape in the vegetative nucleus, in contrast to the euchromatin in sperm cells. At the nuclesome level, they both show a common histone H3.3 variant (HTR5) but also distinct variants, including a gamete-specific variant (HTR10) in the sperm. Whether these variants influence the epigenetic state is not known. At heterochromatin regions, the absence of the SWI/SNF chromatin remodeler DDM1 and the loss of centromeric heterochromatin organization is correlated with derepression of TEs, transcription by RNA Pol IV and production of 21nt small RNAs accumulating in the sperm cells. These siRNAs are thought to reinforce TE silencing in the gametes, possibly via RdDM, to ensure genome integrity. This correlates with enrichment of H3K9me2 at heterochromatic foci, although contradictory immunostaining results were reported [41,42•].

states affecting chromatin modifications and transcription are established soon after cellularization and are inherited post-fertilization [11•]. This dimorphism also correlates with a genome-wide hypomethylation found later during seed development in the endosperm as compared to the embryo, in both *Arabidopsis* and maize [60–62]. Epigenetic dimorphism between the female gametes is further found at the level of nucleosome composition with specific sets of H3.3 isoforms in each cell, as inferred from tagged histone reporters ([46•], Figure 3). Particularly, the egg cell displays only one H3.3 isoform (HTR5) also found in the sperm cells, while the central cells displays two isoforms (HTR8 and HTR14). Whether HTR8 and HTR14 epigenetically distinguish two functional chromatin compartments would be interesting, yet challenging to determine. Interestingly, this nucleosome dimorphism between the egg and central cell is ephemeral and seems remodeled after fertilization, suggesting that it was established solely for the purpose of distinguishing the egg versus central cell chromatin during gametophyte development. This also implies that maternal epigenetic marks associated with these H3 variants are not transmitted to the zygote [46•]. But the possibility remains that some loci escape this remodeling, a situation that would not be detected at the microscopic scale with reporter histones such as used in this study [46•], and leading to the transmission of a maternal epigenetic mark (imprint). Similarly, for some loci, especially those showing parent-of-origin-specific expression following fertilization, the epigenetic states established in the female gametes may be dynamically modified following fertilization [63,64]. For instance, DNA methylation patterns established on the maize *ZmFIE1 and Mee1* loci in the central and egg cells, respectively, are not maintained in the fertilization products [63,64].

But what could explain this epigenetic dimorphism? The distinct transcriptional states support the different requirements of the fertilization products on maternally inherited information: while the endosperm strictly requires *de novo* transcription, the quiescent zygote can undergo 4–5 mitoses in the absence of active PolII [11•]. Initial quiescence in the plant zygote is reminiscent of the situation in animals, where it is a strict requirement for genome reprogramming and the acquisition of totipotency [28]. However, releasing the quiescence in the egg cell is not lethal for the embryo [42•]. Instead, transient defects in patterning are observed [42•], which could be explained by the relative robustness of the plant genome to epigenetic perturbations, possibly mediated by alternative epigenetic pathways [42•,65]. Thus, the relative quiescence in the egg, possibly linked to the selective reprogramming of maternal epigenetic information, might fulfill additional roles. These may be revealed in natural, ecological contexts under selective pressures, by contrast to laboratory conditions where history of the maternal parent does not obviously influence the offspring (at least not detectably). Finally, epigenetic dimorphisms at the level of TE-containing heterochromatic regions may support a similar role to that described in the male gametophyte. In such a model, the central cell is proposed to contribute to protecting genome integrity in the egg by producing and transporting siRNAs reinforcing TE silencing [66,67,68]. This model arose from combined observations made at later stage during seed development, in the endosperm at 7–9 days after pollination. A complex set of maternal small RNAs, including 24nt (and not 21nt as in the pollen [9••]) siRNAs mostly targeting TEs, was found [69•] concomitant with an extensive demethylation of these elements [60]. While very attractive, this model awaits demonstration especially for the presence and mobility of such small RNAs in the central cell. TEs are derepressed in *ago9* but also *cmt3* mutant gametophytes [10••,42•], comforting the hypothesis that small RNAs may preserve genome integrity in the egg cell. However, the expression pattern of AGO9 also suggests that the maternal sporophyte may be involved in this process [10••,25].

Concluding remarks

The past decade of research revealed exciting findings with regard to epigenetic mechanisms controlling developmental processes specific to flowering plants: the determination of sporogenic fate late during development, the differentiation of gametes within multicellular gametophytes, and the distinction of the two female gametes involved in double fertilization. Particularly, there seem to be two levels of epigenetic regulation, both acting at large-scale: one regulatory level targets euchromatic regions where a transcriptionally repressive landscape seems to be established during both sporogenic fate acquisition and egg — and likely sperm — differentiation. This is reminiscent of the quiescence in the animal germ line and early zygote, necessary for the acquisition of totipotency [28]. The involvement of mobile non-coding siRNAs, contributed by the maternal sporophyte or the sporogenic/gametophytic lineage itself, is particularly intriguing and will surely stimulate future research in the field. Furthermore, whether transcriptional quiescence during sporogenesis and gametogenesis plays a similar role in plants and animals remains to be determined. It will be of particular interest to determine whether the epigenetic information related to the maternal history — and thus linked to the environment — is maintained or reset during these phases. On the one hand, the ability to flower acquired following vernalization (winter-mimicking period of cold), and mediated by the epigenetic repression of the flowering control locus FLC, has to be reset in the new diploid generation [70–72]. On the other hand, explaining some maternal effects on the progeny may require the inheritance of maternally acquired epigenetic information [73,74]; although virtually nothing is known about the mechanism. The

second level of epigenetic regulation emerging from recent studies is the control of TEs mostly contained in microscopically visible heterochromatic regions and the centromeres of *Arabidopsis* [59]. In order to preserve genome integrity in the developing embryo, plants seem to sacrifice sister cells of the gametes — the accessory vegetative cell in the pollen and possibly the central cell in the embryo sac — which do not contribute to the next generation. In the current model, which still awaits demonstration, these cells produce mobile siRNAs reinforcing TE silencing in the gametes and possibly in the embryo.

Finally, and on a different note, the recent findings presented in the first section have important implications in the understanding of apomixis, a naturally occurring mode of asexual reproduction through seeds, involving the production of additional unreduced gametophytes in some plant species. Particularly, these findings are consistent with the proposed epigenetic basis for the deregulation of sporogenesis and/or gametogenesis during apomixis [29,75,76].

Acknowledgements

We thank D. Grimanelli for stimulating discussions on topics included in this review. Our work on the epigenetic regulation of plant reproduction is supported by the University of Zürich, a fellowship of the Roche Research Foundation (to MTR), the EPIGENOME Network of Excellence and the European Research Council (to UG), and grants of the Swiss National Science Foundation (to UG and CB).

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Maheshwari P: *An Introduction to the Embryology of Angiosperms*. New York/Toronto/London: McGraw-Hill Book Company; 1950.

2. Walbot V, Evans MM: **Unique features of the plant life cycle and their consequences**. *Nat Rev Genet* 2003, **4**:369-379.

3. • Dickinson HG, Grant-Downton R: **Bridging the generation gap: flowering plant gametophytes and animal germlines reveal unexpected similarities**. *Biol Rev Camb Philos Soc* 2009, **84**:589-615.
This work exhaustively reviews cytological, molecular, and epigenetic aspects of male and female gametophyte development in flowering plants, while drawing interesting comparisons to animal systems.

4. Banks JA: **Gametophyte development in ferns**. *Annu Rev Plant Physiol Plant Mol Biol* 1999, **50**:163-186.

5. Rudall PJ: **How many nuclei make an embryo sac in flowering plants?** *Bioessays* 2006, **28**:1067-1071.

6. Rudall PJ, Bateman RM: **Developmental bases for key innovations in the seed–plant microgametophyte**. *Trends Plant Sci* 2007, **12**:317-326.

7. Dumas C, Rogowsky P: **Fertilization and early seed formation**. *C R Biol* 2008, **331**:715-725.

8. Messing J, Grossniklaus U: **Genomic imprinting in plants**. *Results Probl Cell Differ* 1999, **25**:23-40.

9. •• Slotkin RK, Vaughn M, Borges F, Tanurdzic M, Becker JD, Feijo JA, Martienssen RA: **Epigenetic reprogramming and small RNA silencing of transposable elements in pollen**. *Cell* 2009, **136**:461-472.
This is the first report showing a role for non-coding siRNAs in the regulation of transposable elements (TEs) in the male germ line, that is in *Arabidopsis* pollen. The data support a model where 21nt siRNAs targeting TEs are generated in the vegetative cell and mobilized to the sperm cells where they would reinforce TE silencing, and hence preserve genome integrity.

10. •• Olmedo-Monfil V, Duran-Figueroa N, Arteaga-Vazquez M, Demesa-Arevalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada JP: **Control of female gamete formation by a small RNA pathway in Arabidopsis**. *Nature* 2009, **464**:628-632.
The authors identified that a loss of *ARGONAUTE9* function in *Arabidopsis* deregulates sporogenic fate control with the production of multiple MMCs and ectopic embryo sacs. Furthermore, transposable elements are derepressed in *ago9* embryo sacs, highlighting an additional role in transposable element regulation in the gametes. Expression of *AGO9* in the epidermal cells suggests a non-cell autonomous pathway in both processes.

11. • Pillot M, Baroux C, Vazquez MA, Autran D, Leblanc O, Vielle-Calzada JP, Grossniklaus U, Grimanelli D: **Embryo and endosperm inherit distinct chromatin and transcriptional states from the female gametes in Arabidopsis**. *Plant Cell* 2010, **22**:307-320.
The female gametes involved in double fertilization harbor a stark epigenetic dimorphism at the chromatin and transcriptional level, with the egg showing a relative quiescence reminiscent of the situation in animals. This dimorphism is genetically controlled in the egg by CMT3 directing non-CG methylation and in the central cell by DEMETER-LIKE enzymes mediating removal of methylcytosine residues.

12. •• Garcia-Aguilar M, Michaud C, Leblanc O, Grimanelli D: **Inactivation of a DNA methylation pathway in maize reproductive organs results in apomixis-like phenotypes**. *Plant Cell* 2010, **22**:307–320.
This study identifies four loci involved in gene silencing via DNA methylation that are deregulated between sexual and apomictic maize lines. It further demonstrates a role for two of them, encoding CMT3 and DRM2 homologues, during sporogenesis and gametogenesis and in the establishment of transcriptionnally repressive patterns during these processes.

13. Stahl Y, Simon R: **Plant primary meristems: shared functions and regulatory mechanisms**. *Curr Opin Plant Biol* 2010, **13**:53-58.

14. Rédei GP: **Analysis of the diploid germline of plants by mutational techniques**. *Can J Genet Cytol* 1974, **16**:473-476.

15. Sheridan WF, Golubeva EA, Abrhamova LI, Golubovskaya IN: **The mac1 mutation alters the developmental fate of the hypodermal cells and their cellular progeny in the maize anther**. *Genetics* 1999, **153**:933-941.

16. Nonomura K, Miyoshi K, Eiguchi M, Suzuki T, Miyao A, Hirochika H, Kurata N: **The MSP1 gene is necessary to restrict the number of cells entering into male and female sporogenesis and to initiate anther wall formation in rice**. *Plant Cell* 2003, **15**:1728-1739.

17. Terpstra I, Heidstra R: **Stem cells: the root of all cells**. *Semin Cell Dev Biol* 2009, **20**:1089-1096.

18. Sheridan WF, Avalkina NA, Shamrov II, Batygina TB, Golubovskaya IN: **The mac1 gene: controlling the commitment to the meiotic pathway in maize**. *Genetics* 1996, **142**:1009-1020.

19. Zhao DZ, Wang GF, Speal B, Ma H: **The excess microsporocytes1 gene encodes a putative leucine-rich repeat receptor protein kinase that controls somatic and reproductive cell fates in the Arabidopsis anther**. *Genes Dev* 2002, **16**:2021-2031.

20. • Nonomura K, Morohoshi A, Nakano M, Eiguchi M, Miyao A, Hirochika H, Kurata N: **A germ cell specific gene of the ARGONAUTE family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice**. *Plant Cell* 2007, **19**:2583-2594.
The authors describe the rice mutant *mel1,* affected in an *ARGONAUTE* family member closely related to *AGO1* and *AGO5*, causing early meiotic arrest during sporogenesis. The arrest at leptotene in early prophase I suggests that *MEL1* may be involved in the mitotic-meiotic switch in rice, a key transition during sporogenesis. It further suggests a role for non-coding siRNA-mediated regulation in sporogenesis.

21. Grossniklaus U, Schneitz K: **The molecular and genetic basis of ovule and megagametophyte development**. *Semin Cell Dev Biol* 1998, **9**:227-238.

22. Vaucheret H: **Plant ARGONAUTES**. *Trends Plant Sci* 2008, **13**:350-358.

23. Chan SW: **Inputs and outputs for chromatin-targeted RNAi**. *Trends Plant Sci* 2008, **13**:383-389.

24. Zhou J, Wang X, He K, Charron JB, Elling AA, Deng XW: **Genome-wide profiling of histone H3 lysine 9 acetylation and dimethylation in Arabidopsis reveals correlation between multiple histone marks and gene expression**. *Plant Mol Biol* 2010, **72**:585-595.

25. Duran-Figueroa N, Vielle-Calzada JP: **ARGONAUTE9-dependent silencing of transposable elements in pericentromeric regions of Arabidopsis**. *Plant Signal Behav* 2010:5.

26. Tang F: **Small RNAs in mammalian germline: tiny for immortal**. *Differentiation* 2010, **79**:141-146.

27. Tarutani Y, Shiba H, Iwano M, Kakizaki T, Suzuki G, Watanabe M, Isogai A, Takayama S: **Trans-acting small RNA determines dominance relationships in Brassica self-incompatibility**. *Nature* 2010, **466**:983-986.

28. Seydoux G, Braun RE: **Pathway to totipotency: lessons from germ cells**. *Cell* 2006, **127**:891-904.

29. Koltunow AM, Grossniklaus U: **Apomixis: a developmental perspective**. *Annu Rev Plant Biol* 2003, **54**:547-574.

30. Rodkiewicz B: **Callose in cell walls during megasporogenesis in Angiosperms**. *Planta* 1970, **93**:39-47.

31. Xu XM, Jackson D: **Lights at the end of the tunnel: new views of plasmodesmal structure and function**. *Curr Opin Plant Biol* 2010, **13**:684-692.

32. Ronceret A, Sheehan MJ, Pawlowski WP: **Chromosome dynamics in meiosis**. In *Cell Division Control in Plants*, vol 9. Edited by Zong DPSV. 2010:103-124. Plant Cell Monogr.

33. Berr A, McCallum EJ, Menard R, Meyer D, Fuchs J, Dong A, Shen WH: **Arabidopsis SET DOMAIN GROUP2 is required for H3K4 trimethylation and is crucial for both sporophyte and gametophyte development**. *Plant Cell* 2010, **22**:3232-3248.

34. Hamant O, Ma H, Cande WZ: **Genetics of meiotic prophase I in plants**. *Annu Rev Plant Biol* 2006, **57**:267-302.

35. Wuest SE, Vijverberg K, Schmidt A, Weiss M, Gheyselinck J, Lohr M, Wellmer F, Rahnenfuhrer J, von Mering C, Grossniklaus U: **Arabidopsis female gametophyte gene expression map reveals similarities between plant and animal gametes**. *Curr Biol* 2010, **20**:506-512.

36. Ma H, Sundaresan V: **Development of flowering plant gametophytes**. *Curr Top Dev Biol* 2010, **91**:379-412.

37. Okada T, Endo M, Singh MB, Bhalla PL: **Analysis of the histone H3 gene family in Arabidopsis and identification of the male-gamete-specific variant AtMGH3**. *Plant J* 2005, **44**:557-568.

38. Okada T, Singh MB, Bhalla PL: **Histone H3 variants in male gametic cells of lily and H3 methylation in mature pollen**. *Plant Mol Biol* 2006, **62**:503-512.

39. Ueda K, Kinoshita Y, Xu ZJ, Ide N, Ono M, Akahori Y, Tanaka I, Inoue M: **Unusual core histones specifically expressed in male gametic cells of *Lilium longiflorum***. *Chromosoma* 2000, **108**:491-500.

40. Xu H, Swoboda I, Bhalla PL, Singh MB: **Male gametic cell-specific expression of H2A and H3 histone genes**. *Plant Mol Biol* 1999, **39**:607-614.

41. Cartagena JA, Matsunaga S, Seki M, Kurihara D, Yokoyama M, Shinozaki K, Fujimoto S, Azumi Y, Uchiyama S, Fukui K: **The Arabidopsis SDG4 contributes to the regulation of pollen tube growth by methylation of histone H3 lysines 4 and 36 in mature pollen**. *Dev Biol* 2008, **315**:355-368.

42. • Pillot M, Autran D, Leblanc O, Grimanelli D: **A role for CHROMOMETHYLASE3 in mediating transposon and euchromatin silencing during egg cell reprogramming in Arabidopsis**. *Plant Signal Behav* 2010:5.

In this work, the authors show that CMT3 is, similar to AGO9, involved in transposable element regulation in the female gametophyte. This connects siRNA-mediated silencing and non-CG methylation in this process.

43. Exner V, Aichinger E, Shu H, Wildhaber T, Alfarano P, Caflisch A, Gruissem W, Kohler C, Hennig L: **The chromodomain of LIKE HETEROCHROMATIN PROTEIN 1 is essential for H3K27me3 binding and function during Arabidopsis development**. *PLoS One* 2009, **4**:e5335.

44. Januseck B, Zluvova J, Vyskot B: **Histone H4 acetylation and DNA methylation dynamics during pollen development**. *Protoplasma* 2005, **211**:116-122.

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

46. • Ingouff M, Rademacher S, Holec S, Soljic 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**. *Current Biology* 2010, **20**:2137–2143.

This study report the generation of tagged histone H3 variants covering the entire repertoire of expressed isoforms in *Arabidopsis*. This allowed describing specific sets of variants in the male and female gametes. The authors further show a dynamic remodeling of the tagged histones inherited by either parent after fertilization.

47. Soppe WJ, Jasencakova Z, Houben A, Kakutani T, Meister A, Huang MS, Jacobsen SE, Schubert I, Fransz PF: **DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in Arabidopsis**. *EMBO J* 2002, **21**:6549-6559.

48. Borg M, Brownfield L, Twell D: **Male gametophyte development: a molecular perspective**. *J Exp Bot* 2009, **60**:1465-1478.

49. Brownfield L, Hafidh S, Borg M, Sidorova A, Mori T, Twell D: **A plant germline-specific integrator of sperm specification and cell cycle progression**. *PLoS Genet* 2009, **5**:e1000430.

50. Okada T, Singh MB, Bhalla PL: **Transcriptome profiling of *Lilium longiflorum* generative cells by cDNA microarray**. *Plant Cell Rep* 2007, **26**:1045-1052.

51. Bourc'his D, Voinnet O: **A small-RNA perspective on gametogenesis, fertilization, and early zygotic development**. *Science* 2010, **330**:617-622.

52. Grant-Downton R, Hafidh S, Twell D, Dickinson HG: **Small RNA pathways are present and functional in the angiosperm male gametophyte**. *Mol Plant* 2009, **2**:500-512.

53. Grant-Downton R, Le Trionnaire G, Schmid R, Rodriguez-Enriquez J, Hafidh S, Mehdi S, Twell D, Dickinson H: **MicroRNA and tasiRNA diversity in mature pollen of *Arabidopsis thaliana***. *BMC Genomics* 2009, **10**:643.

54. Le Trionnaire G, Twell D: **Small RNAs in angiosperm gametophytes: from epigenetics to gamete development**. *Genes Dev* 2010, **24**:1081-1085.

55. Lindroth AM, Cao X, Jackson JP, Zilberman D, McCallum CM, Henikoff S, Jacobsen SE: **Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation**. *Science* 2001, **292**:2077-2080.

56. Cao X, Jacobsen SE: **Role of the arabidopsis DRM methyltransferases in de novo DNA methylation and gene silencing**. *Curr Biol* 2002, **12**:1138-1144.

57. Ingouff M, Sakata T, Li J, Sprunck S, Dresselhaus T, Berger F: **The two male gametes share equal ability to fertilize the egg cell in *Arabidopsis thaliana***. *Curr Biol* 2009, **19**:R19-20.

58. Faure JE, Rusche ML, Thomas A, Keim P, Dumas C, Mogensen HL, Rougier M, Chaboud A: **Double fertilization in maize: the two male gametes from a pollen grain have the ability to fuse with egg cells**. *Plant J* 2003, **33**:1051-1062.

59. Fransz P, Soppe W, Schubert I: **Heterochromatin in interphase nuclei of *Arabidopsis thaliana***. *Chromosome Res* 2003, **11**:227-240.

60. Gehring M, Bubb KL, Henikoff S: **Extensive demethylation of repetitive elements during seed development underlies gene imprinting**. *Science* 2009, **324**:1447-1451.

61. Hsieh TF, Ibarra CA, Silva P, Zemach A, Eshed-Williams L, Fischer RL, Zilberman D: **Genome-wide demethylation of Arabidopsis endosperm**. *Science* 2009, **324**:1451-1454.

62. Lauria M, Rupe M, Guo M, Kranz E, Pirona R, Viotti A, Lund G: **Extensive maternal DNA hypomethylation in the endosperm of *Zea mays***. *Plant Cell* 2004, **16**:510-522.

63. Gutierrez-Marcos JF, Costa LM, Dal Pra M, Scholten S, Kranz E, Perez P, Dickinson HG: **Epigenetic asymmetry of imprinted genes in plant gametes**. *Nat Genet* 2006, **38**:876-878.

64. Jahnke S, Scholten S: **Epigenetic resetting of a gene imprinted in plant embryos**. *Curr Biol* 2009, **19**:1677-1681.

65. Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszkowski J: **Transgenerational stability of the Arabidopsis epigenome is coordinated by CG methylation**. *Cell* 2007, **130**:851-862.

66. Martienssen RA: **Heterochromatin, small RNA and post-fertilization dysgenesis in allopolyploid and interploid hybrids of Arabidopsis**. *New Phytol* 2010, **186**:46-53.

67. Springer NM: **Small RNAs: how seeds remember to obey their mother**. *Curr Biol* 2009, **19**:R649-651.

68. Mosher RA, Melnyk CW: **siRNAs and DNA methylation: seedy epigenetics**. *Trends Plant Sci* 2010, **15**:204-210.

69. • Mosher RA, Melnyk CW, Kelly KA, Dunn RM, Studholme DJ, Baulcombe DC: **Uniparental expression of PolIV-dependent siRNAs in developing endosperm of Arabidopsis**. *Nature* 2009, **460**:283-286.
This study reveals the presence of a complex set of maternally derived siRNAs in the endosperm of developed seeds, targeting essentially transposable elements. These siRNAs are dependent on the maternal activity of RNA PolIV.

70. Feng S, Jacobsen SE, Reik W: **Epigenetic reprogramming in plant and animal development**. *Science* 2010, **330**:622-627.

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

72. Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ: **Resetting of FLOWERING LOCUS C expression after epigenetic repression by vernalization**. *Proc Natl Acad Sci U S A* 2008, **105**:2214-2219.

73. Munir J, Dorn LA, Donohue K, Schmitt J: **The effect of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana* (Brassicaceae)**. *Am J Bot* 2001, **88**:1240-1249.

74. Galloway LF, Etterson JR: **Transgenerational plasticity is adaptive in the wild**. *Science* 2007, **318**:1134-1136.

75. Grimanelli D, Garcia M, Kaszas E, Perotti E, Leblanc O: **Heterochronic expression of sexual reproductive programs during apomictic development in Tripsacum**. *Genetics* 2003, **165**:1521-1531.

76. Grossniklaus U, Nogler GA, van Dijk PJ: **How to avoid sex: the genetic control of gametophytic apomixis**. *Plant Cell* 2001, **13**:1491-1498.

77. Baroux C, Pecinka A, Fuchs J, Schubert I, Grossniklaus U: **The triploid endosperm genome of Arabidopsis adopts a peculiar, parental-dosage-dependent chromatin organization**. *Plant Cell* 2007, **19**:1782-1794.

78. Kohler C, Gaudin V, Hennig L: **Green chromatin dynamics in Zurich: meeting summary based on the European Workshop on Plant Chromatin 2009 in Zurich, Switzerland**. *Epigenetics* 2010, **5**:80-83.